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# SCORE Placeholder Sheet for IFW Content

Application Number: 12433412 Document Date: 04/30/2009

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Form Revision Date: February 8, 2006

DocCode – SCORE

# SCORE Placeholder Sheet for IFW Content

Application Number: 12433412 Document Date: 04/30/2009

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- Design Drawing

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- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
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Form Revision Date: February 8, 2006

PTO/SB/05 (08-08)
Approved for use through 06/30/2010. 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.* | 64836DIV(51590) |
| *First Inventor* | Michel Sadelain |
| *Title* | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| *Express Mail Label No.* | |

## APPLICATION ELEMENTS

*See MPEP chapter 600 concerning utility patent application contents.*

*ADDRESS TO:* **Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450**

1. [X] **Fee Transmittal Form** (e.g., PTO/SB/17)
2. [X] **Applicant claims small entity status.** See 37 CFR 1.27.
3. [X] **Specification** [*Total Pages* 25 ]
   Both the claims and abstract must start on a new page
   *(For information on the preferred arrangement, see MPEP 608.01(a))*
4. [X] **Drawing(s)** *(35 U.S.C. 113)* [*Total Sheets* 4 ]
5. **Oath or Declaration** [*Total Sheets* ]
   a. [ ] Newly executed (original or copy)
   b. [X] A 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) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. [X] **Application Data Sheet**. See 37 CFR 1.76
7. [ ] **CD-ROM or CD-R** in duplicate, large table or Computer Program *(Appendix)*
   [ ] Landscape Table on CD
8. **Nucleotide and/or Amino Acid Sequence Submission** *(if applicable, items a. – c. are required)*
   a. [X] 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))
   Name of Assignee
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** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
13. [X] **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 equivalent.
17. [ ] Other:

18. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:*

[ ] Continuation [X] Divisional [ ] Continuation-in-part (CIP) of prior application No.: 10/188,221

*Prior application information: Examiner* Maria Marvich *Art Unit:* 1633

### 19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 65488 *OR* [ ] Correspondence address below

| | | | | | |
|---|---|---|---|---|---|
| Name | | | | | |
| Address | | | | | |
| City | | State | | Zip Code | |
| Country | | Telephone | | Email | |

| | | | |
|---|---|---|---|
| Signature | /Peter C. Lauro/ | Date | April 30, 2009 |
| Name (Print/Type) | Peter C. Lauro, Esq. | Registration No. (Attorney/Agent) | 32,360 |

BOS2 734986.1

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 64836DIV(51590) |
|---|---|---|
| | Application Number | |

| Title of Invention | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
|---|---|

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

## Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

## Applicant Information:

**Applicant 1** [Remove]

| **Applicant Authority** | ◉ Inventor | ○ Legal Representative under 35 U.S.C. 117 | ○ Party of Interest under 35 U.S.C. 118 | |
|---|---|---|---|---|
| **Prefix** | **Given Name** | **Middle Name** | **Family Name** | **Suffix** |
| Mr. | Michel | | Sadelain | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| **City** | New York | **State/Province** | NY | **Country of Residence** i | US |
|---|---|---|---|---|---|
| **Citizenship under 37 CFR 1.41(b)** i | US | | | | |

**Mailing Address of Applicant:**

| **Address 1** | 401 East 89th Street | | |
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**Applicant 2** [Remove]

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| Mr. | Stefano | | Rivella | |

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**Applicant 3** [Remove]

| **Applicant Authority** | ◉ Inventor | ○ Legal Representative under 35 U.S.C. 117 | ○ Party of Interest under 35 U.S.C. 118 | |
|---|---|---|---|---|
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| Mr. | Chad | | May | |

**Residence Information (Select One)** ○ US Residency ◉ Non US Residency ○ Active US Military Service

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 64836DIV(51590) |
|---|---|---|
| | Application Number | |
| Title of Invention | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | |

| | | | |
|---|---|---|---|
| **Citizenship under 37 CFR 1.41(b)** i | US | | |
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| **Address 1** | 220 Avenue A | | |
| **Address 2** | Apt. 3C | | |
| **City** | New York | **State/Province** | NY |
| **Postal Code** | 10009 | **Country**i | US |

