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Complete if Known	
Application Number	TBA
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First Named Inventor	Sadelain
Examiner Name	
Group Art Unit	
Attorney Docket No.	MSK.P-050

METHOD OF PAYMENT

1. ☑ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   - Deposit Account Number: 15-0610
   - Deposit Account Name: Oppedahl & Larson LLP
   - ☑ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   - ☑ Applicant claims small entity status. See 37 CFR 1.27
2. ☐ **Payment Enclosed:** ☐ Check ☐ Credit card ☐ Money Order ☑ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	370.00
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	

SUBTOTAL (1) ($) 370.00

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below	Fee Paid
Total Claims	41	-20** =	21	X	16.00	=378.00
Independent Claims	4	- 3** =	1	X	140.00	=140.00
Multiple Dependent						=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 518.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($)

SUBMITTED BY				Complete *(if applicable)*	
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MSK.P-050
Patent Application

VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Statement Concerning Government Funding

This application was supported by funds provided under NHLBI grant No. HL57612. The United States government may have certain rights in the invention.

Statement Concerning Related Applications

This application claims the benefit of US Provisional Application No. 60/301,861 filed June 29, 2001 and US Provisional Application No. 60/302,852 filed July 2, 2001, both of which are incorporated herein by reference.

Background of the Invention

This application relates to a vector comprising a mammalian, and particularly a human globin gene and to the use thereof in treatment of hemoglobinopathies, including α- and β-thalessemia and sickle-cell disease.

Current treatment modalities for β-thalassemias consist of either red blood cell transfusion plus iron chelation (which extends survival but is cumbersome, expensive and an imperfect therapy), or allogeneic bone marrow transplant (which carries a lethal risk and is not available to the majority of patients). Thus, there is a substantial need for improved therapeutic approaches. The present invention provides a genetic correction in autologous hematopoietic stem cells, thus using gene therapy to provide a less-risky and more effective long-term treatment.

While gene therapy has been proposed for many years, a significant challenge facing efforts to develop gene therapy vectors is the ability to produce therapeutically useful levels of a desired protein or peptide. The present invention provides a vector which is capable of providing therapeutically meaningful levels of human globin for sustained periods of time.

This ability arises from the ability to transmit large genomic regulatory sequences that control expression of the therapeutic gene.

Summary of the Invention

In accordance with the invention, a recombinant lentiviral vector is provided comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control regions which include large portions of DNase I hypersensitive sites HS2, HS3 and HS4. The regions may be the complete site or some lesser site which provides the same functionality as the specific sequences set forth below. This vector provides expression of β-globin when introduced into a mammal, for example a human, *in vivo*. Optionally, the vector further comprises a region encoding a dihydrofolate reductase.

By incorporation of different globin genes, the vector of the invention may be used in treatment of hemoglobinopathies, including α- and β-thalessemia and sickle-cell disease. For example, hematopoietic progenitor or stem cells may be transformed *ex vivo* and then restored to the patient. Selection processes may be used to increase the percentage of transformed cells in the returned population. For example, a selection marker which makes transformed cells more drug resistant than un-transformed cells allows selection by treatment of the cells with the corresponding drug. Selection and/or enrichment may also be carried out *in vivo*, for example using methotrexate or similar antifolates to select for cells rendered resistant by the expression from the vector of a dihydrofolate reductase (DHFR).

Brief Description of the Drawings

Fig. 1 shows the genomic structure of a recombinant onco-retroviral vector in accordance with the invention.

Fig. 2 shows the genomic structure of recombinant onco-retroviral vector within the scope of the invention.

Fig. 3 shows experimental results demonstrating increased mean β-globin expression in transduced MEL cells.

Fig. 4 shows the average vector copy number in peripheral blood cells, measured periodically for 24 weeks, which confirms showed highly efficient gene transfer in cells transduced with the vector of the invention.

Figs. 5A and B show human β-globin expression per endogenous allele 12 days and 22 weeks after introduction of cells transduced with the vector of the invention.

Fig. 6 shows haematocrit level, red blood cell count, reticulocyte count and haemoglobin level fifteen weeks after transplantation with unselected TNS9-transduced $Hbb^{th3/+}$ bone marrow.

Detailed Description of the Invention

In a first aspect of the present invention, a recombinant lentirviral vector is provided comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4.

As used in the specification and claims hereof, the term "recombinant lentiviral vector" refers to an artificially created polynucleotide vector assembled from a lentiviral-vector and a plurality of additional segments as a result of human intervention and manipulation.

The term "functional globin gene" refers to a nucleotide sequence the expression of which leads to a globin that does not produce a hemoglobinopathy phenotype, and which is effective to provide therapeutic benefits to an individual with a defective globin gene. The functional globin gene may encode a wild-type globin appropriate for a mammalian individual to be treated, or it may be a mutant form of globin, preferably one which provides for superior

properties, for example superior oxygen transport properties. The functional globin gene includes both exons and introns, as well as globin promoters and splice doners/acceptors. Suitably, the globin gene may encode α-globin, β-globin, or γ-globin. β-globin promoters may be sued with each of the globin genes.

The recombinant vectors of the invention also include large portions of the locus control region (LCR) which include DNase I hypersensitive sites HS2, HS3 and HS4. In prior studies, smaller nucleotide fragments spanning the core portions of HS2, HS3 and HS4 have been utilized. Sadelain et al. *Proc. Nat'l Acad. Sci. (USA)*92: 6728-6732 (1995); Lebouich et al., *EMBO J.* 13: 3065-3076 (1994). The term "large portions" refers to portions of the locus control region which encompass larger portions of the hypersensitive sites as opposed to previously tested fragments including only the core elements. The regions may be the complete site or some lesser site which provides the same functionality as the specific sequences set forth below. In preferred embodiments of the invention, the large portions of the locus control regions are assembled from multiple fragments, each spanning one of the DNase I hypersensitive sites. In addition, the locus control region has two introduced GATA-1 binding sites at the junction between HS3 and HS4. While not intending to be bound by any specific mechanism, it is believed that the incorporation of these transcription factor binding sites enhances the effectiveness of the vector.

The genomic structure of one embodiment of the vector of the invention (TNS9) is shown in Fig. 1. TNS9 incorporates human β-globin gene (from position -618 to +2484) that includes an extended promoter sequence and a 3'-enhancer element. Optionally, a portion of 3' U3 region of the lentiviral backbone can be deleted for increased safety. In Fig. 1, the exons and introns of the human β-globin gene are represented by filled and open boxes, The locations are indicated for the splice donor (SD), splice acceptor (SA), packaging region (ψ), rev-response element (RRE), human β-globin promoter (P) and 3'-β-globin enhancer (E). Thus, in the vector TNS9, a functional β-globin gene, which includes both the exons and introns of the gene and the relevant control sequences from the human β-globin locus. These are combined with the large

fragments of the locus control region. The 3.2 kb LCR assembled into dTNS9 consists of an 840 bp HS2 fragment (*Sna*BI-*Bst*XI), a 1308 bp HS3 fragment (*Hind*III-*Bam*HI) and a 1069 bp HS4 fragment (*Bam*HI-*Ban*II).

In a further aspect of the invention, the β-globin gene coding sequence can be exchanged and replaced with either the gamma globin gene (for sickle cell disease) or the alpha globin gene (for alpha-thalassemias). In one strategy, a NcoI-Pst I fragment of the β-globin gene is replaced with the corresponding NcoI-HindIII fragment of the gamma globin gene or the NcoI-PstI fragment of the human alpha globin gene. These fragments start at the translational start of each globin gene (spanning the NcoI site) and end past their respective polyadenylation signals. In the second strategy, chimeric genes can be generated by only swapping the coding sequence of each one of the three exons of these genes. Thus, for the gamma globin gene, the result is a vector that comprises the beta globin promoter, the beta globin 5' untranslated region, the gamma exon 1 coding region, the gamma intron 1 the gamma exon 2, the beta intron 2, the gamma exon 3, and the beta 3' untranslated region. Thus all the elements of the TNS9 vector remain in place (promoter, enhancers, 5' and 3' untranslated regions, the LCR elements, the 2 additional GATA-1 binding sites and the introns of the beta globin gene (at least intron 2, which is most important). In a third strategy, the codon usage within exon 3 of the gamma globin gene can be modified so that its sequence will resemble as much as possible that of the beta globin gene. The reason for testing this is that the beta globin gene is always the best expressed.

Additional elements may be included in the vectors of the invention to facilitate utilization of the vector in therapy. For example, the vector may include selectable markers, to confirm the expression of the vector or to provide a basis for selection of transformed cells over untransformed cells, or control markers which allow targeted disruption of transformed cells, and thus the selective removal of such cells should termination of therapy become necessary.

In a further specific embodiment, the vector of the invention includes the mouse PGK promotor and human dihydrofolate reductase (DHFR) cDNA as a transcriptional unit. Mutant forms of DHFR which increase the capacity of the DHFR to confer resistance to drugs

such as methotrexate are suitably used. For example, single and double mutants of DHFR with mutations at amino acids 22 and 31 as described in commonly assigned PCT Publication No. WO 97/33988, which is incorporated herein by reference, may be advantageously utilized.

Fig. 2 shows the genomic structure of specific vector within the scope of the invention. The vector includes a deleted LTR, from -456 to -9 of HIV LTR and the PGK promoter (530 bp) from the murine phosphoglycerate kinase 1 gene. It also includes a DHFR-encoding region encoding human DHFR with s/f mutation at amino acid 22. The locus control region and the β-globin region are the same as in TSN9. This vector is designated dTNS9-PD. This incorporation of DHFR into this vector provides transformed cells with a methotrexate-resistant phenotype. As a result, methotrexate, and other antifolates can be used, both *in vitro* and *in vivo* as a selection to tool to enhance levels of the functional hemoglobin. When hematopoietic stem cells were transformed using dTNS9-PD and reintroduced to mice that were then treated with NMBPR-P (0.5 mg/dose) and TMTX (0.5 mg dose) for five days, observed levels of expressed human β-globin were much higher in mice transduced with dTNS9-PD vectors after treatment with TMTX and NMBPR-P for selection of transduced cells.

The vectors of the invention are used in therapy for treatment of individuals suffering from hemoglobinopathies. In one embodiment of the invention, hematopoietic progenitor or stem cells are transformed *ex vivo* and then restored to the patient. As usedin the specification and claims hereof, the term "hematopoietic progenitor sand stem cells" encompasses hematopoietic cells and non-hematopoietic stem cells, e.g., embryonic stem cells, hematopoietic stem cell precursors, or any of the latter generated by nuclear transfer from a somatic cell. It is know in the art that efficient genes transfer into human embryonic stem cells can be achieved using lentiviral vectors.

Selection processes may be used to increase the percentage of transformed cells in the returned population. For example, a selection marker which makes transformed cells more drug resistant than un-transformed cells allows selection by treatment of the cells with the

corresponding drug. When DHFR is used as a selection marker, it can be used for enrichment of transduced cells *in vitro,* or for *in vivo* selection to maintain the effectiveness of the vector.

The invention will now be further described with reference to the following non-limiting examples.

Example 1

To produce vector TNS9, the human β-globin gene was subcloned from Mβ6L (Sadelain et al. *Proc. Nat'l Acad. Sci. (USA)*92: 6728-6732 (1995)) into lentiviral vector pHR'LacZ (Zuffery et al., Nature 15: 871-875 (1997)) replacing the CMV-LacZ sequence. pHR'eGFP was constructed by replacing LacZ with the eGFP sequence (Clontech). Viral stocks were generated by triple transfection of the recombinant vectors pCMVΔR8.9 (Zuffrey et al.) and pMD.G in 293T cells as previously described in Dull, et al., *J. Virol.* 72: 8463-8471 (1998). The pseudotyped virions were concentrated by ultracentrifugationm resuspended and titrated as described in Gallardo et al., *Blood* 90: 952-957 (1997). For comparison, RSN1 was used which has a similar structure, except that the LCR contains only the core portion of HS2, HS3 and HS4. Northern blot analysis showed full length RNA transcripts, indicating that the recombinant lentiviral genomoes are stable. Southern blot analysis on genomic DNA from transduced cells, digested once in each long terminal repeat (LTR) results in a single band corresponding to the expected size for the vector, indicating that the proviral structure is not rearranged.

Example 2

To investigate the tissue specificity, stage specificity and expression level of the vector-encoded human B-globin gene, we transduced RNS1 and TNS9 into MEL cells, lymphoid Jurkat cells and myeloid HL-60 cells. Cell-free viral supernatant was used to infect C88 MEL cells in the presence of polybrene (8 $\mu g\ ml^{-1}$). Transduced MEL cells were subcloned by limiting dilution, and screened by PCR for transduction[30] using primers that anneal in the human β-globin promoter sequence (βPS, 5' -GTCTAAGTGATGACAGCCGTACCTG-3') and in HS2 (C2A, 5'-

TCAGCCTAGAGT GATGACTCC TATCTG-3'). Vector copy number and integration site analysis was determined by Southern blot analysis[9]. Transduced MEL cells were induced to maturation by 5-day culture in 5 mM N,N'- hexamethylene bisacetamide (HMBA, Sigma).

To induce β-globin transcription, transduced MEL cell pools were differentiated using hexamethylene bisacetamide HMBA). Human β-globin (β^A) and mouse β-globin transcripts were measured by quantitative primer extension. After normalization to vector copy number and to endogenous β-globin expression per allele, human β-globin levels were 14.2 ± 4.7% for RNS1 and 71.3 ± 2.3% for TNS9 in pooled MEL cells (Fig. 2a). MEL, Jurkat and HL-60 cells were transduced with RNS1, TNS9 or control GFP recombinant lentivirus. Human β-globin RNA expression in HMBA induced MEL cells (grey bars) was measured by quantitative primer extension and normalized to mouse β-globin RNA expression per locus. Expression was then normalized to the vector copy number determined by Southern blot. No human β-globin RNA expression was detected in non-induced MEL (black bars), Jurkat (white bars) or HL-60 cells (hatched bars), indicating that globin expression was erythroid- and differentiation-specific. No human β-globin expression was detected in non-induced MEL, Jurkat and HL-60 cells (Fig. 3), indicating that human β-globin expression was appropriately regulated in terms of tissue specificity and state of differentiation. We generated a panel of MEL cell clones that carried a single copy of either vector to distinguish whether the increased expression obtained in HMBA-treated Mel cells transduced with TNS9 rather than RNS1 was the result of an increase in β^A expression per cell or of an increase in the fraction of cells expressing human β-globin. Transduced MEL cells were subcloned by limiting dilution immediately after transduction, avoiding any bias towards favourable chromosomal integration sites as produced by drug selection[5]. The proportion of clones expressing human β-globin varied significantly between the two vectors. One out of ten RNS1 positive clones yielded measurable human β-globin transcripts, in contrast to 12 out of 12 for TNS9 also expressed higher levels of human β-globin than did those bearing RNS1 ($P < 0.01$, Fisher's exact test). Cells bearing TNS9 also expressed higher levels of human β-globin than did those bearing RNS1 ($P < 0.01$, Wilcoxon rank sum

test). These findings established that both the level and probability of expression at random integration sites was increased with the TNS9 vector.

Example 3

Quantification of human β-globin mRNA

Total RNA was extracted from MEL, Jurkat and HL-60 cells, or mouse spleen and blood using TRIzol. Quantitative primer extension assays were done using the Primer Extension System-AMV Reverse Transcriptase kit (Promega) with [^{32}P] dATP end-labelled primers specific for retroviral-derived human β-globin (5' -CAGTAACGGCAGACTTCTCCTC -3') and mouse β-globin (5' -TGATGTCTGTTTCTGGGGTT GTG -3'), with predicted extension products of 90 bp and 53 bp, respectively. The probes yield products of identical length for β^{maj}, β^{min}, β^{s} and β^{t}. Primers were annealed to 4μg of RNA and reactions were run according to manufacturer's protocols. Radioactive bands were quantitated by phosphorimager analysis (BioRad). RNA isolated from A85.68 mice[20] was used as positive control. After correction for primer labelling, the human to mouse RNA signal was 29 ± 1% per gene copy in repeated experiments (n > 8), in agreement with previous findings based on RT-PCR[20]. Values measured in bone marrow chimaeras that were obtained in separate runs were standardized to the value obtained in the A85.68 RNA sample. In Figs. 2 and 3c, d, human β-globin expression is expressed per vector copy and normalized to the endogenous transcripts (accounting for two endogenous alleles). In Fig. 3b, human transcripts are reported as the fraction of total β-globin RNA (Huβ / Huβ + Muβ) to reflect absolute contribution of vector-encoded transcripts.

Example 4

To investigate the function of the vectors *in vivo*, we transduced and transplanted murine bone marrow cells without any selection in syngeneic, lethally irradiated recipient mice. Donor bone marrow was flushed from the femurs of 8- to 16-week-old male C57BL/6 or $Hbb^{th3/+\ mice}$ (Jackson Laboratories) that had been injected intravenously (i.v.) 6 days earlier with 5-flurouracil

(5-FU, Pharmacia; 150 mg kg^{-1} body weight). Bone marrow cells were resuspended in serum-free medium, and supplemented with IL-1α (10 ng ml^{-1}), IL-3 (100 U ml^{-1}), IL-6 (150 U ml^{-1}), Kit ligand (10 ng ml^{-1}) (Genzyme), β-mercaptoethanol (0.5 mM; Sigma), $_L$-glutamine (200 mM), penicillin (100 IU ml^{-1}) and streptomycin (100 μg m^{-1}), and cultured for 18 h. Recipient mice (11- to 14-week-old C57/BL6 or $Hbb^{th3/+}$ mice) were irradiated with 10.5 Gy (split dose 2 x 5.25 Gy) on the day of transplantation. Bone marrow cells were pelleted and resuspended in serum-free medium containing concentrated lentiviral supernatant, and supplemented with polybrene (8μg ml^{-1}), $_L$-glutamine (200 mM), penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1}), and cultured for 6 h. Transduced bone marrow cells (1 x 10^5 or 5 x 10^5) were then i.v. injected into each of the irradiated female recipients to establish short-term and long-term bone marrow chimaeras, respectively.

In short-term studies, spleens were removed 12 d after transplantation to extract total RNA and genomic DNA. To monitor long-term chimaeras, two or three capillary tubes of blood were collected every 4-6 weeks, from which genomic DNA, total RNA and haemoglobin were extracted. To examine vector function reliably in long-term animals, erythroid cell populations were purified from spleen. Single-cell suspensions were incubated with rat anti-mouse TER-119 monoclonal antibody (PharMingen). Sheep anti-Rat IgG dynabeads (M-450, Dynal Inc.) Were added to the antibody-coated spleen cells and purified as recommended by the manufacturer. Vector copy number, integration pattern and chimaerism were determined by Southern blot analysis. The fraction of donor DNA relative to recipient was determined by stripping and reprobing the blot with a [^{32}P] dCTP-labelled probe specific for the Y chromosome and normalizing to an endogenous mouse band. Radioactive bands were quantitated by phosphorimager analysis. Sera from five randomly selected long-term bone marrow chimaeras (30 weeks after transplantation) tested negative for HIV-1 *gag* by RT-PCR using the Amplicor HIV-1 monitor kit (Roche).

Vector copy number and human β-globin RNA transcripts were measured during a 24-week period in mice transplanted with RNS1 (n = 8) or TNS9 (n = 10) transduced bone marrow.

a, Vector copy number was assessed by southern blot analysis of genomic DNA isolated from peripheral blood at weeks 6, 10, 16 and 24. The average vector copy number in peripheral blood cells, measured periodically for 24 weeks (Fig. 4), showed highly efficient gene transfer with both vectors (1.8 ± 0.6 and 0.8 ± 0.6 average vector copies per cell for β-globin transcript levels in the 10-20% range during the same time period. To assess transcriptional activity per vector copy, steady-state RNA transcripts and vector copy number were quantified in pooled CFU-S_{12} and in erythroid TER-119+ spleen cells. Twelve days after transplantation, human β-globin expression per endogenous allele, (Fig. 5a). Twenty weeks later these values were 0.5 ± 0.1% (significantly lower than on day 12, P = 0.02) and 15.8 ± 0.9% respectively (Fig. 5b). These findings established that the larger LCR fragments increased globin expression *in vivo* and, furthermore, suggested that TNS9 is more resistant to transcriptional silencing than is RNS1.

The levels of TNS9-encoded human β-globin could be produced. Haemoglobin tetramers incorporating vector-encoded human β^A and endogenous murine α-globin (designated Hbb^{hu}) were quantitated in peripheral blood red cell lysates after cellulose acetate gel fractionation. Hbb^{hu} levels accounting for up to 13% of total haemoglobin were found 24 weeks after transplantation (Fig. 3e, Table 1 in Supplementary Information). In the same assays, transgenic mice bearing one copy of a 230-kb yeast artificial chromosome (YAC) encompassing the entire human β-globin like gene cluster[20] showed 14% of their total haemoglobin incorporating human β^A. No haemoglobin tetramers containing human β^A were measurble in any of the mice bearing RNS1 (table 1 in Supplementary Information). The proportion of mature peripheral blood red cells expressing human β^A was elevate in most TNS9 bone marrow chimaeras, as shown by dual staining of human β^A and TER-119. In contrast, chimaeras engrafted with RNS1-transduced bone marrow showed highly variable fractions of weakly staining β^A -positive erythrocytes. Normalized to the fraction of circulating β^A -positive mature red cells, the levels of haemoglobin containing lentivirus-encoded β^A were on average 64% of those obtained in the YAC transgenic mice.

Example 5

To ascertain that true HSCs were transduced, we carried out secondary transplants using marrow from primary recipients collected 24 weeks after transplantation. The TNS9 and RNS1 vectors were readily detected in all secondary recipients 13 weeks after transplantation. Human β-globin expression was maintained in all recipients of TNS9-transduced marrow. The successful transduction of HSCs was confirmed by integration site analyses. Southern blot analysis was performed on genomic DNA isolated from bone marrow, spleen and thymus of secondary bone marrow transplant recipients collected 13 weeks after transplant (one representative RNS1, and two representative TNS9 secondary transplant recipients are shown). Two endogenous bands are found in the genomic DNA of C57BL/6 (B6) mice.

Example 6

In view of the high levels of expression, we tested the extent to which the TNS9 vector could correct the phenotype of thalassaemic cells using β^0 -thalassaemic heterozygote mice that lack a copy of their b1 and b2 β-globin genes ($Hbb^{th3/+}$)[21]. These heterozygotes have a clinical phenotype similar to human thalassaemia intermedia and exhibit chronic anaemia (haematocrit 28-30%, haemoglobin 8-9 g dl^{-1}) and anomalies in red cell size (anisocytosis) and shape (poikilocytosis). Fifteen weeks after transplantation with unselected TNS9-transduced $Hbb^{th3/+}$ bone marrow, the haematocrit level, red blood cell count, reticulocyte count and haemoglobin level were markedly improved in five out of five recipient mice (Fig. 6). Anisocytosis and poikilocytosis were markedly reduced in the peripheral blood smears of chimaeras bearing the TNS9 vector. Control mice transplanted with $Hbb^{th3/+}$ bone marrow cells transduced with a vector encoding enhanced green fluorescent protein (eGFP) all remained severely anaemic (n = 5, Fig. 6) and maintained their abnormal red cell morphology. These results establish that levels of circulating haemoglobin obtained with TNS9 were indeed therapeutically relevant.

The combined effect of the high efficiency of gene transfer and the absence of vector rearrangements afforded by the recombinant lentivirus carrying the β-globin gene and LCR

configuration adopted in TNS9 yielded levels of human β^A expression in the therapeutic range. The higher expression obtained with TNS9 compared with RNS1 was associated with a higher fraction of permissive integration sites in MEL cells and a higher fraction of human β^A - containing red blood cells in bone marrow chimaeras. RNS1, which carries a weaker enhancer, silenced over time whereas TNS9 retained undiminished transcriptional activity over the same time period and in secondary transplant recipients.

Higher levels of murine α_2: human $\beta^A{}_2$ tetramers were obtained in peripheral blood samples from recipients of TNS9-transduced $Hbb^{th3/+}$ bone marrow (21 ± 3% of total haemoglobin, n = 5, than with $Hbb^{+/+}$ bone marrow (6 ± 4%, n + 10). The two groups showed comparable peripheral blood vector copy numbers and levels of human β-globin RNA (0.8 ± 0.2 compared with 0.8 ± 0.6, and 16.8 ± 6% compared with 10.8 ± 7%, respectively). This observation is consistent with a competitive advantage of murine β-globin over human β-globin in associating with murine α-globin[22]. In thalassaemic patients, added human β-chain synthesis would improve the α:β chain imbalance and thus increase red cell survival and ameliorate the ineffective erythropiesis in these patients. In patients with sickle cell disease, transduced β^A chains are expected to have an advantage over the β^S chains produced by both endogenous genes in competing for the available α-chains[23]. Given that patients with S/β-thalassaemia whose HbA represents 10-30% of their total haemoglobin are very mildly affected[1,24], the clinical benefit of such an intervention would be highly significant.

Example 7

To investigate long-term expression of the transduced human *β-globin* genes and its therapeutic efficacy, we generated bone marrow chimeras engrafted with TNS9-transduced $Hbb^{th3/+}$ bone marrow cells (n =5) and studied them over a 40-week period.

Donor bone marrow was flushed from the temurs of 8-to 16- week old male c57/BL6 or $Hbb^{th3/+}$ mice [23] obtain from Jackson Laboratories (Bar Harbor , ME) that had been injected intravenously (IV) 6 days earlier with 5-flurouracil (5-FU) 150 mg/kg body weight obtained from

Pharmacia (Piscataway, NJ). Bone marrow cells were resuspended in X-VIVO-15 serum-free medium and supplemented with 10 ng/mL interleukin-1 α (IL-1α) 100 U/mL IL-3, 150 U/mL IL-6, 10ng/mL Kit ligand obtained from Genzyme (Cambridge, MA), 0.5mM β-mercaptoethanol obtained from Sigma (St. Louis, MO), 200-mM $_L$-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Bone marrow cells were ten pelleted and resuspended in serrum-free medium containing concentrated lentiviral supernatant and supplemented with 8 mg/mL polybrene (Sigma), 200mM $_L$-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and cytokines as above, and cultureed for 8 hours. Transduced bone marrow cells (5×10^5) were ten injected IV into each of the irradiated female recipients to establish bone marrow chimeras. Recipients mice (11- to 14-week-old C57/BL6 or $Hbb^{th3/+}$ mice) were irradiated with 10.5 Gy (Split dose 2 × 5.25 Gy) on the day of transplantation.

Age-matched chimeras engrafted with eGFP-transduced $Hbb^{th3/+}$ (n=5) and Hbb $^{+/+}$ (n=5) bone marrow cells served as controls. Vector copy number was monitored in peripheral blood by quantitive Southern blot analysis, and found to remain stable, between 0.5 and 1.0 copy/cell on average (data not shown). Protein expression was assessed by quantitive hemoglobin analysis, to measure the proportion of hemoglobin tetramers that incorporate human β^A (Hbb^{hu}, mu α_2: hu$\beta^A{}_2$) or murine β-globin (Hbb ^{mu}, muα_2:muβ_2), and immunofluorescence, to determine the fraction of mature RBCs that contain human β^A protein. Transgenic mice bearing one copy of a 230-kb yeast artificial chromosome encompassing the entire human β-globin-like gene cluster[28] served as reference, showing 14% of their total hemoglobin incorporating human β^A and 100% β^A+RBCs[19,28] Hbb^{hu} accounted for 19% to 22% of the totalhemoglobim in TNS9 chimeras. These levels remained stable up to 40 weeks after transplantation. Over this same time period, the proportion of mature peripheral RBCs expressing human β^A also remained elevated and stable (about 70% to 80%), as shown by dual staining of human β^A and TER-119.

Example 8

Long-Term amelioration of anemia

The stability of TNS9-encoded β^A expression detected in peripheral blood suggested that long-trem hematologic and systemic therapeutic benefits could be obtained. To investigate whether Hbb^{hu} production would suffice to teart the anemia, we closely monitored hemoglobin parameters over 40 weeks. The marked increase in hemoglobin concentration, RBC counts, and hematocrit was sustained throughout this time period. Control mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells remained severely anemic, indicating that the transplantation procedure itself did not alter the anemic state. The reticulocyte counts decreased to 5% to 8% in TNS9 treated-chimeras, compared to 19% to 21% in control eGFP-treated $Hbb^{th3/+}$ chimeras and age-matched $Hbb^{th3/+}$ mice, suggesting an increase in RBC life span and a decreasein erythropoietic activity.

Example 9

To determine the impact of sustained human β-globin gene expression on hematopoiesis, we studied the degree of splenomegaly (enlargement of the spleen) and EMH in 1-year-old chimeras and age-mateched control mice. Spleen weights measured in Tns9-treated $Hbb^{th3/+}$ chimeraas were indistinguishable from recipients of eGFP-transduced normal bone marrow, as were the total number of cells per spleen. In contrast, mice engrafted with eGFP-transduced $Hbb^{th3/+}$ bone marrow cells showed spleen weights and total cell numbers that were about 3-fold greater. The correction of spleen weight in TNS9 bone marrow chimeras corresponded to a concomitant normalization in total hematopoietic progenitor cell content. Spleen CFU-Es, BFUEs, andCFUs-GM were reduced to levelsmeasured in recipients of eGFP-transduced $Hbb^{th+/+}$ bone marrow, whereas they remained elevated in control chimeras engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells and in age-matched $Hbb^{th3/+}$ mice, as previously observed in another murine modelof β-thalassemis.[29]

The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and age-match controls. Histopathology of spleens of mice that received transplants of eGFP-tranduced $Hbb^{th3/+}$ marrow was virtually identical to that of slpeen from control $Hbb^{th3/+}$ mice. Specifically, the red pulp was significantly expanded, accounting for 80% to 90% of the cross-sectional area, and densely occupied by nucleated erythroid precursors. The white pulp, based on cross-sectional area, wsa relatively decreased and the marginal zones were obscured by the large number of nucleated RBCs, reflecting major expansion of the red pulp and erythroid precursors. In TNS9-treated chimeras, the amount of red pulp was considerably decreased, accounting for olny about 50% to 60% of the cross-sectional area. In addition, the number of nucleated erythroid precursors in the red pulp was decreased. Other immature hematopoietic cells were present in the red pulp, but much less frequently than in the spleens of control $Hbb^{th3/+}$ thalassemic mice. The livers from TNS9-treated chimeras were similar to those of the normal control mice in that no EMH was detected. In contrast, livers from mice engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells showed seral small foci of intrasinusoidal EMH.

Example 10

Toxic iron accumulation in the organs of thalassemic patients is a consequence of RBC destruction and increased gastrointestinal iron uptake. To determine whether sustained expression from the TNS9 vector reduced iron overload, we stuied tissue sections of liver and heart, stained using Gomori iron stain. No iron deposition was seen in the livers of normal $Hbb^{+/+}$ control mice, whereas $Hbb^{th3/+}$ mice showed variable amounts of iron, including some large aggregates. TNS9-trasduced treated chimeras demonstrated low to undetectable levels of iron in the livers, whereas iron was readily detected in the livers of all mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells. No iron accumulation was found in the heart of treated or control mice, as previously observed in another murine model of β-thalassemia,[30] in contrast to what is found in the human disease. [1-3]

Example 11

To assess to efficacy of *in vivo* selection for cells transduced with globin and DHFR-encoding vectors in accordance with the invention, using antifolates the following alternative protocols are used. In protocol 1, the recipient mice are treated daily for 5 days with MTX (25mg/Kg) and NBMPR-P (20mg/Kg), starting 6 weeks after administration of transduced bone marrow cells. In protocol 2, the recipient mice are treated daily for 5 days with TMTX (40mg/Kg) and NBMPR-P (20mg/Kg), starting 6 weeks after administration of transduced bone marrow cells. In protocol 3, the recipient mice, conditioned with busulphan rather than with gamma-irradiation, are treated daily for 5 days with TMTX (40mg/Kg) and NBMPR-P (20mg/Kg), starting 4 weeks after administration of transduced bone marrow cells. (TMTX (Neutrexin; US Bioscience);
>MTX (Methotrexate LPF Sodium; Immunex); NBMPR-P (Nitrobenzylthioinosine 5'-monophpsphate disodium salt; Alberta nucleoside therapeutics). Protocol 3 is in principle the most attractive protocol as the recipients are not irradiated and furthermore not treated with a "myeloablative conditioning regimen". They are treated with a
relatively milder conditioning regimen consisting of a "non-myeloablative" dose of busulphan. It is hoped that, in combination with "in vivo selection" mediated by DHFR/TMTX, the recipients could be satisfactorily engrafted without receiving a
harsh pre-transplant treatment. This would be the way to go for treating subjects with severe hemoglobinopathies.

What is claimed is:

1. A recombinant lentiviral vector comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control region, which include DNase I hypersensitive sites HS2, HS3 and HS4, said vector providing expression of the globin gene when introduced into a mammal *in vivo*.

2. The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

4. The vector of claim 3, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

5. The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. The vector of claim 2, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

8. The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. The vector of claim 1, wherein the functional globin gene encodes human β-globin.

11. The vector of claim 10, further comprising a region encoding a dihydrofolate reductase.

12. The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

13. The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human

dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. The vector of claim 11, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

17. The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19. A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant lentiviral vector comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control region, which include DNase I hypersensitive sites HS2, HS3 and HS4, said vector providing expression of β-globin when introduced into a mammal *in vivo*; and

expressing the functional globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

22. The method of claim 21, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

23. The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

26. The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate,

28. The method of claim 27, wherein the antifolate is methotrexate.

29. The method of claim 19, wherein the globin gene encodes human β-globin.

30. A mammalian hematopoietic progenitor or stem cell transduced with a recombinant lentivector comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4, wherein said cells express the functional β-globin gene.

31. The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

34. The transduced cell of claim 33, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

35. The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

36. The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. The transduced cell of claim 30, wherein the globin gene encodes human β-globin.

39. A method for making a therapeutic composition for treatment of hemoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising:

(a) a region comprising a functional globin gene; and

(b) large portions of the β-globin locus control region, which include DNase I hypersensitive sites HS2, HS3 and HS4, said vector providing expression of the globin gene when introduced into a mammal *in vivo.*, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. The method of claim 39, wherein the globin gene encodes human β-globin.

ABSTRACT OF THE DISCLOSURE

Recombinant lentiviral vectors having a region encoding a functional β-globin gene; and large portions of the β-globin locus control regions which include DNase I hypersensitive sites HS2, HS3 and HS4 provides expression of β-globin when introduced into a mammal, for example a human, *in vivo*. Optionally, the vector further includes a region encoding a dihydrofolate reductase. The vector may be used in treatment of hemoglobinopathies, including β-thalessemia and sickle-cell disease. For example, hematopoietic progenitor or stem cells may be transformed *ex vivo* and then restored to the patient. Selection processes may be used to increase the percentage of transformed cells in the returned population. For example, a selection marker which makes transformed cells more drug resistant than un-transformed cells allows selection by treatment of the cells with the corresponding drug.

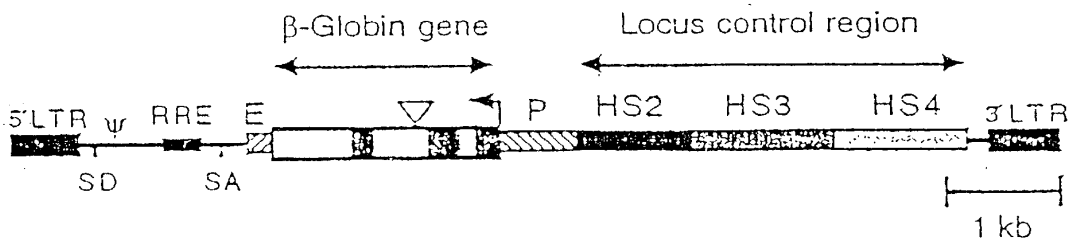

β-Globin gene
Locus control region
5'LTR
ψ
RRE
E
P
HS2
HS3
HS4
3'LTR
SD
SA
1 kb

Fig. 1

Clinical Use of Drug Resistance

In Vivo Selection of Genetically Modified Stem Cells

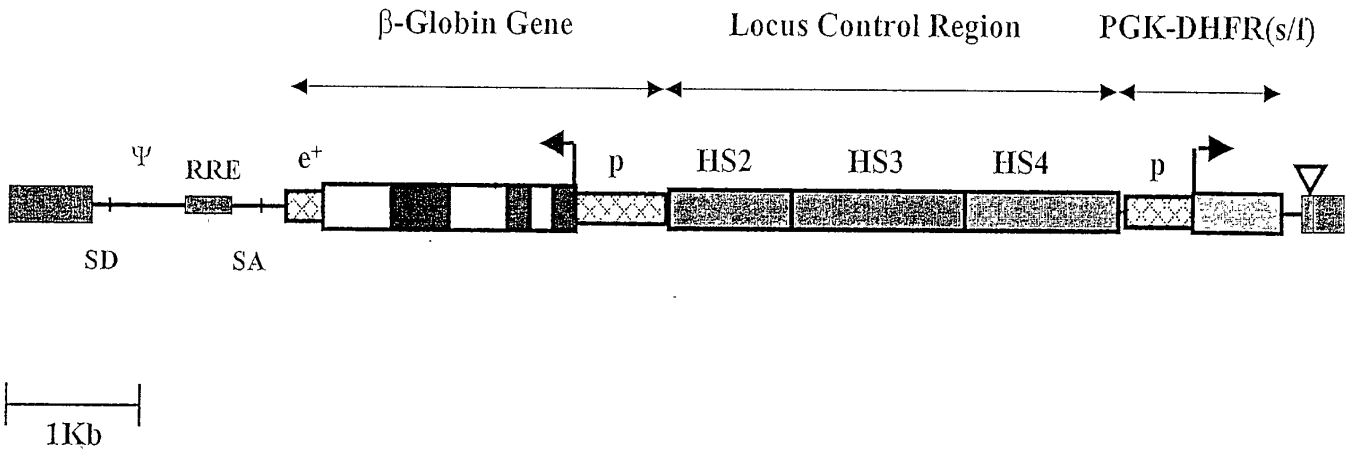
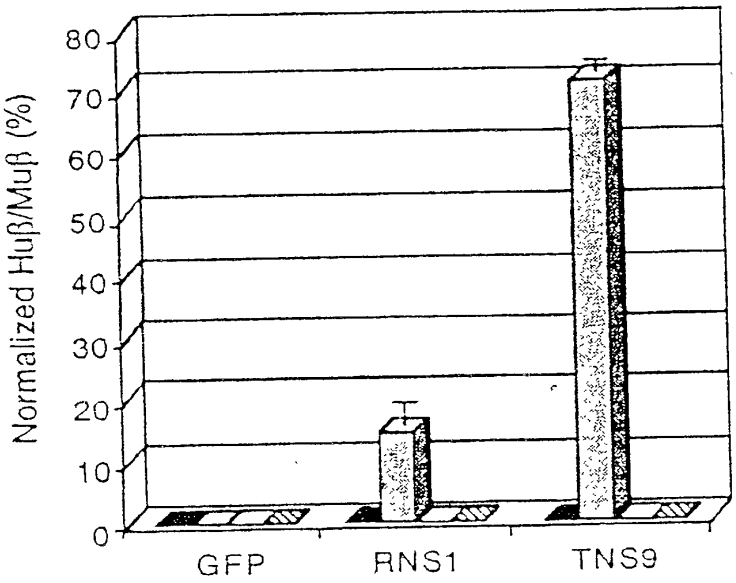


Fig. 2

10193521.070102

3/4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30
20
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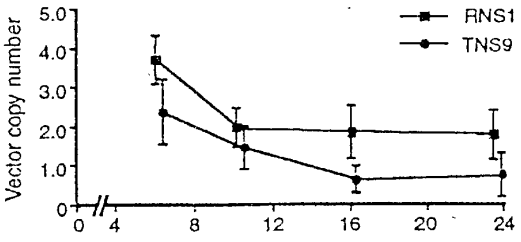
RNS1
TNS9

Fig. 3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12
16
20
24
RNS1
TNS9

Fig. 4

Fig. 5 A

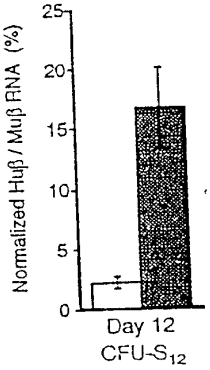
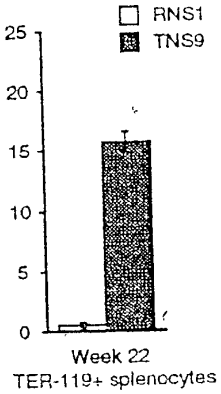


Fig. 5 B



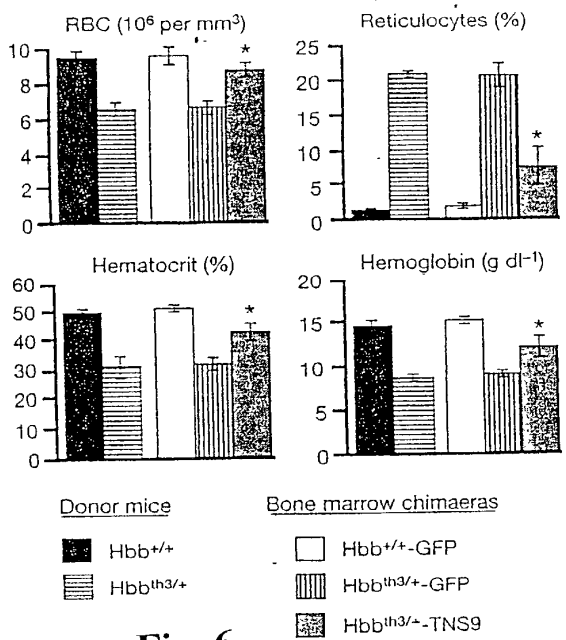


Fig. 6

07-03-02 10188221.070102

PTO/SB/05 (03-01)
Approved for use through 10/31/2002. OMB 0651-0032
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	MSK.P-050
First Inventor	SADELAIN
Title	Vector Encoding Human
Express Mail Label No.	EL556129086US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Box Patent Application, Washington, DC 20231

1. [✔] Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. [✔] Applicant claims small entity status. See 37 CFR 1.27.
3. [✔] Specification [Total Pages 25]
   (preferred arrangement set forth below)
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. [✔] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 4]
5. Oath or Declaration [Total Pages]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63 (d)) *(for continuation/divisional with Box 18 completed)*
   i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1 63(d)(2) and 1.33(b).
6. [ ] Application Data Sheet. See 37 CFR 1.76
7. [ ] CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] paper
   c. [ ] Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. [ ] Assignment Papers (cover sheet & document(s))
10. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
11. [ ] English Translation Document *(if applicable)*
12. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
13. [ ] Preliminary Amendment
14. [ ] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. [ ] Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. [ ] Other: .....

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No.: ____/____

Prior application information: *Examiner:* ____ *Group Art Unit:* ____

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

[✔] *Customer Number or Bar Code Label* (Insert Customer No. or Attach bar code label here) 021121 or [ ] *Correspondence address below*

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Address					
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Country		Telephone		Fax	

Name (Print/Type)	MARINA T. LARSON, PH.D.	Registration No. (Attorney/Agent)	32,038
Signature	Marina T Larson	Date	07/01/2002

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PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

07/10/2002 HLE333 00000055 10188221

01 FC:201	370.00 OP
02 FC:202	42.00 OP
03 FC:203	189.00 OP

PTO-1556
(5/87)

*U.S. Government Printing Office: 2001 — 481-697/59173

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2001

Application or Docket Number: 10/582,21

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	41	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	41 minus 20=	* 21
INDEPENDENT CLAIMS	4 minus 3 =	* 1
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	370.00	OR	BASIC FEE	740.00
X$ 9=	189	OR	X$18=	
X42=	42	OR	X84=	
+140=		OR	+280=	
TOTAL	601	OR	TOTAL	

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/188,221	07/01/2002	Sadelain	MSK.P-050

CONFIRMATION NO. 9026

021121
OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

FORMALITIES LETTER

OC000000008784865

Date Mailed: 09/13/2002

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is missing.
  A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(l) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

Items Required To Avoid Processing Delays:

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$65** for a Small Entity

- **$65** Late oath or declaration Surcharge.

A copy of this notice MUST be returned with the reply.

margaret

Customer Service Center
Initial Patent Examination Division (703) 308-1202

PART 3 - OFFICE COPY

OIPE JC184 OCT 08 2002 PATENT & TRADEMARK OFFICE

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My citizenship, residence and post office address are as listed below next to my name.

I believe I am the original, first and [ ] sole/[X] joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: ***Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies***

the specification of which

(a) [ ] is attached hereto.

(b) [X] was filed on July 1, 2002 as Application Serial No. 10/188221 and was amended on _____.

(c) [ ] was described and claimed in International Application No. _____ filed on _____ and amended on _____.

Acknowledgment of Duty of Disclosure

I hereby state that I have reviewed and understood the content of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

35 U.S.C. § 120

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)

Power of Attorney

I hereby appoint the practitioners at Customer Number 021121 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
[redacted] **021121** PATENT TRADEMARK OFFICE	OPPEDAHL & LARSON LLP (970)468-6600

Claim f r Pri rity

I hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign applications for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), FILED WITHIN TWELVE MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	PRIORITY CLAIMED	CERTIFIED COPY ATTACHED
				YES[ ] NO[ ]	YES[ ] NO[ ]
FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)		

Provisional Application

I hereby claim the benefit under 35 U.S.C § 119(e) of any United States provisional application(s) listed below.

60/301,861 — JUNE 29, 2001
(application number) (filing date)

60/302,852 — JULY 2, 2001
(application number) (filing date)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	SADELAIN	MICHEL	
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE	STATE OR COUNTRY OF RESIDENCE	COUNTRY OF CITIZENSHIP
	New York	New York	USA
POST OFFICE ADDRESS 401 E. 89TH STREET, APT. 9K		CITY New York	STATE/COUNTRY ZIP CODE New York 10028
DATE Aug 23, 2002		SIGNATURE Michel	

[X] Signature for additional joint inventor attached. Numer of Pages 1.
[ ] Signature by Administrator(trix) or legal representative for deceased or incapacitated inventor. Number of Pages ___.
[ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR § 1.47. Number of Pages ___.

SUBSCRIBED AND SWORN TO BEFORE ME
THIS 23 DAY OF August 2002
Marilyn Chibowski
NOTARY PUBLIC

MARILYN CHIBOWSKI
NOTARY PUBLIC, STATE OF NEW YORK
NO. 01CH5076602
COMMISSIONED AND QUALIFIED
IN ROCKLAND COUNTY
MISSION ENDS 4/21/03

For Michel Sadelain

NAME OF SECOND INVENTOR	LAST NAME RIVELLA	FIRST NAME STEFANO	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP Italy
POST OFFICE ADDRESS 736 West End Avenue Apt. 5B		CITY New York	STATE/COUNTRY ZIP CODE New York 10025
DATE 8/23/02		SIGNATURE	

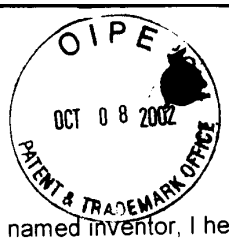
NAME OF THIRD INVENTOR	LAST NAME MAY	FIRST NAME CHAD	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS 220 Avenue A Apt. 3C		CITY New York	STATE/COUNTRY ZIP CODE New York 10009
DATE Aug. 21, 2002		SIGNATURE	

NAME OF FOURTH INVENTOR	LAST NAME BERTINO	FIRST NAME JOSEPH	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS 117 Sunset Hill Dr.		CITY Branford	STATE/COUNTRY ZIP CODE CT 06405
DATE		SIGNATURE	

SUBSCRIBED AND SWORN TO BEFORE ME
THIS 23 DAY OF August 20 02
Marilyn Chibowski
NOTARY PUBLIC

For. Stefano R. Vella

MARILYN CHIBOWSKI
NOTARY PUBLIC, STATE OF NEW YORK
NO. 01CH5076602
COMMISSIONED AND QUALIFIED
IN ROCKLAND COUNTY
MISSION ENDS 4/2/03



COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My citizenship, residence and post office address are as listed below next to my name.

I believe I am the original, first and [ ] sole/[X] joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: ***Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies***

the specification of which

(a) [ ] is attached hereto.

(b) [X] was filed on July 1, 2002 as Application Serial No. 10/188221 and was amended on _____.

(c) [ ] was described and claimed in International Application No. _____ filed on _____ and amended on _____.

Acknowledgment of Duty of Disclosure

I hereby state that I have reviewed and understood the content of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

35 U.S.C. § 120

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)

Power of Attorney

I hereby appoint the practitioners at Customer Number 021121 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
021121 PATENT TRADEMARK OFFICE	OPPEDAHL & LARSON LLP (970)468-6600

Claim for Priority

I hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign applications for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), FILED WITHIN TWELVE MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	PRIORITY CLAIMED	CERTIFIED COPY ATTACHED
				YES[ ] NO[ ]	YES[ ] NO[ ]
FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)		

Provisional Application

I hereby claim the benefit under 35 U.S.C § 119(e) of any United States provisional application(s) listed below.

60/301,861 JUNE 29, 2001
(application number) (filing date)

60/302,852 JULY 2, 2001
(application number) (filing date)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR	LAST NAME SADELAIN	FIRST NAME MICHEL	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS 401 E. 89TH STREET, APT. 9K		CITY New York	STATE/COUNTRY ZIP CODE New York 10028
DATE		SIGNATURE	

[X] Signature for additional joint inventor attached. Numer of Pages 1.
[ ] Signature by Administrator(trix) or legal representative for deceased or incapacitated inventor. Number of Pages ___.
[ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR § 1.47. Number of Pages __.

NAME OF SECOND INVENTOR	LAST NAME RIVELLA	FIRST NAME STEFANO	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP Italy
POST OFFICE ADDRESS 736 West End Avenue Apt. 5B		CITY New York	STATE/COUNTRY ZIP CODE New York 10025
DATE		SIGNATURE	

NAME OF THIRD INVENTOR	LAST NAME MAY	FIRST NAME CHAD	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS 220 Avenue A Apt. 3C		CITY New York	STATE/COUNTRY ZIP CODE New York 10009
DATE		SIGNATURE	

NAME OF FOURTH INVENTOR	LAST NAME BERTINO	FIRST NAME JOSEPH	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE New York	STATE OR COUNTRY OF RESIDENCE New York	COUNTRY OF CITIZENSHIP USA
POST OFFICE ADDRESS 117 Sunset Hill Dr.		CITY Branford	STATE/COUNTRY ZIP CODE CT 06405
DATE 8/26/02		SIGNATURE	

OIPE JC184
OCT 0 8 2002
PATENT & TRADEMARK OFFICE

ATTORNEY DOCKET NO.: MSK.P-050

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sadelain et al.
Serial No.:	10/188,221
Confirmation No.:	9026
Filed:	July 1, 2002
For:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

RESPONSE TO NOTICE TO FILE MISSING PARTS

Hon. Commissioner of Patents
Washington, DC 20231

Sir:

In response to the Notice to File Missing Parts mailed on September 13, 2002, applicant herewith submits the Combined Declaration and Power of Attorney for the above-captioned application. A copy of the Notice to File Missing Parts is enclosed.

Enclosed is Form PTO-2038 in the amount of $65.00 for payment of the declaration surcharge. The Commissioner is authorized to charge any additional fees that might be due to Deposit Account No. 15-0160.

Respectfully,

OPPEDAHL & LARSON LLP

Marina T. Larson

Marina T. Larson, PTO Reg. No. 32,038
P.O. Box 5068
Dillon, CO 80435-5068
Tel: (970) 468-6600
Fax: (970) 468-0104

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)

I hereby certify that this paper and the attachments named herein are being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents, Box Missing Parts, Washington, DC 20231 on September 18, 2002

9-18-02
Date of Signature

Lori South
Lori South

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/188,221	07/01/2002	Sadelain	MSK.P-050

021121
OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

OIPE JC184 OCT 08 2002 PATENT & TRADEMARK OFFICE

CONFIRMATION NO. 9026

FORMALITIES LETTER

OC000000008784865

Date Mailed: 09/13/2002

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is missing.
  A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(l) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

Items Required To Avoid Processing Delays:

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

UMMARY OF FEES DUE:

otal additional fee(s) required for this application is **$65** for a Small Entity

- **$65** Late oath or declaration Surcharge.

A copy of this notice MUST be returned with the reply.

Margaret

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

OIPE JC109
OCT 0 8 2002
PATENT & TRADEMARK OFFICE

T # 0350

ATTORNEY DOCKET NO.: MSK.P-050
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sadelain
Serial No.:	10/188,221
Confirmation No.:	9026
Filed:	July 1, 2002
Title:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

INFORMATION DISCLOSURE STATEMENT

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicant requests that the references listed on Form PTO-1449, which is enclosed, be made of record in the Patent Office file relating to the above-captioned application. Copies of the references are provided herewith. No fee is believed to be due with this paper as we have not received an action on the merits, however the Commissioner is authorized to charge any fees which might be due to Deposit Account No. 15-0610.

Respectfully submitted,

OPPEDAHL & LARSON LLP

Marina T. Larson, Ph.D.
Reg. No. 32,038
P.O. Box 5068
Dillon, Co. 80435-5068

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)

I hereby certify that this paper and the attachments named herein are being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231 on Oct. 3, 2002.

10-3-02
Date of signature

Lori South

OIPE JC109
OCT 0 8 2002
PATENT & TRADEMARK OFFICE

Substitute for form 1449

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Application No.: 10/188,221
Applicant: Sadelain
Filing Date: July 1, 2002
Conf. No.: 9026
Title: Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
Attorney Docket No.: MSK.P-050

U.S. PATENT DOCUMENTS

Examiners Initials	U S Patent No.	Name of Persons or applicant	Date of Publication of Cited Document

FOREIGN PATENT DOCUMENTS

Examiners Initials	Patent No.	Name of Persons or applicant	Date of Publication of Cited Document
	WO 97/33988	Sloan-Kettering Institute for Cancer Research	09/18/1997

OTHER PRIOR ART - NON PATENT LITERATURE DOCUMENTS

Examiner Initials	
	Huang. et al., "Nonadditivity of Mutational Effects at the Folate Binding Site of *Escherichia* coli Dihydrofolate Reductase", *Biochemistry,* Vol. 33, No. 38, pp. 11576 - 11585, 1994
	Ercikan et al., "Effect of codon 22 mutations on substrate and inhibitor binding for human dihydrofolate reductase", *Chemistry and Biology of Pteridines and Folates,* pp 515 - 519, 1993
	May, et al., "Therapeutic haemoglobin synthesis in β-thalassaemic mice expressing lentivirus-encoded human β-globin", *Nature,* Vol. 406, pp. 82 - 86, July 6, 2000
	D. Bodine, "Globin Gene Therapy: One (Seemingly) Small Vector Change, One Giant Leap in Optimism", *Molecular Therapy,* Vol. 2, No. 2, pp. 101 -102, August 2000

This Information Disclosure Citation List is being submitted as a substitute for Form PTO-1449. The Examiner is requested to place his or her initials on the lines adjacent to the citations to indicate that the reference has been considered. The Examiner is further requested to fill in his or her name and the date the information was considered in blocks at the bottom of this substitute for Form PTO-1449.

Examiner Signature _____ Date Considered _____

ATTORNEY DOCKET NO.: MSK.P-050
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sadelain et al.
Serial No.:	10/188,221
Confirmation No.:	9026
Filed:	July 1, 2002
Title:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

SUBMISISON OF SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313

Sir:

Applicant requests that the references listed on Substitute forForm PTO-1449, which is enclosed, be made of record in the Patent Office file relating to the above-captioned application. Copies of the references are provided herewith. No fee is believed to be due with this paper as we have not received an action on the merits, however the Commissioner is authorized to charge any fees which might be due to Deposit Account No. 15-0610.

Respectfully submitted,
OPPEDAHL & LARSON LLP

Marina T Larson

Marina T. Larson, Ph.D.
Reg. No. 32,038
P.O. Box 5068
Dillon, Co. 80435-5068
970-468-6600

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)

I hereby certify that this paper and the attachments named herein are being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner for Patents, PO Box 1450, Alexandria, VA 22313 on NOV 12 2003

11-12-2003
Date of signature

Lori South
Lori South

OIPE JC1900 NOV 17 2003 PATENT & TRADEMARK OFFICE

PTO/SB/08A (06-03)
Approved for use through 07/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	10/188,221
Filing Date	7/1/2002
First Named Inventor	Sadelain et al.
Art Unit	1632
Examiner Name	R. R. Shukla
Attorney Docket Number	MSK.P-050

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of US PTO Patent Documents at www.upsto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

OIPE JC190
NOV 17 2003
PATENT & TRADEMARK OFFICE

PTO/SB/08B (06-03)
Approved for use through 06/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	10/188,221
Filing Date	7/1/2002
First Named Inventor	Sadelain et al.
Art Unit	1632
Examiner Name	R. R. Shukla
Attorney Docket Number	MSK.P-050

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		DZIERZAK ET AL., Lineage-specific expression of a human β-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells., 1988, Page(s) 35-41, Volume 331	
		KALBERER ET AL., Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent extinction of expression of human β-globin in engrafted mice, PNAS, 2000, Page(s) 5411-5415, Volume 97, Number 10	
		MAY ET AL., Successful treatment of murine β-thalassemia intermedia by transfer of the human β-globin gene, Blood, 2002, Page(s) 1902-1908, Volume 99, Number 6	
		RAFTOPOULOS ET AL., Long-Term Transfer and Expression of the Human β-Globin Gene in a Mouse Transplant Model, Blood, 1997, Page(s) 3414-3422, Volume 90, Number 9	
		RIVELLA ET AL., Genetic Treatment of Severe Hemoglobinopathies: The Combat Against Transgene Variegation and Transgene Silencing, Seminars in Hematology, 1998, Page(s) 112-125, Volume 35, Number 2	
		SABATINO ET AL., Long-term expression of γ-globin mRNA in mouse erythrocytes from retrovirus vectors containing the human γ-globin gene.., PNAS, 2000, Page(s) 13294-13299, Volume 97, Number 24	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	MSK.P-050	9026

21121 7590 05/05/2004

OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

EXAMINER	
SHUKLA, RAM R	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 05/05/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Ram R. Shukla	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-41* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-41 are pending.

Election/Restrictions

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-18, drawn to a lentiviral vector comprising a functional globin gene, classified in class 435, subclass 320.1.

II. Claims 19-24 and 29, drawn to a method of treating a hemoglobinopathy in a mammal by introducing into the mammal a lentiviral comprising a globin gene, classified in class 424, subclass 93.1.

III. Claims 19 and 25-28, drawn to a method of treating a hemoglobinopathy in a mammal by introducing into the mammal a cell ex vivo transduced with a lentiviral comprising a globin gene, classified in class 424, subclass 93.21.

IV. Claims 30-41, drawn to a method of making hematopoietic progenitory stem cells transduced with a lentiviral comprising a globin gene, classified in class 435, subclass 325.

3. The inventions are distinct, each from the other because of the following reasons:

Inventions of the groups I-IV are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)).

In the instant case the invention of group I is used for practicing the methods of groups II and III and for making the stem cells of group IV. Additionally, the methods of groups II and III comprise distinct steps which are not coextensive.

Furthermore, methods of groups II-IV can be practiced by using a different vector comprising a globin gene.

Because these inventions are distinct for the reasons given above, have acquired a separate status in the art shown by their different classification and their recognized divergent subject matter, and because each invention requires a separate, non-coextensive search, restriction for examination purposes as indicated is proper.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ram R. Shukla whose telephone number is (571) 272-0735 . The examiner can normally be reached on Monday through Friday from 7:30 am to 4:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached at (571) 272-0804. The fax phone number for TC 1600 is (703) 703-872-9306. Any inquiry of a general nature, formal matters or relating to the status of this application or proceeding should be directed to the William Phillips whose telephone number is (571) 272-0548.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for

published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Ram R. Shukla, Ph.D.
Primary Examiner
Art Unit 1632

RAM R. SHUKLA, PH.D.
PRIMARY EXAMINER

Index of Claims

Application No.	Applicant(s)
10/188,221	SADELAIN ET AL.
Examiner	**Art Unit**
Ram R. Shukla	1632

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	+	Restricted	I	Interference	O	Objected

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al. Application No.: 10/188,221 Filed: 7/1/2002 Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies Attorney Docket No.: MSK.P-050 Customer No.: 021121	Group Art Unit: 1632 Examiner: Ram Shukla Confirmation No: 9026

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Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

Dear Sir:

Responsive to the restriction requirement mailed May 5, 2004 for the above-captioned application, Applicants hereby elect the claims of Group I, Claim 1-18. This election is made without traverse. However, Applicants point out that Groups II - IV are drawn to methods of using the subject matter of Group I. Accordingly, it is respectfully submitted that these claims should be recombined should the claims of Group I be found to be allowable.

Respectfully Submitted,

Marina T Larson

Marina T. Larson, Ph.D
Attorney/Agent for Applicant(s)
Reg. No. 32038
(970) 468 6600

I hereby certify that this paper and any attachments named herein are transmitted to the United States Patent and Trademark Office, Fax number: 703-872-9306 on June 1, 2004 .

Marina T Larson

Marina T. Larson, PTO Reg. No. 32,038

June 1, 2004
Date of Signature

PAGE 1/1 * RCVD AT 6/1/2004 1:36:14 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/4 * DNIS:8729306 * CSID:9704680104 * DURATION (mm-ss):00-58

L Number	Hits	Search Text	DB	Time stamp
1	4934	lentivir$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:08
2	10671	globin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:08
3	10099	locus ADJ control ADJ region or lcr	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:09
4	986	hs2 or hs3 or hs4	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:09
5	2	lentivir$ same globin same (locus ADJ control ADJ region or lcr) same (hs2 or hs3 or hs4)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:12
6	37	globin same (locus ADJ control ADJ region or lcr) same (hs2 or hs3 or hs4)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:13
7	314502	vector or lentiviral NEAR vector	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:13
8	24	globin same (locus ADJ control ADJ region or lcr) same (hs2 or hs3 or hs4) same (vector or lentiviral NEAR vector)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:17
9	0	globin same (locus ADJ control ADJ region or lcr) same (hs2 or hs3 or hs4) same l7.clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:13
10	2119	pgk NEAR promoter	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:18
11	35503	DHFR or dihdrofolate reductase	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:18
12	12046	DHFR or dihdrofolate ADJ reductase	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:18
13	124895	muta$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:19
14	3263	(DHFR or dihdrofolate reductase) SAME (DHFR or dihdrofolate ADJ reductase) same muta$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:19
15	0	(DHFR or dihdrofolate reductase) SAME (DHFR or dihdrofolate ADJ reductase) same muta$ same lentivir$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:19

16	13	(DHFR or dihdrofolate reductase) SAME (DHFR or dihdrofolate ADJ reductase) same muta$ same globin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/19 13:19

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     FILE 'MEDLINE' ENTERED AT 13:25:35 ON 19 AUG 2004
L1          3018 S LENTIVIR?
L2         13425 S GLOBIN
L3        100877 S ?GLOBIN
L4          1457 S LCR OR LOCUS CONTROL REGION
L5             3 S L1 (S) L3 (S) L4
L6           252 S HS2 OR HS3 OR HS3
L7             0 S L1 (S) L3 (S) L4 (S) L6
L8             0 S L1 (L) L3 (L) L4 (L) L6
L9             6 S L1 (L) L3 (L) L4

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, PCTFULL, USPATFULL' ENTERED AT
     13:31:47 ON 19 AUG 2004
L10            6 S L7
L11          631 S L9

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	MSK.P-050	9026

21121 7590 08/25/2004

OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

EXAMINER	
SHUKLA, RAM R	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 08/25/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Ram R. Shukla	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 June 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41* is/are pending in the application.

4a) Of the above claim(s) *19-41* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *07 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11/17/03*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of the invention of group I, claims 1-18 drawn to a retroviral vector in the reply filed on 6/1/04 is acknowledged. In view of applicants' election without traversal, applicants' arguments are moot.

2. Claims 19-41 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 6/1/04.

3. Claims 1-18 are under consideration.

4. Application claims priority to provisional applications 60/301.861 (filed JUNE 29, 2001) and 60/302,852 (filed JULY 2, 2001).

Claim Rejections - 35 USC § 112

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claim 1-18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-18 are indefinite because it is unclear as to what is meant by the term "region" in the claims, for example, in (a) in cliam 1, line 1 claim 2 etc. Applicants are advised to use commonly used term such as "a nucleotide sequence encoding" as appropriate.

Claim 5, 8, 14 17 are indefinite because the metes and bounds of the claimed invention, such as the term "a set of mutations" is not clear and has not been defined in the specification.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1 and 10 are rejected under 35 U.S.C. 102(a) as being anticipated by May et al (Nature 406:82-86, 2000).

May et al teaches a retroviral vector that comprises sequences human beta-globin LCR (HS1, HS2, HS3, HS4 and HS5) and sequences encoding human beta-globin gene (see figure 1).

9. Claims 1 and 10 are rejected under 35 U.S.C. 102(B) as being anticipated by Raftopoulos et al (Blood 90:3414-3422, 1997).

Raftopoulos et al teaches a retroviral vector that comprises sequences human beta-globin LCR (HS2, HS3, and HS4) and sequences encoding human beta-globin gene (see figure 1 and materials and methods section on page 3414, right column).

10. Claims 1-3 and 10-12 are rejected under 35 U.S.C. 102(b) as being anticipated by LeBoulch (US Patent 5,631,162Nature 406:82-86, 2000).

The patent teaches retroviral vectors with LCR derivatives, with or without selectable marker, with or without heterologous enhancer/promoer, with or without 5′ or 3′ viral splice sites, globin gene or derivatives (see figure 1). The patent teaches vectors comprising mouse PGK promoter and DHFR gene (see claims such as claim 9-16).

Claim Rejections - 35 USC § 103

11. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 1-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Raftopoulos et al (Blood 90:3414-3422, 1997) and LeBoulch (US Patent 5,631,162, 5-20-1997) in view of Ercikan et al (Chemistry and Biology of Pteridines and Folates, Edited by J.E. Ayling et al,. Plenum Press, New York, 1993) and Bertino et al (WO 97/33988, 12 March 1997).

Raftopoulos et al teaches a retroviral vector that comprises sequences human beta-globin LCR (HS2, HS3, and HS4) and sequences encoding human beta-globin gene (see figure 1 and materials and methods section on page 3414, right column).

The LeBoulch patent teaches retroviral vectors with LCR derivatives, with or without selectable marker, with or without heterologous enhancer/promoer, with or without 5′ or 3′ viral splice sites, globin gene or derivatives (see figure 1). The patent teaches vectors comprising mouse PGK promoter and DHFR gene (see claims such as claim 9-16). The art also teaches that the vectors have improved viral titer and low frequency of rearrangement. Column 6 describes different parts of the vector and that DHFR is one of the selectable markers and PGK promoter was one of the promoters driving the expression of selectable marker (see lines 45-62).

Neither Raftopulos nor LeBoulch teaches a retroviral vector comprising human DHFR gene and that the human DHFR has a mutation at aa 22 or 31 of the wild type sequence.

Ercikan et al teaches effect of mutations on substrate and inhibitor binding for human DHFR. The art teaches that mutations in codon 22 maty be a hot spot which readily mutates to impart a MTX-resistant phenotype to its host cell (see

introduction on page 515). The art also teaches that these mutants are superior dominant selectable markers in gene transfer technologies (see the last paragraph on page 515).

WO 97/33988 teaches double mutant forms of human DHFR that can be used as selectable markers and for modifying the genome of human cells, such as bone marrow or PBMCs to render them resistant to chemotherapy (see the abstract). On page 7, the art teaches retroviral vector which can incorporate the mutant DHFR which can be used for increasing tolerance of antifolate tolerance in patients (see page 8).

At the time of the invention, it would have been obvious to an artisan of ordinary skill in the art to modify the vector of LeBoulch et al or Raftopoulos et al by replacing the DHFR gene in the vector with the single mutant DHFR of Ercikan or double mutant of WO 97/33988 with a reasonable expectation of success. An artisan of skill would have been motivated to use the mutant DHFR because such would have allowed tolerance to antifolate drugs in patients as taught by WO 97/33988.

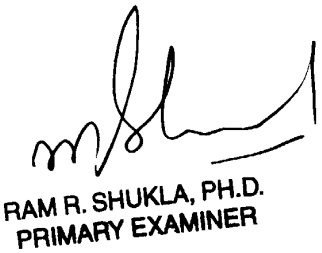
13. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ram R. Shukla whose telephone number is (571) 272-0735 . The examiner can normally be reached on Monday through Friday from 7:30 am to 4:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached at (571) 272-0804. The fax phone number for TC 1600 is (703) 872-9306. Any inquiry of a general nature, formal matters or relating to the status of this application or proceeding should be directed to the Dianiece Jacobs whose telephone number is (571) 272-0532.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR.

Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Ram R. Shukla, Ph.D.
Primary Examiner
Art Unit 1632



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NOV 17 2003
PATENT & TRADEMARK OFFICE

PTO/SB/08A (06-03)
Approved for use through 07/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	10/188,221
Filing Date	7/1/2002
First Named Inventor	Sadelain et al.
Art Unit	1632
Examiner Name	R. R. Shukla
Attorney Docket Number	MSK.P-050

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] -Number[4]- Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

Examiner Signature	RRS	Date Considered	8/20/04

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of US PTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PTO/SB/08B (06-03)
Approved for use through 06/30/2003. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	10/188,221
Filing Date	7/1/2002
First Named Inventor	Sadelain et al.
Art Unit	1632
Examiner Name	R. R. Shukla
Attorney Docket Number	MSK.P-050

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
RRS		DZIERZAK ET AL., Lineage-specific expression of a human β-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells., 1988, Page(s) 35-41, Volume 331	
		KALBERER ET AL., Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent extinction of expression of human β-globin in engrafted mice, PNAS, 2000, Page(s) 5411-5415, Volume 97, Number 10	
		MAY ET AL., Successful treatment of murine β-thalassemia intermedia by transfer of the human β-globin gene, Blood, 2002, Page(s) 1902-1908, Volume 99, Number 6	
		RAFTOPOULOS ET AL., Long-Term Transfer and Expression of the Human β-Globin Gene in a Mouse Transplant Model, Blood, 1997, Page(s) 3414-3422, Volume 90, Number 9	
		RIVELLA ET AL., Genetic Treatment of Severe Hemoglobinopathies: The Combat Against Transgene Variegation and Transgene Silencing, Seminars in Hematology, 1998, Page(s) 112-125, Volume 35, Number 2	
RRS		SABATINO ET AL., Long-term expression of γ-globin mRNA in mouse erythrocytes from retrovirus vectors containing the human γ-globin gene.., PNAS, 2000, Page(s) 13294-13299, Volume 97, Number 24	

Examiner Signature	RRS	Date Considered	8/21/04

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Notice of References Cited	Application/Control No. 10/188,221	Applicant(s)/Patent Under Reexamination SADELAIN ET AL.	
	Examiner Ram R. Shukla	Art Unit 1632	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,631,162	05-1997	LeBoulch et al.	435/320.1
	B	US-			
	C	US-			
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FOREIGN PATENT DOCUMENTS

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NON-PATENT DOCUMENTS

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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims

Application No.	Applicant(s)
10/188,221	SADELAIN ET AL.
Examiner	**Art Unit**
Ram R. Shukla	1632

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Search Notes	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Ram R. Shukla	1632	

SEARCHED			
Class	Subclass	Date	Examiner
435	320.1	8/21/04	RRS

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
Medline, CAPLUS, PCTFUL, USPATFUL, EAST etc. Strategy enclosed	8/21/04	RRS

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Bib Data Sheet

CONFIRMATION NO. 9026

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
10/188,221	07/01/2002 RULE	424	1632	MSK.P-050

APPLICANTS

Michel Sadelain, New York, NY;

Stefano Rivella, New York, NY;
Chad May, New York, NY;Joseph Bertino, New York, NY;

** CONTINUING DATA **********
This appln claims benefit of 60/301,861 06/29/2001 RPS
and claims benefit of 60/302,852 07/02/2001

** FOREIGN APPLICATIONS **********

IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** SMALL ENTITY **
** 09/12/2002

Foreign Priority claimed	STATE OR	SHEETS	TOTAL	INDEPENDENT
☐ yes ☒ no	COUNTRY	DRAWING	CLAIMS	CLAIMS
35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance	NY	4	41	4
Verified and Acknowledged Examiner's Signature RPS Initials				

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DILLON , CO
80435-5068

TITLE
Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

FILING FEE	FEES: Authority has been given in Paper	☐ All Fees
RECEIVED 666	No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

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OCT 0 8 2002
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Application No.: 10/188,221
Applicant: Sadelain
Filing Date: July 1, 2002
Conf. No.: 9026
Title: Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
Attorney Docket No.: MSK.P-050

U.S. PATENT DOCUMENTS

Examiners Initials	U S Patent No.	Name of Persons or applicant	Date of Publication of Cited Document

FOREIGN PATENT DOCUMENTS

Examiners Initials	Patent No.	Name of Persons or applicant	Date of Publication of Cited Document
	WO 97/33988	Sloan-Kettering Institute for Cancer Research	09/18/1997

OTHER PRIOR ART - NON PATENT LITERATURE DOCUMENTS

Examiner Initials	
RRS	Huang. et al., "Nonadditivity of Mutational Effects at the Folate Binding Site of *Escherichia* coli Dihydrofolate Reductase", *Biochemistry*, Vol. 33, No. 38, pp. 11576 - 11585, 1994
	Ercikan et al., "Effect of codon 22 mutations on substrate and inhibitor binding for human dihydrofolate reductase", *Chemistry and Biology of Pteridines and Folates*, pp 515 - 519, 1993
	May, et al., "Therapeutic haemoglobin synthesis in β-thalassaemic mice expressing lentivirus-encoded human β-globin", *Nature*, Vol. 406, pp. 82 - 86, July 6, 2000
RRS	D. Bodine, "Globin Gene Therapy: One (Seemingly) Small Vector Change, One Giant Leap in Optimism", *Molecular Therapy*, Vol. 2, No. 2, pp. 101 -102, August 2000

This Information Disclosure Citation List is being submitted as a substitute for Form PTO-1449. The Examiner is requested to place his or her initials on the lines adjacent to the citations to indicate that the reference has been considered. The Examiner is further requested to fill in his or her name and the date the information was considered in blocks at the bottom of this substitute for Form PTO-1449.

Examiner Signature _____ Date Considered _____

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	
Filed: 7/1/2002	Group Art Unit: 1632
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Examiner: R. Shukla
Attorney Docket No.: MSK.P-050	Confirmation No: 9026
Customer No.: 021121	

Commissioner for Patents

PO Box 1450

Alexandria, VA 22313-1450

AMENDMENT

Dear Sir:

In response to the Office Action of August 25, 2004, please amend this application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Cert. Under 37 CFR 1.8
This paper and the attachments named herein are being deposited with the United States Postal Service with sufficient postage as first class mail and addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on

NOV 23 2004

Name Anne Wagner

Signature (Anne Wagner)

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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (previously presented) A recombinant vector comprising:
(a) a region encoding a functional β-globin gene; and
(b) large portions of the β-globin locus control region, which include complete DNase I hypersensitive sites HS2, HS3 and HS4, said vector providing expression of β-globin when introduced into a mammal *in vivo*.

2. (previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (currently amended) The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

4. (currently amended presented) The vector of claim 3, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

5. (previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (currently amended) The vector of claim 2, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

8. (previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (previously presented) The vector of claim 1, wherein the functional globin gene encodes human β-globin.

11. (previously presented) The vector of claim 10 further comprising a region encoding a dihydrofolate reductase.

12. (currently amended) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

13. (currently amended) The vector of claim 12, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

14. (previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (currently amended) The vector of claim 11, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

17. (previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19. (withdrawn) A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) large portions of the β-globin locus control region, which include complete DNase I hypersensitive sites HS2, HS3 and HS4, said vector providing expression of β-globin when introduced into a mammal *in vivo*; and

expressing the functional β-globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. (withdrawn) The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. (withdrawn) The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

22. (withdrawn) The method of claim 21, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

23. (withdrawn) The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. (withdrawn) The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. (withdrawn) The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

26. (withdrawn) The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. (withdrawn) The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure

of the cells to an antifolate,

28. (withdrawn) The method of claim 27, wherein the antiifolate is methotrexate.

29. (withdrawn) The method of claim 19, wherein the globin gene encodes human β-globin.

30. (withdrawn) A mammalian hematopoietic progenitor or stem cell transduced with a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4, wherein said cells express the functional β-globin gene.

31. (withdrawn) The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. (withdrawn) The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. (withdrawn) The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydroflate reductase.

34. (withdrawn) The transduced cell of claim 33, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

35. (withdrawn) The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

36. (withdrawn) The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. (withdrawn) The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. (withdrawn) The transduced cell of claim 30, wherein the globin gene encodes human β-globin.

39. (withdrawn) A method of making a therapeutic compositions for treatment of henoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising

(a) a region encoding a functional β-globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4, said vector provideing expression of the globin gene when introduced into a mammal *in vivo*, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. (withdrawn) The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. (withdrawn) The method of claim 39, wherein the globin gene encodes human β-globin.

42. (new) The vector of claim 1, wherein the large portions of the β-globin locus control region comprises an 840 bp fragment of HS2 extending between by SmaBI and BstXI restriction sites, a 1308 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and BanII restriction markers.

43. (new) The vector of claim 142, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

REMARKS/ARGUMENTS

This is in response to the Office Action mailed August 25, 2004 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Claims 3, 4, 12, 13, and 16 have been amended to correct a typographical error in the spelling of "dihydrofolate." Claims 42 and 43 have been added dependent of claim 1. These claims are supported on Pages 5 and 4, respectively. The fee for these additional claims is enclosed. The Commissioner is authorized to charge any additional fees or credit any overpayment to Deposit Account No. 15-0610.

The Examiner rejected claims 1-18 under 35 USC 112, second paragraph as indefinite. In particular, the Examiner argues that the term "region" and the phrase "a set of mutations" are indefinite. Applicants respectfully traverse this rejection.

As a first matter, Applicants note that "it is incumbent on the **Examiner** to establish that one having ordinary skill in the art would not have been able to determine the scope of protection defined by the claim when read in light of the specification." In re Cordova, 10 U.S.P.Q. 2d 1949, 1952 (POBAI 1989). Thus, it is not sufficient to say something is indefinite, the Examiner must explain why a person skilled in the art would be unable to determine the meaning of the claim, given the plain meaning of the terms and the specification. Here, that burden has not been met.

Moreover, the claims cannot be shown to be indefinite when the correct legal standard is applied. As to the term "region," the meaning is clear. Part of the vector contains a functional globin gene. The use of the term "region" in this manner is not at all unusual in the art. Vectors may be said to have promoter regions, and coding regions so the basis for the Examiner's rejection is not understood. Similarly, the plain meaning of the phrase "set of mutations" is that the DHFR is different from the wild type as a result of one or more mutations, and these mutations are referred to collectively, for convenience in wording, as a "set of mutations." Thus, Applicants submit that this rejection should be withdrawn.

Claims 1 and 10 stand rejected as anticipated over May et al. This paper reflects the work of the inventors of this application. To overcome this rejection, Applicants enclose Katz-type declarations signed by each inventor.

The Examiner rejected claims 1 and 10 as anticipated by Raftopoulos et al or LeBoulch (patent or article). These references all disclose the same vector, and therefore are addressed as one. In order for a reference to anticipate the claimed invention, the reference must disclose each and every element of the claims. In this case, in characterizing the teaching of the reference, the

Examiner has left out an important element, namely that the vector contains "large portions of the β-globin locus control region" that include the "complete DNase I hypersentisive regions."

Since the Examiner has not addressed the requirement in the claim that the vector contain large portions of the β-globin locus control regions, including the **complete** hypersenstive regions, the basis for the rejection is not clear. It is noted that the Leboulch references is specifically mentioned on Page 4 of the specification, and said to not meet this limitation because is has only the core elements of the hypersensitive regions. Furthermore, the LeBoulch et al. patent discloses at Col. 6, lines 30-44 the sequences of the LCR fragments used in the p141 vector. These are all significantly shorter than the LCR pieces used in the present invention, as illustrated in the following table:

LCR-segment	LeBoulch (bp)	RNS1/May (bp)	application (bp)
HS2	374	423	840
HS3	287	280	1308
HS4	243	283	1069

Thus, the references do not disclose large portions as presently claimed. The anticipation rejection should therefore be withdrawn.

Claims 1-18 are also rejected as obvious over Raftopoulos and LeBoulch in view of Ercikan and Bertino. In making this rejection, the Examiner still has not addressed the limitation in the claim that the vector contains "large portions" of the LCR and thus has failed to make a prima facie rejection under 35 USC § 103. Furthermore, as is made clear in the application, this difference is significant to the performance of the vector. In the examples, TNS9 is a vector with large portions of the LCR. RNS1 has small portions of the LCR (see the cited May paper) similar to those of LeBoulch (RNS1 in Table above). The results clearly show the superiority of the vector of the invention. (See Figs 3-5). Thus, the vector of claim 1 is not obvious, and the secondary references which relate to limitations in dependent claims do not overcome the deficiency of the base references. Thus, the rejections under 35 USC § 103 should be withdrawn.

For these reasons, this application is now considered to be in condition for allowance and such action is earnestly solicited. Further, recombining the non-elected claims is considered appropriate.

Applicants also enclose a Supplemental Information Disclosure Statement together with the appropriate fee and copies of the mentioned non-patent references. Entry of the Information Disclosure Statement and consideration of the references is requested.

Respectfully submitted,

Marina T. Larson Ph.D.
PTO Reg. No. 32,038
Attorney for Applicant
(970) 468-6600

Enclosures:
Declaration (four copies)
Information Disclosure Statement (PTO 1449)
References (3)
Credit Carrd Payment Form

OIPE JC106
NOV 2 6 2004
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	Group Art Unit: 1632
Filed: 7/1/2002	Examiner: Ram Shukla
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Confirmation No: 9026
Attorney Docket No.: MSK.P-050	
Customer No.: 021121	

DECLARATION UNDER RULE 132

The undersigned inventors each hereby individually declare as follows:

1. I am a named inventor of the above-captioned application, and as such I am familiar with the application, including the claims.

2. I understand that the Examiner has cited May et al. *Nature* 406: 82-86 (1997) against this application. This publication represents the work of the inventors of the present application. To the extent that others are named as co-authors of the paper, they are co-named in recognition of non-inventive contributions to the paper. They are not inventors of the invention claimed in this application.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

Katz/132 Declaration

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: Nov 15, 2004

Michel Sadelain

Dated: _____

Stefano Rivella

Dated: _____

Chad May

Dated: _____

Joseph Bertino

OIPE JC106 NOV 26 2004 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	
Filed: 7/1/2002	Group Art Unit: 1632
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Examiner: Ram Shukla
Attorney Docket No.: MSK.P-050	Confirmation No: 9026
Customer No.: 021121	

DECLARATION UNDER RULE 132

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3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

Katz/132 Declaration

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

dated: _____

Michel Sadelain

dated: 11/09/2004

Stefano Rivella

dated: _____

Chad May

dated: _____

Joseph Bertino

OIPE JC105
NOV 26 2004
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	
Filed: 7/1/2002	Group Art Unit: 1632
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Examiner: Ram Shukla
Attorney Docket No.: MSK.P-050	Confirmation No: 9026
Customer No.: 021121	

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punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

dated: _____

_____

Michel Sadelain

dated: _____

_____

Stefano Rivella

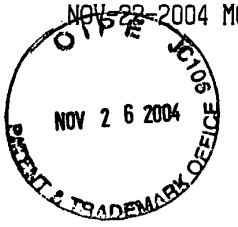
dated: Nov. 11, 2004

Chad May

dated: _____

_____

Joseph Bertino



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	
Filed: 7/1/2002	Group Art Unit: 1632
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Examiner: Ram Shukla
Attorney Docket No.: MSK_P-050	Confirmation No: 9026
Customer No.: 021121	

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3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

dated: _____ _____

Michel Sadelain

dated: _____ _____

Stefano Rivella

dated: _____ _____

Chad May

dated: 11/22/04 _____

Joseph Bertino

Subst. Form PTO-1449

INFORMATION DISCLOSURE CITATION IN AN APPLICATION

(Use several sheets if necessary)

OIPE JC106 NOV 2 6 2004 PATENT & TRADEMARK OFFICE

Docket Number (Optional)	Application Number
Y1979-0003	10/188,221
Applicant	
Sadelain, et al.	
Filing Date	**Group Art Unit**
July 1, 2002	1632

U. S. PATENT DOCUMENTS

EXAMINER INTIIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	5,126,260	6/30/92	Tuan et al.			
	5,631,162	5/20/97	LeBoulch et al.			
	5,834,256	11/10/98	Finer, et al.			
	5,858,740	1/12/99	Finer et al.			
	5,981,276	11/9/99	Sodroski et al.			
	5,994,136	11/30/99	Naldini et al.			
	6,103,516	1/11/00	Verma et al.			
	6,218,187	4/17/01	Finer et al.			
	6,294,165	9/25/01	Lever et al.			
	6,312,682	11/6/01	Kingsman et al.			
	6,428,953	8/6/02	Naldini et al.			
	6,524,851	2/25/03	Ellis			
	6,544,771	4/8/03	Rivière et al.			

FOREIGN PATENT DOCUMENTS

	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	Translation YES	Translation NO

OTHER DOCUMENTS (*Including Author, Title, Date, Pertinent Pages, Etc.*)

		Dull *et al.* (1998) J. Virol. 72:8463-8471, "A Third-Generation Lentivirus Vector with a Conditional Packaging System"
		Naldini et al. (1996) Science 272:263-267, "In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector"
		Sadelain et al. (1995) Proc. Natl. Acad. Sci. 92:6728-6732, "Generation of a high-titer retroviral vector capable of expressing high levels of the human ß-globin gene"

EXAMINER	DATE CONSIDERED

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP § 609; Draw line through citation if not in conformance and not considered. Include copy with next communication to applicant.

NY\264242.1

11/29/2004 EAREGNY1 00000016 10188221
02 FC:1806 180.00 OP

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2003

Application or Docket Number: 10/188,221

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS		
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	minus 20=	*
INDEPENDENT CLAIMS	minus 3 =	*
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	385.00	OR	BASIC FEE	770.00
X$ 9=		OR	X$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL		OR	TOTAL	

TF

CLAIMS AS AMENDED - PART II

AMENDMENT A	(Column 1)		(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Filed 11-26-04				
Total	* 43	Minus	** 41	= 2
Independent	* 4	Minus	*** 4	= 0
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=	18	OR	X$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B	(Column 1)		(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C	(Column 1)		(Column 2)	(Column 3)
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

CLAIMS ONLY

SERIAL NO.	FILING DATE
10/188,221	
APPLICANT(S)	

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
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TOTAL IND.	4					
TOTAL DEP.	39					
TOTAL CLAIMS	43					

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TOTAL CLAIMS						

* MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS

FORM PTO-2022 (1-99)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

Freeform Search

Database: US Pre-Grant Publication Full-Text Database; US Patents Full-Text Database; US OCR Full-Text Database; EPO Abstracts Database; JPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L4 same l3

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

Search Clear Interrupt

Search History

DATE: Monday, February 28, 2005 Printable Copy Create Case

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L5	L4 same l3	9	L5
L4	hypersensitive	3652	L4
L3	L2 with l1	149	L3
L2	lentivi$ or retrovir$	43741	L2
L1	beta globin	5312	L1

END OF SEARCH HISTORY

First Hit Fwd Refs Previous Doc Next Doc Go to Doc#
Generate Collection Print

L5: Entry 3 of 9 File: USPT Aug 29, 2000

DOCUMENT-IDENTIFIER: US 6110666 A
** See image for Certificate of Correction **
TITLE: Locus control subregions conferring integration-site independent transgene expression abstract of the disclosure

Brief Summary Text (5):
The complete .beta.-globin LCR comprises four DNase I hypersensitive sites (HS) on a 20 kbp fragment that is too large to be incorporated into retrovirus or adeno-associated virus (AAV) vectors designed for integration into the mammalian genome. Individual hypersensitive sites, in particular the 5'HS2 associated element, have been studied for the ability to regulate transduced globin genes (Novak et al., Proc. Natl. Acad. Sci. USA 87:3386-3390, 1990; Chang et al., Proc. Natl. Acad. Sci. USA 89:3107-3110, 1992; Miller et al., Blood 82:1900-1906, 1993). However, it has proven to be difficult to obtain stable high-titer viruses bearing these sequences.

Previous Doc Next Doc Go to Doc#

L5: Entry 7 of 9 File: USPT Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780447 A
TITLE: Recombinant adeno-associated viral vectors

Detailed Description Text (80):
10. Takekoshi K. J., Oh Y. I., Westerman K. W., London I. M., Leboulch P.: Retroviral transfer of a human beta-globin/delta-globin hybrid gene linked to the beta locus control region hypersensitive site 2 aimed at the gene therapy of sickle cell disease. Proc Natl Acad Sci USA 92: 3014, 1995

L5: Entry 8 of 9 File: USPT May 20, 1997

DOCUMENT-IDENTIFIER: US 5631162 A
TITLE: Retroviral vectors for transducing .beta.-globin gene and .beta.-locus control region derivatives

Brief Summary Text (4):
Gene transfer experiments have previously shown that the proximal cis-acting elements of the human .beta.-globin gene are insufficient for gene therapy applications because they provide a very low, integration site-dependent expression of the human .beta.-globin transgene (less than 1 to 5% of human .beta.-globin/murine.beta..sub.maj -globin mRNA ratio) (Cone et al., Mol. Cell Biol. 7:887-897 (1987); Dzierzak et al., Nature 331:35-41 (1988); Karlsson et al., Proc. Natl. Acad. Sci. USA 78:3629-3633 (1988); Miller et al., J. Virol., 62:4337-4345 (1988); Bender et al., Mol. Cell. Biol., 9:1426-1434 (1989)). The discovery of major hypersensitive sites (HS) far upstream of the human .beta.-globin gene locus, constituting the .beta.-Locus Control region (.beta.-LCR), has given new hope for successful gene therapy of human .beta.-globin gene disorders. (Tuan and London, Proc. Natl. Acad. Sci. USA 81:2718-2722 (1984); Tuan et al., Proc. Natl. Acad. Sci. USA 82:6384-6388 (1985); Forrester et al., Proc. Natl. Acad. Sci. USA 89:1968-1972 (1986); Grosveld et al., Cell 51:975-985 (1987)). LCR derivatives are able to confer erythroid-specific, high, integration site-independent expression of a linked .beta.-globin gene in transgenic mice and murine erythroleukemia (MEL) cells, which mimic adult erythroid differentiation (Grosveld et al., Cell 51:975-985 (1987)). Because the activity of each HS site has now been localized to small DNA fragments (U.S. Pat. No. 5,126,260; Curtin et al., Proc. Natl. Acad. Sci. USA 86:7082-7086 (1989); Forrester et al., Proc. Natl. Acad. Sci. USA 86:5439-5443 (1989); Ryan et al., Genes Dev. 3:314-323 (1989); Tuan et al., Proc. Natl. Acad. Sci. USA, 86:2554-2558 (1989); Collis et al., EMBO J., 9:233-240 (1990); Ney et al., Genes Dev. 4:993-1006 (1990); Philipsen et al., EMBO J., 9:2159-2167 (1990); Talbot et al., EMBO J., 9:2169-2178 (1990); Pruzina et al., Nucleic Acids Res., 19:1413-1419 (1991); Walters et al., Nucleic Acids Res., 19:5285-5393 (1991)), it has become possible to construct retroviral vectors transducing .beta.-LCR derivatives linked to the human .beta.-globin gene and its proximal cis-acting elements (Novak et al., Proc. Natl. Acad. Sci., USA, 87:3386-3390 (1990); Chang et al., Proc. Natl. Acad. Sci. USA, 89:3107-3110 (1992)). However, these [.beta.-globin/LCR] retroviruses have low titer, are very unstable with multiple rearrangements upon transmission of the proviral structure, and provide a relatively modest and highly variable enhancement of .beta.-globin gene expression in infected murine erythroleukemia (MEL) cells (Novak et al., Proc. Natl. Acad. Sci. USA 87:3386-3390 (1990); Chang et al., Proc. Natl. Acad. Sci. USA 89:3107-3110 (1992)).

Brief Summary Text (5):
U.S. Pat. No. 5,126,260 describes DNAaseI hypersensitive sites that constitute the .beta.-LCR and, in particular, identifies the HS2 enhancer within the .beta.-LCR structure. U.S. Pat. No. 5,126,260 also claims the use of .beta.-LCR and HS2 derivatives in gene transfer protocols, including retrovirus-mediated gene transfer, to obtain high expression level of the human .beta.-globin gene. However, U.S. Pat. No. 5,126,260 does not identify specific means by which stable proviral transmission of [.beta.-globin/LCR] retroviruses can be achieved.

Previous Doc Next Doc Go to Doc#

Freeform Search

Database: US Pre-Grant Publication Full-Text Database; US Patents Full-Text Database; US OCR Full-Text Database; EPO Abstracts Database; JPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: l12 same l11 same l9

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

Search Clear Interrupt

Search History

DATE: Tuesday, March 01, 2005 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
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L13	L12 with l11 with l9	1	L13
L12	globin	11737	L12
L11	gata	1980	L11
L10	L9 same L8	9	L10
L9	hypersensitive	3654	L9
L8	L7 with L6	149	L8
L7	lentivi$ or retrovir$	43773	L7
L6	beta globin	5314	L6
L5	5766584.pn.	2	L5
L4	5776584.pn.	2	L4
L3	5576658.pn.	2	L3
L2	5576684.pn.	2	L2
L1	5576584.pn.	2	L1

END OF SEARCH HISTORY

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End of Result Set

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L13: Entry 1 of 1 File: PGPB Feb 3, 2005

PGPUB-DOCUMENT-NUMBER: 20050028230
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050028230 A1

TITLE: Non-human animals expressing heterologous complement receptor type 1 (CR1) molecules on erythrocytes and uses therefor

PUBLICATION-DATE: February 3, 2005

US-CL-CURRENT: 800/18; 435/320.1, 435/354

APPL-NO: 10/ 843038 [PALM]
DATE FILED: May 10, 2004

RELATED-US-APPL-DATA:
Application is a non-provisional-of-provisional application 60/469262, filed May 9, 2003,

RELATED APPLICATION

[0001] This application claims priority to U.S. provisional application Ser. No. 60/469,262 filed May 9, 2003, the contents of which are entirely incorporated by reference.

Previous Doc Next Doc Go to Doc#

First Hit Fwd Refs Previous Doc Next Doc Go to Doc#
End of Result Set

Generate Collection Print

L14: Entry 4 of 4 File: USPT Sep 3, 2002

DOCUMENT-IDENTIFIER: US 6444421 B1
TITLE: Methods for detecting intermolecular interactions in vivo and in vitro

Detailed Description Text (199):
A locus control region (LCR) confers high level, position-independent expression of a linked gene (see, Orkin (1995) Eur J Biochem 231:271-81). The best characterized among these is the .beta.-globin LCR which serves as the master regulatory element for the expression of the globin family of genes in a locus that spans almost 100 kb (Orkin, id.). The expression of the globin family of genes in erythroid cells are developmentally regulated. In human, the .epsilon.-globin gene is expressed first in the embryo, followed by the gamma globin genes in the fetus, and the .beta.-globin gene at birth and throughout life. Proper expression of all these genes is dependent on the LCR, which resides in four DNase 1 hypersensitive sites (5'HS1-5) located upstream of the .epsilon.-globin gene. The complete set of these hypersensitive sites is required for full position-independent expression suggesting that they act synergistically. These DNaseI hypersensitive sites contain a number of binding sites for CACCC and E box binding factors, GATA-1 and NFE-2 as well as yet uncharacterized factors (Talbot and Grosveld (1991) EMBO J. 10:1391-8).

Previous Doc Next Doc Go to Doc#

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	MSK.P-050	9026

21121 7590 03/31/2005

OPPEDAHL AND LARSON LLP
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DILLON, CO 80435-5068

EXAMINER	
NGUYEN, DAVE TRONG	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 03/31/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Dave T. Nguyen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 December 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-43* is/are pending in the application.

4a) Of the above claim(s) *19-41* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18,42 and 43* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11/26/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Claims 3, 4, 12, 13, 16 have been amended, claims 42 and 43 have been added by the amendment filed November 23, 2004. This application is now assigned to a new examiner.

Claims 19-41 have been withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected claimed invention.

Claims 1-18, 42, and 43, to which the following grounds of rejection are applicable, are pending.

This application contains sequence disclosures (see page 8 and page 9, for example) that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Applicant must comply with the requirements of the sequence rules (37 CFR 1.821 - 1.825) should this application be issued with an allowable claim.

In view of applicant's remarks in the response, particularly page 7 bridging page 8, the prior art rejections of record have been replaced by new grounds of prior art rejection, which would accurately reflect the nature of the invention and its obviousness over the prior art of record.

The invention is drawn to a recombinant vector having a region encoding a

functional.beta.-globin gene; and large portions of the .beta.-globin locus control regions which include DNase I hypersensitive sites HS2, HS3 and HS4 provides expression of .beta.-globin when introduced into a mammal, for example a human, in vivo. The as-filed application on par. 0021 states:

> The recombinant vectors of the invention also include large portions of the locus control region (LCR) which include DNase I hypersensitive sites HS2, HS3 and HS4. In prior studies, smaller nucleotide fragments spanning the core portions of HS2, HS3 and HS4 have been utilized. Sadelain et al. Proc. Nat'l Acad. Sci. (USA)92: 6728-6732 (1995); Lebouich et al., EMBO J. 13: 3065-3076 (1994). The term "large portions" refers to portions of the locus control region which encompass larger portions of the hypersensitive sites as opposed to previously tested fragments including only the core elements. The regions may be the complete site or some lesser site which provides the same functionality as the specific sequences set forth below. In preferred embodiments of the invention, the large portions of the locus control regions are assembled from multiple fragments, each spanning one of the DNase I hypersensitive sites. In addition, the locus control region has two introduced GATA-1 binding sites at the junction between HS3 and HS4. While not intending to be bound by any specific mechanism, it is believed that the incorporation of these transcription factor binding sites enhances the effectiveness of the vector.

Thus, the issue, when read light of the as-filed application and applicant's remark, is whether or not the mere recitation of "large portion of the β-globin locus control region", notwithstanding the fact showing that previously tested fragments including "only the core elements" are well-known in the prior art (LeBoulch), is sufficient to render the presently claimed invention patentable. A close review of the as-filed application only shows that by constructing a lentiviral vector comprising essentially of the 3.2 kb LCR, which consists of an 840 bp HS2 fragment (SnaBI-BstXI), a 1308 bp HS3 fragment (HindIII-BamHI) and a 1069 bp

HS4 fragment (BamHI-BanII), unexpected levels of expression of the human beta-globin gene were produced both *in vitro* and *in vivo*. See figures 3, 5 and 6.

However and on the basis of one single unexpected finding with regard to the make and use of the TNS9 vector, the as-filed application attempts to claim any recombinant vector expressing a functional β-globin gene and such a "large portion of the β-globin locus control region". While it could be reasonably argued that the prior art such as LeBoulch (US patent 5,631,162) does not anticipate the claimed invention because of the "large portion" limitation and because of the recitation of the patent itself as the benchmark of the "core elements", such is not sufficient to render the claimed invention, as broadly claimed, non-obvious over the prior art of record. This is precisely because 1) It is well-established in the prior art of record that recombinant viral vectors comprising more than the core portions the β-globin locus control region could be routinely made to drive expression of the human beta-globin gene when introduced into a mammal *in vivo;* and 2) the claimed invention could reasonably construed as to embrace any further extension of any portion of nucleotide residues flanking the core portions the β-globin locus control region, *e.g.,* > two nucleotide residues up to portions that can reasonably inserted into a recombinant viral vector, *e.g.,* retroviral vector, gutless recombinant adenovirus vector (up to 30 kb insert).

In view of 1) and 2) and in view of the fact that one single unexpected finding of the TNS9 vector is not commensurate in scope of the claimed invention, the following grounds of rejection are properly applicable:

Claims 1-3, and 10-12 are rejected under 35 USC 103 as being unpatentable over LeBoulch (US Pat No. 5,631,162) or Sadelain *et al.* (PNAS, Vol 92, pp. 6728-6732, 1995), each of which taken with either Grosveld (US 6,110,666) or Chung (US Pat No. 6,444,421 B1).

The patent teaches retroviral vectors with LCR derivatives, with or without selectable marker, with or without heterologous enhancer/promoter, with or without 5/ or 3/ viral splice sites, globin genes or derivatives (see Figure 1). The patent teaches vectors comprising mouse PGK promoter and DHFR gene (see claims such as claims 9-16. Column 6 of the patent discloses that the 374 bp-HS2, 287 bp-HS3 and the 243-bp HS4.

Sadelain *et al.* teaches that as long as HS2 and HS3 are oriented in the same directions as the beta-globin gene and adjacent to its promoter, that a full intron 1, a 476-bp intron 2 are present, and the 1-kb LCR fragment comprising the 478-bp HS2, 260-bp HS3, and 283-bp HS4 core sequences, a recombinant vector with necessary elements (page 6731, column 2) b such as the Mβ6LK vector (page 6730, columns 1 and 2) can be constructed to direct elevated beta-Globin expression.

In view of applicant's loose definition of the "large portion", and given applicant's remark on page 8, applicant appears to assert that any prior art teaching only the core elements of the hypersensitive regions is outside of the scope of the claimed invention. Hence, a claimed invention that could reasonably comprise any portion of nucleotide residues flanking the core element

is clearly within the scope of the claimed invention, which merely recites a "large portion of the β-globin locus control region".

Within the above context, as exemplified by either LeBoulch or Sadelain, Grosveld *et al.* teaches on column 4 that as long as a fully functional or complete LCR includes a chromatin opening domain, and on column 7 that, for example, the 5'HS3 and 5'HS2 based construct comprises the 1.9 kB DNA sequence is sufficient to drive high level of transgene expression.

Likewise, Chung teaches that the beta-globin LCR is well-studied, and is known to confer high level, position-independent expression of a linked gene (column 37). In fact, it is conventional and/or routine for one of ordinary skill in the art to construct and make constructs that have portions larger than the core elements necessary to drive expression of a linked gene such as the human beta-globin gene, as long as promoter enhancing regions and/or transcription factor bindings sites are retained, wherein these sites are well taught in the prior art of record. More specifically, column 39, second full par. discloses that a 0.85 kb HS3, a 1.25 kb HS4 and together a 4.63 kb fragment that contains HS2, 3, and 4 of the globin LCR can be constructed into a cloning vector.

As such, it would have been obvious for one of ordinary skill in the art to construct and employ any additional nucleotides residues flanking the core elements as disclosed in LeBoulch or Sadelain and/or any additional well-known elements such as enhancers, promoters, selectable markers, as a matter of suitable use of cloning techniques so long as the essential elements such as

necessarily elements of a recombinant viral vector, promoter enhancing regions, a chromatin opening domains, and desired transcription factor binding sites are retained, thereby enhancing the expression of the transgene.. One would have been motivated to employ portions larger than the core elements because of the teaching of Grosveld, which teaches that as long as a fully functional or complete LCR includes a chromatin opening domain, *e.g,* the 5'HS3 and 5'HS2 based construct comprises the 1.9 kB-HS3 DNA sequence, is constructed, such is sufficient to drive high level of transgene expression. One also would have been motivated to employ a larger portion than the core elements as set froth in LeBoulch or Seadelain because Chung discloses that a 0.85 kb HS3, a 1.25 kb HS4 and together a 4.63 kb fragment that contains HS2, 3, and 4 of the globin LCR can be constructed into a cloning vector, and thus, shows that such is also suitable for use to drive high expression of a transgene in a target cell.

Thus, the claimed invention was *prima facie obvious.*

Claims 1 and 42 are rejected under 35 USC 103 as being unpatentable over LeBoulch (US Pat No. 5,631,162) or Sadelain *et al.* (PNAS, Vol 92, pp. 6728-6732, 1995), each of which taken with Chung (US Pat No. 6,444,421 B1), and further in view of Grosveld (US 6,110,666).

The patent teaches retroviral vectors with LCR derivatives, with or without selectable marker, with or without heterologous enhancer/promoter, with or without 5/ or 3/ viral splice sites, globin genes or derivatives (see Figure 1). The

patent teaches vectors comprising mouse PGK promoter and DHFR gene (see claims such as claims 9-16. Column 6 of the patent discloses that the 374 bp-HS2, 287 bp-HS3 and the 243-bp HS4.

Sadelain *et al.* teaches that as long as HS2 and HS3 are oriented in the same directions as the beta-globin gene and adjacent to its promoter, that a full intron 1, a 476-bp intron 2 are present minuse the 3' enhancer, and the 1-kb LCR fragment comprising the 478-bp HS2, 260-bp HS3, and 283-bp HS4 core sequences, a recombinant vector with necessary elements (page 6731, column 2) b such as the Mβ6LK vector (page 6730, columns 1 and 2) can be constructed to direct elevated beta-Globin expression.

In view of applicant's loose definition of the "large portion", and given applicant's remark on page 8, applicant appears to assert that any prior art teaching only the core elements of the hypersensitive regions is outside of the scope of the claimed invention. Hence, a claimed invention that could reasonably comprise any portion of nucleotide residues flanking the core element is clearly within the scope of the claimed invention, which merely recites a "large portion of the β-globin locus control region".

Within the above context, Chung teaches that the beta-globin LCR is well-studied and known to confer high level, position-independent expression of a linked gene (column 37). In fact, it is conventional and/or routine for one of ordinary skill in the art to construct and make constructs that have portions larger than the core elements necessary to drive expression of a linked gene such as

the human beta-globin gene, as long as promoter enhancing regions and/or transcription factor bindings sites are retained, wherein these sites are well taught in the prior art of record. More specifically, column 39, second full par. discloses that a 0.85 kb HS3, a 1.25 kb HS4 and together a 4.63 kb fragment that contains HS2, 3, and 4 of the globin LCR can be constructed into a cloning vector.

As such, it would have been obvious for one of ordinary skill in the art to construct and employ any additional nucleotides residues flanking the core elements as disclosed in LeBoulch or Sadelain and/or any additional well-known elements such as enhancers, promoters, selectable markers, as a matter of suitable use of cloning techniques so long as the essential elements such as necessarily elements of a recombinant viral vector, promoter enhancing regions, a chromatin opening domains of the LCR, and desired transcription factor binding sites of the LCR are retained, thereby enhancing the expression of the transgene.. One would have been motivated to employ portions larger than the core elements, such as the 4.63 fragment of Chung, because Chung teaches that it is conventional and/or routine for one of ordinary skill in the art to construct and make constructs that have portions larger than the core elements necessary to drive expression of a linked gene such as the human beta-globin gene, as long as promoter enhancing regions and/or transcription factor bindings sites are retained, wherein these sites are well taught in the prior art of record. Accordingly, this teaching is further exemplified by the make and use of the 4.63 fragment of the LCR of Chung, which comprises the 840-bp HS2, the 1069 bp

fragment of HS4, and the 0.85 kb HS2. One would have a reasonable expectation of success of making a suitable modification because of the teaching of the totality of the prior art of record. This totality of the prior art of record would also include Applicant's own work (Sadelain *et al*), which teaches that as long as HS2 and HS3 are oriented in the same directions as the beta-globin gene and adjacent to its promoter, that a full intron 1, a 476-bp intron 2 are present minuse the 3' enhancer, and the 1-kb LCR fragment comprising the 478-bp HS2, 260-bp HS3, and 283-bp HS4 core sequences, a recombinant vector with necessary elements (page 6731, column 2) b such as the Mβ6LK vector (page 6730, columns 1 and 2) can be constructed to direct elevated beta-Globin expression.

The combined cited references do not teach a further incorporation of the larger fragment than the one described in Chung, such as a HS3 fragment comprising a 1308 bp fragment.

However, at the time the invention was made, Grosveld *et al.* teaches on column 4 that as long as a fully functional or complete LCR includes a chromatin opening domain, and on column 7 that, for example, the 5'HS3 and 5'HS2 based construct comprises the 1.9 kB HS3 based DNA sequence is sufficient to drive high level of transgene expression.

One of ordinary skill in the art would also have been motivated to employ a larger portion than the HS3 fragment, as set forth in the combined based references, in a recombinant vector constructed for expression of a globin gene. One would have been motivated to employ a HS3 fragment that comprises a

chromatin opening domain such as one described in Grosveld because Grosveld teaches on column 4 that as long as a fully functional or complete LCR includes a chromatin opening domain, and on column 7 that, for example, the 5'HS3 and 5'HS2 based construct comprises the 1.9 kB DNA sequence is sufficient to enhance a high level of transgene expression in a desired cell *in vivo*.

Thus, the claimed invention was *prima facie obvious*.

Claims 1-18 are rejected under 35 USC 103 as being unpatentable over LeBoulch (US Pat No. 5,631,162) or Sadelain *et al.* (PNAS, Vol 92, pp. 6728-6732, 1995), each of which taken with either Grosveld (US 6,110,666) or Chung (US Pat No. 6,444,421 B1), and further in view of Ercikan (Chemistry and Biology of Pteridines, and Folats, 1993) and Bertino (WO 97/33988).

The rejection of the base claims, as being unpatentable over LeBoulch (US Pat No. 5,631,162) or Sadelain *et al.* (PNAS, Vol 92, pp. 6728-6732, 1995), each of which taken with either Grosveld (US 6,110,666) or Chung (US Pat No. 6,444,421 B1), is applied here as indicated above.

The combined cited references do not teach a retroviral vector comprising human DHFR gene and that the human DHFR has a mutation at aa 22 or 31 of the wild type sequence.

Ercikan teaches effect of mutations on substrate and inhibitor binding site for human DHFR. The art teaches that mutations in codon 22 may be a hot spot

which readily mutates to impart a MTX-resistant phenotype to its host cell (see page 515). The art also teaches that these mutants are superior dominant selectable markers in gene transfer technologies (see the last par. On page 515).

The '988 reference teaches double mutant forms of human DHFR that can be used as selectable markers and for modifying the genome of human cells, such as bone marrow of PBMCs to render them resistant to chemotherapy (abstract). On page 7, the art teaches retroviral vector which can incorporate the mutant DHFR which can be used for increasing tolerance of antifolate tolerance in patients (see page 8).

At the time the invention was made, it would have been obvious to an artisan of ordinary skill in the art to modify the vector of the based combined cited references by replacing or incorporating the DHFR gene into the vector with the single mutant DHFR of Ercikan or double mutant of the '988 reference with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to use the mutant DHFR mutant because such would have allowed tolerance to antifolate drugs in patients as taught by the '988 reference.

Thus, the claimed invention was *prima facie* obvious.

Applicant's response (pages 8 and 9, particularly page 9) has been considered by the examiner, but is either moot or not persuasive because of the new grounds of rejection and remarks as set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 43 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 43 is indefinite because the claim is dependent on a base claim (claim 142) which is not recited any where on record. Thus, the metes and bounds of the claim can not be determined. Clarification is requested.

Applicant is suggested to amend the claims limited to a lentiviral vector comprising essentially of the 3.2 kb LCR, which consists of an 840 bp HS2 fragment (SnaBI-BstXI), a 1308 bp HS3 fragment (HindIII-BamHI) and a 1069 bp HS4 fragment (BamHI-BanII). Such claim amendment would obviate all outstanding prior art rejection because of applicant's unexpected finding as described above.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner *Dave Nguyen* whose telephone number is **571-272-0731**.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, *Ram Shukla*, may be reached at **571-272-0735.**

Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Central Fax number, which is **571-273-8300**.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR)

can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Dave Nguyen
Primary Examiner
Art Unit: 1632

DAVE TRONG NGUYEN
PRIMARY EXAMINER

Notice of References Cited	Application/Control No. 10/188,221	Applicant(s)/Patent Under Reexamination SADELAIN ET AL.	
	Examiner Dave T. Nguyen	Art Unit 1632	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-6,110,666	08-2000	Grosveld et al.	435/6
	B	US-6,444,421	09-2002	Chung, Jay H.	435/6
	C	US-6,524,851 B1	02-2003	Ellis, James	435/325
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Subst. Form PTO-1449

INFORMATION DISCLOSURE CITATION IN AN APPLICATION

(Use several sheets if necessary)

OIPE JC106 NOV 26 2004 PATENT & TRADEMARK OFFICE

Docket Number (Optional)	Application Number
Y1979-0003	10/188,221
Applicant	
Sadelain, et al.	
Filing Date	**Group Art Unit**
July 1, 2002	1632

U. S. PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	5,126,260	6/30/92	Tuan et al.			
	5,631,162	5/20/97	LeBoulch et al.			
	5,834,256	11/10/98	Finer, et al.			
	5,858,740	1/12/99	Finer et al.			
	5,981,276	11/9/99	Sodroski et al.			
	5,994,136	11/30/99	Naldini et al.			
	6,103,516	1/11/00	Verma et al.			
	6,218,187	4/17/01	Finer et al.			
	6,294,165	9/25/01	Lever et al.			
	6,312,682	11/6/01	Kingsman et al.			
	6,428,953	8/6/02	Naldini et al.			
	6,524,851	2/25/03	Ellis			
	6,544,771	4/8/03	Rivière et al.			

FOREIGN PATENT DOCUMENTS

	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	Translation YES	Translation NO

OTHER DOCUMENTS (*Including Author, Title, Date, Pertinent Pages, Etc.*)

	Dull *et al.* (1998) J. Virol. 72:8463-8471, "A Third-Generation Lentivirus Vector with a Conditional Packaging System"
	Naldini et al. (1996) Science 272:263-267, "In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector"
	Sadelain et al. (1995) Proc. Natl. Acad. Sci. 92:6728-6732, "Generation of a high-titer retroviral vector capable of expressing high levels of the human ß-globin gene"

EXAMINER

DATE CONSIDERED 3/5/05

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP § 609; Draw line through citation if not in conformance and not considered. Include copy with next communication to applicant.

NY\264242.1

11/29/2004 EAREGAY1 00000016 10188221
02 FC:1806 180.00 OP

Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination	
10/188,221	SADELAIN ET AL.	
Examiner	**Art Unit**	
Dave T. Nguyen	1632	

SEARCHED			
Class	Subclass	Date	Examiner
same as	set forth previously	3/5/2005	DTN

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
West 2.0 searched	3/5/2005	DTN

Considered
3/15/05

OIPE JC106 NOV 26 2004 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	
Filed: 7/1/2002	Group Art Unit: 1632
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Examiner: Ram Shukla
Attorney Docket No.: MSK.P-050	Confirmation No: 9026
Customer No.: 021121	

DECLARATION UNDER RULE 132

The undersigned inventors each hereby individually declare as follows:

1. I am a named inventor of the above-captioned application, and as such I am familiar with the application, including the claims.

2. I understand that the Examiner has cited May et al. *Nature* 406: 82-86 (1997) against this application. This publication represents the work of the inventors of the present application. To the extent that others are named as co-authors of the paper, they are so-named in recognition of non-inventive contributions to the paper. They are not inventors of the invention claimed in this application.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

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JUN 30 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al. Application No.: 10/188,221 Filed: 7/1/2002 Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies Attorney Docket No.: MSK.P-050 Customer No.: 021121	Group Art Unit: 1632 Examiner: R. Shukla Confirmation No: 9026

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Dear Sir:

In response to the Office Action of March 31, 2005, please amend this application as follows:

07/01/2005 TL0111 00000001 10188221
01 FC:2201 100.00 OP
02 FC:2202 75.00 OP

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 9 of this paper.

I hereby certify that this paper and any attachments named herein are transmitted to the United States Patent and Trademark Office, Fax number: 703-872-9306 on June 30, 2005.

Marina T. Larson

Marina T. Larson, PTO Reg. No. 32,038

June 30, 2005
Date of Signature

PAGE 1/15 * RCVD AT 6/30/2005 12:10:16 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID:9704680104 * DURATION (mm-ss):27-12

Appln No.: 10/188,221
Amendment Dated: June 30, 2005
Reply to Office Action of March 31, 2005

Amendments to the Specification

Please amend the paragraph bridging Pages 7-8 as follows:

To investigate the tissue specificity, stage specificity and expression level of the vector-encoded human B-globin gene, we transduced RNS1 and TNS9 into MEL cells, lymphoid Jurkat cells and myeloid HL-60 cells. Cell-free viral supernatant was used to infect C88 MEL cells in the presence of polybrene (8 $\mu g\ ml^{-1}$). Transduced MEL cells were subcloned by limiting dilution, and screened by PCR for transduction[30] using primers that anneal in the human β-globin promoter sequence (βPS, 5' -GTCTAAGTGATGACAGCCGTACCTG-3', Seq ID No.: 1) and in HS2 (C2A, 5'- TCAGCCTAGAGT GATGACTCC TATCTG-3', Seq ID No.: 2). Vector copy number and integration site analysis was determined by Southern blot analysis[9]. Transduced MEL cells were induced to maturation by 5-day culture in 5 mM N,N'- hexamethylene bisacetamide (HMBA, Sigma).

Page 9, please amend the first full paragraph after the titles in Example 3 as follows:

Total RNA was extracted from MEL, Jurkat and HL-60 cells, or mouse spleen and blood using TRIzol. Quantitative primer extension assays were done using the Primer Extension System-AMV Reverse Transcriptase kit (Promega) with [^{32}P] dATP end-labelled primers specific for retroviral-derived human β-globin (5' -CAGTAACGGCAGACTTCTCCTC -3', Seq ID No.: 3) and mouse β-globin (5' -TGATGTCTGTTTCTGGGGTT GTG -3', Seq ID No.: 4), with predicted extension products of 90 bp and 53 bp, respectively. The probes yield products of identical length for β^{maj}, β^{min}, β^{s} and β^{t}. Primers were annealed to 4μg of RNA and reactions were run according to manufacturer's protocols. Radioactive bands were quantitated by phosphorimager analysis (BioRad). RNA isolated from A85.68 mice[20] was used as positive control. After correction for primer labelling, the human to mouse RNA signal was 29 ± 1% per gene copy in repeated experiments (n > 8), in agreement with previous findings based on RT-PCR[20]. Values measured in bone marrow chimaeras that were obtained in separate runs were standardized to the value obtained in the A85.68 RNA sample. In Figs. 2 and 3c, d, human β-globin expression is expressed per vector copy and normalized to the endogenous transcripts (accounting for two endogenous alleles). In Fig. 3b, human transcripts are reported as the fraction of total β-globin RNA (Huβ / Huβ + Muβ) to reflect absolute contribution of vector-encoded transcripts.

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Appln No.: 10/188,221
Amendment Dated: June 30, 2005
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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A recombinant vector comprising:
(a) a region encoding a functional β-globin gene; and
(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~, said vector providing expression of β-globin when introduced into a mammal *in vivo*.

2. (previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (previously presented) The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

4. (previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

8. (previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type

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human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (previously presented) The vector of claim 1, wherein the functional globin gene encodes human β-globin.

11. (previously presented) The vector of claim 10 further comprising a region encoding a dihydrofolate reductase.

12. (previously presented) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

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19. (withdrawn, currently amended) A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase Hypersensitive sites HS2, HS3 and HS4~~, said vector providing expression of β-globin when introduced into a mammal *in vivo*; and

expressing the functional β-globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. (withdrawn) The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. (withdrawn, currently amended) The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

22. (withdrawn, currently amended) The method of claim 21, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

23. (withdrawn) The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. (withdrawn) The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. (withdrawn) The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

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26. (withdrawn) The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. (withdrawn) The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate,

28. (withdrawn) The method of claim 27, wherein the antiifolate is methotrexate.

29. (withdrawn) The method of claim 19, wherein the globin gene encodes human β-globin.

30. (withdrawn) A mammalian hematopoietic progenitor or stem cell transduced with a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4, wherein said cells express the functional β-globin gene.

31. (withdrawn) The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. (withdrawn) The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. (withdrawn, currently amended) The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

34. (withdrawn, currently amended) The transduced cell of claim 33, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

35. (withdrawn) The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

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36. (withdrawn) The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. (withdrawn) The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. (withdrawn) The transduced cell of claim 30, wherein the globin gene encodes human β-globin.

39. (withdrawn, currently amended) A method of making a therapeutic compositions for treatment of henoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising

(a) a region encoding a functional β-globin gene; and

(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~,, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. (withdrawn) The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. (withdrawn) The method of claim 39, wherein the globin gene encodes human β-globin.

42. (canceled)

43. (currently amended) The vector of claim ~~142~~ 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (new) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (new) A recombinant vector comprising:

(a) a region encoding a functional β-globin gene;

(b) portions of the β-globin locus control region, which include as three separate fragments complete DNase I hypersensitive sites HS2, HS3 and HS4, and

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(c) two GATA-1 binding sites at the junction between the HS3 and HS4 fragments, said vector providing expression of β-globin when introduced into a mammal *in vivo*.

46. (new) The vector of claim 45, wherein the vector is a lentiviral vector.

47. (new) The vector of claim 45, wherein the vector is pTNS9.

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Appln No.: 10/188,221
Amendment Dated: June 30, 2005
Reply to Office Action of March 31, 2005

REMARKS/ARGUMENTS

This is in response to the Office Action mailed March 31, 2005 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Applicants note that the Information Disclosure Statements filed in this case on October 8, 2002 and December 16, 2002 have not been returned with initials indicating consideration by the Examiner. The October 8, 2002 Information Disclosure Statement is present in the Image File Wrapper as reflected in Private PAIR. A further copy of the December 16, 2002 IDS, which was filed electronically, together with the acknowledgment receipt is attached. No fee should be due for this submission, since it was filed prior to the mailing of the first Official Action. Consideration of the references from the two disclosure statements and return of an initialed copy of the 1449 with the next paper are requested.

The Examiner indicated that a sequence listing is required. Applicants have amended the specification to identify sequence ID Nos. and have electronically filed a Sequence Listing consistent with these numbers. A paper copy of the Sequence Listing is enclosed, and the undersigned certifies that this paper listing has the same content as the electronically filed sequence listing.

Claim 1 has been amended to specify that the LCR portion of the vector consists essentially of three restriction fragments, namely the 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, the 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and the 1069 bp fragment of HS4 extending between BamHI and BanII restriction sites. These fragments contain the complete HS2, HS3 and HS4 DNaseI hypersensitive sites, respectively, and may appear in any order. In the Official Action, the Examiner suggested that the claims be amended "to a lentiviral vector comprising essentially of the 3.2 kb LCR which consists of" these fragments. Applicants believe that the language adopted embodies this concept but more clearly describes the LCR portion of the vector. The Examiner also suggested adding a limitation to a lentiviral vector. Applicants have added claim 44 with this limitation, but do not see why this restriction is required to overcome the art rejections.

Claims 45 and 46 have been added and are drawn to a vector having complete HS2, HS3 and HS4 sites, arranged in a contiguous fashion but each on a separate fragment, and having two GATA-I binding sites inserted at the HS3/HS4 fragment junction. Nothing in the art cited by the Examiner discloses such binding sites (previously referenced in claim 42) and Applicants therefore submit that these claims are also allowable over the cited art.

Claim 47 has been added directed to the specific vector made in Example 1, pTNS9.

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Appln No.: 10/188,221
Amendment Dated: June 30, 2005
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As a result of this amendment, three additional claims, and on additional independent claim are presented. Applicants enclose a credit card authorization for the additional claims fees. The Commissioner is authorized to charge any additional fees or credit any overpayments to Deposit Account No. 15-0610.

In view of the foregoing, Applicants submit that this application is in form for allowance. Favorable reconsideration and allowance are respectfully urged.

Respectfully submitted,

Marina T. Larson Ph.D.
PTO Reg. No. 32,038
Attorney for Applicant
(970) 468-6600

Enclosures:
Copy of IDS filed 12/16/2002
Paper Sequence Listing
Credit Card Payment form

PAGE 10/15 * RCVD AT 6/30/2005 12:10:16 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID:9704680104 * DURATION (mm-ss):27-12

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Bertino, Joseph
Rivella, Stefano

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Acknowl[illegible]gment Receipt

SUBMISSION TYPE: **Information Disclosure Statement**
APPLICATION NUMBER: **10188221**
FIRST NAMED INVENTOR: **Michel Sadelain**
TITLE OF INVENTION: **Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies**
ATTORNEY DOCKET NUMBER: **MSK.P-050**

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Electronic Information Disclosure Statement

Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

Application:	*10/188221*
	10/188221
Confirmation:	9026
Applicant(s):	Michel Sadelain
Docket Number:	MSK.P-050
Group Art Unit:	1646
Examiner:	
search string:	(5610053 or 6090608 or 5631162).pn.

US Patent Documents

Note: Applicant is not required to submit a paper copy of cited US Patent Documents

Init	Citation No.	Patent Number	Date	Bar Code	Patentee	Class	Subclass
	P01	5610053	1997-03-11	*5610053*	Chung et al.	435	172.3
	P02	6090608	2000-07-18	*6090608*	Oppenheim et al.	435	235.1
	P03	5631162	1997-05-20	*5631162*	LeBoulch et al.	435	320.1

Signature

Examiner Name	Date

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2003

Application or Docket Number: 10/188,221

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS		
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	minus 20=	*
INDEPENDENT CLAIMS	minus 3 =	*
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

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CLAIMS AS AMENDED - PART II

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FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

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FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

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AMENDMENT C	(Column 1)		(Column 2)	(Column 3)
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*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
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[redacted]

IFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al. Application No.: 10/188,221 Filed: 7/1/2002 Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies Attorney Docket No.: MSK.P-050 Customer No.: 021121	Group Art Unit: 1632 Examiner: R. Shukla Confirmation No: 9026

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Dear Sir:

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I hereby certify that this paper and any attachments named herein are transmitted to the United States Patent and Trademark Office, Fax number: 703-872-9306 on June 30, 2005.

Marina T. Larson, PTO Reg. No. 32,038 — June 30, 2005, Date of Signature


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Acknowledgment Receipt

SUBMISSION TYPE: Information Disclosure Statement
APPLICATION NUMBER: 10188221
FIRST NAMED INVENTOR: Michel Sadelain
TITLE OF INVENTION: Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
ATTORNEY DOCKET NUMBER: MSK.P-050

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al. Application No.: 10/188,221 Filed: 7/1/2002 Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies Attorney Docket No.: MSK.P-050 Customer No.: 021121	Group Art Unit: 1632 Examiner: R. Shukla Confirmation No: 9026

Commissioner for Patents

PO Box 1450

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Dear Sir:

In response to the Office Action of March 31, 2005, please amend this application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 9 of this paper.

I hereby certify that this paper and any attachments named herein are transmitted to the United States Patent and Trademark Office, Fax number: 703-872-9306 on June 30, 2005.

Marina T. Larson, PTO Reg. No. 32,038

June 30, 2005
Date of Signature

07/13/2005 LWONDIM1 00000102 10188221

01 FC:2201 100.00 OP
02 FC:2202 75.00 OP

Appln No.: 10/188,221
Amendment Dated: June 30, 2005
Reply to Office Action of March 31, 2005

Amendments to the Specification

Please amend the paragraph bridging Pages 7-8 as follows:

To investigate the tissue specificity, stage specificity and expression level of the vector-encoded human B-globin gene, we transduced RNS1 and TNS9 into MEL cells, lymphoid Jurkat cells and myeloid HL-60 cells. Cell-free viral supernatant was used to infect C88 MEL cells in the presence of polybrene (8 μg ml^{-1}). Transduced MEL cells were subcloned by limiting dilution, and screened by PCR for transduction[30] using primers that anneal in the human β-globin promoter sequence (βPS, 5' -GTCTAAGTGATGACAGCCGTACCTG-3', Seq ID No.: 1) and in HS2 (C2A, 5'- TCAGCCTAGAGT GATGACTCC TATCTG-3', Seq ID No.: 2). Vector copy number and integration site analysis was determined by Southern blot analysis[9]. Transduced MEL cells were induced to maturation by 5-day culture in 5 mM N,N'- hexamethylene bisacetamide (HMBA, Sigma).

Page 9, please amend the first full paragraph after the titles in Example 3 as follows:

Total RNA was extracted from MEL, Jurkat and HL-60 cells, or mouse spleen and blood using TRIzol. Quantitative primer extension assays were done using the Primer Extension System-AMV Reverse Transcriptase kit (Promega) with [^{32}P] dATP end-labelled primers specific for retroviral-derived human β-globin (5' -CAGTAACGGCAGACTTCTCCTC -3', , Seq ID No.: 3) and mouse β-globin (5' -TGATGTCTGTTTCTGGGGTT GTG -3', Seq ID No.: 4), with predicted extension products of 90 bp and 53 bp, respectively. The probes yield products of identical length for β^{maj}, β^{min}, β^{s} and β^{t}. Primers were annealed to 4μg of RNA and reactions were run according to manufacturer's protocols. Radioactive bands were quantitated by phosphorimager analysis (BioRad). RNA isolated from A85.68 mice[20] was used as positive control. After correction for primer labelling, the human to mouse RNA signal was 29 ± 1% per gene copy in repeated experiments (n > 8), in agreement with previous findings based on RT-PCR[20]. Values measured in bone marrow chimaeras that were obtained in separate runs were standardized to the value obtained in the A85.68 RNA sample. In Figs. 2 and 3c, d, human β-globin expression is expressed per vector copy and normalized to the endogenous transcripts (accounting for two endogenous alleles). In Fig. 3b, human transcripts are reported as the fraction of total β-globin RNA (Huβ / Huβ + Muβ) to reflect absolute contribution of vector-encoded transcripts.

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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A recombinant vector comprising:
(a) a region encoding a functional β-globin gene; and
(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~, said vector providing expression of β-globin when introduced into a mammal *in vivo*.

2. (previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (previously presented) The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

4. (previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

8. (previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type

human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (previously presented) The vector of claim 1, wherein the functional globin gene encodes human β-globin.

11. (previously presented) The vector of claim 10 further comprising a region encoding a dihydrofolate reductase.

12. (previously presented) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

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19. (withdrawn, currently amended) A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~, said vector providing expression of β-globin when introduced into a mammal *in vivo*; and

expressing the functional β-globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. (withdrawn) The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. (withdrawn, currently amended) The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

22. (withdrawn, currently amended) The method of claim 21, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

23. (withdrawn) The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. (withdrawn) The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. (withdrawn) The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

26. (withdrawn) The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. (withdrawn) The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate,

28. (withdrawn) The method of claim 27, wherein the antiifolate is methotrexate.

29. (withdrawn) The method of claim 19, wherein the globin gene encodes human β-globin.

30. (withdrawn) A mammalian hematopoietic progenitor or stem cell transduced with a recombinant vector comprising:

(a) a region encoding a functional β-globin gene; and

(b) large portions of the β-globin locus control regions, which include DNase I hypersensitive sites HS2, HS3 and HS4, wherein said cells express the functional β-globin gene.

31. (withdrawn) The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. (withdrawn) The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. (withdrawn, currently amended) The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a ~~dihydroflate~~ dihydrofolate reductase.

34. (withdrawn, currently amended) The transduced cell of claim 33, wherein the dihydrofolate reductase is a human ~~dihdrofolate~~ dihydrofolate reductase.

35. (withdrawn) The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

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36. (withdrawn) The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. (withdrawn) The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. (withdrawn) The transduced cell of claim 30, wherein the globin gene encodes human β-globin.

39. (withdrawn, currently amended) A method of making a therapeutic compositions for treatment of henoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising

(a) a region encoding a functional β-globin gene; and

(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~,, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. (withdrawn) The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. (withdrawn) The method of claim 39, wherein the globin gene encodes human β-globin.

42. (canceled)

43. (currently amended) The vector of claim ~~142~~ 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (new) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (new) A recombinant vector comprising:

(a) a region encoding a functional β-globin gene;

(b) portions of the β-globin locus control region, which include as three separate fragments complete DNase I hypersensitive sites HS2, HS3 and HS4, and

(c) two GATA-1 binding sites at the junction between the HS3 and HS4 fragments, said vector providing expression of β-globin when introduced into a mammal *in vivo*.

46. (new) The vector of claim 45, wherein the vector is a lentiviral vector.

47. (new) The vector of claim 45, wherein the vector is pTNS9.

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Amendment Dated: June 30, 2005
Reply to Office Action of March 31, 2005

REMARKS/ARGUMENTS

This is in response to the Office Action mailed March 31, 2005 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Applicants note that the Information Disclosure Statements filed in this case on October 8, 2002 and December 16, 2002 have not been returned with initials indicating consideration by the Examiner. The October 8, 2002 Information Disclosure Statement is present in the Image File Wrapper as reflected in Private PAIR. A further copy of the December 16, 2002 IDS, which was filed electronically, together with the acknowledgment receipt is attached. No fee should be due for this submission, since it was filed prior to the mailing of the first Official Action. Consideration of the references from the two disclosure statements and return of an initialed copy of the 1449 with the next paper are requested.

The Examiner indicated that a sequence listing is required. Applicants have amended the specification to identify sequence ID Nos. and have electronically filed a Sequence Listing consistent with these numbers. A paper copy of the Sequence Listing is enclosed, and the undersigned certifies that this paper listing has the same content as the electronically filed sequence listing.

Claim 1 has been amended to specify that the LCR portion of the vector consists essentially of three restriction fragments, namely the 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, the 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and the 1069 bp fragment of HS4 extending between BamHI and BanII restriction sites. These fragments contain the complete HS2, HS3 and HS4 DNaseI hypersensitive sites, respectively, and may appear in any order. In the Official Action, the Examiner suggested that the claims be amended "to a lentiviral vector comprising essentially of the 3.2 kb LCR which consists of" these fragments. Applicants believe that the language adopted embodies this concept but more clearly describes the LCR portion of the vector. The Examiner also suggested adding a limitation to a lentiviral vector. Applicants have added claim 44 with this limitation, but do not see why this restriction is required to overcome the art rejections.

Claims 45 and 46 have been added and are drawn to a vector having complete HS2, HS3 and HS4 sites, arranged in a contiguous fashion but each on a separate fragment, and having two GATA-I binding sites inserted at the HS3/HS4 fragment junction. Nothing in the art cited by the Examiner discloses such binding sites (previously referenced in claim 42) and Applicants therefore submit that these claims are also allowable over the cited art.

Claim 47 has been added directed to the specific vector made in Example 1, pTNS9.

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Amendment Dated: June 30, 2005
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As a result of this amendment, three additional claims, and on additional independent claim are presented. Applicants enclose a credit card authorization for the additional claims fees. The Commissioner is authorized to charge any additional fees or credit any overpayments to Deposit Account No. 15-0610.

In view of the foregoing, Applicants submit that this application is in form for allowance. Favorable reconsideration and allowance are respectfully urged.

Respectfully submitted,

Marina T. Larson Ph.D.
PTO Reg. No. 32,038
Attorney for Applicant
(970) 468-6600

Enclosures:
Copy of IDS filed 12/16/2002
Paper Sequence Listing
Credit Card Payment form

SEQUENCE LISTING

<110> Sadelain, Michel
May, Chad
Bertino, Joseph
Rivella, Stefano

<120> Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies

<130> MSK.P-050

<140> US 10/188,221
<141> 2002-07-01

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RAW SEQUENCE LISTING

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Application Serial Number: 10/188,221
Source: 1600-EFS
Date Processed by STIC: 7-1-05

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IFW16

RAW SEQUENCE LISTING DATE: 07/01/2005
PATENT APPLICATION: **US/10/188,221** TIME: 13:57:48

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      4       May, Chad
      5       Bertino, Joseph
      6       Rivella, Stefano
      8 <120> TITLE OF INVENTION: Vector Encoding Human Globin Gene and Use Thereof in
Treatment of
      9       Hemoglobinopathies
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VERIFICATION SUMMARY DATE: 07/01/2005
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JUL 0 1 2005
JC118
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Electronic Filing System (EFS) Data

Electronic Patent Application Submission

USPTO Use Only

EFS ID:	87365
Application ID:	10188221
Title of Invention:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
First Named Inventor:	Michel Sadelain
Domestic/Foreign Application:	Domestic Application
Filing Date:	2002-07-01
Effective Receipt Date:	2005-06-30
Submission Type:	BIO Sequence Filing
Filing Type:	
Confirmation number:	9026
Attorney Docket Number:	MSK.P-050

Total Fees Authorized:

Digital Certificate Holder: cn=Marina T. Larson,ou=Registered Attorneys,ou=Patent and Trademark Office,ou=Department of Commerce,o=U.S. Government,c=US
Certificate Message Digest: 6cb683a4ab0469c6e027d7c3cb3f8648047ddea6

JUL 0 1 2005
PATENT & TRADEMARK OFFICE 1618

TRANSMITTAL

Electronic Version v1.1
Stylesheet Version v1.1.0

Title of Invention	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

Application Number: 10/188221
Date: 2002-07-01
First Named Applicant: Michel Sadelain

Confirmation Number: 9026
Attorney Docket Number: MSK.P-050

I hereby certify that the use of this system is for OFFICIAL correspondence between patent applicants or their representatives and the USPTO. Fraudulent or other use besides the filing of official correspondence by authorized parties is strictly prohibited, and subject to a fine and/or imprisonment under applicable law.

I, the undersigned, certify that I have viewed a display of document(s) being electronically submitted to the United States Patent and Trademark Office, using either the USPTO provided style sheet or software, and that this is the document(s) I intend for initiation or further prosecution of a patent application noted in the submission. This document(s) will become part of the official electronic record at the USPTO.

Submitted by:	Elec. Sign.	Sign. Capacity
Marina Larson Registered Number: 32038	/marina/	Attorney

JUL 0 1 2005
PATENT & TRADEMARK OFFICE

Documents being submitted	Files
us-bio-seq-trans	eseq-usbios.xml
	us-bio-seq-trans.dtd
	us-bio-seq-trans.xsl
sequence-listing	msk50us.ST25.txt

Comments

This submission is made in partial response to the Official Action mailed March 31, 2005 for this application.

JUL 0 1 2005
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JC118

AMINO ACID AND/OR NUCLEOTIDE SEQUENCE LISTING SUBMISSION

Electronic Version v13
Stylesheet Version v01

This is a request for filing the electronic Computer Readable Form copy of a sequence listing via the Electronic Filing System for a patent application under 37 CFR 1.821-1.825 instead of via one of the physical media specified in 37 CFR 1.824(c).

This communication has an attached file which is an electronic copy of the amino acid and/or nucleotide sequence listing for the previously mentioned United States patent application.

The electronic copy submitted herewith is the Computer Readable Form (CRF), as required by 1.821(e).

Any applicable fees associated with the filing of the electronic copy have been paid.

This submission does not go beyond the disclosure of the application as originally filed (i.e., contains no new matter). It may be in addition to an original CRF, filed to comply with the sequence rules.

This submission in electronic form comprises only the CRF of 37 CFR 1.821(e). I acknowledge that I am responsible for all additional requirements of amino acid and/or nucleotide sequence listing submissions as specified in 37 CFR 1.821 - 1.825.

This submission does not go beyond the disclosure of the application as originally filed (i.e., contains no new matter), and/or is in addition to an original CRF filed to comply with the sequence rules. If not made to comply with an originally filed CRF, it is identical to the sequences disclosed in the application as originally filed and/or the paper copy of the sequence listing as originally filed.

I hereby certify that this correspondence is being transmitted to the United States Patent and Trademark Office on the following date: 2005-06-30

Name: Marina Larson
Electronic Signature Mark: /marina/

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Compression software used:

JUL 0 1 2005
PATENT & TRADEMARK OFFICE
JC118

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May, Chad
Bertino, Joseph
Rivella, Stefano

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JC118
JUL 0 1 2005
PATENT & TRADEMARK OFFICE

10/188,221

PTO/SB/06 (08-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number

CLAIMS AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
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INDEPENDENT CLAIMS (37 CFR 1.16(b))	minus 3 =	*	X $____ =		OR	X $____ =	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(d))			+ $____ =		OR	+ $____ =	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL		OR	TOTAL	

CLAIMS AS AMENDED – PART II

6/30/05

AMENDMENT A

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AMENDMENT C

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* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

2.

JUL 01 2005
PATENT & TRADEMARK OFFICE

Electronic Filing System (EFS) Data

Electronic Patent Application Submission

USPTO Use Only

EFS ID:	87365
Application ID:	10188221
Title of Invention:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
First Named Inventor:	Michel Sadelain
Domestic/Foreign Application:	Domestic Application
Filing Date:	2002-07-01
Effective Receipt Date:	2005-06-30
Submission Type:	BIO Sequence Filing
Filing Type:	
Confirmation number:	9026
Attorney Docket Number:	MSK.P-050

Total Fees Authorized:

Digital Certificate Holder: cn=Marina T. Larson,ou=Registered Attorneys,ou=Patent and Trademark Office,ou=Department of Commerce,o=U.S. Government,c=US
Certificate Message Digest: 6cb683a4ab0469c6e027d7c3cb3f8648047ddea6

Adjustment date: 09/15/2005 SDIRETA1
07713/2005 LWONDIM1 00000102 10188221
01 FC:2201 -100.00 OP
02 FC:2202 -75.00 OP

Refund Ref:
09/15/2005 0030025427

Credit Card Refund Total: $175.00

Am Exp..: XXXXXXXXXX6019

APP_ID=10188221

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JUL 0 1 2005
U.S. TRADEMARK OFFICE 810

TRANSMITTAL

Electronic Version v1.1
Stylesheet Version v1.1.0

Title of Invention	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

Application Number: 10/188221
Date: 2002-07-01
First Named Applicant: Michel Sadelain

Confirmation Number: 9026
Attorney Docket Number: MSK.P-050

I hereby certify that the use of this system is for OFFICIAL correspondence between patent applicants or their representatives and the USPTO. Fraudulent or other use besides the filing of official correspondence by authorized parties is strictly prohibited, and subject to a fine and/or imprisonment under applicable law.

I, the undersigned, certify that I have viewed a display of document(s) being electronically submitted to the United States Patent and Trademark Office, using either the USPTO provided style sheet or software, and that this is the document(s) I intend for initiation or further prosecution of a patent application noted in the submission. This document(s) will become part of the official electronic record at the USPTO.

Submitted by:	Elec. Sign.	Sign. Capacity
Marina Larson Registered Number: 32038	/marina/	Attorney

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JUL 0 1 2005
PATENT & TRADEMARK OFFICE BLDG

Documents being submitted	Files
us-bio-seq-trans	eseq-usbios.xml
	us-bio-seq-trans.dtd
	us-bio-seq-trans.xsl
sequence-listing	msk50us.ST25.txt

Comments

This submission is made in partial response to the Official Action mailed March 31, 2005 for this application.

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2005 JUL 20 AM 9:14

US PATENT & TRADEMARK OFFICE

Attorney Docket MSK.P050
U.S. PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Sadelain, et al.
Serial No:	10/188,221
Filing Date:	07/01/2002
Title:	Vector Encoding Human Globin Gene and Use Thereof in Treatment Of Hemoglobinopathies

REQUEST FOR REFUND

Via Facsimile
Director of U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313

Attn: Refunds
fax: ~~703-308-6778~~ 571-273-6500

Sir:

Attached hereto is a copy of a page from our American Express statement showing two identical charges of $175.00 for the above-referenced application, one on July 1, 2005 and the other on July 13, 2005. The charge of $175.00 was the government fee for an amendment filed on June 30, 2005. On July 13th we were asked by Michelle Terrell with the USPTO to re-fax the amendment that was faxed over on June 30, 2005. One of our assistants re-faxed the document on July 13th, prior to finding out that Marina Larson, attorney for this application, had already re-faxed it on June 30th, 2005. We then called and found out from Michelle that what had been sent on July 13th had already been processed. Since we have been charged twice for filing the amendment, we are requesting a refund of the $175.00 charged to our American Express account on July 13th, 2005.

Respectfully submitted,

OPPEDAHL & LARSON LLP

Marina T. Larson, Ph.D.
Reg. No. 32,038
P.O. Box 5068
Dillon, CO 80435-5068
970-468-6600

Enclosure

PAGE 1/2 * RCVD AT 7/20/2005 1:13:12 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-6/25 * DNIS:2736500 * CSID:9704680104 * DURATION (mm-ss):00-54

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PATENT MAIL DIVISION
2005 JUL 20 AM 9:14
US PATENT & TRADEMARK OFFICE

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06/28/2005	US PATENT TRADEMARK 703-3054631 VA 11154111 WWW.USPTO.GOV	40.00
06/30/2005	US PATENT TRADEMARK 703-3054631 VA 10537280 WWW.USPTO.GOV	65.00
06/30/2005	US PATENT TRADEMARK 703-3054631 VA 60521555 WWW.USPTO.GOV	120.00
06/30/2005	US PATENT TRADEMARK 703-3054631 VA 10331163 WWW.USPTO.GOV	60.00
07/01/2005	US PATENT TRADEMARK 703-3054631 VA 10188221 WWW.USPTO.GOV	175.00
07/01/2005	US PATENT TRADEMARK 703-3054631 VA 60521592 WWW.USPTO.GOV	120.00
07/07/2005	OFFICEMAX, INC. 0716DENVER CO 071611104 OFFICE SUPPLIES 80239	430.38
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07/08/2005	ARAPAHOE CAFE DILLON CO 900069161 FOOD-BEV	170.62
07/08/2005	US PATENT TRADEMARK 703-3054631 VA 6323205 WWW.USPTO.GOV	515.00
07/11/2005	US PATENT TRADEMARK 703-3054631 VA 10524122 WWW.USPTO.GOV	-100.00
07/12/2005	US PATENT TRADEMARK 703-3054631 VA 770261 WWW.USPTO.GOV	-800.00
07/14/2005	QUALCOMM INC 800-2383672 CA 069750008 COMPUTER EQUIP/SOFTWARE	53.77
07/13/2005	COREL CORPORATION 800-772-6735 NH 00136605 SOFTWARE	36.00
07/13/2005	US PATENT TRADEMARK 703-3054631 VA 09869365 WWW.USPTO.GOV	290.00
07/13/2005	US PATENT TRADEMARK 703-3054631 VA 10188221 WWW.USPTO.GOV	175.00

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  (FILE 'HOME' ENTERED AT 06:07:46 ON 02 OCT 2005)

     FILE 'MEDLINE, CAPLUS, SCISEARCH' ENTERED AT 06:08:01 ON 02 OCT 2005
L1          2118 S GLOBIN AND (LCR OR LOCUS (A) CONTROL (A) REGION)
L2            17 S L1 AND (SADELIN?/AI OR RIVELLA?/AU OR MAY?/AU OR BERTINO?/AU)
L3            11 DUP REM L2 (6 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 06:14:20 ON 02 OCT 2005

     FILE 'MEDLINE, CAPLUS, SCISEARCH' ENTERED AT 06:16:16 ON 02 OCT 2005
L4            17 S L1 AND (SADELAIN?/AI OR RIVELLA?/AU OR MAY?/AU OR BERTINO?/AU
L5            21 S L1 AND (SADELAIN?/AU )
L6            21 S L5 AND L1
L7             0 S L6 AND VETOR
L8            18 S L6 AND VECTOR
L9            12 DUP REM L8 (6 DUPLICATES REMOVED)
L10            5 S L9 NOT L3

     FILE 'STNGUIDE' ENTERED AT 06:17:54 ON 02 OCT 2005

     FILE 'MEDLINE, CAPLUS, SCISEARCH' ENTERED AT 06:22:01 ON 02 OCT 2005

=> s globin and hs2 and hs3 and hs4
L11           90 GLOBIN AND HS2 AND HS3 AND HS4

=> s globin and hs2 (s) hs3 (s) hs3
L12          148 GLOBIN AND HS2 (S) HS3 (S) HS3

=> s globin and hs2 (s) hs3 (s) hs4
L13           66 GLOBIN AND HS2 (S) HS3 (S) HS4

=> s globin and hs2 (s) hs3 (s) hs4 (s) vector
L14            6 GLOBIN AND HS2 (S) HS3 (S) HS4 (S) VECTOR

=> s globin and hs2 (s) hs3 (s) hs4 (s) plasmid
L15            0 GLOBIN AND HS2 (S) HS3 (S) HS4 (S) PLASMID

=> dup rem l14
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Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6	"5610053".pn. or "6090608".pn. or "5631162".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/10/02 06:35
L2	6	l1 and globin	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/10/02 06:35
L3	4	l1 and globin and (LCR or locus adj control adj region)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/10/02 06:37
L4	1	l1 and globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/10/02 06:37
S1	3	"9733988"	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/10/02 06:34
S2	1	10/188221 and functional	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:00
S3	8840	Sadelin.in. or Rivella.in. or May.in. or Bertino.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:01
S4	2	S3 and globin.clm.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:02
S5	12344	globin	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:02

S6	89	globin and hemoglobinopathy	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:03
S7	23	vector same globin and hemoglobinopathy	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:03
S8	229	globin same (lcr or locus adj control adj region)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:03
S9	8	globin same (lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:05
S10	3	globin same (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:10
S11	3	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:10
S12	1190	(lentivirus or lentiviral) and globin	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:10
S13	2	(lentivirus or lentiviral) same globin same lcr	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:11
S14	1	10/298491	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2005/09/30 14:32

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	MSK.P-050	9026

21121 7590 10/04/2005

OPPEDAHL AND LARSON LLP
P O BOX 5068
DILLON, CO 80435-5068

EXAMINER	
MARVICH, MARIA	
ART UNIT	**PAPER NUMBER**
1633	

DATE MAILED: 10/04/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Maria B. Marvich, PhD	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 June 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41 and 43-47* is/are pending in the application.

4a) Of the above claim(s) *19-41* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18 and 43-47* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/30/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This office action is in response to an amendment filed 6/30/05. An IDS filed 6/30/05 has been received. The documents have been considered and the signed eIDS accompanies this office action. Claim 42 has been cancelled. Claims 1, 19, 21, 22, 33, 34 and 43 have been amended. Claims 44-47 have been added. Claims 1-41 and 43-47 are pending in this application. Claims 19-41 have been withdrawn. Therefore, claims 1-18 and 43-47 are under examination in the application.

Response to Amendment

Any rejection of record in the previous action not addressed in this office action is withdrawn. There are new grounds of rejection herein that were not necessitated by applicants amendment and therefore, this action is not final.

Specification

The disclosure is objected to because of the following informalities: on page 4, line 6, the phrase "which confirms showed highly" is grammatically incorrect. On page 6, line 12, the phrase "selection to tool to enhance" is grammatically incorrect. On page 6, line 19, "usedin" requires a space between "used" and "in" and in line 23, the word "genes" should be singular. On page 14, line 8, "then" is misspelled as "ten". On page 15, line 4 "term" is misspelled as "trem", in line 12, "descreasein" requires a space between "decrease" and "in", line 20, "matched" is misspelled as "mateched", in line 21, "chimeras" is misspelled as "chimeraas" and in line 26, "levelsmeasured" requires a space between the words "levels" and "measured" and in

line 29, "modelof" requires a space between the words "model" and "of". On page 16, line 3, "spleen" is misspelled as "slpeen" and in line 19, "studied" is misspelled as "stuied".

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-18 and 43-47 are rejected under 35 USC 112, 1st paragraph, as failing to comply with the written description requirement. The claims contains subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Applicants recite a genus of recombinant vectors comprising mutant human dihydrofolate reductase (DHFR) that has increased resistance to antifolate as compared to wild-type DHFR.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.

Applicants recite a recombinant vector comprising a region encoding a functional b-globin gene. The specification teaches that the term "functional globin gene" includes both exons and introns, as well as globin promoters and splices donors/acceptors and can be

expressed. Specifically, The specification discloses two recombinant lentiviral vectors, TNS9 and dTNS9-PD. TNS9 vector incorporates –618 to +2484 of the human β-globin gene. This encompasses the coding sequence and an extended portion of the promoter and enhancer region. dTNS9-PD comprises a deletion in the LTR and includes a PGK promoter form the murine phosphoglycerate kinase 1 gene and a mutant DHFR region with a mutation at amino acid 22. The specification also teaches that the β-globin gene coding sequence can be replaced with fragments of gamma globin, alpha globin. Each of the fragments encompasses Met to the polyadenylation sequence, which essentially are the coding sequences of the alpha and gamma genes. Other than the β-globin gene from position –618 to +2484 that includes an extended promoter and 3' enhancer element, applicants do not disclose the genomic sequence of "functional globin genes" that can be used in the recombinant vector for expression of β-globin when introduced into a mammal *in vivo*. However, there is no reference sequence provided in the specification or the claims. Furthermore, all of the components of the gene as regulation sequences, introns, and exons must be determined empirically in order to generate the recombinant vector for expression *in vivo* in mammals. Furthermore, it is unclear what components are required such that it is "functional". Applicants claim the gene without any disclosure about its structure. Given that applicants recite any functional β-globin gene but disclose only –618 to +2484 of the human β-globin gene for which no reference sequence is provided, given that the genomic structures of any β-globin gene such that is "functional" are unknown, it is concluded that the invention must be empirically determined. In an unpredictable art, the disclosure of one species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

In the instant case, applicants recite that the vector also comprises DHFR as a selection marker. Specifically in dependent claims 5, 8, 14 and 17, applicants claim a genus of DHFR mutant forms that comprise mutations that increase the capacity of the DHFR to confer resistance to drugs. Applicants recite a single mutant form that comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence. However, there is no reference sequence provided in the specification or the claims. Rather, the specification discloses that double and single mutants of DHFR with mutations at amino acids 22 and 31 are described in commonly owned WO document 97/33988. Both the specification and WO 97/33988 teach that the reference sequence is human DHFR. As to the genus of DHFR mutants, the specification and prior art teach DHFR with mutations at amino acids 22 and 31. However, the structural requirements of these mutants are not provided. As to the functional requirements, the prior art teaches that the effect of the DHFR mutants is due to an increase in the rate of methotrexate dissociation. However, neither the prior art nor the specification teach that any other amino acids are capable of mediating the same effect. Given the large size and diverse nature of h DHFR proteins with mutations and the inability to determine which will also possess the ability to confer increased resistance to drugs, it is concluded that the invention must be empirically determined. In an unpredictable art, the disclosure of two species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

Conclusion

Claims 1-18 and 43-47 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B. Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, David Nguyen, PhD can be reached on (571)-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Maria B Marvich, PhD
Examiner
Art Unit 1633

September 14, 2005

JAMES KETTER
PRIMARY EXAMINER

Electronic Information Disclosure Statement

Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies

Application:	*10/188221*
	10/188221
Confirmation:	9026
Applicant(s):	Michel Sadelain
Docket Number:	MSK.P-050
Group Art Unit:	1646
Examiner:	
search string:	(5610053 or 6090608 or 5631162).pn.

US Patent Documents

Note: Applicant is not required to submit a paper copy of cited US Patent Documents

Init	Citation No.	Patent Number	Date	Bar Code	Patentee	Class	Subclass
MM	P01	5610053	1997-03-11	*5610053*	Chung et al.	435	172.3
MM	P02	6090608	2000-07-18	*6090608*	Oppenheim et al.	435	235.1
MM	P03	5631162	1997-05-20	*5631162*	LeBoulch et al.	435	320.1

Signature

MMunich 9/30/05

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
10/188,221	SADELAIN ET AL.
Examiner	**Art Unit**
Maria B. Marvich, PhD	1633

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

Claim		Date								
Final	Original	9/30/05								
	1	√								
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Claim		Date								
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Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination	
10/188,221	SADELAIN ET AL.	
Examiner	**Art Unit**	
Maria B. Marvich, PhD	1633	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
East, PALM inventor search	9/30/2005	MM
East databases- USPAT, PGPUB, EPO, JPO, Derwent, IBM-IDB search notes attached	9/30/2005	MM
STN databases- Caplus, Scisearch, Medline search notes attached	9/30/2005	MM

UNITED STATES PATENT AND TRADEMARK OFFICE

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www.uspto.gov

APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	FILE WRAPPER LOCATION
10/188,221		1633	16M1

Correspondence Address / Fee Address Change

The following fields have been set to Customer Number 52334 on 10/12/2005

- Correspondence Address
- Maintenance Fee Address

The address of record for Customer Number 52334 is:
OPPEDAHL & LARSON LLP - MSK
P. O. BOX 5068
DILLON,CO 80435-5068

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al. Application No.: 10/188,221 Filed: 7/1/2002 Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies Attorney Docket No.: MSK.P-050 Customer No.: 021121	Group Art Unit: 1632 Examiner: R. Shukla Confirmation No: 9026

Commissioner for Patents

PO Box 1450

Alexandria, VA 22313-1450

AMENDMENT

Dear Sir:

In response to the Office Action of October 4, 2005, please amend this application as follows:

Amendment to the Specification begin on Page 2.

Amendments to the Claims are reflected in the listing of claims which begins on page 5 of this paper.

Remarks/Arguments begin on page 11 of this paper.

Amendments to the Specification

Page 3, amend the paragraph starting on line 5 as follows:

Fig. 4 shows the average vector copy number in peripheral blood cells, measured periodically for 24 weeks, which confirms ~~showed~~ highly efficient gene transfer in cells transduced with the vector of the invention.

Page 6, amend the paragraphs starting on line 4 as follows:

Fig. 2 shows the genomic structure of specific vector within the scope of the invention. The vector includes a deleted LTR, from -456 to -9 of HIV LTR and the PGK promoter (530 bp) from the murine phosphoglycerate kinase 1 gene. It also includes a DHFR-encoding region encoding human DHFR with s/f mutation at amino acid 22. The locus control region and the β-globin region are the same as in TSN9. This vector is designated dTNS9-PD. This incorporation of DHFR into this vector provides transformed cells with a methotrexate-resistant phenotype. As a result, methotrexate, and other antifolates can be used, both *in vitro* and *in vivo* as a selection ~~to tool~~ tool to enhance levels of the functional hemoglobin. When hematopoietic stem cells were transformed using dTNS9-PD and reintroduced to mice that were then treated with NMBPR-P (0.5 mg/dose) and TMTX (0.5 mg dose) for five days, observed levels of expressed human β-globin were much higher in mice transduced with dTNS9-PD vectors after treatment with TMTX and NMBPR-P for selection of transduced cells.

The vectors of the invention are used in therapy for treatment of individuals suffering from hemoglobinopathies. In one embodiment of the invention, hematopoietic progenitor or stem cells are transformed *ex vivo* and then restored to the patient. As ~~usedin~~ used in the specification and claims hereof, the term "hematopoietic progenitor sand stem cells" encompasses hematopoietic cells and non-hematopoietic stem cells, e.g., embryonic stem cells, hematopoietic stem cell precursors, or any of the latter generated by nuclear transfer from a somatic cell. It is know in the art that efficient gene ~~genes~~ transfer into human embryonic stem cells can be achieved using lentiviral vectors.

Page 15, amend the paragraphs beginning at line 3 as follows:

The stability of TNS9-encoded ^{A} expression detected in peripheral blood suggested that ~~long-trem~~ long-term hematologic and systemic therapeutic benefits could be obtained. To investigate whether Hbb^{hu} production would suffice to ~~teart~~ treat the anemia, we closely monitored hemoglobin parameters over 40 weeks. The marked increase in hemoglobin concentration, RBC counts, and hematocrit was sustained throughout this time period. Control mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells remained severely

anemic, indicating that the transplantation procedure itself did not alter the anemic state. The reticulocyte counts decreased to 5% to 8% in TNS9 treated-chimeras, compared to 19% to 21% in control eGFP-treated $Hbb^{th3/+}$ chimeras and age-matched $Hbb^{th3/+}$ mice, suggesting an increase in RBC life span and a ~~decreasein~~ decrease in erythropoietic activity.

Example 9

To determine the impact of sustained human -globin gene expression on hematopoiesis, we studied the degree of splenomegaly (enlargement of the spleen) and EMH in 1-year-old chimeras and ~~age-matecheed~~ age-matched control mice. Spleen weights measured in Tns9-treated $Hbb^{th3/+}$ ~~chimeraas~~ chimeras were indistinguishable from recipients of eGFP-transduced normal bone marrow, as were the total number of cells per spleen. In contrast, mice engrafted with eGFP-transduced $Hbb^{th3/+}$ bone marrow cells showed spleen weights and total cell numbers that were about 3-fold greater. The correction of spleen weight in TNS9 bone marrow chimeras corresponded to a concomitant normalization in total hematopoietic progenitor cell content. Spleen CFU-Es, BFUEs, andCFUs-GM were reduced to ~~levelsmeasured~~ levels measured in recipients of eGFP-transduced $Hbb^{th+/+}$ bone marrow, whereas they remained elevated in control chimeras engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells and in age-matched $Hbb^{th3/+}$ mice, as previously observed in another murine ~~modelof~~ model of -thalassemis.[29]

Page 15, amend the paragraphs starting on line 1 as follows:

The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and age-match controls. Histopathology of spleens of mice that received transplants of eGFP-tranduced $Hbb^{th3/+}$ marrow was virtually identical to that of ~~slpeen~~ spleen from control $Hbb^{th3/+}$ mice. Specifically, the red pulp was significantly expanded, accounting for 80% to 90% of the cross-sectional area, and densely occupied by nucleated erythroid precursors. The white pulp, based on cross-sectional area, ~~wsa~~ was relatively decreased and the marginal zones were obscured by the large number of nucleated RBCs, reflecting major expansion of the red pulp and erythroid precursors. In TNS9-treated chimeras, the amount of red pulp was considerably decreased, accounting for olny about 50% to 60% of the cross-sectional area. In addition, the number of nucleated erythroid precursors in the red pulp was decreased. Other immature hematopoietic cells were present in the red pulp, but much less frequently than in the spleens of control $Hbb^{th3/+}$ thalassemic mice. The livers from TNS9-treated chimeras were similar to those of the normal control mice in that no EMH was detected. In contrast, livers from mice engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells showed seral small foci of intrasinusoidal EMH.

Example 10

Toxic iron accumulation in the organs of thalassemic patients is a consequence of RBC destruction and increased gastrointestinal iron uptake. To determine whether sustained expression from the TNS9 vector reduced iron overload, we ~~stuied~~ studied tissue sections of liver and heart, stained using Gomori iron stain. No iron deposition was seen in the livers of normal $Hbb^{+/+}$ control mice, whereas $Hbb^{th3/+}$ mice showed variable amounts of iron, including some large aggregates. TNS9-trasduced treated chimeras demonstrated low to undetectable levels of iron in the livers, whereas iron was readily detected in the livers of all mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells. No iron accumulation was found in the heart of treated or control mice, as previously observed in another murine model of - thalassemia,[30] in contrast to what is found in the human disease. [1-3]

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A recombinant vector comprising:
(a) a region comprising ~~encoding~~ a functional globin ~~β-globin~~ gene; and
(b) portions of the -globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, said vector providing expression of ~~-globin~~ globin when introduced into a mammal *in vivo*.

2. (previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (previously presented) The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

4. (previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

8. (previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (previously presented) The vector of claim 1, wherein the functional globin gene encodes human -globin.

11. (previously presented) The vector of claim 10 further comprising a region encoding a dihydrofolate reductase.

12. (previously presented) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19. (withdrawn, currently amended) A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional globin ~~-globin~~ gene; and

(b) portions of the -globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, said vector providing expression of ~~-globin~~ globin when introduced into a mammal *in vivo*; and

expressing the functional ~~-globin~~ globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. (withdrawn) The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. (withdrawn) The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

22. (withdrawn) The method of claim 21, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

23. (withdrawn) The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. (withdrawn) The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. (withdrawn) The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

26. (withdrawn) The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. (withdrawn) The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate,

28. (withdrawn) The method of claim 27, wherein the antiifolate is methotrexate.

29. (withdrawn) The method of claim 19, wherein the globin gene encodes human -globin.

30. (withdrawn, currently amended) A mammalian hematopoietic progenitor or stem cell transduced with a recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional ~~-globin~~ globin gene; and

(b) ~~large~~ portions of the -globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~, wherein said cells express the functional ~~-globin~~ globin gene.

31. (withdrawn) The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. (withdrawn) The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. (withdrawn) The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

34. (withdrawn) The transduced cell of claim 33, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

35. (withdrawn) The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

36. (withdrawn) The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. (withdrawn) The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. (withdrawn) The transduced cell of claim 30, wherein the globin gene encodes human -globin.

39. (withdrawn, currently amended) A method of making a therapeutic composition ~~compositions~~ for treatment of henoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising

(a) a region comprising ~~encoding~~ a functional ~~-globin~~ globin gene; and

(b) portions of the -globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. (withdrawn) The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. (withdrawn) The method of claim 39, wherein the globin gene encodes human -globin.

42. (canceled)

43. (previously presented) The vector of claim 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (previously presented) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (currently amended) A recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional ~~-globin~~ globin gene;

(b) portions of the -globin locus control region, which include as three separate fragments complete DNase I hypersensitive sites HS2, HS3 and HS4, and

(c) two GATA-1 binding sites at the junction between the HS3 and HS4 fragments, said vector providing expression of ~~-globin~~ globin when introduced into a mammal *in vivo*.

46. (previously presented) The vector of claim 45, wherein the vector is a lentiviral vector.

47. (previously presented) The vector of claim 45, wherein the vector is pTNS9.

REMARKS/ARGUMENTS

This is in response to the Office Action mailed October 4, 2005 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Applicants again note that the Information Disclosure Statement filed in this case on October 8, 2002 has not been returned with initials indicating consideration by the Examiner. The October 8, 2002 Information Disclosure Statement is present in the Image File Wrapper as reflected in Private PAIR. Consideration of the references from the disclosure statement and return of an initialed copy of the 1449 with the next paper are requested.

Applicants have amended the specification to correct the typographical errors noted by the Examiner. In addition, withdrawn claim 30 has been amended to conform to the previous amendment of claim 1 so that it can be recombined with claim 1 should withdrawal of the restriction requirement be considered appropriate upon allowance of the elected claims.

Applicants have also amended the independent claims to refer to "globin" as opposed to "β-globin." This language is consistent with the language in the claims as originally filed. At some point, the β was inserted in the claims in error, and no amendment to the claims was noted.

The Examiner has rejected claims 1-18 and 43-47 under 35 USC § 112, first paragraph, as lacking a written description. The focus of the rejection is on the reference to mutant human dihydrofolate reductase, and the reference to a functional globin gene. As a first matter, Applicants have amended to claims to refer to a region comprising a functional globin gene. rather than encoding.

The Examiner has rejected the claims for lack of written description based on the number of examples in the specification for the individual components of the claimed invention. Based on the recently decided case of *Capon v Eshhar*, 76 USPQ2d 1078 (Fed. Cir. 2005), Applicants submit that this rejection is in error

Capon is based on an interference proceeding in which both parties claimed chimeric genes comprising a gene segment encoding a single-chain (scFv) antibody, and a second gene encoding a cytoplasmic signaling domain. Expression of a chimeric gene resulted in cells that expressed a cell surface marker that produced cell signaling in response to binding of an antigen to the scFV antibody. The Board of Patent and Appeals and Interferences had held that both specifications were lacking in written description because of a limited number of examples, and the absence of a complete sequence of at least one chimeric gene within the scope of the claims.

In vacating and remanding the holding of the Board of Appeals, the Federal Circuit observed:

> The "written description" requirement must be applied in the context of the particular invention and the state of the knowledge. The Board's rule that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known, is an inappropriate generalization. When the prior art includes the nucleotide information, precedent does not set a per se rule that the information must be determined afresh.
>
> * * *

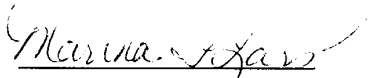
76 USPQ2d at 1084-5. In the present case, the "particular invention" is not the sequence of globin genes that produce functional proteins, and it is not the sequence of mutants of DHFR. As in *Capon*, the "particular invention" is the combination of known elements into a particular structure. However, unlike *Capon*, the present claims are far less broad.

Globin genes have been the subject of intense study, and sequences and structures for defective and functional proteins are well known. For example, *A Syllabus of Human Hemoglobin Variants* (1996), by Titus H.J. Huisman, Marianne F.H. Carver, and Georgi D. Efremov, was published by The Sickle Cell Anemia Foundation in Augusta, GA, USA. As stated in the on-line version of this publication, "this is a comprehensive listing of all known human hemoglobin variants, including variants of the alpha-, beta-, gamma-, and delta-globin chains." http://globin.cse.psu.edu/html/huisman/variants/. Thus, the structure of suitable globin genes was well-known when the application was filed, and re-presentation of these sequences is not required by the written description requirement.

With respect to mutant DHFR, Applicants have cited in the specification documents describing known sequences of such mutants, with reference to a prior publication of the sequences. *Capon* indicates that no more should be required. As to the statement that the ability to confer drug-resistance must be empirically determined, Applicants note that this is not their invention. Their invention is a combination of elements, one of which is a gene encoding a DHFR mutant that is known (as a result of prior work or future invention) to have this property. To deny coverage to the full scope of Applicants invention because someone might develop a new DHFR mutant, thereby allowing that person to freely misappropriate Applicants' invention of the combination, is not what the written description requirement was intended to accomplish.

For the foregoing reasons, Applicants submit that this case is now in form for allowance. Favorable reconsideration and allowance of all claims is respectfully requested. Further, recombination of the withdrawn claims is requested.

Respectfully submitted,



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(970) 262-1800

PTO/SB/22 (12-04)
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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2005 (Fees pursuant to the Consolidated Appropriation Act, 2005 (H.R. 4818).)	Docket Number (Optional) **MSK.P-050**
Application Number **10/188,221**	Filed **7/1/2002**
For **Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies**	
Art Unit **1633**	Examiner **Marcia Marich**

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

	Fee	Small Entity Fee	
[x] One month (37 CFR 1.17(a)(1))	$120	$60	$ 60.00
[ ] Two months (37 CFR 1.17(a)(2))	$450	$225	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,020	$510	$
[ ] Four months (37 CFR 1.17(a)(4))	$1,590	$795	$
[ ] Five months (37 CFR 1.17(a)(5))	$2,160	$1,080	$

[ ] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check in the amount of the fee is enclosed.

[x] Payment by credit card.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[ ] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number _____. I have enclosed a duplicate copy of this sheet.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant/inventor

[ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

[X] attorney or agent of record. Registration Number 32038

[ ] attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____.

Marina T. Larson — Signature

February 6, 2006 — Date

Marina T. Larson, Ph.D — Typed or printed name

970 262 1800 — Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[ ] Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	10188221
Filing Date:	01-Jul-2002
Title of Invention:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
First Named Inventor:	Michel Sadelain
Filer:	Marina T. Larson
Attorney Docket Number:	MSK.P-050

Filed as Small Entity

Utility Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issurance:				
Extension-of-Time:				
Extension for response within 1st month	2251	1	60	60

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**60**

Electronic Acknowledgement Receipt

EFS ID:	1004302
Application Number:	10188221
Confirmation Number:	9026
Title of Invention:	Vector encoding human globin gene and use thereof in treatment of hemoglobinopathies
First Named Inventor:	Michel Sadelain
Customer Number:	52334
Filer:	Marina T. Larson
Filer Authorized By:	
Attorney Docket Number:	MSK.P-050
Receipt Date:	06-FEB-2006
Filing Date:	01-JUL-2002
Time Stamp:	17:24:06
Application Type:	Utility

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	
RAM confirmation Number	23

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part	Pages
1		20060206_amendment.pdf	629590	yes	13

	Multipart Description		
	Doc Desc	**Start**	**End**
	Amendment - After Non-Final Rejection	1	1
	Specification	2	4
	Claims	5	10
	Applicant Arguments or Remarks Made in an Amendment	11	13

Warnings:					
2	Extension of Time	20060206_extension.pdf	60754	no	1
Warnings:					
3	Fee Worksheet (PTO-875)	fee-info.pdf	6759	no	2
Warnings:					
		Total Files Size (in bytes):	697103		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

10/188,224

PTO/SB/06 (08-03)
Approved for use through 7/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number

CLAIMS AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE (37 CFR 1.16(a))				$____	OR		$____
TOTAL CLAIMS (37 CFR 1.16(c))	minus 20 =	*	X $____ =		OR	X $____ =	
INDEPENDENT CLAIMS (37 CFR 1.16(b))	minus 3 =	*	X $____ =		OR	X $____ =	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(d))			+ $____ =		OR	+ $____ =	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL		OR	TOTAL	

CLAIMS AS AMENDED – PART II

6/30/05

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	* 47	Minus	** 46	= 1	X $____ =	25	OR	X $____ =	
Independent (37 CFR 1.16(b))	* 5	Minus	*** 5	=	X $____ =	0	OR	X $____ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $____ =		OR	+ $____ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

2/6/06

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	* 46	Minus	** 47	=	X $____ =		OR	X $____ =	
Independent (37 CFR 1.16(b))	* 5	Minus	*** 5	=	X $____ =		OR	X $____ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $____ =		OR	+ $____ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total (37 CFR 1.16(c))	*	Minus	**	=	X $____ =		OR	X $____ =	
Independent (37 CFR 1.16(b))	*	Minus	***	=	X $____ =		OR	X $____ =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $____ =		OR	+ $____ =	
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

2

OIPE IAP62
MAR 13 2006
PATENT & TRADEMARK OFFICE

IFW 1633

PTO/SB/21 (09-04)
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket Number	64836(51590)

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [x] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Power of Attorney and Correspondence Address Indication Form
Statement Under 37 CFR 3.73(b)
Return Receipt Postcard

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	EDWARDS ANGELL PALMER & DODGE LLP		
Signature	Amy Leahy		
Printed name	Amy M. Leahy		
Date	March 9, 2006	Reg. No.	47,739

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: March 9, 2006 Signature: Denise Kacinski (Denise Kacinski)

207097

OIPE IAP62
MAR 13 2006
PATENT & TRADEMARK OFFICE

PTO/SB/82 (04-05)
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REVOCATION OF POWER OF ATTORNEY WITH NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket Number	64836(51590)

I hereby revoke all previous powers of attorney given in the above-identified application.

[X] A Power of Attorney is submitted herewith.

OR

[ ] I hereby appoint the practitioners associated with the Customer Number: [ ]

[ ] Please change the correspondence address for the above-identified application to:

[ ] The address associated with Customer Number: [ ]

OR

[ ] Firm *or* Individual Name

Address					
City					
Country		State		Zip	
Telephone		Email			

I am the:

[ ] Applicant/Inventor.

[X] Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature	James S Quirk		
Name	James S. Quirk		
Date	3/29/06	Telephone	212 639 6181

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of 1 forms are submitted.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 9, 2006 Signature: Denise Kacinski (Denise Kacinski)

206307

OIPE IAP62 MAR 13 2006 PATENT & TRADEMARK OFFICE

PTO/SB/81 (04-05)
Approved for use through 11/30/2005. OMB 0651-0035
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POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Title	VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT, etc.
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket No.	64836(51590)

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

[x] Practitioners associated with the Customer Number: 21874

OR

[ ] Practitioner(s) named below:

Name	Registration Number	Name	Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

[x] The address associated with the above-mentioned Customer Number:

OR

[ ] The address associated with Customer Number:

OR

[ ] Firm or Individual Name: Amy Leahy, EDWARDS ANGELL PALMER & DODGE LLP

Address	P.O. Box 55874				
City	Boston	State	MA	Zip	02205
Country	US	Telephone	(203) 975-7505	Email	aleahy@eapdlaw.com

I am the:

[ ] Applicant/Inventor.

[x] Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature	James S Quirk	Date	3/7/06
Name	James S. Quirk	Telephone	212 639 6181
Title and Company	Authorized Signer for Assignee		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of 1 forms are submitted.

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 9, 2006 Signature: Denise Kacinski (Denise Kacinski)

OIPE IAP62
MAR 13 2006
PATENT & TRADEMARK OFFICE

PTO/SB/96 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Michel Sadelain et al.

Application No./Patent No.: 10/188,221 Filed/Issue Date: July 1, 2002

Entitled: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Memorial Sloan-Kettering Cancer Center , a _____
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest; or

2. [ ] an assignee of less than the entire right, title and interest.
The extent (by percentage) of its ownership interest is _____ %

in the patent application/patent identified above by virtue of either:

A. [X] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 013256 , Frame 0983 , or for which a copy thereof is attached.

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as shown below:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____ , Frame _____ , or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____ , Frame _____ , or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____ , Frame _____ , or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] Copies of assignments or other documents in the chain of title are attached.
[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, if the assignment is to be recorded in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

James S. Quirk — Signature
3/7/06 — Date
James S. Quirk — Printed or Typed Name
212 639 6181 — Telephone Number
Authorized Signer for Assignee — Title

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: March 9, 2006 Signature: Denise Kacinski (Denise Kacinski)

206308

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	64836(51590)	9026

21874 7590 04/04/2006

EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
MARVICH, MARIA	
ART UNIT	**PAPER NUMBER**
1633	

DATE MAILED: 04/04/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Notice of Non-Compliant Amendment (37 CFR 1.121)	Application No. 10/188,221	Applicant(s) SADELAIN ET AL.	
	Examiner Maria B. Marvich, PhD	Art Unit 1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

The amendment document filed on _____ is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121 or 1.4. In order for the amendment document to be compliant, correction of the following item(s) is required.

THE FOLLOWING MARKED (X) ITEM(S) CAUSE THE AMENDMENT DOCUMENT TO BE NON-COMPLIANT:

- ☐ 1. Amendments to the specification:
  - ☐ A. Amended paragraph(s) do not include markings.
  - ☐ B. New paragraph(s) should not be underlined.
  - ☐ C. Other _____.
- ☐ 2. Abstract:
  - ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
  - ☐ B. Other _____.
- ☐ 3. Amendments to the drawings:
  - ☐ A. The drawings are not properly identified in the top margin as "Replacement Sheet," "New Sheet," or "Annotated Sheet" as required by 37 CFR 1.121(d).
  - ☐ B. The practice of submitting proposed drawing correction has been eliminated. Replacement drawings showing amended figures, without markings, in compliance with 37 CFR 1.84 are required.
  - ☐ C. Other _____.
- ☒ 4. Amendments to the claims:
  - ☐ A. A complete listing of all of the claims is not present.
  - ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
  - ☐ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following status identifiers: (Original), (Currently amended), (Canceled), (Previously presented), (New), (Not entered), (Withdrawn) and (Withdrawn-currently amended).
  - ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
  - ☒ E. Other: *See Continuation Sheet*.
- ☐ 5. Other (e.g., the amendment is unsigned or not signed in accordance with 37 CFR 1.4):

_____

For further explanation of the amendment format required by 37 CFR 1.121, see MPEP § 714.

TIME PERIODS FOR FILING A REPLY TO THIS NOTICE:

1. Applicant is given **no new time period** if the non-compliant amendment is an after-final amendment or an amendment filed after allowance. If applicant wishes to resubmit the non-compliant after-final amendment with corrections, the **entire corrected amendment** must be resubmitted.
2. Applicant is given **one month**, or thirty (30) days, whichever is longer, from the mail date of this notice to supply the correction, if the non-compliant amendment is one of the following: a preliminary amendment, a non-final amendment (including a submission for a request for continued examination (RCE) under 37 CFR 1.114), a supplemental amendment filed within a suspension period under 37 CFR 1.103(a) or (c), and an amendment filed in response to a *Quayle* action. If any of above boxes 1. to 4. are checked, the correction required is only the **corrected section** of the non-compliant amendment in compliance with 37 CFR 1.121.

   Extensions of time are available under 37 CFR 1.136(a) only if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action.

   Failure to timely respond to this notice will result in:
   Abandonment of the application if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action; or
   Non-entry of the amendment if the non-compliant amendment is a preliminary amendment or supplemental amendment.

_____ _____

Legal Instruments Examiner (LIE), if applicable Telephone No.

Continuation of 4(e) Other: Claims 1, 19, 30, 39 and 45 no longer include reference to beta prior to globin. It is unclear if this is meant to indicate that all reference to the term has been deleted.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/188,221	07/01/2002	Michel Sadelain	MSK.P-050

CONFIRMATION NO. 9026

OC000000018454026

52334
Marina Larson & Associates LLC
re: MSK
P. O. BOX 4928
DILLON, CO 80435-4928

Date Mailed: 04/04/2006

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/13/2006.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

TRUWORK GEBRE
PTOSS (703) 308-9150

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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/188,221	07/01/2002	Michel Sadelain	64836(51590)

CONFIRMATION NO. 9026

21874
EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205

OC000000018454041

Date Mailed: 04/04/2006

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 03/13/2006.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

TRUWORK GEBRE
PTOSS (703) 308-9150

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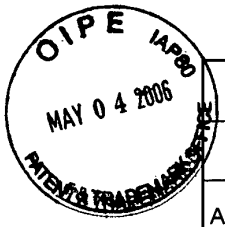
5-5-06 IFW 1632



AMENDMENT TRANSMITTAL LETTER

Docket No. 64836(51590)

Application No.	Filing Date	Examiner	Art Unit
10/188,221-Conf. #9026	July 1, 2002	Not Yet Assigned	N/A

Applicant(s): Michel Sadelain et al.

Invention: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

TO THE COMMISSIONER FOR PATENTS

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated and is transmitted as shown below.

CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest Number Previously Paid	Number Extra Claims Present	Rate	
Total Claims	46	- 47 =		x	
Independent Claims	5	- 5 =		x	
Multiple Dependent Claims (check if applicable)		☐			
Other fee (please specify):					
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT:					0.00

☐ Large Entity ☒ Small Entity

☒ No additional fee is required for this amendment.

☐ Please charge Deposit Account No. _____ in the amount of $ _____. A duplicate copy of this sheet is enclosed.

☐ A check in the amount of $ _____ to cover the filing fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director is hereby authorized to charge and credit Deposit Account No. 04-1105 as described below. A duplicate copy of this sheet is enclosed.

☒ Credit any overpayment.

☒ Charge any additional filing or application processing fees required under 37 CFR 1.16 and 1.17.

Amy M. Leahy

Dated: May 4, 2006

Amy M. Leahy
Attorney/Agent Reg. No.: 47439

EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(203) 975-7505

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EV749290191US, on the date shown below in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: May 4, 2006 Signature: Denise Kacinski (Denise Kacinski)

209862

OIPE IAP80 MAY 04 2006 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sadelain, et al.	
Application No.: 10/188,221	Group Art Unit: 1632
Filed: 7/1/2002	Examiner: Maria B. Marvich
Title: Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies	Confirmation No: 9026
Attorney Docket No.: MSK.P-050	
Customer No.: 021121	

CERTIFICATE OF EXPRESS MAIL

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service in an envelope addressed to MS Amendment, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 as "Express Mail Post Office to Addressee" Mailing Label No. EV 749290191 US on May 4, 2006.

Date: May 4, 2006

Denise Kacinski

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Dear Sir:

In response to the Office Action of April 4, 2006, please amend this application as follows:

Amendments to the Specification begin on Page 2.

Amendments to the Claims are reflected in the listing of claims which begins on page 5 of this paper.

Remarks/Arguments begin on page 11 of this paper.

Amendments to the Specification

Page 3, amend the paragraph starting on line 5 as follows:

Fig. 4 shows the average vector copy number in peripheral blood cells, measured periodically for 24 weeks, which confirms ~~showed~~ highly efficient gene transfer in cells transduced with the vector of the invention.

Page 6, amend the paragraphs starting on line 4 as follows:

Fig. 2 shows the genomic structure of specific vector within the scope of the invention. The vector includes a deleted LTR, from -456 to -9 of HIV LTR and the PGK promoter (530 bp) from the murine phosphoglycerate kinase 1 gene. It also includes a DHFR-encoding region encoding human DHFR with s/f mutation at amino acid 22. The locus control region and the β-globin region are the same as in TSN9. This vector is designated dTNS9-PD. This incorporation of DHFR into this vector provides transformed cells with a methotrexate-resistant phenotype. As a result, methotrexate, and other antifolates can be used, both *in vitro* and *in vivo* as a selection ~~to tool~~ tool to enhance levels of the functional hemoglobin. When hematopoietic stem cells were transformed using dTNS9-PD and reintroduced to mice that were then treated with NMBPR-P (0.5 mg/dose) and TMTX (0.5 mg dose) for five days, observed levels of expressed human β-globin were much higher in mice transduced with dTNS9-PD vectors after treatment with TMTX and NMBPR-P for selection of transduced cells.

The vectors of the invention are used in therapy for treatment of individuals suffering from hemoglobinopathies. In one embodiment of the invention, hematopoietic progenitor or stem cells are transformed *ex vivo* and then restored to the patient. As ~~usedin~~ used in the specification and claims hereof, the term "hematopoietic progenitor sand stem cells" encompasses hematopoietic cells and non-hematopoietic stem cells, e.g., embryonic stem cells, hematopoietic stem cell precursors, or any of the latter generated by nuclear transfer from a somatic cell. It is know in the art that efficient gene ~~genes~~ transfer into human embryonic stem cells can be achieved using lentiviral vectors.

Page 15, amend the paragraphs beginning at line 3 as follows:

The stability of TNS9-encoded β^A expression detected in peripheral blood suggested that ~~long-trem~~ long-term hematologic and systemic therapeutic benefits could be obtained. To investigate whether Hbb^{hu} production would suffice to ~~teart~~ treat the anemia, we closely monitored hemoglobin parameters over 40 weeks. The marked increase in hemoglobin concentration, RBC counts, and hematocrit was sustained throughout this time period. Control mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells remained severely anemic, indicating that the transplantation procedure itself did not alter the anemic state. The reticulocyte counts decreased to 5% to 8% in TNS9 treated-chimeras, compared to 19% to 21% in control eGFP-treated $Hbb^{th3/+}$ chimeras and age-matched $Hbb^{th3/+}$ mice, suggesting an increase in RBC life span and a ~~decreasein~~ decrease in erythropoietic activity.

Example 9

To determine the impact of sustained human β-globin gene expression on hematopoiesis, we studied the degree of splenomegaly (enlargement of the spleen) and EMH in 1-year-old chimeras and ~~age-matecheđ~~ age-matched control mice. Spleen weights measured in Tns9-treated $Hbb^{th3/+}$ ~~chimeraas~~ chimeras were indistinguishable from recipients of eGFP-transduced normal bone marrow, as were the total number of cells per spleen. In contrast, mice engrafted with eGFP-transduced $Hbb^{th3/+}$ bone marrow cells showed spleen weights and total cell numbers that were about 3-fold greater. The correction of spleen weight in TNS9 bone marrow chimeras corresponded to a concomitant normalization in total hematopoietic progenitor cell content. Spleen CFU-Es, BFUEs, andCFUs-GM were reduced to ~~levelsmeasured~~ levels measured in recipients of eGFP-transduced $Hbb^{th+/+}$ bone marrow, whereas they remained elevated in control chimeras engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells and in age-matched $Hbb^{th3/+}$ mice, as previously observed in another murine ~~modelof~~ model of β-thalassemis.[29]

Page 15, amend the paragraphs starting on line 1 as follows:

The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and age-match controls. Histopathology of spleens of mice that received transplants of eGFP-tranduced $Hbb^{th3/+}$ marrow was virtually identical to that of ~~slpeen~~ spleen from control $Hbb^{th3/+}$ mice. Specifically, the red pulp was significantly expanded, accounting for 80% to 90% of the cross-sectional area, and densely occupied by nucleated erythroid precursors. The white pulp, based on cross-sectional area, ~~wsa~~ was relatively decreased and the marginal zones were obscured by the large number of nucleated RBCs, reflecting major expansion of the red pulp and erythroid precursors. In TNS9-treated chimeras, the amount of red pulp was considerably decreased, accounting for olny about 50% to 60% of the cross-sectional area. In addition, the number of nucleated erythroid precursors in the red pulp was decreased. Other immature hematopoietic cells were present in the red pulp, but much less frequently than in the spleens of control $Hbb^{th3/+}$ thalassemic mice. The livers from TNS9-treated chimeras were similar to those of the normal control mice in that no EMH was detected. In contrast, livers from mice engrafted with eGFP-trasduced $Hbb^{th3/+}$ bone marrow cells showed ~~seral~~ several small foci of intrasinusoidal EMH.

Example 10

Toxic iron accumulation in the organs of thalassemic patients is a consequence of RBC destruction and increased gastrointestinal iron uptake. To determine whether sustained expression from the TNS9 vector reduced iron overload, we ~~stuied~~ studied tissue sections of liver and heart, stained using Gomori iron stain. No iron deposition was seen in the livers of normal $Hbb^{+/+}$ control mice, whereas $Hbb^{th3/+}$ mice showed variable amounts of iron, including some large aggregates. TNS9-trasduced treated chimeras demonstrated low to undetectable levels of iron in the livers, whereas iron was readily detected in the livers of all mice that received transplants of eGFP-transduced $Hbb^{th3/+}$ bone marrow cells. No iron accumulation was found in the heart of treated or control mice, as previously observed in another murine model of β-thalassemia,[30] in contrast to what is found in the human disease. [1-3]

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A recombinant vector comprising:
(a) a region comprising ~~encoding~~ a functional globin ~~β-globin~~ gene; and
(b) portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, said vector providing expression of ~~β-globin~~ globin when introduced into a mammal *in vivo*.

2. (previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (previously presented) The vector of claim 2, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

4. (previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

8. (previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (previously presented) The vector of claim 1, wherein the functional globin gene encodes human β-globin.

11. (previously presented) The vector of claim 10 further comprising a region encoding a dihydrofolate reductase.

12. (previously presented) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19. (withdrawn, currently amended) A method for treatment of hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy comprising the steps of:

introducing to the mammalian individual a recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional globin ~~β-globin~~ gene; and

(b) portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, said vector providing expression of ~~β-globin~~ globin when introduced into a mammal *in vivo*; and

expressing the functional ~~β-globin~~ globin gene in the mammal, thereby providing a therapeutic benefit to the mammalian individual.

20. (withdrawn) The method of claim 19, wherein the vector further comprises a region encoding a dihydrofolate reductase.

21. (withdrawn) The method of claim 20, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

22. (withdrawn) The method of claim 21, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

23. (withdrawn) The method of claim 22, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

24. (withdrawn) The method of claim 23, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

25. (withdrawn) The method of claim 19, wherein the step of introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and then restoring the transformed cells to the mammalian individual.

26. (withdrawn) The method of claim 25, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to restoring the cells to the mammalian individual.

27. (withdrawn) The method of claim 26, wherein the vector further comprises a region encoding a dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate,

28. (withdrawn) The method of claim 27, wherein the antiifolate is methotrexate.

29. (withdrawn) The method of claim 19, wherein the globin gene encodes human β-globin.

30. (withdrawn, currently amended) A mammalian hematopoietic progenitor or stem cell transduced with a recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional ~~β-globin~~ globin gene; and

(b) ~~large~~ portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites ~~include complete DNase I hypersensitive sites HS2, HS3 and HS4~~, wherein said cells express the functional ~~β-globin~~ globin gene.

31. (withdrawn) The transduced cell of claim 30, wherein the mammalian hematopoietic progenitor or stem cell is a human cell.

32. (withdrawn) The transduced cell of claim 31, wherein the vector further comprises a region encoding a dihydrofolate reductase.

33. (withdrawn) The transduced cell of claim 32, wherein the vector further comprises a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

34. (withdrawn) The transduced cell of claim 33, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

35. (withdrawn) The transduced cell of claim 34, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

36. (withdrawn) The transduced cell of claim 35, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

37. (withdrawn) The transduced cell of claim 30, wherein the dihydrofolate reductase is a human dihdrofolate reductase.

38. (withdrawn) The transduced cell of claim 30, wherein the globin gene encodes human β-globin.

39. (withdrawn, currently amended) A method of making a therapeutic composition ~~compositions~~ for treatment of ~~henoglobinopathy~~ hemoglobinopathy in a mammalian individual, comprising the steps of preparing a recombinant lentiviral vector comprising

(a) a region comprising ~~encoding~~ a functional ~~β-globin~~ globin gene; and

(b) portions of the β-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BAMHI and BanII restriction sites, obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

40. (withdrawn) The method of claim 39, further comprising the step of performing an *ex vivo* selection using an antifolate.

41. (withdrawn) The method of claim 39, wherein the globin gene encodes human β-globin.

42. (canceled)

43. (previously presented) The vector of claim 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (previously presented) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (currently amended) A recombinant vector comprising:

(a) a region comprising ~~encoding~~ a functional ~~β-globin~~ globin gene;

(b) portions of the β-globin locus control region, which include as three separate fragments complete DNase I hypersensitive sites HS2, HS3 and HS4, and

(c) two GATA-1 binding sites at the junction between the HS3 and HS4 fragments, said vector providing expression of ~~β-globin~~ globin when introduced into a mammal *in vivo*.

46. (previously presented) The vector of claim 45, wherein the vector is a lentiviral vector.

47. (previously presented) The vector of claim 45, wherein the vector is pTNS9.

REMARKS/ARGUMENTS

This is in response to the Office Action mailed April 4, 2006 and October 4, 2005 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Applicants again note that the Information Disclosure Statement filed in this case on October 8, 2002 has not been returned with initials indicating consideration by the Examiner. The October 8, 2002 Information Disclosure Statement is present in the Image File Wrapper as reflected in Private PAIR. Consideration of the references from the disclosure statement and return of an initialed copy of the 1449 with the next paper are requested.

Applicants have amended the specification to correct the typographical errors noted by the Examiner. In addition, withdrawn claim 30 has been amended to conform to the previous amendment of claim 1 so that it can be recombined with claim 1 should withdrawal of the restriction requirement be considered appropriate upon allowance of the elected claims.

Applicants have also amended the independent claims to refer to "globin" as opposed to "β-globin." This language is consistent with the language in the claims as originally filed. At some point, the β was inserted in the claims in error, and no amendment to the claims was noted.

The Examiner has rejected claims 1-18 and 43-47 under 35 USC § 112, first paragraph, as lacking a written description. The focus of the rejection is on the reference to mutant human dihydrofolate reductase, and the reference to a functional globin gene. As a first matter, Applicants have amended to claims to refer to a region comprising a functional globin gene. rather than encoding.

The Examiner has rejected the claims for lack of written description based on the number of examples in the specification for the individual components of the claimed invention. Based on the recently decided case of *Capon v Eshhar*, 76 USPQ2d 1078 (Fed. Cir. 2005), Applicants submit that this rejection is in error

Capon is based on an interference proceeding in which both parties claimed chimeric genes comprising a gene segment encoding a single-chain (scFv) antibody, and a second gene encoding a cytoplasmic signaling domain. Expression of a chimeric gene resulted in cells that expressed a cell surface marker that produced cell signaling in response to binding of an antigen to the scFV antibody. The Board of Patent and Appeals and Interferences had held that both specifications were lacking in written description because of a limited number of examples, and the absence of a complete sequence of at least one chimeric gene within the scope of the claims.

In vacating and remanding the holding of the Board of Appeals, the Federal Circuit observed:

> The "written description" requirement must be applied in the context of the particular invention and the state of the knowledge. The Board's rule that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known, is an inappropriate generalization. When the prior art includes the nucleotide information, precedent does not set a per se rule that the information must be determined afresh.
>
> * * *

76 USPQ2d at 1084-5. In the present case, the "particular invention" is not the sequence of globin genes that produce functional proteins, and it is not the sequence of mutants of DHFR. As in *Capon*, the "particular invention" is the combination of known elements into a particular structure. However, unlike *Capon*, the present claims are far less broad.

Globin genes have been the subject of intense study, and sequences and structures for defective and functional proteins are well known. For example, *A Syllabus of Human Hemoglobin Variants* (1996), by Titus H.J. Huisman, Marianne F.H. Carver, and Georgi D. Efremov, was published by The Sickle Cell Anemia Foundation in Augusta, GA, USA. As stated in the on-line version of this publication, "this is a comprehensive listing of all known human hemoglobin variants, including variants of the alpha-, beta-, gamma-, and delta-globin chains." http://globin.cse.psu.edu/html/huisman/variants/. Thus, the structure of suitable globin genes was well-known when the application was filed, and re-presentation of these sequences is not required by the written description requirement.

With respect to mutant DHFR, Applicants have cited in the specification documents describing known sequences of such mutants, with reference to a prior publication of the sequences. *Capon* indicates that no more should be required. As to the statement that the ability to confer drug-resistance must be empirically determined, Applicants note that this is not their invention. Their invention is a combination of elements, one of which is a gene encoding a DHFR mutant that is known (as a result of prior work or future invention) to have this property. To deny coverage to the full scope of Applicants invention because someone might develop a new DHFR mutant, thereby allowing that person to freely misappropriate Applicants' invention of the combination, is not what the written description requirement was intended to accomplish.

For the foregoing reasons, Applicants submit that this case is now in form for allowance. Favorable reconsideration and allowance of all claims is respectfully requested. Further, recombination of the withdrawn claims is requested.

Respectfully submitted,

Date May 4, 2006

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PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number: 10188824

CLAIMS AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE (37 CFR 1.16(a))				$	OR		$
TOTAL CLAIMS (37 CFR 1.16(c))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(b))	minus 3 =	*	X $ =		OR	X $ =	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(d))			+ $ =		OR	+ $ =	
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL		OR	TOTAL	

CLAIMS AS AMENDED – PART II

6/30/05

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
AMENDMENT A	Total (37 CFR 1.16(c))	* 47	Minus	** 46	* 1	X $ =	25	OR	X $ =	
	Independent (37 CFR 1.16(b))	* 5	Minus	*** 5	=	X $ =	0	OR	X $ =	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

2.6.06

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
AMENDMENT B	Total (37 CFR 1.16(c))	* 46	Minus	** 47	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(b))	* 5	Minus	*** 5	=	X $ =		OR	X $ =	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

5/4/06

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
AMENDMENT C	Total (37 CFR 1.16(c))	* 46	Minus	** 47	= 0	X $ =	0	OR	X $ =	
	Independent (37 CFR 1.16(b))	* 5	Minus	*** 5	= 0	X $ =	0	OR	X $ =	
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(d))					+ $ =		OR	+ $ =	
						TOTAL ADD'L FEE	0	OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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2

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S20	8	(lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 06:13
S22	1	09/899479 and einerhand	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 06:33
S21	9	(lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4) and (lcr or locus adj control adj region)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 10:32
L2	5	(lcr or locus adj control adj region) same restriction adj map	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 10:49
L1	0	(lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4) same restriction adj map	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 10:49
L3	0	(lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4) and restriction adj map	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 10:49
S3	8840	Sadelin.in. or Rivella.in. or May.in. or Bertino.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30
L4	10032	Sadelin.in. or Rivella.in. or May.in. or Bertino.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
S18	1	S15 and globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30

EAST Search History

S17	4	S15 and globin and (LCR or locus adj control adj region)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30
L9	1	L7 and globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
L8	4	L7 and globin and (LCR or locus adj control adj region)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
S9	8	globin same (lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30
L11	9	globin same (lcr or locus adj control adj region) adj20 (hs2 and hs3 and hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
S11	3	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30
L6	4	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
L5	4	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30
S13	2	(lentivirus or lentiviral) same globin same lcr	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30
L10	3	(lentivirus or lentiviral) same globin same lcr	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2006/07/18 11:30

EAST Search History

L7	6	"5610053".pn. or "6090608".pn. or "5631162".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2006/07/18 11:30

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	64836(51590)	9026

21874 7590 07/31/2006

EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
MARVICH, MARIA	

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 07/31/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Maria B. Marvich, PhD	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41 and 43-47* is/are pending in the application.

4a) Of the above claim(s) *19-41* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18 and 43-47* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *07 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This office action is in response to an amendment filed 6/30/06 and 2/6/06. Claim 42 has been cancelled. Claims 1, 19 and 45 have been amended. Claims 1-41 and 43-47 are pending in this application. Claims 19-41 have been withdrawn. Therefore, claims 1-18 and 43-47 are under examination in the application.

Response to Amendment

Any rejection of record in the previous action not addressed in this office action is withdrawn. There are no new grounds of rejection herein and therefore, this action is final.

Specification

The disclosure is objected to because of the following informalities: On page 14, line 8, "then" is misspelled as "ten". **This objection is maintained for reasons of record in the office action mailed 10/4/05.**

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-18 and 43-47 are rejected under 35 USC 112, 1st paragraph, as failing to comply with the written description requirement. The claims contains subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the

inventors, at the time the application was filed, had possession of the claimed invention. **This rejection is maintained for reasons of record in the office action mailed 10/4/05 and restated below.**

Applicants recite a genus of recombinant vectors comprising functional globin gene and a mutant human dihydrofolate reductase (DHFR) that has increased resistance to antifolate as compared to wild-type DHFR.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.

Applicants recite a recombinant vector comprising a region encoding a functional b-globin gene. The specification teaches that the term "functional globin gene" includes both exons and introns, as well as globin promoters and splices donors/acceptors and can be expressed. Specifically, The specification discloses two recombinant lentiviral vectors, TNS9 and dTNS9-PD. TNS9 vector incorporates –618 to +2484 of the human β-globin gene. This encompasses the coding sequence and an extended portion of the promoter and enhancer region. dTNS9-PD comprises a deletion in the LTR and includes a PGK promoter form the murine phosphoglycerate kinase 1 gene and a mutant DHFR region with a mutation at amino acid 22. The specification also teaches that the β-globin gene coding sequence can be replaced with fragments of gamma globin, alpha globin. Each of the fragments encompasses Met to the

polyadenylation sequence, which essentially are the coding sequences of the alpha and gamma genes. Other than the β-globin gene from position –618 to +2484 that includes an extended promoter and 3' enhancer element, applicants do not disclose the genomic sequence of "functional globin genes" that can be used in the recombinant vector for expression of β-globin when introduced into a mammal *in vivo*. However, there is no reference sequence provided in the specification or the claims. Furthermore, all of the components of the gene as regulation sequences, introns, and exons must be determined empirically in order to generate the recombinant vector for expression *in vivo* in mammals. Furthermore, it is unclear what components are required such that it is "functional". Applicants claim the gene without any disclosure about its structure. Given that applicants recite any functional globin gene but disclose only –618 to +2484 of the human β-globin gene for which no reference sequence is provided, given that the genomic structures of any globin gene are unknown, it is concluded that the invention must be empirically determined. In an unpredictable art, the disclosure of one species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

In the instant case, applicants recite that the vector also comprises DHFR as a selection marker. Specifically in dependent claims 5, 8, 14 and 17, applicants claim a genus of DHFR mutant forms that comprise mutations that increase the capacity of the DHFR to confer resistance to drugs. Applicants recite a single mutant form that comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence. However, there is no reference sequence provided in the specification or the claims. Rather, the specification discloses that double and single mutants of DHFR with mutations at amino acids 22 and 31 are

described in commonly owned WO document 97/33988. Both the specification and WO 97/33988 teach that the reference sequence is human DHFR. As to the genus of DHFR mutants, the specification and prior art teach DHFR with mutations at amino acids 22 and 31. However, the structural requirements of these mutants are not provided. As to the functional requirements, the prior art teaches that the effect of the DHFR mutants is due to an increase in the rate of methotrexate dissociation. However, neither the prior art nor the specification teach that any other amino acids are capable of mediating the same effect. Given the large size and diverse nature of h DHFR proteins with mutations and the inability to determine which will also possess the ability to confer increased resistance to drugs, it is concluded that the invention must be empirically determined. In an unpredictable art, the disclosure of two species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

Response to Argument

Applicants traverse the claim rejections under 35 U.S.C. 112, first paragraph on pages 11-13 of the amendment filed 2/6/06 and 5/4/06. Applicants argue that the claims have been amended to recite that the construct comprises a region comprising a functional globin gene rather than encoding it and therefore overcome the rejection. Applicants argue that the specification has cited documents that describe known sequences of such mutants and based upon *Capon* no more is required.

Applicants' arguments have been fully considered but they are not persuasive. The Guidelines for Written Description state "The claimed invention as a whole may not be

adequately described if the claims require an essential or critical element which is not adequately described in the specification and which is not conventional in the art". In the instant case, the specification has specifically defined a "functional globin gene" as including both exons and introns, as well as globin promoters and splices donors/acceptors. However, the isolation of such genes is not possible because all of these components are not known in the art nor are they disclosed in the specification. Applicants' reference *Capon* as evidence that a rejection under lack of Written Description is inappropriate when based upon the number of examples in the specification. This fact pattern was not used to reject the instant claims. In fact, the rejection is based upon the lack of description in the specification as to the structural requirements of any of the genes that are functional globin and therefore, the specification has failed to describe the genes such that the nexus of structure and function is apparent. Secondly, applicants claim a genus of mutant DHFR that have increased resistance to antifolates. While generation of mutant human DHFR is exemplified in the art as disclosed by the specification, mutagenesis has only demonstrated a single amino acid mutation that generates a single mutant DHFR that is of increased resistance to antifolates. As stated in *Capon* "The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science." *Capon* also states "It is well recognized that in the "unpredictable" fields of science, it is appropriate to recognize the variability in the science

in determining the scope of the coverage to which the inventor is entitled. Such a decision usually focuses on the exemplification in the specification". In this case, the analysis of the field of functional globin gene isolation and cloning and isolation of DHFR mutants with increased resistance to antifolates is highly unpredictable as demosntrated by the art and requires more description than isolation and cloning of the coding sequences or demonstration of a single mutant within a genus of genes and mutants.

Conclusion

Claims 1-18 and 43-47 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B. Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, David Nguyen, PhD can be reached on (571)-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Maria B Marvich, PhD
Examiner
Art Unit 1633

September 14, 2005

DAVE TRONG NGUYEN
SUPERVISORY PATENT EXAMINER

Notice of References Cited	Application/Control No. 10/188,221	Applicant(s)/Patent Under Reexamination SADELAIN ET AL.	
	Examiner Maria B. Marvich, PhD	Art Unit 1633	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Sorrentino et al, Localization and characterization of the DNase I-hypersensitive site II (HS II) enhancer. A critical regulatory element within the beta-globin locus-activating region, Ann NY Acad Sci, 1990;612:141-51.
	V	Collis et al, Definition of the minimal requirements within the human beta-globin gene and the dominant control region for high level expression, EMBO J, 1990 Jan;9(1):233-40.
	W	Molete et al, Sequences flanking hypersensitive sites of the beta-globin locus control region are required for synergistic enhancement, MCB, 2001 May;21(9):2969-80.
	X	Genbank NG-000007, priority date 6/19/2006, downloaded 7/24/06

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Maria B. Marvich, PhD	1633	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
East, PALM inventor search	9/30/2005	MM
East databases- USPAT, PGPUB, EPO, JPO, Derwent, IBM-IDB search notes attached	9/30/2005	MM
STN databases- Caplus, Scisearch, Medline search notes attached	9/30/2005	MM
East, STN search history updated, search ntoes attached	7/17/2006	MM

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	FILE WRAPPER LOCATION
10/188,221		1633	16M1

Correspondence Address / Fee Address Change

The following fields have been set to Customer Number 21874 on 08/03/2006

- Correspondence Address

The address of record for Customer Number 21874 is:
EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON,MA 02205

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	64836(51590)	9026

21874 7590 10/23/2006

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BOSTON, MA 02205

EXAMINER	
MARVICH, MARIA	

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 10/23/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Interview Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Maria Marvich	1633	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Dave T. Nguyen*.

(2) *Maria Marvich*.

(3)*Amy Leahy*.

(4)*Lisa Wilson*.

Date of Interview: *11 October 2006*.

Type: a)☐ Telephonic b)☐ Video Conference
c)☒ Personal [copy given to: 1)☒ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☒ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☒ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *Agreed to change functional globin gene to DNA encoding functional globin to overcome rejection. Evidence overcoming rejection of DHFR mutants presented. Finally, source of LCR restriction sites requested*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

M Marvich
Examiner's signature, if required

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)
In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

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PATENT & TRADEMARK OFFICE

12-1-06

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AMENDMENT TRANSMITTAL LETTER

Docket No. 64836(51590)

Application No.	Filing Date	Examiner	Art Unit
10/188,221-Conf. #9026	July 1, 2002	Maria Marvich	1633

Applicant(s): Michel Sadelain et al.

Invention: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

TO THE COMMISSIONER FOR PATENTS

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated and is transmitted as shown below.

CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest Number Previously Paid	Number Extra Claims Present	Rate	
Total Claims	23	- 47 =		x	
Independent Claims		- 3 =		x	
Multiple Dependent Claims (check if applicable)	☐				
Other fee (please specify):	Extension for response within first month;				60.00
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT:					60.00

☐ Large Entity ☒ Small Entity

☐ No additional fee is required for this amendment.

☒ Please charge Deposit Account No. 04-1105 in the amount of $ 60.00. A duplicate copy of this sheet is enclosed.

☐ A check in the amount of $ _____ to cover the filing fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☒ The Director is hereby authorized to charge and credit Deposit Account No. 04-1105 as described below. A duplicate copy of this sheet is enclosed.

☒ Credit any overpayment.

☒ Charge any additional filing or application processing fees required under 37 CFR 1.16 and 1.17.

Amy M. Leahy

Dated: November 30, 2006

Amy M. Leahy
Attorney/Agent Reg. No.: 47,739

EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(203) 975-7505

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EV814886281US, on the date shown below in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: November 30, 2006 Signature: Denise Kacinski (Denise Kacinski)

220491

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NOV 30 2006
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Sadelain *et al.*

Serial No.: 10/188,221

Filed: July 1, 2002

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Attorney Docket No.: 64836(51590)

Confirmation No.: 9026

Group Art Unit: 1633

Examiner: Maria Marvich

Mail Stop: Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATION UNDER 37 C.F.R. §1.10 FOR "EXPRESS MAIL"

Date of Deposit: November 30, 2006 Mailing Label Number: EV 814886281 US

I hereby certify that this document is being deposited with the United States Postal Service on the date indicated abve in an envelope as "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 and addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Denise Kacinski
Name of Person Mailing Paper

Signature of Person Mailing Paper

AMENDMENT AND RESPONSE TO OFFICE ACTION UNDER 37 C.F.R. § 1.111

Dear Sir:

This paper is being submitted in response to the non-final Office Action mailed on July 31, 2006, which set a three-month shortened statutory period for reply ending on October 31, 2006. A Petition for a one-month extension of time accompanies this response. The Commissioner is authorized to charge any additional fees occasioned by this paper, or credit any overpayment of such fees, to Deposit Account No. 04-1105.

Amendments to the specification begin on page 2.

Amendments to the claims are reflected in the listing of claims beginning on page 3.

Remarks/arguments begin on page 7 of this paper.

AMENDMENT

It is respectfully requested that the claims and specification be amended without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

In the Specification:

Kindly replace the paragraph beginning at page 13, line 24 and ending at page 14, line 17 with the following paragraph:

--Donor bone marrow was flushed from the ~~temurs~~ tumors of 8- to 16-week old male c57/BL6 or $Hbb^{th3/+}$ mice[23] obtain from Jackson Laboratories (Bar Harbor, Me.) that had been injected intravenously (IV) 6 days earlier with 5-flurouracil (5-FU) 150 mg/kg body weight obtained from Pharmacia (Piscataway, N.J.). Bone marrow cells were resuspended in X-VIVO-15 serum-free medium and supplemented with 10 ng/mL interleukin-1α (IL-1 α 100 U/mL IL-3, 150 U/mL IL-6, 10 ng/mL Kit ligand obtained from Genzyme (Cambridge, Mass.), 0.5 mM β-mercaptoethanol obtained from Sigma (St. Louis, Mo.), 200-mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Bone marrow cells were ~~ten~~ then pelleted and resuspended in ~~serrum~~ serum-free medium containing concentrated lentiviral supernatant and supplemented with 8 mg/mL polybrene (Sigma), 200 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and cytokines as above, and ~~cultureed~~ cultured for 8 hours. Transduced bone marrow cells (5×10^5) were ~~ten~~ then injected IV into each of the irradiated female recipients to establish bone marrow chimeras. Recipients mice (11- to 14-week-old C57/BL6 or $Hbb^{th3/+}$ mice) were irradiated with 10.5 Gy (Split dose 2 X 5.25 Gy) on the day of transplantation.--

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A recombinant vector comprising:

(a) a region comprising a nucleotide sequence encoding a functional globin ~~gene~~; and

(b) portions of the ß-globin locus control region, which consist essentially of an 840 bp fragment of HS2 extending between SnaBI and BstXI restriction sites, a ~~1309~~1308 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between ~~BAMHI~~BamHI and BanII restriction sites, said vector providing expression of globin when introduced into a mammal *in vivo*.

2. (Previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (Currently Amended) The vector of claim 2, further comprising a mouse PGK promoter~~, wherein the mouse PGK promoter controls~~ to control the expression of the ~~region encoding a~~ dihydroflate reductase.

4. (Previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (Previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (Previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (Previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a

human dihydrofolate reductase.

8. (Previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (Previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (Currently Amended) The vector of claim 1, wherein the functional globin ~~gene encodes~~is human ß-globin.

11. (Previously presented) The vector of claim 10, further comprising a region encoding a dihydrofolate reductase.

12. (Previously presented) The vector of claim 11, further comprising a mouse PGK promoter, wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (Previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (Previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (Previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence

and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (Previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (Previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (Previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19-42. (Canceled)

43. (Previously presented) The vector of claim 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (Previously presen ted) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (Currently Amended) A recombinant vector comprising:
(a) a region comprising a nucleotide sequence encoding a functional globin ~~gene~~;
(b) portions of the ß-globin locus control region, which include as three separate fragments complete DNase I hypersensitivity sites HS2, HS3 and HS4, and
(c) two GATA-1 binding sites at the junction between the HS3 and HS4 fragments, said vector providing expression of globin when introduced into a mammal *in vivo*.

46. (Previously presented) The vector of claim 45, wherein the vector is a lentiviral vector.

47. (Previously presented) The vector of claim 45, wherein the vector is pTNS9.

REMARKS

Claims 1-41 and 43-47 are pending. Claims 19-41 are canceled herein. No new claims have been added. Accordingly, claims 1-18 and 43-47 will be pending upon entry of this Response. The claim cancellations have been made solely to expedite prosecution of the present application and should not be construed as an acquiescence to any of the rejections of record in the prosecution of the instant application. Applicants reserve the right to pursue the subject matter of the claims as originally filed in one or more subsequent applications.

Claims 1, 3, 10 and 45 are currently amended. Support for the recitation of "a 1308 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and BanII restriction sites" in Claim 1 can be found on page 5, lines 1-3 of the specification. Claims 1 and 45 are also amended to recite a "nucleotide sequence encoding a functional globin." Support for this recitation can be found on page 3, lines 24-26 of the specification. Claim 10 depends from Claim 1 and is amended to preserve the antecedent basis of the language in Claim 1. The changes to Claim 3 are partly grammatical in nature and partly to more accurately reflect that the PGK promoter controls expression of the DHFR (as opposed to the region encoding the DHFR).

An interview with Examiner Marvich and Supervisory Examiner David Nguyen was held on October 11, 2006. The Examiners are thanked for their helpful comments during the interview. In accord with their suggestions, Claims 1 and 45 have been amended to include the recitation of a "nucleotide sequence encoding a functional globin."

The Office Action Summary indicates that this action is non-final. However, page 2 of the Office Action indicates that the Action is final, yet no declaration of finality appears in the Conclusion on page 7 of the Response. During the interview, the Examiners were informed that the Applicants have relied upon the Office Action Summary and the Conclusion in determining that the Action is non-final. Confirmation that the Action is regarded by the Office as a non-final Action is respectfully requested.

During the interview, the Examiners inquired as to the location of the restriction sites that delineate the claimed fragments of the DNase I hypersensitive sites HS2, HS3, and HS4 within the Locus Control Region ("LCR") (e.g., the SnaBI and BstXI restriction sites of HS2, the HindIII and BamHI restriction sites of HS3, and the BamHI and BanII restriction sites of HS4). The sequence information and location of the hypersensitive sites was known in the art at the

time that the instant application was filed (see GenBank Accession Number NG_000007 for exemplary sequence information) which was available to the public before the earliest filing date of the present application.[1] Accordingly, sequence information providing the location of the restriction sites within the LCR was readily accessible to one skilled in the art using conventional sequence analysis techniques. In response to the Examiners' inquiry, the precise location of the restriction sites are summarized: the SnaBI and BstXI restriction sites of HS2 are located at positions 17,093 and 16,240, respectively; the BamHI and HindIII restriction sites of HS3 are located at positions 12,065 and 13,360, respectively; and the BamHI and BanII restriction sites of HS4 are located at positions 8,496 and 9,576, respectively.

In view of the evidence made of record by this Response, which is discussed further below, and the amendment to claims 1 and 45, it is believed that a tentative agreement was reached to withdraw the rejections of record against the instant application. It is respectfully submitted that the amendments and remarks herein place the application in condition for allowance.

I. The objections to the specification are overcome

An objection to the specification is made on the basis of a typographical error. Correction of the paragraph containing this error has been made by amendment to the specification. Accordingly, reconsideration and withdrawal of the present objection to the specification are respectfully requested.

II. The rejections under 35 U.S.C. § 112, first paragraph are overcome

The claims 1-18 and 43-47 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement on the basis that the rejected claims allegedly contain subject matter that was not described in the specification in such a way to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention at the time the application was filed. It is respectfully submitted that the conclusions of the Federal Circuit in the recently decided case of Falkner v. Inglis, 448 F.3d 1357, 1379 (Fed. Cir. 2006), further confirm that the presently claimed invention fulfills the written description requirement.

[1] GenBank Accession Number NG_000007 is currently at version 3 (v3) and the nucleotide numbering used herein is based on that sequence. The earliest version thereof (v1) was produced from sequences that were published no later than 1985 as is clear from the attached printout of the relevant pages from v1 (and which is available on the internet

Accordingly, Applicants respectfully disagree with the rejections of the present Office Action under Section § 112, first paragraph, and traverse as follows.

In Falkner, the Federal Circuit heard an appeal from a decision of the Board of Patent Appeals and Interferences (the "Board") in Interference No. 105,187 between Falkner et al. and Inglis et al., in which the Board entered judgment against Falkner. The court reviewed "whether the Inglis benefit applications [i.e. the priority applications] adequately describe and enable a poxvirus-based vaccine." *Id.* At 1363.

Relevant to this case, the court held "there is no *per se* rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure" and particularly recognized, when considering whether Inglis's failure to provide any DNA sequences of the poxvirus genome or the locations of "essential" genetic regions to be deleted fails the written description requirement, that no such disclosure was required because the sequences were already known in the art. *Id.* at 1366-1367.

The court further explained that "a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement," and ultimately held that "where . . . accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences (here "essential genes"), satisfaction of the written description requirement does not require either the recitation or incorporation by reference (where permitted) of such genes and sequences." *Id.* at 1358.

The facts and issues in Falkner are similar to those raised by the rejections of the present Office Action. Accordingly, Applicants respectfully submit that the holdings of Falkner necessitate reconsideration and withdrawal of the rejections under 35 U.S.C. § 112.

First, the basis provided in the Office Action for alleging a lack of written description to support the phrase "a functional globin gene" fails in view of Falkner. A vector comprising the –618 to +2484 region of the human beta-globin gene is described and exemplified in the specification. *See* page 4, lines 19-27 of the specification, Figure 1 and Examples 1-10. Moreover, the specification indicates that the globin gene can encode, for example, α-globin, β-globin and γ-globin. *See* page 4, lines 1-3 of the specification.

Globin genes were well known at the time of filing the instant application. Indeed, globin genes have been the subject of intense study for many years, and sequences and structures for

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at http. ://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=13907843) (Tab 1).

many defective and functional globins are well known in the art. In addition to GenBank Accession Number NG_000007, discussed herein above, the Examiner is respectfully pointed to the following references which further establish the ready availability of sequences of functional globin genes at the time of filing the instant application. References 1-4 relate to alpha-type globin sequences and references 4-12 relate to beta-type globin sequences (including beta and gamma globin sequences).

(1) GenBank Accession No. Z84721 (March 19, 1997): This reference describes a 43,058 bp nucleotide sequence encompassing the human genomic sequence of the alpha globin gene cluster, including alpha globin 1 (33234-34311), alpha globin 2 (37038-38118). The sequence presumably contains exons and introns, globin promoters and globin splice donor/acceptor sites as it represents a contiguous genomic DNA sequence.

(2) GenBank Accession No. NM_000517 (October 31, 2000): This reference describes a 575 bp human mRNA alpha globin 2 sequence.

(3) Hardison et al., J. Mol. Biol. (1991) 222(2):233-249. This reference reports a contiguous nucleotide sequence of 10,621 bp (indicated on page 237 as GenBank Accession No. M35026) of the mouse alpha globin gene cluster. This reference also reports an alignment with the corresponding human sequence (page 240). The sequence presumably contains exons and introns, globin promoters and globin splice donor/acceptor sites as it represents a contiguous genomic DNA sequence.

(4) A Syllabus of Human Hemoglobin Variants (1996), by Titus et al., published by The Sickle Cell Anemia Foundation in Augusta, Georgia (available online at http://globin.cse.psu.edu). This reference describes "a comprehensive listing of all known human hemoglobin variants, including variants of the alpha-, beta-, gamma-, and delta-globin chains." In particular, this reference describes globin mutants having superior oxygen transport properties.

(5) GenBank Accession No. J00179 (August 26, 1993). This reference describes a human 73,326 bp nucleotide sequence encompassing "all of the known beta genes in the cluster on chromosome 11" (see page 8). This genomic DNA region contains the beta and gamma globin genes, and presumably exons, introns, globin promoters and globin splice donor/acceptor sites. The reference specifically indicates that the beta globin promoter is present 5' upstream of the beta gene (see page 11 of 40, line 14). In addition, the

reference specifically indicates the presence of an exon/intron border in the beta gene (see page 11 of 40, line 33).

(6) Tagle et al., Genomics (1992) 13(3):741-760. This reference discloses the 41,101 bp nucleotide sequence of the beta globin gene cluster of a prosimian (see Figure 2). The reference further discloses results of alignments with corresponding regions from human, rabbit and mouse. The sequence presumably contains exons and introns, globin promoters and globin splice donor/acceptor sites as it represents a contiguous genomic DNA sequence.

(7) Grovsfeld et al., Cell (1987) 51(6):975-985. This reference describes the structure of the human beta globin gene cluster. It further includes a description of the regulatory regions required for appropriate expression of the beta globin gene, including upstream and downstream promoters and enhancers (see page 975, left column, last paragraph through right column, first paragraph).

(8) Li et al., Blood (1999) 93(7):2208-2216. This reference discloses vectors used for testing and optimizing expression cassettes for physiologic human beta globin expression. The report describes the construction of a variety of vectors using different portions or regions of the gamma and beta promoters to drive expression (see Methods and Materials: plasmid constructs). The report also states that "the promoters from the gamma and beta globin genes have been intensively studied for many years" (see page 2214, first column, first paragraph of discussion).

(9) Gorman et al., J. Biol. Chem. (2000) 275(46):35914-35919. This reference reports on the use of ribonucleoprotein particles as carriers for antisense repair of faulty splicing of target genes. In particular, the reference tests the approach using the human IVS2-705 pre-mRNA (a known thalassemic beta-globin pre-mRNA which is defective in splicing). Cell lines are used which stably express the IVS2-705 pre-mRNA (see Methods and Materials).

(10) Slightom et al., Cell (1980) 21(3):627-638. This paper reports the complete nucleotide sequence of human gamma-G and gamma-A beta-type globin genes, including the promoter TATA box region 5' to the transcription start site (see sequences on page 631).

(11) Fritsch et al., Cell (1980) 19(4): 959-972. This reference reports the isolation of the entire human beta-globin chromosomal region and its fine detail mapping of each of the

fetal and adult beta-type genes and their intergenic regions (see page 959, right column, last paragraph of introduction).

(12) Marotta et al., J. Biol. Chem. (1977). 252(14):5040-5053 This paper reports the nucleotide sequence of human mRNA beta-globin on page 5047-5048 and the cloning, mapping and sequencing strategies used to obtain same.

Thus, the sequences of suitable globin genes, as defined in the specification, were well known when the application was filed, and re-presentation of these sequences is not required by the written description requirement. Indeed, Falkner observed that "the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification" and that "a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement." *Id.* at 1368. Thus, where Inglis was not required to disclose specific DNA sequences for essential genetic elements of the claimed poxviruses, the presently claimed invention should not be required to have contained specific DNA sequences for functional globin genes as they were readily available in the art.

Second, the basis provided in the Office Action to allege a lack of written description for the phrase "a mutant human dihydrofolate reductase (DHFR)" also fails in view of Falkner. In particular, the Office Action indicates that while the claims recite a genus of DHFR mutants that comprise mutations that increase the capacity of the DHFR to confer resistance to drugs, the specification expressly teaches only two DHFR mutants (human DHFR with mutated amino acid 22 or human DHFR with mutated amino acids 22 and 31) and does not provide any reference sequence nor any explanation as to particular structural requirements of the mutants or which particular mutants will provide the effect of increased drug resistance.

During the interview, several references showing DHFR mutants known in the art were discussed, and it was agreed that the references would be made of record in order to overcome the present rejection. *See* Interview Summary dated October 11, 2006. Accordingly, in view of the references presented herein, it is respectfully submitted that sequence information for mutant DHFR genes having increased capacity to confer drug resistance was well known in the art at the time that the present application was filed. The specification cites WO 97/33988 as a specific example of single and double mutants of DHFR having increased resistance. Mutant forms of DHFR disclosed in WO 97/33988 include Ser31Tyr22, Ser31Phe22, Gly31Tyr22, Gly31Phe22, Ala31Tyr22 and Ala31Phe22. Moreover, reference can be made to Thillet et al., "Site-directed

mutagenesis of mouse dihydrofolate reductase. Mutants with increased resistance to methotrextate and trimethoprim," J. Biol. Chem. (1988), 263: 12500-12508 as evidence that such DHFR mutant sequences were well-known in the art at the time the application was filed. In particular, the reference discusses the preparation of a vector containing a 920-base pair *Eco*RI-*Bgl*II fragment harboring the mouse dihydrofolate reductase gene under the control of a β-lactamase promoter (see page 12501, left column, line 37-41), which is then used as a basis for site-directed mutagenesis to create specific mutations in the DHFR gene. The study concludes the it "is possible to obtain mutants of mouse dihydrofolate reductase with increased resistance to inhibitor." (see page 12507, right column, last paragraph).

The Examiner is respectfully pointed to the following additional references which further exemplify the ready availability of sequences of dihydrofolate reductase genes at the time of the present invention.

(1) GenBank Accession No. J00140 (December 31, 1994). Discloses wildtype human dihydrofolate reductase mRNA sequence.

(2) GenBank Accession No. 1DLR (September 4, 1998). Discloses a human dihydrofolate reductase mutant wherein leucine at position 22 is replaced by phenylalanine (L22F). This mutant is known to have increased resistance to inhibition by antifolates including methotrexate. (see background of WO 97/33988, page 1, lines 20-27 to page 2, lines 1-2).

(3) GenBank Accession No. 1DLS (September 4, 1998). Discloses a human dihydrofolate reductase mutant wherein leucine at position 22 is replaced by tyrosine (L22Y). This mutant is known to have increased resistance to inhibition by antifolates including methotrexate. (see background of WO 97/33988, page 1, lines 20-27 to page 2, lines 1-2).

(4) Blakley et al., Hum. Mutat. (1998) 11(4):259-263. This reference is a review of DHFR mutations that confer increased resistance to methotrexate and other antifolates. Specific DHFR mutations are discussed throughout. Table 1, on page 260, for example, provides a list of specific mutations in mouse, hamster, and human DHFR genes that result in resistance to methotrexate. Table 2, on page 261, in another example, provides a list of human DHFR variants that have been engineered by site-directed mutagenesis.

(5) McIvor R.S., Bone Marrow Transplant. (1996) 18 Suppl. 3:S50-4. This reference is a

review of DHFR mutations that confer increased resistance to methotrexate and other antifolates. Reference is made to known DHFR variants, e.g. see page S50, right column, last paragraph.

(6) Banerjee D. et al. Acta. Biochim. Pol. (1995) 42(4):457-464. This reference is a review of DHFR mutations that confer increased resistance to methotrexate and other antifolates. Reference is made to known DHFR variants, e.g. see page 459, right column, last paragraph.

Accordingly, the sequences of DHFR mutants would have been well known to the person of ordinary skill in the art as that person would have had available to him or her literature sources that would clearly provide such mutants. Therefore, the lack of such sequences in the disclosure does not form a valid basis for a rejection under 35 U.S.C. § 112, first paragraph. Falkner, 448 F.3d 1357, 1358.

Copies of the references discussed herein above do not accompany this response, as it is understood from discussions held during the interview that such copies are not necessary. The Examiner is invited to call the undersigned for a further description of the sequence information or copies of any reference of record in this response.

Reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph, are respectfully requested, as the amendments and remarks herein place the application in condition for allowance.

CONCLUSION

In view of the foregoing amendments and remarks presented herein, reconsideration and withdrawal of all rejections and allowance of the instant application with all pending claims are respectfully solicited. If any issue remains as an impediment to allowance, a further interview with the Examiner and SPE are respectfully requested; and, the Examiner is additionally requested to contact the undersigned to arrange a mutually convenient time and manner for such an interview.

Respectfully submitted,

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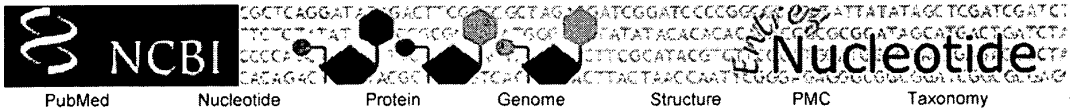
Dated: November 30, 2006



☐ **1:** NG_000007. Reports ...[gi:13907843] The record has been replaced by NG_000007.2

Comment Features Sequence

```
LOCUS       NG_000007              73308 bp    DNA     linear   PRI 25-APR-2001
DEFINITION  Homo sapiens genomic beta globin region (HBB@) on chromosome 11.
ACCESSION   NG_000007
VERSION     NG_000007.1  GI:13907843
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 73308)
  AUTHORS   Tuan,D., Solomon,W., Li,Q. and London,I.M.
  TITLE     The 'beta-like-globin' gene domain in human erythroid cells
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 82 (19), 6384-6388 (1985)
   PUBMED   3879975
REFERENCE   2  (bases 1 to 73308)
  AUTHORS   Vanin,E.F., Henthorn,P.S., Kioussis,D., Grosveld,F. and Smithies,O.
  TITLE     Unexpected relationships between four large deletions in the human
            beta-globin gene cluster
  JOURNAL   Cell 35 (3), 701-709 (1983)
   PUBMED   6652684
REFERENCE   3  (bases 1 to 73308)
  AUTHORS   Ley,T.J. and Nienhuis,A.W.
  TITLE     A weak upstream promoter gives rise to long human beta-globin RNA
            molecules
  JOURNAL   Biochem. Biophys. Res. Commun. 112 (3), 1041-1048 (1983)
   PUBMED   6303333
REFERENCE   4  (bases 1 to 73308)
  AUTHORS   Orkin,S.H., Kazazian,H.H. Jr., Antonarakis,S.E., Goff,S.C.,
            Boehm,C.D., Sexton,J.P., Waber,P.G. and Giardina,P.J.
  TITLE     Linkage of beta-thalassaemia mutations and beta-globin gene
            polymorphisms with DNA polymorphisms in human beta-globin gene
            cluster
  JOURNAL   Nature 296 (5858), 627-631 (1982)
   PUBMED   6280057
REFERENCE   5  (bases 1 to 73308)
  AUTHORS   Grosveld,F., Busslinger,M., Grosveld,G., Groffen,J., DeKleine,A.
            and Flavell,R.A.
  TITLE     The structure and expression of the haemoglobin genes
  JOURNAL   Adv. Exp. Med. Biol. 158, 65-80 (1982)
   PUBMED   6297279
REFERENCE   6  (bases 1 to 73308)
  AUTHORS   Efstratiadis,A., Posakony,J.W., Maniatis,T., Lawn,R.M.,
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O'Connell,C., Spritz,R.A., DeRiel,J.K., Forget,B.G., Weissman,S.M., Slightom,J.L., Blechl,A.E., Smithies,O., Baralle,F.E., Shoulders,C.C. and Proudfoot,N.J.
TITLE The structure and evolution of the human beta-globin gene family
JOURNAL Cell 21 (3), 653-668 (1980)
PUBMED 6985477
REFERENCE 7 (bases 1 to 73308)
AUTHORS Fritsch,E.F., Lawn,R.M. and Maniatis,T.
TITLE Molecular cloning and characterization of the human beta-like globin gene cluster
JOURNAL Cell 19 (4), 959-972 (1980)
PUBMED 6155216
REFERENCE 8 (bases 1 to 73308)
AUTHORS Marotta,C.A., Forget,B.G., Cohne-Solal,M., Wilson,J.T. and Weissman,S.M.
TITLE Human beta-globin messenger RNA. I. Nucleotide sequences derived from complementary RNA
JOURNAL J. Biol. Chem. 252 (14), 5019-5031 (1977)
PUBMED 873928
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U01317.1.
[WARNING] On Jan 23, 2002 this sequence was replaced by gi:18266749.

The five beta-like globin genes are found within a 45 kb cluster on chromosome 11 in the following order:

5' epsilon (HBE1), gamma G (HBG2), gamma A (HBG1), beta 1 pseudogene (HBBP1), delta (HBD), beta (HBB) -3'

These embryonic, fetal and adult beta-like genes have the same overall exonic structure, leading to the conclusion that they are derived from one ancestral gene. In particular, they have many consensus sequences and repetitive sequences in common.

The promoter region sequences 'ccaat', 'ata' and 'cttccg' are characteristic of all human beta-like genes, as well as of some other mammalian genes, and are thought to influence initiation of transcription and translation.

Epsilon Gene: The epsilon globin gene (HBE1 below) is normally expressed in the embryonic yolk sac: two epsilon chains together with two zeta chains (an alpha-like globin) constitute the embryonic hemoglobin Hb Gower I; two epsilon chains together with two alpha chains form the embryonic Hb Gower II. Both of these embryonic hemoglobins are normally supplanted by fetal, and later, adult hemoglobin. However, at least nine alternative cap sites which do not possess these conserved sequences have been found upstream from the so-called canonical cap sites at 19486 and 19488.

G-gamma and A-gamma genes: The gamma globin genes (HBG2 and HBG1 below) are normally expressed in the fetal liver, spleen and bone marrow. Two gamma chains together with two alpha chains constitute fetal hemoglobin (HbF) which is normally replaced by adult hemoglobin (HbA) at birth. In some beta-thalassemias and related conditions (HPFH or 'hereditary persistence of fetal hemoglobin'), gamma chain production continues into adulthood.

Pseudo-beta-1: The pseudogene structure was deduced through

OIPE TAP50
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PTO/SB/17 (07-06)
Approved for use through 01/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2006

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 60.00

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Examiner Name	Maria Marvich
Art Unit	1633
Attorney Docket No.	64836(51590)

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[X] Deposit Account Deposit Account Number: 04-1105 Deposit Account Name: Memorial Sloan-Kettering Cancer Center

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[X] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[X] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
[X] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fees Paid ($)**
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	**Fee ($)**	**Small Entity Fee ($)**
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
23 - 47 =		x	=

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
- 3 =		x	=

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims

Fee ($)	**Fee Paid ($)**

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
- 100 =	/50	(round up to a whole number) x		=

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 2251 Extension for response within first month	60.00

SUBMITTED BY

Signature	/Amy M. Leahy/	Registration No. (Attorney/Agent)	47,739	Telephone	(203) 975-7505
Name (Print/Type)	Amy M. Leahy			Date	November 30, 2006

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EV814886281US, on the date shown below in an envelope addressed to:
MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: November 30, 2006 Signature: /Denise Kacinski/ (Denise Kacinski)

220493

OIPE TA 950
NOV 30 2006
PATENT & TRADEMARK OFFICE

PTO/SB/22 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless if displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2006 (Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)	Docket Number (Optional) 64836(51590)
Application Number 10/188,221-Conf. #9026	Filed July 1, 2002
For VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES	
Art Unit 1633	Examiner Maria Marvich

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	
[X]	One month (37 CFR 1.17(a)(1))	$120	$60	$ 60.00
[ ]	Two months (37 CFR 1.17(a)(2))	$450	$225	$
[ ]	Three months (37 CFR 1.17(a)(3))	$1020	$510	$
[ ]	Four months (37 CFR 1.17(a)(4))	$1590	$795	$
[ ]	Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

[X] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 04-1105 . I have enclosed a duplicate copy of this sheet.

I am the

[ ] applicant/inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

[X] attorney or agent of record. Registration Number 47,739

[ ] attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

Amy M. Leahy — Signature

November 30, 2006 — Date

Amy M. Leahy — Typed or printed name

(203) 975-7505 — Telephone Number

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[ ] Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EV814886281US, on the date shown below in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: November 30, 2006 Signature: Denise Kacinski (Denise Kacinski)

12/04/2006 EAREGAY1 00000080 041105 10188221

01 FC:2251 60.00 DA

220496

OIPE
NOV 30 2006
PATENT & TRADEMARK OFFICE

Application No. (if known): 10/188,221 Attorney Docket No.: 64836(51590)

Certificate of Express Mailing Under 37 CFR 1.10

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MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on November 30, 2006
Date

Denise Kacinski
Signature

Denise Kacinski
Typed or printed name of person signing Certificate

Registration Number, if applicable

(203) 975-7505
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Amendment Transmittal Letter
Petition for Extension of Time
Fee Transmittal Form
Charge $60.00 to Deposit Account No. 04-1105

PTO/SB/06 (12-04)
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PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number: 10/188221

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY FEE ($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))							
SEARCH FEE (37 CFR 1.16(k), (l), or (m))							
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))							
TOTAL CLAIMS (37 CFR 1.16(i))	41 minus 20 =	*	X =		OR	X =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	*	X =			X =	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

11/30/04

APPLICATION AS AMENDED – PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 23	Minus	** 47	= 0	X =		OR	X =	
Independent (37 CFR 1.16(h))	* 2	Minus	*** 5	= 0	X =		OR	X =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	0

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	SMALL ENTITY ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X =		OR	X =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X =		OR	X =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2

CLAIMS ONLY

Application Number: 10188221

Filing Date:

Applicant(s):

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
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46		1				
47						
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50						
Total Indep	2					
Total Depend	21					
Total Claims	23					

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	Indep	Depend	Indep	Depend	Indep	Depend
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Total Indep						
Total Depend						
Total Claims						

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DEC 0 8 2006
PATENT & TRADEMARK OFFICE

IFW

1633

PTO/SB/21 (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission

Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket Number	64836(51590)

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [x] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Fee Transmittal Form
Charge $180.00 to Deposit Account No. 04-1105
Return Receipt Postcard

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	EDWARDS ANGELL PALMER & DODGE LLP		
Signature	Amy M. Leahy		
Printed name	Amy M. Leahy		
Date	December 5, 2006	Reg. No.	47,739

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: December 5, 2006 Signature: Denise Kacinski (Denise Kacinski)

220510

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PATENT & TRADEMARK OFFICE

PTO/SB/17 (07-06)
Approved for use through 01/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2006

[x] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Examiner Name	Maria Marvich
Art Unit	1633
Attorney Docket No.	64836(51590)

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 04-1105 Deposit Account Name: Memorial Sloan-Kettering Cancer Center

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[x] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[x] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
[x] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fee ($)**	**Fees Paid ($)**
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	**Fee ($)**	**Small Entity Fee ($)**
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
23 - 47 =		x	=

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
- 3 =		x	=

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
- 100 =	/50	(round up to a whole number) x		=

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement	180.00

SUBMITTED BY

Signature	Amy M. Leahy	Registration No. (Attorney/Agent)	47,739	Telephone	(203) 975-7505
Name (Print/Type)	Amy M. Leahy			Date	December 5, 2006

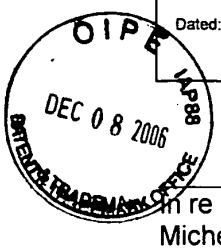
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as First Class Mail, on the date shown below in an envelope addressed to:
MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: December 5, 2006 Signature: Denise Kacinski (Denise Kacinski)

220493

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as First Class Mail, on the date shown below in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: December 5, 2006 Signature: Denise Kacinski
(Denise Kacinski)



Docket No.: 64836(51590)
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michel Sadelain et al.

Application No.: 10/188,221 Confirmation No.: 9026

Filed: July 1, 2002 Art Unit: 1633

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Examiner: Maria Marvich

INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 CFR 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is filed after the mailing date of the first Office Action on the merits, whichever occurs first, but before the mailing date of a Final Office Action or Notice of Allowance (37 CFR 1.97(c)).

In accordance with 37 CFR 1.98(a)(2)(ii), Applicant has not submitted copies of U.S. patents and U.S. patent applications. Applicant submits herewith copies of foreign patents and non-patent literature in accordance with 37 CFR 1.98(a)(2).

12/08/2006 HNGUYEN1 00000039 041105 10188221
01 FC:1806 180.00 DA

220492

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 CFR 1.98 and the Examiner is respectfully requested to consider the listed references.

Please charge our Deposit Account No. 04-1105 in the amount of $180.00 covering the fees set forth in 37 CFR 1.17(a)(1) and 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105, under Order No. 64836(51590).

Dated: December 5, 2006

Respectfully submitted,

By Amy M. Leahy

Amy M. Leahy
Registration No.: 47,739
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(203) 975-7505
Attorneys/Agents For Applicant

OIPE IAP88 DEC 08 2006 PATENT & TRADEMARK OFFICE

PTO/SB/08A/B (09-06)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket Number	64836(51590)

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	GATLIN et al. "In Vitro Selection of Lentivirus Vector-Transduced Human CD34+ Cells." Human Gene Therapy 11: 1949-1957 (2000)	
	CB	SADELAIN "Genetic Treatment of the Haemogloinopathies Recombinations and New Combinations." British Journal of Haematology 98: 247-253 (1997)	
	CC	TISDALE et al. "Towards Gene Therapy for Disorders of Golbin Synthesis." Seminars in Hematology 38:4 382-392 (2001)	
	CD	RIVELLA et al. "Globin Gene Transfer: a Paradigm for Transgenic Regulation and Vector Safety." Gene Therapy and Regulation 00:0; 1-27 (2003)	
	CE	SADELAIN et al. Issues in the Manufacture and Transplantation of Genetically Modified Hematopoietic Stem Cells." Current Opinion in Hematology 7: 364-377 (2000)	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature		Date Considered	

220499

OIPE IAP88
DEC 08 2006
PATENT & TRADEMARK OFFICE

Application No. (if known): 10/188,221 Attorney Docket No.: 64836(51590)

Certificate of Mailing Under 37 CFR 1.10

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MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on December 5, 2006
Date

Denise Kacinski
Signature

Denise Kacinski
Typed or printed name of person signing Certificate

Registration Number, if applicable

(203) 975-7505
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Information Disclosure Statement (IDS)
IDS Form PTO SB-08 (5 References)
Charge $1800.00 to Deposit Account No. 04-1105

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S31	1	S29 and globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:11
L8	2	globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4 and lentivirus	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:11
L7	12	globin and (LCR or locus adj control adj region) and HS2 same hs3 same hs4	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:11
L6	3	l3 and l5	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:10
L5	7	globin adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:10
L4	4	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:10
S11	3	globin and (lcr or locus adj control adj region) adj20 (hs2 adj10 hs3 adj10 hs4)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/03/03 08:08
S3	8840	Sadelin.in. or Rivella.in. or May.in. or Bertino.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/03/03 08:08
S1	3	"9733988"	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/03/03 08:08

EAST Search History

L3	10565	Sadelin.in. or Rivella.in. or May.in. or Bertino.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/03/03 08:08
L2	1	NG adj "000007"	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/03/03 08:08
L1	2	NG000007	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/03/03 08:08
S55	1	10/188221 and (dtns9 and tns9)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/26 08:55
S54	1	10/188221 and (dtns9 andtns9)	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/26 08:55
S53	1	10/188221 and tns9	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/26 08:55
S52	1	10/188221 and hs2	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/26 08:54
S51	1	10/188221 and hs2	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/02/26 08:53
S2	1	10/188221 and functional	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/02/26 08:53
S50	1	10/188221 and human	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/23 13:40

EAST Search History

S49	1	10/188221 and lcr	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/23 13:40
S47	1	10/188221 and tns9	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/23 13:40
S48	0	10/188221 and ptns9	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/23 13:22
S46	1	10/188221 and gata	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2007/02/23 13:22
S45	1	10/188221 and gata	US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2007/02/23 13:22

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	64836(51590)	9026

21874 7590 03/12/2007

EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
MARVICH, MARIA	
ART UNIT	PAPER NUMBER
1633	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/12/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/188,221	SADELAIN ET AL.	
	Examiner	Art Unit	
	Maria B. Marvich, PhD	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 November 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-18 and 43-47* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) *1-18 and 43-47* is/are allowed.

6)☒ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *12/8/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Any rejection of record in the previous action not addressed in this office action is withdrawn. There are no new grounds of rejection herein and therefore, this action is final.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1-18 and 43-47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **This rejection is a new rejection necessitated by applicants' amendment filed 6/30/05.**

Claims 1 and 45 are vague and indefinite in that the metes and bounds of the recitation of specific restriction fragments, which are inherent in the plasmid TNS9 are unclear. The claims recite that the HS2, HS3 and HS4 regions or portions are various cleavage fragments, which implies that there is a reference sequence or specific source for the B-globin LCR DNA required to practice the claimed invention. However, there is no reference sequence or source sequence provided. Hence the metes and bounds of the recited regions and corresponding vector TNS0 are unclear.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-18 and 43-47 are rejected under 35 USC 112, 1st paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. **This rejection is a new rejection necessitated by applicants' amendment filed 6/30/05.**

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

1) Nature of invention. The instant claims are drawn to recombinant vectors comprising a nucleotide sequence encoding a functional globin and essential elements from the β-globin LCR.

2) Scope of the invention. The scope of the invention is unclear in that the β-globin LCR is disclosed and recited to consist of an 840 bp fragment of HS2 extending between SnaBl and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and Banll restriction sites. The specification fails to specify the details of the fragments such that one of skill in the art can produce the recited vectors. More particularly, claims 1-14 and 43-47 are drawn to or

encompass use of specific LCR fragments used to drive expression of globin coding sequence. As such, this application discloses vectors that are encompassed by the definitions for biological material set forth in 37 C.F.R. 1.801. Because it is apparent that this biological material is essential for practicing the claimed invention, it must be obtainable by a reproducible method set forth in the specification or otherwise be known and readily available to the public as detailed in 37 C.F.R. 1.801 through 1.809.

3) Number of working examples and guidance. The specification discloses only two recombinant lentiviral vectors, TNS9 and dTNS9-PD. TNS9 vector is said to incorporate –618 to +2484 of the human β-globin gene and encompasses the coding sequence and an extended portion of the promoter and enhancer region as well as large portions of the LCR of b-globin. These large portions are said to be the same as those in dTNS9-PD, which further comprises a deletion in the LTR and includes a PGK promoter from the murine phosphoglycerate kinase 1 gene and a mutant DHFR region with a mutation at amino acid 22. The LCR region in both is said to consist of an 840 bp fragment of HS2 extending between SnaBl and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and Banll restriction sites. However, there is no disclosure of a reference sequence to generate the recited restriction fragments.

4) State of the art. Direct support for the LCR regions of TNS9 and dTNS9 from the specification is limited to teaching that the LCR region has been obtained from a vector M β6L found in Sandelain et al, PNAS, 1995). However, these teachings do not provide any indication of the sequence, source or organism from which the LCR is obtained. As well, Sandelain did not teach the LCR regions comprising a 1309 bp fragment of HS3 extending between HindIII and

BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and Banll restriction sites. Applicants have stated on pages 7 and 8 of the amendment filed 11/30/06, " The sequence information and location of the hypersensitive sites was known in the art at the time that the instant application was filed (see GenBank Accession Number NG000007 for exemplary sequence information) which was available to the public before the earliest filing date of the present application. Accordingly, sequence information providing the location of the restriction sites within the LCR was readily accessible to one skilled in the art using conventional sequence analysis techniques. In response to the Examiners' inquiry, the precise location of the restriction sites are summarized: the SnaBI and BstXI restriction sites of HS2 are located at positions 17,093 and 16,240, respectively; the BamHI and HindlII restriction sites of HS3 are located at positions 12,065 and 13,360, respectively; and the BamHI and BanII restriction sites of HS4 are located at positions 8,496 and 9,576, respectively."

5) Unpredictability of the art. The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non-enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). (see MPEP 2164.08(b).

It is unclear that the fragments recited in claims 1-14 and found in the vector recited in claims 43-47 were or will be readily available to the public or that the written instructions are sufficient to reproducibly construct this biological material from starting materials known and readily available to the public. Therefore, in order for a deposit to meet all criteria set forth in 37

C.F.R. 1.801 through 1.809, Applicant or Assignee must provide assurance of compliance with provisions of 37 C.F.R. 1.801-1.809 in the form of a declaration or Applicant's representative must provide a statement. The content of such a declaration or statement is suggested by the encoded attachment. Because such deposit will not have been made prior to the effective filing date of the instant application, Applicant is required to submit a verified statement from a person in a position to corroborate the statement that the biological material which had been deposited is the biological material specifically identified in the applicants as filed (37 C.F.R. 1.804). Such a statement need not be verified if the person is an agent or attorney registered to practice before the Office. Applicant is also reminded that the specification must contain reference to the deposit, including deposit (accession) number, date of deposit, name and address of the depository, and the complete taxonomic description.

Specifically, TNS9 and dTNS9 are lentivirus in which the β-globin gene is expressed under control of a region consisting of an 840 bp fragment of HS2 extending between SnaBl and BstXI restriction sites, a 1309 bp fragment of HS3 extending between HindIII and BamHI restriction sites and a 1069 bp fragment of HS4 extending between BamHI and Banll restriction sites. However, a reference sequence to generate these fragments is not provided for in the specification such that a person of skill in the art would be able to make and hence use the recited recombinant vectors. Applicants argue that the location of the restriction sites were well known in the art and point to GenBank Accession Number NG_000007 in which the HS2 sites are found at 17,093 (SnaBI) and 16,240 (BstXI), the HS3 sites are found at 12,065 (BamHI) and 13,360 (HindIII) and the HS4 sites are found at 8,496 (BamHI) and 9576 (BanII). However, a search of the sequence listing from May 2001 (attached) did not demonstrate that the recited

restriction sites could be identified at the locations applicants have provided. Nonetheless, should support be found in a sequence, there is no mention of this sequence in the specification and as such the LCR is not even limited in origin.

This rejection can be overcome by making a deposit of TNS9.

6) Amount of Experimentation Required. Given the lack of guidance in the specification, the specific nature of the fragments and vectors recited and the highly unpredictable nature of the art of determining where to isolate the recited fragments, it is concluded that a person of skill in the art would have had to conduct undue experimentation in order to practice the claimed invention.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

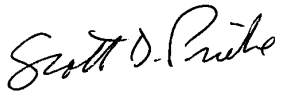
A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B. Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (7:00-4:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, PhD can be reached on (571)-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Maria B Marvich, PhD
Examiner
Art Unit 1633



SCOTT D. PRIEBE, PH.D
PRIMARY EXAMINER

OIPE
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PTO/SB/08A/B (09-06)
Approved for use through 03/31/2007. OMB 0651-0031
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	Maria Marvich
Attorney Docket Number	64836(51590)

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	GATLIN et al. "In Vitro Selection of Lentivirus Vector-Transduced Human CD34+ Cells." Human Gene Therapy 11: 1949-1957 (2000)	
	CB	SADELAIN "Genetic Treatment of the Haemogloinopathies Recombinations and New Combinations." British Journal of Haematology 98: 247-253 (1997)	
	CC	TISDALE et al. "Towards Gene Therapy for Disorders of Golbin Synthesis." Seminars in Hematology 38:4 382-392 (2001)	
	CD	RIVELLA et al. "Globin Gene Transfer: a Paradigm for Transgenic Regulation and Vector Safety." Gene Therapy and Regulation 00:0; 1-27 (2003)	
	CE	SADELAIN et al. Issues in the Manufacture and Transplantation of Genetically Modified Hematopoietic Stem Cells." Current Opinion in Hematology 7: 364-377 (2000)	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature		Date Considered	

220499

Notice of References Cited	Application/Control No. 10/188,221	Applicant(s)/Patent Under Reexamination SADELAIN ET AL.	
	Examiner Maria B. Marvich, PhD	Art Unit 1633	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
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	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	NG_000007 (GI:13907843), Homo sapiens genomic beta globin region on chormosome 11, U.S. National Library of Medicine, Bethesda, MD, USA, May 2001, accessed by PTO on 3/2/07.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination
	10/188,221	SADELAIN ET AL.
	Examiner	Art Unit
	Maria B. Marvich, PhD	1633

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
East, PALM inventor search	9/30/2005	MM
East databases- USPAT, PGPUB, EPO, JPO, Derwent, IBM-IDB search notes attached	9/30/2005	MM
STN databases- Caplus, Scisearch, Medline search notes attached	9/30/2005	MM
East, STN search history updated, search ntoes attached	7/17/2006	MM
East, STN search history updated, search ntoes attached	2/25/2007	MM

OIPE IAP22
AUG 02 2007
PATENT & TRADEMARK OFFICE

Docket No.: 64836(51590)
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michel Sadelain et al.

Application No.: 10/188,221 | Confirmation No.: 9026

Filed: July 1, 2002 | Art Unit: 1633

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | Examiner: M. Marvich

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT (IDS)

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In accordance with 37 CFR 1.97, Applicant(s) hereby make of record the following additional document. A PTO Form SB/08 and a full copy of the document required under 37 CFR 1.98(a)(2) accompany this statement.

Applicant(s) have become aware of the following document, cited in a European Examination Report issued July 9, 2007, during the prosecution of European Patent Application No. 02749753.6, which corresponds to the above referenced application, and in accordance with 37 CFR 1.97(c) and (e)(1) or (b)(3), hereby submit(s) this document for the Examiner's consideration. This document is cited on the enclosed PTO Form SB/08, and a copy of the European Examination Report and of the document required under 37 CFR 1.98(a)(2) cited thereon are enclosed as well.

This statement is not to be interpreted as a representation that the cited document is material, that an exhaustive search has been conducted, or that no other relevant information exists. Nor shall the citation of any document herein be construed

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01 FC:1806 233077 180.00 DA

per se as a representation that such document is prior art. Moreover, Applicant(s) understand(s) the Examiner will make an independent evaluation of the cited document.

This Information Disclosure Statement is filed after the mailing date of a Final Office Action or Notice of Allowance, whichever occurred first, but on or before payment of the Issue Fee (37 CFR 1.97(d)). Applicant(s) hereby petition(s) that the Information Disclosure Statement be considered.

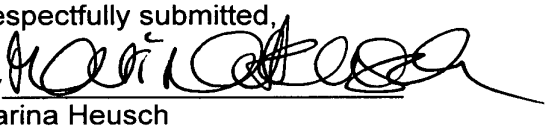
I hereby certify, pursuant to 37 CFR 1.97(e)(1), that each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Information Disclosure Statement.

Furthermore, in accordance with 37 CFR 1.704(d), Applicant(s) note(s) that to our knowledge this communication was not received by any individual designated in 1.56(c) more than thirty days prior to the filing of this statement.

Please charge our Deposit Account No. 04-1105 in the amount of $180.00 covering the fee set forth in 37 CFR 1.17(p). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105, under Order No. 64836(51590). A duplicate copy of this paper is enclosed.

Dated: July 31, 2007

Respectfully submitted,

By 

Marina Heusch
Registration No.: 47,647
Peter C. Lauro
Registration No.: 32,360
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(203) 353-6840
Attorneys/Agents For Applicant

OIPE IAP22
AUG 02 2007
PATENT & TRADEMARK OFFICE

Used in Lieu of PTO/SB/08A/B
(Based on PTO 04-07 version)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	M. Marvich
Attorney Docket Number	64836(51590)

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CA	ZUFFEREY et al., "Self-Inactivating Lentivirus Vector for Safe and Efficient In Vivo Gene Delivery", Journal of Virology, Dec. 1998, Vol. 72, No. 12, pp. 9873-9880.	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

Examiner Signature		Date Considered	

233076

OIPE IAP22 AUG 02 2007 PATENT & TRADEMARK OFFICE

PTO/SB/17 (06-07)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2007

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Examiner Name	M. Marvich
Art Unit	1633
Attorney Docket No.	64836(51590)

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[X] Deposit Account Deposit Account Number: 04-1105 Deposit Account Name: Memorial Sloan-Kettering Cancer Center

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[X] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[X] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
[X] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	**Fee ($)**	**Small Entity Fee ($)**	**Fee ($)**	**Small Entity Fee ($)**	**Fee ($)**	**Small Entity Fee ($)**	**Fees Paid ($)**
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	**Fee ($)**	**Small Entity Fee ($)**
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims		**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____	- =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____	- =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims

Fee ($)	**Fee Paid ($)**
_____	_____

3. APPLICATION SIZE FEE

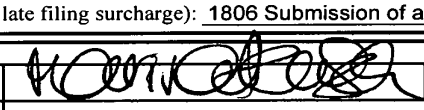
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
_____	- 100 =	_____	/50 = _____ (round up to a whole number) x	_____	= _____

4. OTHER FEE(S) **Fees Paid ($)**

Non-English Specification, $130 fee (no small entity discount)

Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement 180.00

SUBMITTED BY					
Signature		Registration No. (Attorney/Agent)	47,647	Telephone	(203) 353-6840
Name (Print/Type)	Marina Heusch			Date	July 31, 2007

233078

OIPE IAP22
AUG 02 2007
PATENT & TRADEMARK OFFICE

PTO/SB/92 (04-07)
Approved for use through 09/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application No. (if known): 10/188,221 Attorney Docket No.: 64836(51590)

Certificate of Mailing under 37 CFR 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on July 31, 2007
Date

Deborah Clark
Signature

Deborah Clark
Typed or printed name of person signing Certificate

Registration Number, if applicable

(617) 439-4444
Telephone Number

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

Fee Transmittal (1 page)
IDS (Citation) by Applicant (1 Reference) (1 page)
Supplemental Information Disclosure Statement (2 pages)
Charge $180.00 to deposit account 04-1105

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/188,221	07/01/2002	Michel Sadelain	64836(51590)	9026

21874 7590 08/10/2007

EDWARDS ANGELL PALMER & DODGE LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
MARVICH, MARIA	
ART UNIT	**PAPER NUMBER**
1633	

MAIL DATE	DELIVERY MODE
08/10/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKETT NO.
10/188221		Sandelian	

EXAMINER	
Marvich	
ART UNIT	**PAPER NUMBER**
1633	

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Wilson (3) Maria Marvich

(2) Amy Leahy (4) Joe Woitach

Date of interview 7/25/07

Type: ☑ Telephonic ☑ Personal (copy is given to ☑ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: sequence & reference w/ sequences pertaining to restriction fragments from ck

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed:

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed written description in regard to beta globin & human was acknowledged as well known. Claim language discussed to overcome 112 1st as well as obviate potential prior art

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

☐ 1. It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph below has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ 2. Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the substance of the interview unless box 1 above is also checked.

M Marvich
Examiner's Signature

PTOL-413 (REV. 2 -93)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)
In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

OIPE IAP35 SEP 12 2007 PATENT & TRADEMARK OFFICE

09-13-08

RCE/1633

PTO/SB/30 (04-07)
Approved for use through 09/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Request for Continued Examination (RCE) Transmittal

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Art Unit	1633
Examiner Name	M. Marvich
Attorney Docket Number	64836(51590)

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).
   - a. [ ] Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.
     - i. [ ] Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
     - ii. [ ] Other _____
   - b. [X] Enclosed
     - i. [X] Amendment/Reply
     - ii. [X] Affidavit(s)/Declaration(s)
     - iii. [ ] Information Disclosure Statement (IDS)
     - iv. [X] Other Notice of Appeal
2. Miscellaneous
   - a. [ ] Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)
   - b. [ ] Other _____
3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
   - a. [X] The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments to Deposit Account No. 04-1105 . I have enclosed a duplicate copy of this sheet.
     - i. [X] RCE fee required under 37 CFR 1.17(e)
     - ii. [X] Extension of time fee (37 CFR 1.136 and 1.17)
     - iii. [ ] Other _____
   - b. [ ] Check in the amount of $ _____ enclosed
   - c. [ ] Payment by credit card (Form PTO-2038 enclosed)

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature	Peter C. Lauro	Date	September 12, 2007
Name (Print/Type)	Peter C. Lauro	Registration No.	32,360

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EM 053203019 US, on the date shown below in an envelope addressed to:
MS RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: September 12, 2007 Signature: Peter C. Lauro (Peter C. Lauro)

09/13/2007 FMETEKI1 00000066 041105 10188221
01 FC:2801 395.00 DA

OIPE
SEP 19 2007
PATENT & TRADEMARK OFFICE

PTO/SB/22 (04-07)
Approved for use through 09/30/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless if displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) FY 2006 (Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).)	Docket Number (Optional) 64836(51590)
Application Number 10/188,221-Conf. #9026	Filed July 1, 2002
For VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES	
Art Unit 1633	Examiner M. Marvich

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	
[ ]	One month (37 CFR 1.17(a)(1))	$120	$60	$
[ ]	Two months (37 CFR 1.17(a)(2))	$450	$225	$
[X]	Three months (37 CFR 1.17(a)(3))	$1020	$510	$ 510.00
[ ]	Four months (37 CFR 1.17(a)(4))	$1590	$795	$
[ ]	Five months (37 CFR 1.17(a)(5))	$2160	$1080	$

[X] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 04-1105 . I have enclosed a duplicate copy of this sheet.

I am the

[ ] applicant/inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

[X] attorney or agent of record. Registration Number 32,360

[ ] attorney or agent under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

Signature — September 12, 2007 (Date)

Peter C. Lauro (Typed or printed name) — (617) 517-5509 (Telephone Number)

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

[ ] Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EM 053203019 US, on the date shown below in an envelope addressed to: MS RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: September 12, 2007 Signature: _____ (Peter C. Lauro)

09/13/2007 FMETEKI1 00000066 041105 10188221
02 FC:2253 510.00 DA

OIPE IAP35 SEP 12 2007 PATENT & TRADEMARK OFFICE

PTO/SB/17 (06-07)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person are required to respond to a collection of information unless it displays a valid OMB control number.

Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2007

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 1,155.00

Complete if Known	
Application Number	10/188,221-Conf. #9026
Filing Date	July 1, 2002
First Named Inventor	Michel Sadelain
Examiner Name	M. Marvich
Art Unit	1633
Attorney Docket No.	64836(51590)

METHOD OF PAYMENT (check all that apply)

[ ] Check [ ] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[X] Deposit Account Deposit Account Number: 04-1105 Deposit Account Name: Memorial Sloan-Kettering Cancer Center

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

[X] Charge fee(s) indicated below
[ ] Charge fee(s) indicated below, **except for the filing fee**
[X] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
[X] Credit any overpayments

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fee ($)	Small Entity Fee ($)	Fees Paid ($)
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	200	100
Multiple dependent claims	360	180

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
22 - 47 =		x	=

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
1 - 3 =		x	=

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
- 100 =		/50 = (round up to a whole number) x		=

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): 2253 Extension for response within third month	510.00
2801 Request for continued examination (RCE) (see 37 ...	395.00
2401 Notice of appeal	250.00

SUBMITTED BY

Signature	Peter C. Lauro	Registration No. (Attorney/Agent) 32,360	Telephone (617) 517-5509
Name (Print/Type)	Peter C. Lauro		Date September 12, 2007

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Application No. (if known): 10/188,221 Attorney Docket No.: 64836(51590)

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Fee Transmittal (1 page)
Three Month Request for Extension of Time Under 37 CFR 1.136(a) (1 page)
Request for Continued Examination Transmittal (1 page)
Amendment and Response with Enclosures (20 pages)
Amendment Transmittal (1 page)
Declaration of Jason W. Plotkin with Exhibits 1-11 (76 pages)
Notice of Appeal (1 page)
Charge $1,155.00 to deposit account 04-1105

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AMENDMENT TRANSMITTAL LETTER

Docket No. 64836(51590)

Application No.	Filing Date	Examiner	Art Unit
10/188,221-Conf. #9026	July 1, 2002	M. Marvich	1633

Applicant(s): Michel Sadelain et al.

Invention: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

TO THE COMMISSIONER FOR PATENTS

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated and is transmitted as shown below.

CLAIMS AS AMENDED					
	Claims Remaining After Amendment	Highest Number Previously Paid	Number Extra Claims Present	Rate	
Total Claims	22	- 47 =	0	x 25.00	0.00
Independent Claims	1	- 3 =	0	x 100.00	0.00
Multiple Dependent Claims (check if applicable)	☐				
Other fee (please specify):					
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT:					0.00

☐ Large Entity ☒ Small Entity

☒ No additional fee is required for this amendment.

☐ Please charge Deposit Account No. _____ in the amount of $ _____. A duplicate copy of this sheet is enclosed.

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Dated: September 12, 2007

Peter C. Lauro
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Dated: September 12, 2007 Signature: (Peter C. Lauro)

Docket No.: 64836(51590)
(PATENT)

SEP 12 2007 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Sadelain, *et al.*

Serial No.: 10/188,221

Filed: July 1, 2002

For: *VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES*

Confirmation No.: 9026

Group Art Unit: 1633

Examiner: Maria Marvich

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P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE
TO FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.116

Dear Sir:

Applicants submit this paper in response to the final Office Action mailed on March 12, 2007, which set a three-month shortened statutory period for reply ending on June 12, 2007. Applicants submit concurrently herewith a Request for a Three-Month Extension of Time, a Notice of Appeal, a Request for Continued Examination and the required fees for each based on small entity status. The Commissioner is authorized to charge any additional fees occasioned by this paper, or credit any overpayment of such fees, to Deposit Account No. 04-1105, under Order No. 64836(51590).

Amendments to the claims are reflected in the listing of claims beginning on page 2.

Remarks/Arguments begin on page 6.

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 46 and 47 and please cancel claim 45 without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents. This following listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A recombinant vector comprising:

(a) a region comprising a nucleotide sequence encoding a functional globin; and

(b) a 3.2-kb portion ~~portions~~ of ~~the~~ a human ß-globin locus control region (LCR) which consists essentially of ~~an 840 bp~~ an HS2-spanning nucleotide fragment ~~of HS2~~ extending between BstXI and SnaBI ~~and BstXI~~ restriction sites of said LCR, ~~a 1308 bp~~ an HS3-spanning nucleotide fragment ~~of HS3~~ extending between BamHI and HindIII ~~and BamHI~~ restriction sites of said LCR and ~~a 1069 bp~~ an HS4-spanning nucleotide fragment ~~of HS4~~ extending between BamHI and BanII restriction sites of said LCR, said vector providing expression of globin when introduced into a mammal *in vivo*.

2. (Previously presented) The vector of claim 1, further comprising a region encoding a dihydrofolate reductase.

3. (Previously presented) The vector of claim 2, further comprising a mouse PGK promoter, to control the expression of the dihydroflate reductase.

4. (Previously presented) The vector of claim 3, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

5. (Previously presented) The vector of claim 4, wherein the human dihydrofolate reductase is a

mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

6. (Previously presented) The vector of claim 5, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

7. (Previously presented) The vector of claim 2, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

8. (Previously presented) The vector of claim 7, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

9. (Previously presented) The vector of claim 8, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

10. (Previously presented) The vector of claim 1, wherein the functional globin is human ß-globin.

11. (Previously presented) The vector of claim 10, further comprising a region encoding a dihydrofolate reductase.

12. (Previously presented) The vector of claim 11, further comprising a mouse PGK promoter,

wherein the mouse PGK promoter controls the expression of the region encoding a dihydrofolate reductase.

13. (Previously presented) The vector of claim 12, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

14. (Previously presented) The vector of claim 13, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

15. (Previously presented) The vector of claim 14, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

16. (Previously presented) The vector of claim 11, wherein the dihydrofolate reductase is a human dihydrofolate reductase.

17. (Previously presented) The vector of claim 16, wherein the human dihydrofolate reductase is a mutant form having increased resistance to antifolates as compared to wild-type human dihydrofolate reductase, said mutant form differing in amino acid sequence from wild-type human dihydrofolate reductase as a result of a set of mutations.

18. (Previously presented) The vector of claim 17, wherein the set of mutations comprises a mutation at an amino acid corresponding to amino acid 22 of the wild-type sequence and a mutation at an amino acid corresponding to amino acid 31 of the wild type sequence.

19-42. (Canceled)

43. (Previously presented) The vector of claim 1, further comprising 2 GATA-1 binding sites at the junction between the HS3 and HS4 fragments.

44. (Previously presented) The vector of claim 1, wherein the vector is a lentiviral vector.

45. (Canceled)

46. (Currently Amended) The vector of claim ~~45~~1, wherein the vector is a lentiviral vector.

47. (Currently Amended) The vector of claim ~~45~~1, wherein the vector is pTNS9.

REMARKS

Claims 1-18 and 43-47 have been examined. Claims 1, 46 and 47 have been amended and Claim 45 has been cancelled. Accordingly, Claims 1-18, 43-44, 46 and 47 will be pending upon entry of the amendments presented herein. Applicants respectfully request favorable reconsideration and withdrawal of all pending rejections and allowance of the application with claims 1-18, 43-44, 46 and 47 presented herein.

The changes in Claim 1 are in part (b) and relate to the three restriction fragments as discussed below. The amendments to Claims 46 and 47 revise the dependencies. Support for the amendments can be found throughout the specification and claims as filed. No new matter has been added.

In particular, Claim 1, as amended in part (b), now recites that the recombinant vector comprises a 3.2-kb portion of a human β-globin locus control region (LCR) consisting essentially of 3 restriction fragments. Each fragment spans a particular DNase I hypersensitive site (HS) and each fragment's end is identified by particular restriction enzyme recognition sites (listed in 5' to 3' order). With respect to written description support, the phrase "portion of the . . . LCR" (or a similar phrase, *e.g.*, "large portions") is used throughout the specification. Support for the overall size being 3.2 kb is found in the last sentence of ¶ 21 of the specification. Support for the source of the LCR as human is also provided throughout the specification, *see, e.g.*, Example 1 and ¶ 21.

Amendment and cancellation of the claims are not to be construed as acquiescence to any rejections/objections set forth in the instant Office Action and/or any previous Office Action and were done solely to expedite prosecution of the application. Applicants hereby reserve the right to prosecute the subject matter of the claims as originally filed or similar claims in one or more subsequent patent applications.

At the outset, Applicants wish to thank Examiners Marvich and Woitach for the courteous and productive interview extended to Lisa Wilson and Amy Leahy on July 25, 2007. During the interview the Examiners acknowledged that the human β-globin region is well known

in the art. In support of that discussion and this response to the outstanding rejections, Applicants submit the Declaration of Jason W. Plotkin under 37 C.F.R. § 1.132 ("Plotkin").

The globin genes were among the first ever sequenced at the nucleotide level. By the early to mid 1980's, the sequence and genomic organization of the beta family of human globin genes on chromosome 11 were known for greater than 70 kb ("the human β-globin region"). The details thereof, including a list of more than 80 citations to the literature dating between 1974-1985, are provided with the annotated sequence and commentary available under GenBank Accession No. U01317 (which was publically available in 1994; partial copy attached, p.10). This sequence and those provided by GenBank Accession No. NG_000007 (publically available as version 1 as early as April 2001)[1] are the reference sequences for the human β-globin region and are well known to those of skill in the art. Moreover, these sequences, while not directly cited in the specification, are clearly the ones to which those of the skill in the art are directed by the specification (as shown in detail below). Plotkin, ¶¶ 8-13 and 19-22.

The present Office Action contains two outstanding rejections under 35 U.S.C. § 112, one for indefiniteness and the other for lack of enablement. The responses to these rejections are interrelated, requiring explanation of (1) how the foregoing sequences and the references are well known and available, and (2) how to identify the location of the restriction fragments. The former is addressed in response to the indefiniteness rejection, the latter in response to the enablement rejection.

[1] Two subsequent versions were assigned GenBank Accession No. NG_000007. The dates of creation of each version and links from one to another are provided in the annotations to the sequences. When accessing NG_000007 at present, one obtains version 3, which in relevant part includes an additional approximately 9 kb upstream of the version 1. Hence the numbering of the nucleotides is offset between the versions, and can be further slightly offset by sequence polymorphisms and minor variations.

Claims Rejection – 35 U.S.C. §112, Second Paragraph

Claims 1-18 and 43-47 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly vague and indefinite. In particular, the Examiner has alleged that the metes and bounds of the restriction fragments recited in Claims 1 and 45 (now canceled) are unclear for lack of a reference sequence or specific source for the β-globin locus control region (LCR).

In this regard, Claim 1 has been amended to recite the source of the LCR as human. Further, as stated, the complete sequence for the human β-globin region is readily known and available in the art. The LCR is an approximately 20-30 kb region upstream from the ε-globin gene.

With respect to finding the sequences and ascertaining the claimed LCR, for all practical purposes at the time of the invention (and now), the skilled artisan would have immediately turned to the GenBank database to obtain the desired sequence, using a simple key word search without any need to know the accession numbers. Plotkin, ¶¶ 6&7. Mapping the restriction sites would be done with any of the myriad of available software for analyzing sequence data. By knowing the overall genomic organization, the sequence of the β-globin region, that the terminology in this art "speaks" in restriction fragments[2] and the size of the claimed LCR, and by having the guidance from the specification, the skilled artisan can ascertain the claimed fragments of the LCR without ambiguity (as detailed below).

Although the simplest route to finding the sequence is as outlined above, the skilled artisan can arrive at the same GenBank sequences by following the teaching and guidance in the specification. Plotkin, ¶¶ 8-13. Reviewing the specification and following one trail of references, the skilled artisan finds that:

- "restriction fragments spanning the core portions of HS2, HS3 and HS4" are reported in Sadelain (Plotkin, ¶ 9);

---

[2] It is customary to refer to restriction fragments without mentioning the source of the actual sequence, knowing that those sites are in a published sequence (generally cited as a reference in the paper and, unless specifically noted, is the publically available sequence as deposited in the database.

- Sadelain describes the three restriction fragments and containing the core sequences for HS2, HS3 and HS4, referencing, among others, Ryan (Plotkin, ¶ 10);
- Ryan states that "[s]everal years ago, Tuan . . . mapped sites that were super-hypersensitive to DNase I digestion . . . located 6-22 kb upstream of the ε-globin gene" (Plotkin, ¶ 11);
- Fig. 1 of Tuan shows a restriction map of the "human β-like-globin gene cluster" as determined from the sequencing data of Li (Plotkin, ¶ 12); and
- Li shows 21 kb of uninterrupted sequence in Fig. 2 that begins at an EcoRI site, sets coordinate 0 as the initiation codon of the ε-globin gene and extends through three ε-globin exons. Approximately 19.5 kb of the published sequence is located upstream of that initiation codon. This 19.5 kb upstream region contains most of the human β-globin LCR and includes the complete HS2, HS3 and HS4 sites. Plotkin, ¶ 13.

Although Li does not provide a GenBank accession number for the sequence presented in Fig.2, the GenBank database cites Li as the source of those sequences in U01713 and NG_000007. Plotkin, ¶14. Moreover, by finding the Li reference in the PubMed data base (www.pubmed.gov) and displaying that result in "Nucleotide Links" format, one obtains a direct link to a single sequence, U01317. Consequently, one of skill in the art, having found the sequence, either on paper or by searching the GenBank database, is assured of finding the same sequence that has been known since 1985. Plotkin, ¶ 15.

Accordingly, the skilled artisan has a human reference sequence bearing the recited restriction sites, the prior published references teach the location of the HS2, HS4 and HS4 core fragments, and the claimed LCR is assembled from three restriction fragments with an overall size of 3.2 kb (as now recited in Claim 1), so that one can unambiguously determine the scope of the claimed invention. Because the foregoing establishes the availability of a reference sequence and the source of the LCR as human, Applicants submit that the metes and bounds of the claims are clear and unambiguous and this rejection is obviated.

Hence, the rejection of the claims under 35 U.S.C. § 112, second paragraph, is obviated and Applicants respectfully request reconsideration and withdrawal thereof.

Claims Rejection – 35 U.S.C. §112, First Paragraph

Claims 1-18 and 43-47 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

The present invention provides a recombinant vector comprising (a) a region encoding a functional globin; and (b) a 3.2-kb portion of an LCR derived from human β-globin sequences, such that the vector provides expression of the globin when introduced into a mammal *in vivo*. In particular, Claim 1 recites that the 3.2-kb portion consists essentially of an HS2-spanning nucleotide fragment extending between BstXI and SnaBI restriction sites, an HS3-spanning nucleotide fragment extending between BamHI and HindIII restriction sites and an HS4-spanning nucleotide fragment extending between BamHI and BanII restriction sites, wherein the fragments are from a human ß-globin locus control region (ß-globin LCR).

As mentioned, the human ß-globin LCR is a 20-30 kb region extending upstream from the start of the ε-globin gene. The region has been extensively analyzed and the scientific literature reports a variety of expression studies with a 20-kb "minilocus," a 6.5-kb microlocus" and a 1-kb fragment with core DNAase I hypersensitive site. See, Philipsen, 1990, Fig. 1 and Introduction, p. 2159 (Exhibit 7 of Plotkin). To Applicants' knowledge, no previous studies have been conducted with a 3.2-kb portion of a human ß-globin LCR as claimed herein.

With respect to enablement, one skilled in the art must be able to make and use the claimed invention without undue experimentation from the disclosure in the specification and information known in the art at the time of the invention. Traditionally, such determinations are obtained from weighing an analysis of the multiple Wands factors. This response addresses those factors, especially those mentioned in the rejection, and clearly sets forth how to identify the 3.2-kb portion with the recited the restriction fragments based on (1) the information in the specification and the scientific literature and (2) the reference sequences available as of the

earliest filing date accorded the present application (from U.S. Serial No. 60/301,861, filed June 29, 2001). Although this analysis is necessarily tedious and painstaking, it yields clear and unambiguous results and does not require any undue experimentation.

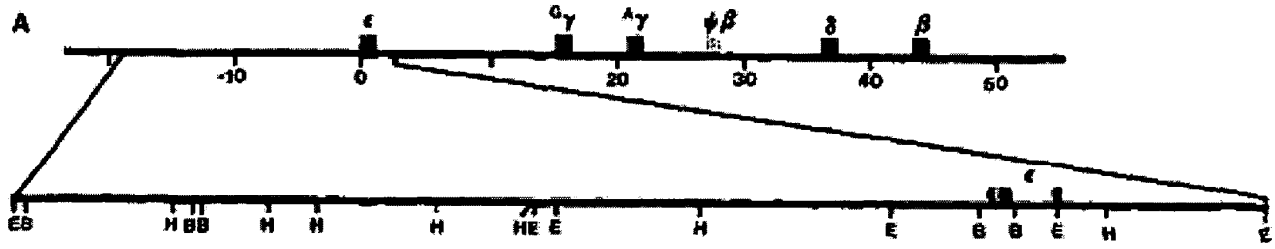
Wands Factors

1) State of the Art

"State of the Art" is considered first because this factor provides the background knowledge from which those of skill in the art would ascertain enablement.

As previously mentioned, the globin genes were among the first ever sequenced and the field developed using restriction fragments as the nomenclature of choice to identify (and even name) various elements of interest. Importantly, any skilled artisan with an interest in the present invention would be aware of this and familiar with the "workings" of the globin literature, including naming differences that arose when competing groups published on common subject matter. By at least 1993, the field had agreed upon a uniform system of nomenclature and that is the system used herein and in the claims. Plotkin, ¶¶ 20-21.

Long before the present invention, the genomic organization and sequence of the human β-globin region on chromosome 11 was well known, with the exact locations and order of each expressed globin gene being 5'—ε—Gγ—Aγ—δ—β—3' as shown in restriction map below (Li, Fig. 1).



The 5'-most EcoRI site is the first nucleotide in the sequence provided by U01317 and NG_000007.1 (the version of NG_000007 that was publically available at the earliest priority date of the present application; the current version is NG_000007.3 and extends about 9 kb further upstream from that EcoRI site). Plotkin, ¶¶ 22-23.

In 1985, Tuan indicated the locations of HS2, HS3 and HS4 as being -11 kb, -14.5 kb and -17.5 kb upstream of the ε-gobin gene, using that point as coordinate 0. Plotkin, ¶ 24.

In 1995, Sadelain described the core sequences for these three DNase hypersensitive sites as follows:

- HS2 being on a 478-bp HindIII-SnaBI fragment including the known HindIII-XbaI fragment;
- HS3 being on a 260-bp fragment including the Hph1-Fnu4HI segment which is 225 bp and is also located on a 1.9-kb HindIII-HindIII fragment; and
- HS4 being on a 283-bp SacI-AvaI fragment.

Plotkin, ¶ 25.

Although presented in chronological order, the foregoing description of the state of the art from Sadelain back to Li represents a synopsis of the knowledge on the state of the globin art imparted from citation of Sadelain in the specification. Such an analytical approach is entirely consistent with case law, which states that which is known in the art need not be included in the specification, and is preferably omitted. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367 (Fed. Cir. 1986); MPEP §2163(II)(A)(3)(a); pages 2100-173.

So although the Examiner has suggested that the specification is lacking in support for an indication of the sequence, source and organism from which the LCR is obtained, this information is actually available from the citation to the Sadelain reference.

2) Nature of the Invention

The nature of the invention identifies the field of the endeavor–here a recombinant vector for treating hemoglobinopathies by expressing a functional globin *in vivo* using the claimed 3.2-kb portion of a human β-globin LCR.

As an aside, under this section of the present Office Action, the Examiner characterized the three fragments in the LCR as "essential elements from the β-globin LCR." Applicants wish to clarify this remark as it is not a term of art and is somewhat misleading. The literature describes core HS sites as small fragments, and these core sequences might be considered as "essential" or "minimal" since they are the smallest fragments that can effect globin expression. In point of fact, the present invention resides in having more than these small core sequences, namely, the invention resides in having the larger, specific HS-containing fragments in the vector and obtaining a level of globin expression not previously possible *in vivo*.

3) Scope of the Invention & Predictability of the Art

The scope of the invention refers to the breadth of embodiments covered by the claims. In the Scope section of the Office Action, the Examiner stated that the specification fails to specify sufficient details to allow one of skill in the art to reproduce the recited vector and suggested depositing the biological material as one method to enable reproducibility. The Unpredictability section ended by indicating that a deposit of TNS9 would overcome this rejection.

Applicants appreciate this indication, and should it be necessary, are willing to make a deposit. However, Applicants firmly believe that the information in the specification, the availability of the sequence of the human β-globin LCR, as well as the extensive knowledge of the core HS sites, render a deposit unnecessary. The basis for this belief is the ability to provide a detailed analysis to identify unambiguously the three HS-spanning restriction fragments of the claimed invention. This analysis is set out in detail in Plotkin's declaration (¶¶ 16-47).

The analysis begins with obtaining the nucleotide sequences for U01317 and NG_000007.1, which are identical with respect to restriction enzymes being analyzed. Plotkin, ¶ 27.

For example, one can download the U01317 or NG_000007.1 sequence from the NCBI website and use any convenient software to analyze the restriction sites. For this analysis, the New England BioLabs program NEBcutter was used (available on the NEB website, www.neb.com). The program allows direct input of the GenBank accession number and determines restriction sites and fragment sizes for any restriction enzyme or combination of enzymes. Plotkin, ¶ 28.

For simplicity, NG7 is used to refer to the reference sequence NG_000007.1, which was the sequence used in this analysis. The results are the same with U01317 but are shifted by about 9000 nucleotides for NG_000007.3 (since that deposited sequence has approximately 9000 more nucleotides upstream from nucleotide 1 of NG7). Plotkin, ¶ 29.

Plotkin describes the initial steps for using NG7 to determine the location and number of restriction enzyme cut sites for BstXI and SnaBI (HS2), for BamHI and HindIII (HS3) and for BamHI and BanII (HS4). The analysis is simplified by considering only the restriction sites located upstream from the start site for the ε-globin gene: the ε-globin transcript begins at nucleotide 19289 and its coding sequence at nucleotide 19541. Knowing this reference point and that HS2 is located about 11 kb upstream from ε-globin gene, that HS3 is about 14.5 kb upstream and that HS4 is about 17.5 kb upstream, enables one of skill in the art to map the approximate location for each HS site. Plotkin, ¶¶ 30-33.

From this background information, Plotkin proceeds to describe how to identify the HS2-spanning fragment (Plotkin, ¶¶ 34-38) followed by how to identify the HS4-spanning fragment (Plotkin, ¶¶ 39-44). Finally, building on these identifications and using the size requirement for the claimed portion of the LCR, Plotkin describes how to identify the HS3-spanning fragment Plotkin, ¶¶ 45-46.

Based on the foregoing, the HS2-spanning fragment in NG7 is located at nucleotides 8055-8911 and has a size of 857 bp; the HS4-spanning fragment in NG7 is located at nucleotides 308-1388 and has a size of 1080 bp and the HS3-spanning fragment in NG7 is located at nucleotides 3878-5172 and has a size of 1295 bp. Plotkin, ¶¶ 38, 44 and 46, respectively.

In conclusion, the teaching of the specification and the language of the claim when taken in combination with well-known and publically available information, can be and has been used to identify a 3.2-kb portion of a human ß-globin LCR that consists essentially of an HS2-spanning nucleotide fragment extending between BstXI and SnaBI restriction sites of the LCR, an HS3-spanning nucleotide fragment extending between BamHI and HindIII restriction sites of the LCR and an HS4-spanning nucleotide fragment extending between BamHI and BanII restriction sites of the LCR (Plotkin, ¶ 47).

4) Number of Working Examples

The specification provides two working examples of vectors (TNS9 and dTNS9-PD; Figs. 1 and 2) comprising the claimed portions of the LCR, and includes data showing therapeutic benefit for treating thalassemia in mice (e.g., Fig. 6). In this section, the Examiner alleged that the specification failed to provide a reference sequence. As established above, a reference sequence was well known at the time of the invention and the specification teaches those of skill in the art how to find those sequences.

As apparent from the reference sequence, and as known in the art, the three fragments that form the 3.2-kb portion of the LCR are assembled from non-contiguous portions of the LCR. In this regard, it should be recognized that these fragments can be joined in either 5'-3' or3'-5' orientation using any of numerous techniques known to those of skill in the art to provide further vector examples. Once assembled into a vector, the fragments need not be cleavable nor must the entire restriction recognition site be present. For example, one skilled in the art will readilty appreciate that the full restriction site might not be present if the fragment is blunt-ended before ligation, yet it may be present if the cut site is filled before ligation. Hence, each HS-spanning

fragment has those LCR sequences that are present between the respective restriction enzyme recognition (cut) sites.

5) Amount of Experimentation

In this section, the Examiner suggested that the "highly unpredictable nature of determining where to isolate the recited fragments" created undue experimentation to practice the present invention. In view of the amendments to the claims to provide the source of the fragments as human and the explanation that shows the three restriction fragments can be located without ambiguity to form a 3.2-kb portion of a human ß-globin LCR, Applicants submit that the experimentation needed to practice is reasonable and does not require any further guidance than what has been provided in the specification and the state of the art.

In summary, upon considering all the Wands factors, Applicants have shown that those of skill in the art can unambiguously identify the claimed subject matter using the reference sequence. With that information, one can unambiguously identify the same fragments from any polymorphic variant of the reference sequence simply by aligning the reference sequences with those of the candidate sequence, since the relative location of restriction sites being constant, once located, those points in the sequence can be found without ambiguity based on matching the surrounding sequences.

Hence, the rejection under 35 U.S.C. § 112, first paragraph, has been overcome and Applicants respectfully request reconsideration and withdrawal thereof.

CONCLUSION

In view of the foregoing amendments and remarks presented herein, reconsideration and withdrawal of all rejections and allowance of the instant application with all pending claims are respectfully solicited. If a telephone conversation with Applicants' attorney(s) would help to expedite the prosecution of the above-identified application, the Examiner is invited to contact the undersigned.

Dated: September 12, 2007

Respectfully submitted,

By _____

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Enclosure (GenBank Accession No. U01317, 2 pages)

1: U01317. Reports Human beta globin...[gi:455025] Links

Comment Features Sequence

```
LOCUS       HUMHBB                  73308 bp    DNA     linear   PRI 09-FEB-2005
DEFINITION  Human beta globin region on chromosome 11.
ACCESSION   U01317 J00093 J00094 J00096 J00158-J00175 J00177 J00178 J00179
            K01239 K01890 K02544 M18047 M19067 M24868 M24886
VERSION     U01317.1  GI:455025
KEYWORDS    Alu repeat; HPFH; KpnI repetitive sequence; RNA polymerase III;
            allelic variation; alternate cap site; beta-1 pseudogene;
            beta-globin; delta-globin; epsilon-globin; gamma-globin; gene
            duplication; globin; polymorphism; promoter mutation; pseudogene;
            repetitive sequence; thalassemia.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 62409 to 62631; 63482 to 63610)
  AUTHORS   Marotta,C.A., Forget,B.G., Weissman,S.M., Verma,I.M.,
            McCaffrey,R.P. and Baltimore,D.
  TITLE     Nucleotide sequences of human globin messenger RNA
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 71 (6), 2300-2304 (1974)
   PUBMED   4135409
REFERENCE   2  (bases 63602 to 63646)
  AUTHORS   Forget,B.G., Marotta,C.A., Weissman,S.M. and Cohen-Solal,M.
  TITLE     Nucleotide sequences of the 3'-terminal untranslated region of
            messenger RNA for human beta globin chain
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 72 (9), 3614-3618 (1975)
   PUBMED   1059150
REFERENCE   3  (bases 63593 to 63626)
  AUTHORS   Proudfoot,N.J. and Brownlee,G.G.
  TITLE     Nucleotide sequences of globin messenger RNA
  JOURNAL   Br. Med. Bull. 32 (3), 251-256 (1976)
   PUBMED   788834
REFERENCE   4  (bases 63673 to 63743)
  AUTHORS   Proudfoot,N.J. and Longley,J.I.
  TITLE     The 3' terminal sequences of human alpha and beta globin messenger
            RNAs: comparison with rabbit globin messenger RNA
  JOURNAL   Cell 9 (4 PT 2), 733-746 (1976)
   PUBMED   1035137
REFERENCE   5  (sites)
  AUTHORS   Proudfoot,N.J. and Brownlee,G.G.
  TITLE     3' non-coding region sequences in eukaryotic messenger RNA
  JOURNAL   Nature 263 (5574), 211-214 (1976)
   PUBMED   822353
REFERENCE   6  (bases 63596 to 63743)
  AUTHORS   Proudfoot,N.J.
  TITLE     Complete 3' noncoding region sequences of rabbit and human
            beta-globin messenger RNAs
  JOURNAL   Cell 10 (4), 559-570 (1977)
   PUBMED   67897
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JOURNAL EMBO J. 3 (11), 2641-2645 (1984)
PUBMED 6210198
REFERENCE 70 (bases 61557 to 61623)
AUTHORS Semenza,G.L., Malladi,P., Surrey,S., Delgrosso,K., Poncz,M. and Schwartz,E.
TITLE Detection of a novel DNA polymorphism in the beta-globin gene cluster
JOURNAL J. Biol. Chem. 259 (10), 6045-6048 (1984)
PUBMED 6327661
REFERENCE 71 (sites)
AUTHORS Orkin,S.H., Antonarakis,S.E. and Kazazian,H.H. Jr.
TITLE Base substitution at position -88 in a beta-thalassemic globin gene. Further evidence for the role of distal promoter element ACACCC
JOURNAL J. Biol. Chem. 259 (14), 8679-8681 (1984)
PUBMED 6086605
REFERENCE 72 (bases 45336 to 47463)
AUTHORS Chang,L.Y. and Slightom,J.L.
TITLE Isolation and nucleotide sequence analysis of the beta-type globin pseudogene from human, gorilla and chimpanzee
JOURNAL J. Mol. Biol. 180 (4), 767-784 (1984)
PUBMED 6098690
REFERENCE 73 (sites)
AUTHORS Grindlay,G.J., Lanyon,W.G., Allan,M. and Paul,J.
TITLE Alternative sites of transcription initiation upstream of the canonical cap site in human gamma-globin and beta-globin genes
JOURNAL Nucleic Acids Res. 12 (4), 1811-1820 (1984)
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REFERENCE 74 (sites)
AUTHORS Stoeckert,C.J. Jr., Collins,F.S. and Weissman,S.M.
TITLE Human fetal globin DNA sequences suggest novel conversion event
JOURNAL Nucleic Acids Res. 12 (11), 4469-4479 (1984)
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AUTHORS Mager,D.L. and Henthorn,P.S.
TITLE Identification of a retrovirus-like repetitive element in human DNA
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 81 (23), 7510-7514 (1984)
PUBMED 6095301
REFERENCE 76 (bases 19120 to 61776)
AUTHORS Collins,F.S. and Weissman,S.M.
TITLE The molecular genetics of human hemoglobin
JOURNAL Prog. Nucleic Acid Res. Mol. Biol. 31, 315-462 (1984)
PUBMED 6397774
REFERENCE 77 (bases 34276 to 34276; 34282 to 34282; 34321 to 34321)
AUTHORS Gilman,J.G. and Huisman,T.H.
TITLE DNA sequence variation associated with elevated fetal G gamma globin production
JOURNAL Blood 66 (4), 783-787 (1985)
PUBMED 2412616
REFERENCE 78 (sites)
AUTHORS Ruskin,B., Greene,J.M. and Green,M.R.
TITLE Cryptic branch point activation allows accurate in vitro splicing of human beta-globin intron mutants
JOURNAL Cell 41 (3), 833-844 (1985)
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AUTHORS Lang,K.M. and Spritz,R.A.
TITLE Cloning specific complete polyadenylylated 3'-terminal cDNA segments
JOURNAL Gene 33 (2), 191-196 (1985)
PUBMED 2581851
REFERENCE 80 (bases 1 to 16593)
AUTHORS Li,Q., Powers,P.A. and Smithies,O.
TITLE Nucleotide sequence of 16-kilobase pairs of DNA 5' to the human epsilon-globin gene
JOURNAL J. Biol. Chem. 260 (28), 14901-14910 (1985)

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AUTHORS Prchal,J.T., Cashman,D.P. and Kan,Y.W.
TITLE Hemoglobin Long Island is caused by a single mutation (adenine to cytosine) resulting in a failure to cleave amino-terminal methionine
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 83 (1), 24-27 (1986)
PUBMED 3455755
REFERENCE 93 (bases 59589 to 59718; 72211 to 72340)
AUTHORS Gilman,J.G. and Abraham,J.
TITLE DNA sequence analysis of the Dutch beta-0-thalassemia deletion
JOURNAL Biomed. Biochim. Acta 46, 131-135 (1987)
REFERENCE 94 (bases 43723 to 50721)
AUTHORS Miyamoto,M.M., Slightom,J.L. and Goodman,M.
TITLE Phylogenetic relations of humans and African apes from DNA sequences in the psi eta-globin region
JOURNAL Science 238 (4825), 369-373 (1987)
PUBMED 3116671
REFERENCE 95 (sites)
AUTHORS Fei,Y.J., Stoming,T.A., Efremov,G.D., Efremov,D.G., Battacharia,R., Gonzalez-Redondo,J.M., Altay,C., Gurgey,A. and Huisman,T.H.
TITLE Beta-thalassemia due to a T----A mutation within the ATA box
JOURNAL Biochem. Biophys. Res. Commun. 153 (2), 741-747 (1988)
PUBMED 3382401
REFERENCE 96 (bases 55038 to 55052)
AUTHORS Atweh,G.F., Brickner,H.E., Zhu,X.X., Kazazian,H.H. Jr. and Forget,B.G.
TITLE New amber mutation in a beta-thalassemic gene with nonmeasurable levels of mutant messenger RNA in vivo
JOURNAL J. Clin. Invest. 82 (2), 557-561 (1988)
PUBMED 3403716
REFERENCE 97 (sites)
AUTHORS Engelke,D.R., Hoener,P.A. and Collins,F.S.
TITLE Direct sequencing of enzymatically amplified human genomic DNA
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 85 (2), 544-548 (1988)
PUBMED 3267215
REFERENCE 98 (sites)
AUTHORS Guidal,C., Lapoumeroulie,C., Krishnamoorthy,R. and Elion,J.
TITLE Sequence correction and reassignment of the TaqI polymorphic site in the human inter-gamma-globin gene region, an African-specific marker
JOURNAL Unpublished
REFERENCE 99 (bases 16594 to 19305)
AUTHORS Trepicchio,W.L., Dyer,M.A., Hardison,R.G. and Baron,M.H.
TITLE Upstream regulatory region of the human embryonic beta-like globin gene, epsilon
JOURNAL Unpublished (1993)
REFERENCE 100 (bases 1 to 73308)
AUTHORS Teixeira,A., Tahiri-Alaoui,A., West,S., Thomas,B., Ramadass,A., Martianov,I., Dye,M., James,W., Proudfoot,N.J. and Akoulitchev,A.
TITLE Autocatalytic RNA cleavage in the human beta-globin pre-mRNA promotes transcription termination
JOURNAL Nature 432 (7016), 526-530 (2004)
PUBMED 15565159
COMMENT On Feb 16, 1994 this sequence version replaced gi:183807.
[1] cRNA fragments.
[2] mRNA.
[5] sites; polyadenylation signal and site for the beta gene.
[4] cDNA from normal and thalassemic mRNAs.
[6] cDNA.
[9] mRNA and cDNA fragments.
[10] mRNA.
[7] cDNA.
[13] see comment below for 35854 to 35946 - may be 40770 to 40862.
[19] sites; amber mutation at codon 17 of the beta chain.
[22] sites; gene order for the beta-like globin cluster.
[27] sites; consensus sequences in the promoter regions.

PTO/SB/31 (04-07)
Approved for use through 09/30/2007. OMB 0651-0031
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NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES	Docket Number (Optional) 64836(51590)

In re Application of
Michel Sadelain et al.

Application Number	Filed
10/188,221-Conf. #9026	July 1, 2002

For VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Art Unit	Examiner
1633	M. Marvich

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 500.00

[X] Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ 250.00

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director has already been authorized to charge fees in this application to a Deposit Account. I have enclosed a duplicate copy of this sheet.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 04-1105 . I have enclosed a duplicate copy of this sheet.

[ ] A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

I am the

[ ] applicant /inventor.

[ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

[X] attorney or agent of record.
Registration number 32,360

[ ] attorney or agent acting under 37 CFR 1.34.
Registration number if acting under 37 CFR 1.34. _____

Signature

Peter C. Lauro
Typed or printed name

(617) 517-5509
Telephone number

September 12, 2007
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of 1 forms are submitted.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service as Express Mail, Label No. EM 053203019 US, on the date shown below in an envelope addressed to:
MS AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: September 12, 2007 Signature: _____ (Peter C. Lauro)

OIPE IAP35
SEP 12 2007
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Sadelain *et al.*

Serial No.: 10/188,221

Filed: July 1, 2002

For: VECTOR ENCODING HUMAN GLOBIN GENE AND EREOF IN TREATMENT OF HEMOGLOBINOPATHIES

Attorney Docket No.: 64836(51590)

Confirmation No.: 9026

Group Art Unit: 1633

Examiner: Maria Marvich

Mail Stop: AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JASON W. PLOTKIN UNDER 37 C.F.R. § 1.132

1. I, Jason W. Plotkin, declare as follows:
2. I am a United States citizen and reside in Queens, New York.
3. I received a B.S. degree in biochemistry from the State University of New York (SUNY), Stonybrook in 1991 and an M.A. in biochemistry from SUNY Buffalo in 1995. My CV is attached (Exhibit 1)
4. I am currently employed as a Research Assistant in the laboratory of Dr. Michel Sadelain at Department of Medicine, Memorial Sloan-Kettering Cancer Center (MSKCC), New York, New York. I have been employed at MSKCC since 2000 and by Dr. Sadelain since 2005. Prior to working at MSKCC, I was an Assistant for Research at The Rockefeller Univerisity, New York, New York from 1995-2000; and as a Laboratory Technician at the School of Medicine, SUNY Buffalo in 1995.
5. I am familiar with and have reviewed the specification of U.S. Serial No. 10/188,221, filed July 1, 2002 ("the '221 application").

Obtaining an LCR Reference Sequence

6. At present (as well as before June 2001), a simple and quick way for a person wishing to determine a reference nucleotide sequence for the human β-globin locus control region (LCR), would be to conduct an appropriate keyword search in the GenBank database, which is a well-known database that has been publically available since the 1980's. The GenBank database can be accessed via the Database section of the NCBI website (www.ncbi.nlm.nih.gov).
7. Such a search finds the reference nucleotide sequences for the human β-globin LCR to be GenBank accession numbers U01713 and NG_000007, which LCR sequences, as established below, were published in 1985.
8. Additionally, the '221 application teaches that the nucleotide sequences for the HS-spanning restriction fragments and the human β-globin LCR are well known and available in the scientific literature.
9. In particular, the '221 application at ¶ 21 states that the "restriction fragments spanning the core portions of HS2, HS3 and HS4" are reported in Sadelain *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:6728-6732 ("Sadelain;" Exhibit 2).
10. Sadelain describes the three restriction fragments containing the core HS2, HS3 and HS4 sequences in detail and cites several scientific references as authority on these points, including, among others, Ryan *et al.* (1989) *Genes Dev.* 3:314-323 ("Ryan;" Exhibit 3) [Sadelain, p. 6730, left col., first full ¶, first sentence, ref. 11].
11. Ryan states that "[s]everal years ago, Tuan . . . mapped sites that were super-hypersensitive to DNase I digestion . . . located 6-22 kb upstream of the ε-globin gene" [Ryan, p. 314, rt. col., first and second full sentences], citing Tuan *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:6384-6388 ("Tuan;" Exhibit 4).
12. Fig. 1 of Tuan shows a restriction map of the "human β-like-globin gene cluster" as determined from the sequencing data of Li *et al.* (1985) *J. Biol. Chem.* 260: 14901-14910 ("Li;" Exhibit 5) [Tuan, p. 6385, Fig.1, caption].
13. Published in 1985, Fig. 2 of Li shows 21 kb of uninterrupted sequence that begins at an EcoRI site, sets coordinate 0 as the initiation codon of the ε-globin gene and extends through three ε-globin exons. Approximately 19.5 kb of the published sequence is

located upstream of that initiation codon. This 19.5 kb upstream region contains most of the human β-globin LCR and includes the complete HS2, HS3 and HS4 sites.

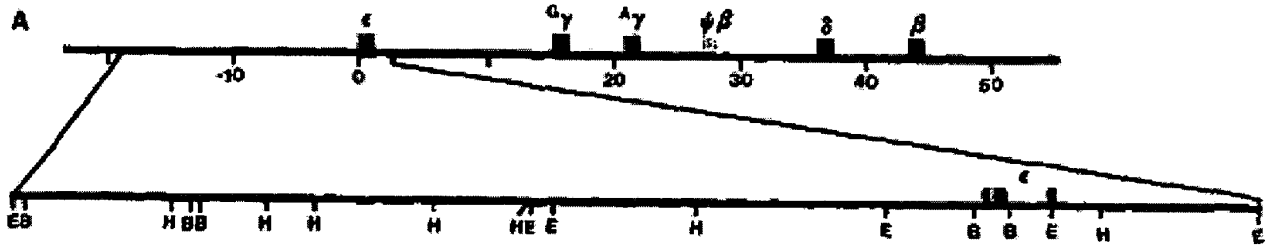
14. Li does not provide a GenBank accession number for the sequence published in Fig. 2. However, the GenBank database, under U01713 and NG_000007, cites Li as the source of the deposited sequences for that region of sequence.
15. Consequently, it is straightforward to ascertain the sequence of the human β-globin LCR by beginning with the information in the '221 application and following the string of citations to Li and the published sequence for the human β-globin LCR .

Mapping the HS-Spanning Restriction Fragments

16. As I have been told and understand, Claim 1 of the '221 application (Exhibit 6) is presently directed to a recombinant vector comprising (a) a region encoding a functional globin; and (b) a 3.2-kb portion of a human β-globin LCR composed of three specific restriction fragments so that the vector provides expression of the globin when introduced into a mammal *in vivo*.
17. In particular, Claim 1 recites that the 3.2-kb portion consists essentially of an HS2-spanning nucleotide fragment extending between BstXI and SnaBI restriction sites, an HS3-spanning nucleotide fragment extending between BamHI and HindIII restriction sites and an HS4-spanning nucleotide fragment extending between BamHI and BanII restriction sites, and that these fragments are from a human ß-globin LCR.
18. Below I describe how to identify and map the three recited restriction fragments based on the information in the specification, the scientific literature and the reference sequences available as of June 29, 2001, which I have been told is the earliest filing date of the '221 application.
19. The human ß-globin LCR is a 20-30 kb region extending upstream from the start of the ε-globin gene.
20. The globin genes were among the first ever sequenced and the field developed using restriction fragments as the nomenclature of choice to identify (and even name) genetic elements of interest.

21. To understand the relevant globin-related literature, prior to 1993, the "locus control region" was also termed the "dominant control region." Importantly, in the papers using the LCR nomenclature (which include Tuan, Sadelain and the present application), the hypersensitive sites were numbered sequentially moving upstream from the ε-globin gene so that HS1 is closest to the ε-globin gene and HS5 is the farthest away. For the papers using the DCR nomenclature, however, the reverse was true (and the most upstream site HS5 was not included). The dual nomenclature means that publications that refer to DCR site 1 relate to HS4, those that refer to DCR site 2 relate to HS3 and those that refer to DCR site 3 relate to HS2. Eventually the field adopted the LCR system of nomenclature.

22. The genomic organization and sequence of the human β-globin region on chromosome 11 is well known, with the exact locations and order of each expressed globin gene being 5'—ε—Gγ—Aγ—δ—β—3' as shown in restriction map below (Li, Fig. 1).



23. The top line shows the overall genomic organization of this approximately 70-75 kb region. The bottom line represents an expanded restriction map for the upstream β-globin LCR showing the locations of the BamHI (B), EcoRI, (E) and HindIII (H) restriction sites. The map also indicates the three exons of the ε-globin gene (solid boxes). The 5'-most EcoRI site is the first nucleotide in the sequence provided by U01317 and NG_000007.1 (which is the version of NG_000007 that was publically available at the earliest filing date of the present application; the current version is NG_000007.3 and extends about 9 kb further upstream from that EcoRI site).

24. In 1985, Tuan reported the locations of HS2, HS3 and HS4 as being approximately -11 kb, -14.5 kb and -17.5 kb upstream of the ε-gobin gene coding sequence, respectively. [Tuan, p. 6387, right col., second full paragraph, lines 8, 11-12 and 18].
25. In 1995, Sadelain described the core sequences for these three DNase hypersensitive sites as follows:
    a. HS2 being on a 478-bp HindIII-SnaBI fragment including the known HindIII-XbaI fragment;
    b. HS3 being on a 260-bp fragment including the Hph1-Fnu4HI segment, which fragment is 225 bp and located on a 1.9-kb HindIII-HindIII fragment (as determined in refs. 27 and 28 cited by Sadelain); and
    c. HS4 being on a 283-bp SacI-AvaI fragment

    [Sadelain, p. 6730, left col., first full paragraph, lines 13-19]. Refs. 27 and 28 cited by Sadelain are Philipsen *et al.* (1990) *EMBO J.* 9:2159-2167 (Exhibit 7) and Philipsen *et al.* (1993) *EMBO J.* 12:1077-1085 (Exhibit 8), respectively.
26. With the foregoing information, and as described below, one can unambiguously identify the three claimed HS-spanning restriction fragments on the reference sequence of the human β-globin LCR.
27. The analysis begins with obtaining the nucleotide sequences for U01317 and NG_000007.1, which are identical with respect to restriction enzymes being analyzed.
28. For example, one can download the U01317 or NG_000007.1 sequence from the NCBI website and use any convenient software to analyze the restriction sites. Many such programs are available via the internet and most laboratories using recombinant DNA techniques have such software. For purposes of this analysis, the New England BioLabs program NEBcutter was used (available on the NEB website, www.neb.com). The program allows direct input of the GenBank accession number and determines restriction sites and fragment sizes for any restriction enzyme or combination of enzymes.
29. For simplicity, NG7 is used to refer to the reference sequence NG_000007.1, which was the sequence used in this analysis. The results would be the same with U01317 but shifted by about 9000 nucleotides for NG_000007.3 (since that deposited sequence has approximately 9000 more nucleotides upstream from nucleotide 1 of NG7.

30. Using NG7, one determines the location and number of restriction enzyme cut sites for BstXI and SnaBI (HS2), for BamHI and HindIII (HS3) and for BamHI and BanII (HS4). The resulting list of restriction sites and fragments sizes for the foregoing enzymes as well as the enzymes relevant to analyzing the core sequences (¶ 25) are provided in Exhibit 9 for HS2, Exhibit 10 for HS3 and Exhibit 11 for HS4.
31. To narrow the analysis to the relevant restriction sites, one finds the nucleotide start site for the ε-globin gene and only considers those restriction sites located upstream of that point.
32. The ε-globin gene start site is available in the literature, but is easily found in the commentary to GenBank nucleotide sequences. For NG7, the ε-globin transcript begins at nucleotide 19289 and its coding sequence at nucleotide 19541.
33. From this reference point and knowing that HS2 is located about 11 kb upstream from ε-globin gene, that HS3 is about 14.5 kb upstream and that HS4 is about 17.5 kb upstream, one determines the approximate location for each HS site. Hence, the general location of HS2 should be at or near nucleotide 8200, the general location of HS3 should be at or near nucleotide 4800 and the general location of HS4 should be at or near nucleotide 1800.
34. The location of the HS2-spanning fragment is determined first because there are only 3 SnaBI sites in the entire sequence and only one of them is upstream from the ε-globin gene, namely the site at nucleotide 8911.
35. From this information, the location of the HS2 core sequences must be on the HindIII-SnaBI fragment at nucleotides 8487-8911 and on the HindIII-XbaI fragment at nucleotides 8487-8911. Moreover, this means the SnaBI site forms the 3' end of the HS2-spanning fragment.
36. To find the BstXI, it must be (i) upstream from the SnaBI site based on the known location for the core sequences, and (ii) may not create a fragment that exceeds about 2.8 kb otherwise it would exceed the overall size limit to provide a 3.2-kb portion of the LCR. The size of 2.8 kb is selected by assuming that the HS3- and HS4-spanning fragments are at least as big as their corresponding core sequences, which total about 0.5 kb. One knows, in fact, that they must be larger.

37. Only the first BstXI site upstream of the uniquely-determined SnaBI site satisfies this criteria, namely the site at nucleotide 8055. Hence there is only one restriction fragment that spans HS2 and extends between BstXI and SnaBI restriction sites in the human ß-globin LCR.
38. **Thus, the HS2-spanning fragment in NG7 is located at nucleotides 8055-8911 and has a size of 857 bp (per the NEBCutter software).**
39. The location of the BamHI sites used for HS3 and HS4 are determined next. Building on the foregoing information, *i.e.*, that the BamHI sites must be located further upstream, only three sites can possibly be used, namely those at 308, 3714 and 3877. These sites are depicted above in the figure from Li (¶ 22). Knowing that the HS3 site should be around nucleotide 4800 and the HS4 site should be around nucleotide 1800, it means that the BamHI site at 308 forms the 5' end of HS4-spanning fragment and that one of the other two sites form the 5' end of the H3-spanning fragment.
40. Next, one locates the HS4-spanning fragment. As a point of reference, the HS4 core sequences are in the SacI-AvaI fragment at nucleotides 956-1234.
41. Because BanII cuts the LCR sequence many times, one realizes that the digestion must have been done under partial digestion conditions since a complete enzymatic digestion would not provide a single BamHI-BanII fragment that spanned the HS4 core. Thus, there are only two possible BanII sites that yield a BamHI-BanII fragment spanning the HS4 core, namely those sites at 1388 and 2203. Of these two choices, the latter creates an HS4-spanning fragment of 1891 bp, which when combined with the 857 bp HS2 fragment means that the remaining HS3-spanning fragment could be no greater than about .45 kb (from 3.2 less .86 and 1.89). This possibility seems unlikely and is so confirmed by determining the minimum size for the HS3-spanning fragment to be greater than 1 kb.
42. In this regard, the HS3 core is located on a 1.9 kb HindIII fragment at nucleotides 3267-5172. As expected, that fragment contains the 225 HphI-Fnu4HI fragment (nucleotides 4550-4773). Based on this data, the HindIII site at 5172 must form the 3' end of the HS3-spanning fragment because the next HindIII restriction site, at nucleotide 6159 going in the 3' direction, would create a fragment too large to provide an overall size of

3.2 kb. This is true regardless of which BamHI site is used (i.e., the size of the HS3 fragment using the HindIII site at 6159 is 2.2 kb for the closest BamHI site (3878) and 163 bases longer with the other BamHI site). Using the HindIII site at 5172 (and either BamHI site), means that the HS3-spanning fragment is either 1.30 or 1.46 kb.

43. Accordingly, since the HS3-spanning fragment is greater than 1 kb, the HS4-spanning fragment must be the smaller of the two possible BamHI-BanII fragments to meet the overall size requirement of 3.2 kb. That BanII site is located at nucleotide 1388.
44. **Thus, the HS4-spanning fragment in NG7 is located at nucleotides 308-1388 and has a size of 1080 bp (per the NEBCutter software).**
45. Finally, one determines which BamHI site forms the 5'end of the HS3-spanning fragment by summing the sizes of the three fragments and selecting the set that sums to 3.2 kb. As stated in ¶37, the 3' end of this fragment is the HindIII site at 5172 so that the HS3-spanning fragment can be nucleotides 3715-5172 (1.46 kb) or nucleotides 3878-5172 (1.30 kb). The sizes of the three combined HS-spanning fragments are

$$0.86 + \mathbf{1.30} + 1.08 = 3.24 \quad \text{OR} \quad 0.86 + \mathbf{1.46} + 1.08 = 3.40.$$

Since the smaller set satisfies the requirement that the overall size of the claimed LCR portion be 3.2 kb, the BamHI site must be that at nucleotide 3878.

46. **Thus, the HS3-spanning fragment in NG7 is located at nucleotides 3878-5172 and has a size of 1295 bp (per the NEBCutter software).**
47. In conclusion, the teaching of the specification and the language of the claim when taken in combination with well-known and publically available information, has been used to unambiguously identify a 3.2-kb portion of a human ß-globin LCR with an HS2-spanning nucleotide fragment extending between BstXI and SnaBI restriction sites of the LCR, an HS3-spanning nucleotide fragment extending between BamHI and HindIII restriction sites of the LCR and an HS4-spanning nucleotide fragment extending between BamHI and BanII restriction sites of the LCR.
48. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that the

undersigned acknowledges that any false statements and the like so made are punishable by fine or imprisonment under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patent that may issue from U.S. Application Serial No. 10/188,221.

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Publications:

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Genetics

Generation of a high-titer retroviral vector capable of expressing high levels of the human β-globin gene

(hemoglobinopathies/gene therapy/locus control region)

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ABSTRACT **Retrovirus-mediated gene transfer into hematopoietic cells may provide a means of treating both inherited and acquired diseases involving hematopoietic cells. Implementation of this approach for disorders resulting from mutations affecting the β-globin gene (e.g., β-thalassemia and sickle cell anemia), however, has been hampered by the inability to generate recombinant viruses able to efficiently and faithfully transmit the necessary sequences for appropriate gene expression. We have addressed this problem by carefully examining the interactions between retroviral and β-globin gene sequences which affect vector transmission, stability, and expression. First, we examined the transmission properties of a large number of different recombinant proviral genomes which vary both in the precise nature of vector, β-globin structural gene, and locus control region (LCR) core sequences incorporated and in the placement and orientation of those sequences. Through this analysis, we identified one specific vector, termed MβδL, which carries both the human β-globin gene and core elements HS2, HS3, and HS4 from the LCR and faithfully transmits recombinant proviral sequences to cells with titers greater than 10^6 per ml. Populations of murine erythroleukemia (MEL) cells transduced by this virus expressed levels of human β-globin transcript which, on a per gene copy basis, were 78% of the levels detected in an MEL-derived cell line, Hu11, which carries human chromosome 11, the site of the β-globin locus. Analysis of individual transduced MEL cell clones, however, indicated that, while expression was detected in every clone tested ($n = 17$), the levels of human β-globin treatment varied between 4% and 146% of the levels in Hu11. This clonal variation in expression levels suggests that small β-globin LCR sequences may not provide for as strict chromosomal position-independent expression of β-globin as previously suspected, at least in the context of retrovirus-mediated gene transfer.**

The successful treatment of β-globin disorders by gene therapy will likely require the efficient and stable introduction of a functional β-globin gene into self-renewing hematopoietic stem cells. While we and others had established that retroviral vectors encoding the human β-globin structural gene could direct the erythroid-specific synthesis of β-globin in mice engrafted with genetically modified bone marrow cells (1–4), these studies suffered from two important limitations. First, the retroviral titers generally obtained with those vectors made it difficult to generate long-term bone marrow chimeras engrafted with efficiently transduced cells. A second problem was the variable and generally low level of human β-globin expression *in vivo* observed in the reconstituted animals.

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The prospects for increasing the level of β-globin expression obtainable in retroviral vectors were dramatically improved by the recent finding that DNA sequences located considerable distances from the human β-globin structural gene on chromosome 11, termed locus control region (LCR) sequences (5, 6), play a critical role in the transcriptional control of genes within the β-globin-like gene cluster (reviewed in refs. 7–9). Specifically, transgenic experiments from a number of laboratories have indicated that the linkage of LCR sequences to the human β-globin structural gene dramatically increases the level of expression of β-globin observed in erythroid cells (10–13). Some studies further suggested that LCR sequences may differ from classical transcriptional enhancer sequences in that they provide for the chromosomal position-independent expression of linked genes (10–16). Smaller fragments, more suitable for insertion into vectors, have recently been defined, which have been reported to retain partial LCR activity (17–29). Recent efforts by several groups to incorporate LCR subfragments into β-globin retroviral vectors, however, have resulted in vector rearrangements (ref. 30 and our observations), poor titers (31), or very low expression of the β-globin gene (32).

We report here a systematic study of the β-globin gene, LCR core sites, and retroviral sequences that control vector transmission. This analysis has led to the generation of a high-titer, genomically stable retroviral vector bearing the human β-globin gene and the LCR core sites HS2, HS3, and HS4. This vector confers elevated and erythroid-specific expression of the β-globin gene. Expression in different clones of transduced murine erythroleukemia (MEL) cells is variable, however, raising questions about the ability of small LCR sequences to confer position-independent gene expression, at least in the context of a retroviral vector.

MATERIALS AND METHODS

Generation of Vectors and Packaging Cell Lines. The retroviral vectors pSG and pMFG are described elsewhere (33, 34). The β-globin gene was subcloned from pSVX-Neo (1). The β-globin coding sequence (βCS) consists of a 444-bp *Nco* I-linker-containing β-globin sequence from the translational start to the stop codon derived from the cDNA. The HS2, HS3, and HS4 fragments (see text and refs. 19–22 and 27–29) were, respectively, given *Hin*dIII, *Eco*RI, and *Mlu* I linkers and subcloned in all possible orientations in a *Sac* II–*Hin*dIII–

Abbreviations: LCR, locus control region; LTR, long terminal repeat; MEL, murine erythroleukemia; MLV, murine leukemia virus.

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*Eco*RI–*Mlu* I–*Sac* II polylinker. The vectors described herein, lacking any selectable marker, were introduced by calcium phosphate cotransfection with pSV2Neo (35) into the ψ-CRE packaging cell line (36). After 10 days of selection in G418 (Geneticin, Sigma) at 1 mg/ml, the G418-resistant colonies (>50 per transfection) were pooled by trypsinization and grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, penicillin, and streptomycin without G418.

Fibroblast Infection and Viral Transmission Assay. Five milliliters of supernatant harvested from pooled producer cells was applied onto 10^6 NIH 3T3 fibroblasts. Polybrene (Sigma) was added at 8 μg/ml. After 4 hr, the conditioned medium was removed. Three days later, genomic DNA was extracted from the confluent NIH 3T3 fibroblasts. For Southern analysis, 10-μg samples of genomic DNA from the producer and the corresponding 3T3 target cells were digested overnight with the restriction enzyme *Nhe* I [which cuts in both long terminal repeats (LTRs)] and run side by side on a 1% agarose gel. [^{32}P]dCTP-labeled β-globin probes were generated by using the human *Nco* I–*Eco*RI genomic template. Band intensity was quantified by using a phosphorimager (Fuji Bio-Imaging). Vector signal intensity was normalized to that of the endogenous band. Very even loading in Figs. 1–3 allows for direct reading of the gels as shown.

MEL Cell Infection and Screening. C88 MEL cells were grown in RPMI medium/10% fetal bovine serum/penicillin/streptomycin. Infection was performed by a 48-hr cocultivation of 10^5 MEL cells, treated for the previous 18 hr with tunicamycin (Sigma) at 0.2 μg/ml, on the ecotropic producer in the presence of Polybrene at 4 μg/ml. After cocultivation, MEL cells were subcloned onto 96-well plates at 0.2 cell per well. Single clones were then expanded and genomic DNA was prepared. Infected clones were screened by PCR using the primers GCAAGAAAGTGCTCGGTG in exon 2 and TCTGATAGGCAGCCTGCA in exon 3. The DNA of positive clones was further analyzed by Southern blotting for copy number and integration site.

MEL Cell Induction and Globin mRNA Quantification. After 4 days in 2% (vol/vol) dimethyl sulfoxide, RNA was extracted from each MEL cell clone by using the lithium chloride/urea procedure (37). This induction-extraction procedure was repeated at least once. RNA was quantified by RNase protection (38) using probes described in ref. 39 for human β-globin and a 180-nucleotide *Pst* I–*Bam*HI genomic fragment (40) for mouse α-globin. One to 2 μg of RNA per extract was incubated with both admixed probes, which, after RNase digestion, were electrophoresed on a 6% polyacrylamide/urea gel. Signal intensity for each band (the α-globin serving as internal control for both induction and loading onto the gel) was determined by using the phosphorimager.

RESULTS

Features of Retroviral Vector Design Which Affect Transmission of the Human β-Globin Structural Gene. To assess the titer and genomic stability of different retroviral vectors bearing the human β-globin gene, we first developed a direct assay to characterize and quantitate vector transmission. This assay is based on a quantitative Southern blot analysis of vector copy number in both transfected packaging cells and infected 3T3 fibroblasts. Supernatant is harvested from a pool of stable virus-producing cell clones and used to infect the target fibroblasts under defined conditions (see *Materials and Methods*). Southern blot analysis of vector DNA copy number in the polyclonal producer and target cells allows for a direct evaluation of average transmission for any retroviral construct. This is achieved by direct comparison of signal intensity in both genomic DNA extracts run side by side and therefore does not rely on indirect readouts dependent on gene expression (such as G418 resistance). This method provides not only for a direct measurement of gene transfer but also for the detection of rearrangements of any given construction. As shown in Fig. 1, transmission of a minimal 1.8-kb β-globin gene and promoter (−129 to +1650) cloned in reverse orientation in the pSG vector (33) was not detectable in this assay (lanes A). Transmission of the larger 2.8-kb *Sph* I–*Pst* I (−615 to +2163) globin fragment was also not detectable (data not shown). In contrast, the β-globin coding sequence alone transmitted very well, showing comparable signals in the packaging and target cell DNAs (lanes B). The high titer of the latter vector, however, was greatly reduced by inclusion of the core sites HS2, HS3, and HS4 of the LCR (see below), which were introduced in the vector upstream of the globin sequence in either orientation (lanes C and D).

To determine which β-globin genomic sequences were responsible for the dramatic reduction in titer, we constructed a set of recombinant vectors bearing systematic deletions of untranslated regions of the gene. As shown in Fig. 2, deletion of the 5′ untranslated region and intron 1, either alone or in combination, failed to increase viral titers (lanes A–D). Deletion of intron 2 (lanes E) increased transmission to levels similar to the coding sequence vector (lanes I), yet further combined deletions did not increase titer any more (lanes F–H). Because β-globin DNA templates lacking intron 2 direct poor β-globin expression (refs. 24 and 41–44 and data not shown), we examined transmission of two subintronic deletions (from +518 to +1288 and +679 to +953). Both yielded similar titers, which were still 10-fold down from the intronless gene (shown for the first deletion, lanes J, Fig. 2). Therefore, intron 2 alone accounts for reduced titers of β-globin gene, and deletions within intron 2, while increasing titer, still transmit less well than intronless constructions.

Recombinant Human β-Globin Genes Yield Higher Titers in the pMFG Vector. Since decreased viral transmission may result from a negative interaction between β-globin and retroviral sequences, which is only partially alleviated by intron 2 subdeletions, we compared transmission of β-globin sequences in different vectors. Modifications of the length of gag sequence, replacement of the Moloney murine leukemia virus (MLV) packaging signal by that of Ha-Ras MLV, and mutation of the splice donor site present in pSG failed to increase vector transmission (data not shown). Subcloning recombinant β-globin fragments into the pMFG vector (34), however, significantly increased transmission (Fig. 3). Lanes A and D show transmission of the full β-globin fragment; lanes B and E, the genomic sequence bearing the intron 2 deletion shown

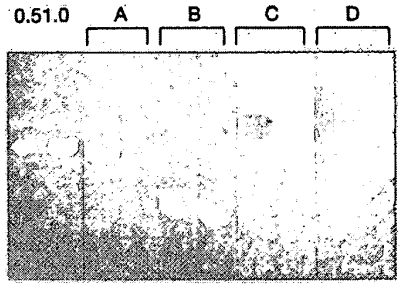

FIG. 1. Comparison of vector transmission by Southern blot analysis of vector copy number in the packaging cell (left lane) and target cell (right lane) genomic DNA. In lanes A, 1.8-kb β-globin gene and promoter; in lanes B, βCS; in lanes C and D, βCS and the three core elements HS2, HS3, and HS4 in sense and antisense orientation, respectively. See text for sequence description. Genomic DNA was digested with *Nhe* I and blots were probed with a β-globin probe. Control lanes show signal intensity for one proviral copy per cell (1.0) and one copy per two cells (0.5). Even loading (data not shown) allows for directly comparing signal intensity between lanes.

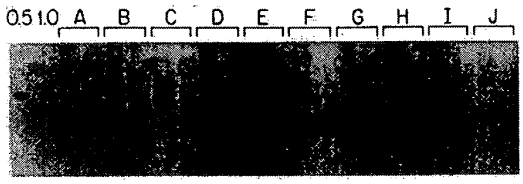

FIG. 2. Effect of β-globin gene untranslated sequences on vector transmission. Southern blot analysis of packaging cell (ψ-CRE, left lane) and target cell (NIH 3T3, right lane) genomic DNA. Lanes: A, 1.8-kb β-globin gene and promoter; B, 5′ untranslated region deletion; C, intron 1 deletion; D, B and C combined deletions; E, intron 2 deletion; F, B and E combined deletions; G, C and E combined deletions; H, B, C, and E combined deletions; I, β-globin coding sequence only; J, 770-bp intron 2 deletion. See Fig. 1 legend and text for sequences and methods.

in lanes J of Fig. 2; and lanes C and F, the globin coding sequence, in the vectors pSG and pMFG, respectively. Comparison between lanes B and E shows a gain in titer of about 5-fold when the pMFG vector is used, showing that altering vector sequences can affect transmission.

The Core Sites of HS2, HS3, and HS4 Can Be Incorporated into a High-Titer Genomically Stable Retroviral Vector. Previous studies of LCR sequences had indicated that a "minilocus" approximately 20 kb in size, comprising sites HS1, HS2, HS3, and HS4, possessed close to complete LCR activity (10, 11, 14). However, because of the inability to transmit sequences of such size in retrovirus vectors, we chose to attempt to incorporate "core elements" of HS2, HS3, and HS4, which are markedly reduced in size and have been shown to retain at least partial LCR activity, into vectors carrying the β-globin structural gene. The following sequences were employed for our studies, based on studies in transgenic animals (17–22, 27–29): (*i*) the 283-bp *Sac* I–*Ava* I core HS4 fragment (29); (*ii*) the 260-bp core HS3, including the *Hph* I–*Fnu*4HI segment (27, 28) and the upstream NF-E2 site, which encompasses all sites footprinted *in vivo* (45, 46); and (*iii*) a 478-bp *Hin*dIII–*Sna*BI HS2 fragment encompassing all sites footprinted *in vivo* (45, 47) in the core *Hin*dIII–*Xba* I fragment (19–22) and most of the adjacent alternating purine-pyrimidine stretch (21, 48). As shown previously in Fig. 1, one specific orientation of the sites greatly reduced proviral transmission. Accordingly, the three sites were subcloned in such a way that all eight permutations of relative arrangement were generated. The resulting cassettes were introduced, in sense and antisense orientations upstream of the β-globin sequence in the high-titer β-globin vector shown in lanes B of Fig. 1. As shown in Fig. 4, transmission of these vectors shows significant variations with regard to copy number and genomic stability (e.g., the two species transmitted in lanes H). Among these 16 constructs, we identified four combinations which were transmitted at high titer without rearrangements (lanes A, E, L, and N). All bear HS2 in the antisense orientation relative to retroviral transcription. The reason for the lack of the endogenous band in lanes E is unclear.

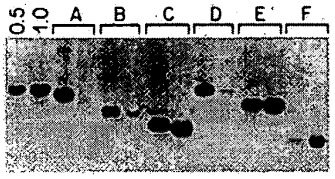

FIG. 3. Comparison of transmission of β-globin sequences in two different vectors. Lanes A–C, pSG vector; lanes D–F, pMFG vector. Lanes A and D, β-globin gene (as in Fig. 1, lanes A); lanes B and E, intron 2 deleted minigene (as in Fig. 2, lanes J); lanes C and F, βCS (as in Fig. 1, lanes B). See Figs. 1 and 2 for nomenclature and methods. Signal intensity between lanes can be directly compared owing to very even loading (data not shown) except for lanes F, which were loaded with 5 μg.

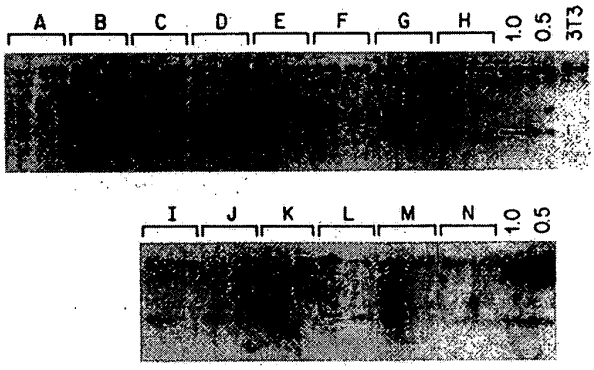

FIG. 4. Effect of HS orientation on vector transmission. Southern blot analysis of packaging cell (ψ-CRE, left lane) and target cell (NIH 3T3, right lane) genomic DNA (see previous figure legends for methods). The top band corresponds to the endogenous globin genes. HS orientation (S, sense; s, antisense) in the vector is (in the order HS4-HS3-HS2): A, Sss; B, reversed A; C, sss; D, reversed C; E, SSs; F, reversed E; G, sSs; H, reversed G; I, SsS; J, reversed I; K, ssS; L, reversed K; M, sSS; and N, reversed M. Lane 3T3, NIH 3T3.

For further studies, we selected the combination with the highest titer and in which HS2 and HS3 were oriented in the same direction as the β-globin gene and adjacent to its promoter. The resulting vector, Mβ6L, is shown in Fig. 5. In summary, this construct contains a 2150-bp *Sna*BI–*Pst*I β-globin fragment including a full intron 1 and a 476-bp intron 2 and lacking the 3′ enhancer [on the basis of other studies that suggest it is redundant in the presence of the LCR (24)]. The 1-kb LCR fragment comprises the HS2, HS3, and HS4 core sequences described above (Fig. 5*A*). Southern blot analysis of NIH 3T3 cells transduced by Mβ6L indicates that approximately one proviral copy is transferred per cell (Fig. 5*B*). On the basis of previous comparisons of proviral copy number and titers measured by selectable marker expression (unpublished results), the titer of Mβ6L is approximately 1×10^6 per ml.

Mβ6L Directs Elevated β-Globin Expression, Detectable at All Integration Sites in MEL Cells. To test the biological activity of Mβ6L, we chose MEL cells as target cells for gene transfer, as those cells have been extensively employed in studies of β-globin transcription (10, 14–16, 23–26). A panel of MEL cell clones infected with Mβ6L was generated by cocultivation of C88 MEL cells and virus-producing cells and subsequent subcloning by limiting dilution (see *Materials and Methods*). Southern blot analyses confirmed that intact vector copies were integrated at different chromosomal positions for each clone (data not shown). Expression of human β-globin was quantitated by RNase protection using total RNA extracted from dimethyl sulfoxide-induced clones. Levels of human β-globin mRNA transcripts were measured by using the phosphorimager and normalized to endogenous α- or β-major globin transcripts. This analysis is summarized in Table 1, where values are normalized to human β-globin expression in Hu11, a MEL cell containing human chromosome 11 under selective pressure (49), based on the comparison of their respective human β to murine α mRNA ratios (calculations based on human β/murine β-major yielded very similar results). In a pool of 10^5 unselected MEL cells infected with the parent vector lacking the LCR, Mβ6, the mean level of expression was 2.2% of that measured in Hu11. Mean expression was 78% in MEL cell pools infected with Mβ6L. Human β-globin transcripts were detected in NIH 3T3 fibroblasts but at levels about 1/100 of the level measured from the equal amount of RNA from induced MEL cells (Table 1). This

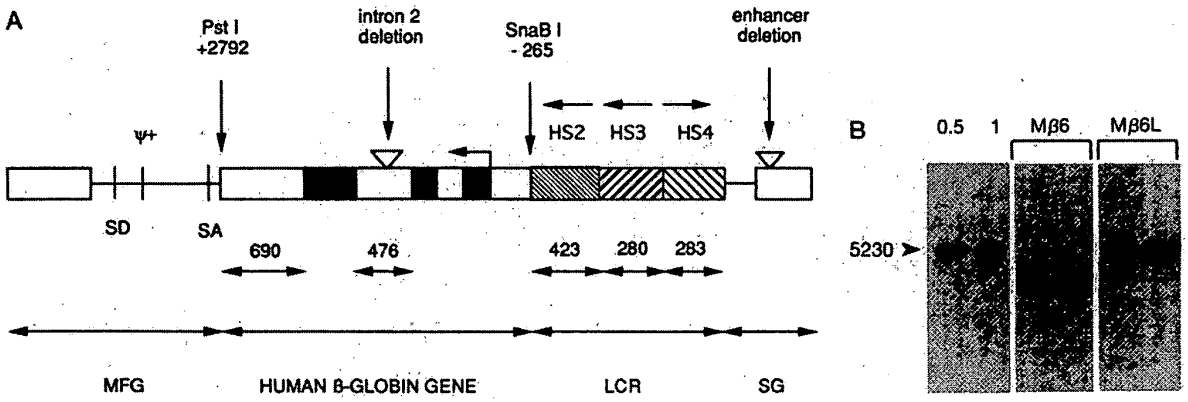

FIG. 5. (*A*) Schematic representation of the Mβ6L vector. Black boxes represent the β-globin exons. Arrows above the HS boxes indicate their orientations. Numbers below delineated segments represent fragment length in bp. ψ+, packaging signal. White boxes at ends represent LTRs. See text for exact sequences. SD, splice donor; SA, splice acceptor; MFG, 5′ sequences derived from the MFG vector; SG, 3′ sequences derived from the SG vector. (*B*) Transmission characteristics of vectors Mβ6L and Mβ6, the parent β-globin gene vector lacking the LCR fragment. Southern blot analysis is carried out as in the previous figures. Length on left is in bp.

finding is consistent with other studies of β-globin expression in transfected fibroblasts (14, 15, 23, 24). In subcloned MEL cells, the expression values ranged from 4% to 146% (ML1–ML7, Table 1). The level measured for each clone was very stable upon repeated measurement as well as upon reinduction. Importantly, in this set of clones and others with slightly different β-globin gene boundaries (MS and RCM, unpublished observations), which represent a total of 17 clones, human β-globin expression, albeit variable in amount, was readily detected in all cases. This is in contrast to clones infected with the vector lacking LCR sequences (Mβ6), in which human β-globin expression was undetectable in 3 of 4 clones (M1–M4; Table 1).

Table 1. Human β-globin expression in dimethyl sulfoxide-induced MEL cells and in NIH 3T3 fibroblasts

Cell	LCR	Huβ gene copy number	Huβ RNA/Mα RNA per vector copy, %
MEL	−	0	0
Hu11	+	1	100
MEL/Mβ6	−	0.5	2.2
MEL/Mβ6L	+	1.6	78
3T3/Mβ6	−	1.9	0.2
3T3/Mβ6L	+	1.8	0.6
M1	−	1	2
M2	−	1	<0.1
M3	−	1	<0.1
M4	−	1	<0.1
ML1	+	1	61
ML2	+	1	101
ML3	+	1	146
ML4	+	1	4
ML5	+	3	28
ML6	+	4	35
ML7	+	5	74

Protected RNA transcripts were fractionated on a 6% polyacrylamide/urea gel and their radioactivities were measured by using the phosphorimager. Results are expressed as human β-globin mRNA/endogenous murine α-globin mRNA divided by vector copy number, normalized to the values measured in the Hu11 clone (see text). Values in NIH 3T3 cells are expressed as human β-globin RNA divided by vector copy number in 1 μg of total RNA normalized to the human β-globin signal in 1 μg of total RNA from dimethyl sulfoxide-induced Hu11 cells. In the first six lines, data from a large polyclonal cell population; in the rest of the table, data from individual MEL cell clones.

DISCUSSION

In this study, we report the resolution of two major obstacles to the implementation of retrovirus-based genetic treatment of β-globin disorders: first, the generation of a high-titer retroviral vector suitable for obtaining the efficient transduction of hematopoietic stem cells and, second, the generation of vectors capable of expressing high levels of the β-globin gene in an erythroid-specific fashion. Our systematic study of vector transmission indicated problems associated with incorporation of both the β-globin structural gene itself and the LCR core sequences. In the case of the β-globin structural gene, our results parallel those of Miller *et al.* (43), who had observed that the poor transmission of retroviral vectors carrying the β-globin structural gene was caused by both untranslated sequences in the 5′ and 3′ regions of the *Hpa* I–*Xba* I β-globin fragment and sequences within intron 2. Our results differ slightly from those of that study in that we found that sequences within intron 2 alone primarily account for the poor transmission of β-globin vectors (Fig. 2). We found that features of retroviral vector design also affected proviral transmission of β-globin sequences. For example, modifications of the vector sequences juxtaposed to the 3′ end of the β-globin gene indicated that including the Moloney MLV splice acceptor region from pMFG (34) resulted in a 5-fold increase in titer. Deletion of the U3 region of the 3′ LTR (33) did not decrease titer and, in fact, led to a moderate (2-fold) increase (data not shown).

We also confirmed the studies of Novak *et al.* (30) that suggested that LCR sequences can lead to significant vector instability (data not shown). In our studies, even the core fragments incorporated together led to decreased transmission and vector instability in particular sequence arrangements (Figs. 1 and 4). To overcome this problem, we examined the transmission of all 16 permutations of the core sites, and we identified combinations that result in stable transmission and the highest titers (Fig. 4, lanes A, E, L, and N).

The LCR was first functionally defined *in vivo* in transgenic mice by using a 20-kb LCR fragment (10, 11), a 6.5-kb fragment (15), or single HS sites (17–22, 27–29). Studies in animals suggested that the LCR confers position-independent expression, based on the calculation that β-globin expression per transgene copy is relatively constant (7). However, it should be noted that transgenic studies involving either complete LCR or core sequences generally relied on the analysis of cells possessing more than one copy per cell. In contrast, our use of retrovirus-mediated gene transfer made it possible to examine the issue of position independence under conditions

where one copy of the transcription unit per cell is integrated in a precise reproducible way and in the absence of any selection (50). In a panel of MEL cell clones representing independent integration sites, we found that expression was detectable in all clones, yet ranged from 4% to 146% (Table 1) of human β-globin expression in the Hu11 cell line in single-copy clones (the variability decreases, as expected, in multicopy clones). Our data therefore suggest that the LCR, in this configuration, acts more like a classical enhancer than the proposed function of an LCR (reviewed in refs. 7–10 and 51). The absence of strictly position-independent expression (10, 14, 15) we observe raises the issues of whether the LCR can truly confer position-independent expression when present at one copy per cell, whether the compact arrangement of the transcription and chromatin regulators within our vector leads to suboptimal interactions and inefficient LCR activity, or whether it is specifically the combination of core elements which we have employed which lack this ability (see refs. 17–22 for HS2, 27 and 28 for HS3, and 29 for HS4). Whatever the explanation, our data strongly suggest that it is important to carefully reevaluate the characteristics of larger LCR-containing sequences in the context of single-copy insertions.

For the purposes of gene therapy the most critical test of the LCR sequences will be the introduction of the virus genomes described above into hematopoietic cells followed by their transplantation. The inability to obtain chromosome position-independent expression of the human β-globin gene in the context of single-copy insertions would make the prospects for effective genetic treatment of hemoglobinopathies less likely than previously believed.

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A single erythroid-specific DNase I super-hypersensitive site activates high levels of human β-globin gene expression in transgenic mice

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Erythroid-specific DNase I super-hypersensitive (HS) sites that are normally located far upstream of the human β-globin locus were inserted immediately upstream of a 4.1-kb fragment containing the human β-globin gene. These constructs (HS β) and a construct containing the β-globin gene alone (β) were microinjected into fertilized mouse eggs, and expression was analyzed in erythroid fetal liver and brain of day-16 embryos that developed. Only 7 of 23 animals that contained the β gene alone expressed human β-globin mRNA in erythroid tissue, and the average level of expression per gene copy was 0.3% of endogenous mouse β-globin mRNA. In contrast, 50 of 51 transgenic mice that contained various HS β constructs expressed the transgene specifically in erythroid tissue. The average level of expression per gene copy for constructs containing all five upstream HS sites was 109% of endogenous mouse β-globin mRNA. Constructs that contained a single super-hypersensitive site (HS II β) expressed 40% as much human β-globin as mouse β-globin mRNA per gene copy. These results demonstrate that the HS VI site, normally located downstream of the human β-globin locus, is not required for high-level expression. Furthermore, the results demonstrate that high levels of human β-globin gene expression can be obtained in transgenic mice even when a relatively small fragment of DNA (1.9 kb) containing erythroid-specific super-hypersensitive site II (HS II) is inserted upstream of the human β-globin gene.

[*Key Words*: DNase I super-hypersensitive sites; locus activation sequences; tissue specificity]

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The human β-like globin genes are regulated precisely in three important ways: They are expressed only in erythroid tissue, only during defined stages of development, and at high levels. To determine the sequences responsible for these three levels of control, we and others have microinjected human globin gene constructs into fertilized mouse eggs and analyzed their expression in animals that developed (Costantini et al. 1985; Townes et al. 1985a,b; Kollias et al. 1986). These studies have demonstrated that sequences upstream, within, and downstream of the human β-globin gene are involved in adult, erythroid-specific expression (Behringer et al. 1987; Kollias et al. 1987; Trudel and Costantini 1987).

Although transgenic mice that express human β-globin mRNA at levels equivalent to mouse β-globin mRNA have been produced with relatively small human β-globin gene constructs, many of the animals express the transgene at low levels and others do not express the gene at all. Also, the highest expressors generally have the highest number of transgenes per cell. Recently, Grosveld et al. (1987) demonstrated that high levels of human β-globin gene expression can be obtained in animals with a single copy of the transgene if sequences at the extreme ends of the human β-globin locus are included in the injected constructs. Several years ago, Tuan et al. (1985) and Forrester et al. (1986) mapped sites that were super-hypersensitive to DNase I digestion in these regions. These DNase I super-hypersensitive (HS) sites are located 6–22 kb upstream of the ε-globin gene and 19 kb downstream of the β-globin gene. The sites are present specifically in erythroid tissue at all stages of development. Figure 1 depicts the locations of these sites in the human β-globin locus.

The structure of mutant loci from patients with several hemoglobinopathies suggests that the upstream HS sites are required for efficient β-globin gene expression in humans (Bunn and Forget 1986; Stamatoyannopoulos et al. 1987). English and Dutch γδβ-thalassemias result from deletions that remove all of the upstream HS sites (Fig. 1). Although the β-globin gene is intact in these patients, no β-globin mRNA is produced from the mutant alleles. On the other hand, several other dele-

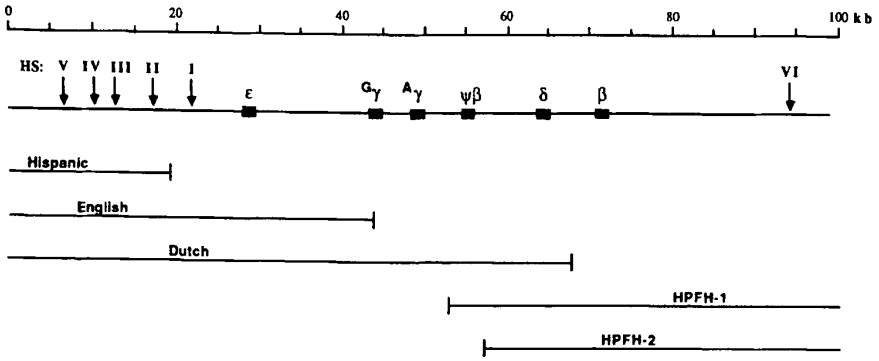

Figure 1. Human β-globin locus. A 100-kb region of human chromosome 16 containing the β-like globin genes is illustrated. Erythroid-specific HS sites located 6–22 kb upstream of ε and 19 kb downstream of β are marked by arrows (Tuan et al. 1985; Forrester et al. 1986). The lines beneath the locus represent deletions involved in Hispanic (C. Driscoll, pers. comm.), English, and Dutch γδβ-thalassemias and two deletion forms of HPFH (Bunn and Forget 1986; Stamatoyannopoulos et al. 1987). The Hispanic patient described by C. Driscoll et al. (pers. comm.) has a β^S allele on the affected chromosome but does not make any sickle hemoglobin.

tions suggest that the downstream site may not be essential. High levels of human γ-globin gene expression are observed in patients with two deletion forms of hereditary persistence of fetal hemoglobin (HPFH-1 and HPFH-2). Both of these mutant alleles lack the downstream super-hypersensitive site (HS VI; see Fig. 1). To determine whether the downstream site is required for high level expression of the human β-globin gene, we have made constructs containing all of the upstream sites but lacking the downstream site. These constructs were microinjected into fertilized mouse eggs, and the levels of human β-globin mRNA were analyzed in mice that developed. Constructs containing only HS I and HS II or HS II alone were also tested to determine the minimum number of HS sites required for high level expression of the human β-globin gene in transgenic mice.

Results

Production of HS β-globin transgenic mice

Figure 2 illustrates the seven constructs that were puri-

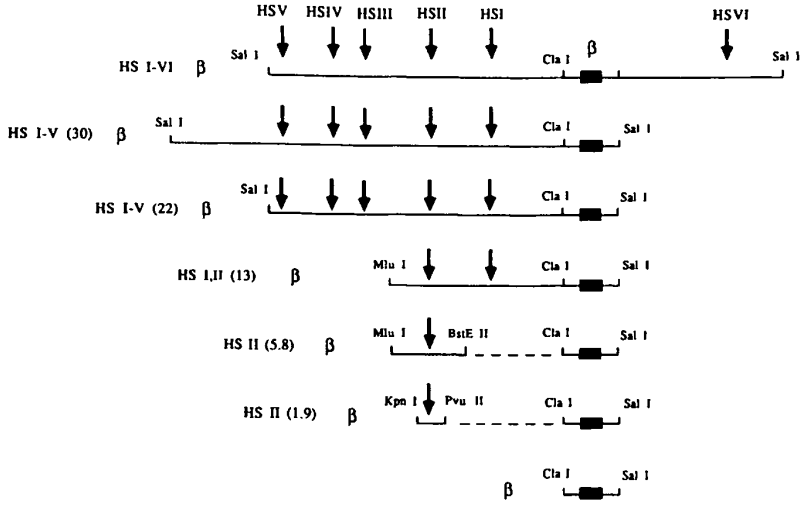

Figure 2. HS β and β constructs injected into fertilized mouse eggs. HS I–VI β, HS I–V (30) β, and HS I–V (22) β were constructed from λ clone fragments containing the HS sequences (Li et al. 1985; D. Flenor and R. Kaufman, unpubl.). The numbers in parentheses represent the sizes of the upstream fragments in kilobase pairs. These fragments and a fragment containing the human β-globin gene were inserted into the cosmid vector pCV001, as described in Methods. HS I,II (13) β and HS I (7.0) β were derived from the HS I–V (22) β cosmid clone. HS II (5.8) β and HS II (1.9) β were cloned as plasmids, as described in Methods. The β-globin gene in all of these constructs is on a 4.1-kb *HpaI–XbaI* fragment containing 815 bp of 5′-flanking sequence and 1700 bp of 3′-flanking sequence. The *HpaI* site was changed to *ClaI*, and the *XbaI* site was changed to *SalI* in all constructs except HS I–VI β, where the *XbaI* site was changed to *XhoI*. In all cases, the fragments were cut out of cosmid or plasmid clones, purified from vector sequences on low gelling temperature agarose gels, and microinjected into fertilized mouse eggs as described by Brinster et al. (1985).

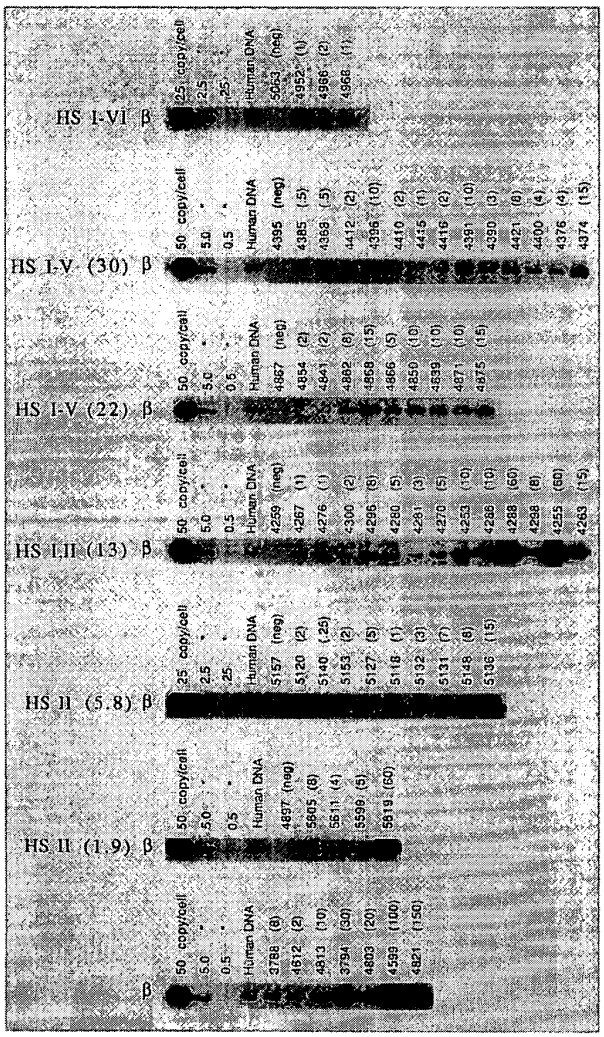

Figure 3. Southern blot analysis of HS β and β transgenic mice. Ten micrograms of fetal liver DNA or control DNA was digested with *Bam*HI and *Pst*I and separated on 1.0% agarose gels. After blotting onto nitrocellulose, the samples were hybridized with a human β-globin-specific probe derived from the second intron. (Lanes *1*–3) Herring sperm DNA spiked with the equivalent of 50, 5.0, and 0.5 or 25, 2.5, and 0.25 copies per cell of the respective construct; (lane *4*) human DNA; (lane *5*) a nontransgenic mouse control. The single 1.7-kb band observed in all of the samples, except the negative controls, represents the fragment generated from digestion of the *Bam*HI site in the second exon of the human β-globin gene and the *Pst*I site located 559 bp downstream of the poly(A) site. The intensity of this band was compared to the standards in lanes *1*–*4* to determine transgene copy number. The number of copies per cell of the transgene is listed in parenthesis after each sample number.

fied from vector sequences and injected into fertilized mouse eggs. These eggs were transferred into the uteri of psuedopregnant foster mothers, and the embryos were removed after 16 days of development. Total nucleic acids were prepared from the erythroid fetal livers and from brains, and transgenic mice were identified by DNA dot hybridization with β-globin and HS II-specific probes. Fetal liver DNA from positive animals was then analyzed by Southern blotting to determine transgene copy number and integrity. Figure 3 illustrates the Southern blots used to determine transgene copy number. Lanes 1–3 of each blot are herring sperm DNA spiked with the equivalent of 50, 5.0, and 0.5 or 25, 2.5, and 0.25 copies per cell of the respective construct; lane 4 is human DNA; and lane 5 is a nontransgenic mouse control. The controls and fetal liver DNA from each sample were digested with *Bam*HI and *Pst*I, and blots were hybridized with a human β-globin IVS 2 probe. A single 1.7-kb band was observed in all of the samples except the negative controls. The intensity of this band was compared to the standards in lanes 1–4 to determine transgene copy number. The number of copies per cell of the transgene is listed in parenthesis after each sample number. These values ranged from 0.25 to 150 copies per cell. Mice that contained less than one copy per cell are probably mosaics that integrated the transgene at the two- or four-cell stage. All of the samples were cut with several other enzymes and Southern blots were probed with various HS site and β-globin probes to determine transgene integrity (data not shown). All of the animals contained intact constructs except for samples 5140 and 5153 of HS II (5.8) β. Although the human β-globin gene was intact, the HS II site in both of these samples was rearranged.

Expression of human β-globin mRNA in HS β-transgenic mice

Human and mouse β-globin mRNA levels were determined for each fetal liver and brain sample by solution hybridization with oligonucleotide probes as described previously (Townes et al. 1985b). In addition, fetal liver RNA was analyzed for correctly initiated human β-globin and mouse α- and β-globin mRNAs by primer extension. Mice switch directly from embryonic to adult hemoglobin synthesis when fetal liver becomes the major site of erythropoiesis at 13–17 days of development. Therefore, 16-day fetal liver is considered an adult erythroid tissue. Figure 4 illustrates the primer extension gel of fetal liver RNA from HS I–VI β transgenic mice. The HS I–VI construct contains all five upstream and one downstream DNase I HS sites flanking the human β-globin gene (Fig. 2). Lane 1 is human reticulocyte RNA, and lane 2 is fetal liver RNA from a nontransgenic mouse control. The authentic human β-globin primer extension product is 98 bp, and the correct