**Applicant 4** [Remove]

| | | | | |
|---|---|---|---|---|
| **Applicant Authority** | (●) Inventor | ( ) Legal Representative under 35 U.S.C. 117 | ( ) Party of Interest under 35 U.S.C. 118 | |
| **Prefix** | **Given Name** | **Middle Name** | **Family Name** | **Suffix** |
| Mr. | Joseph | | Bertino | |

| | | | |
|---|---|---|---|
| **Residence Information (Select One)** | ( ) US Residency | (●) Non US Residency | ( ) Active US Military Service |
| **City** | Branford | **Country Of Residence**i | US |
| **Citizenship under 37 CFR 1.41(b)** i | US | | |
| **Mailing Address of Applicant:** | | | |
| **Address 1** | 1117 Sunset Hill Dr. | | |
| **Address 2** | | | |
| **City** | Branford | **State/Province** | CT |
| **Postal Code** | 06405 | **Country**i | US |

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. [Add]

## Correspondence Information:

**Enter either Customer Number or complete the Correspondence Information section below.**
**For further information see 37 CFR 1.33(a).**

☐ **An Address is being provided for the correspondence Information of this application.**

| | | |
|---|---|---|
| **Customer Number** | 65488 | |
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## Application Information:

| | | | |
|---|---|---|---|
| **Title of the Invention** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | | |
| **Attorney Docket Number** | 64836DIV(51590) | **Small Entity Status Claimed** | ☒ |
| **Application Type** | Nonprovisional | | |
| **Subject Matter** | Utility | | |
| **Suggested Class (if any)** | | **Sub Class (if any)** | |
| **Suggested Technology Center (if any)** | 1633 | | |
| **Total Number of Drawing Sheets (if any)** | 4 | **Suggested Figure for Publication (if any)** | |

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 64836DIV(51590) |
|---|---|---|
| | Application Number | |

| Title of Invention | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
|---|---|

## Publication Information:

| | |
|---|---|
| ☐ | Request Early Publication (Fee required at time of Request 37 CFR 1.219) |
| ☐ | **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S. C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing. |

## Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

| Please Select One: | ◉ Customer Number | ○ US Patent Practitioner | ○ Limited Recognition (37 CFR 11.9) |
|---|---|---|---|
| Customer Number | 65488 | | |

## Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

| Prior Application Status | Pending | | Remove |
|---|---|---|---|
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| | Division of | 10188221 | 2002-07-01 |
| Prior Application Status | Pending | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| 10188221 | non provisional of | 60301861 | 2001-06-29 |
| Prior Application Status | Pending | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing Date (YYYY-MM-DD) |
| 10188221 | non provisional of | 60302852 | 2001-07-02 |

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

## Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

| | | | Remove |
|---|---|---|---|
| Application Number | Country i | Parent Filing Date (YYYY-MM-DD) | Priority Claimed |
| | | | ○ Yes ○ No |

| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 64836DIV(51590) |
|---|---|---|
| | Application Number | |

| Title of Invention | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
|---|---|

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## Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

**Assignee 1** Remove

If the Assignee is an Organization check here. ☒

| Organization Name | Memorial Sloan-Kettering Cancer Center | | |
|---|---|---|---|
| **Mailing Address Information:** | | | |
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| Address 2 | | | |
| **City** | New York City | **State/Province** | NY |
| **Country** i | US | Postal Code | 10021 |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Assignee Data may be generated within this form by selecting the **Add** button. Add

## Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

| **Signature** | /Peter C. Lauro/ | | | Date (YYYY-MM-DD) | 2009-04-30 |
|---|---|---|---|---|---|
| First Name | Peter | Last Name | Lauro | Registration Number | 32360 |

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

# 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 ($\beta^A$) 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 $\beta^A$ 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 $\beta^{maj}$, $\beta^{min}$, $\beta^{s}$ and $\beta^{t}$. 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 ($1.8 \pm 0.6$ and $0.8 \pm 0.6$ 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 $0.5 \pm 0.1\%$ (significantly lower than on day 12, $P = 0.02$) and $15.8 \pm 0.9\%$ 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 $\beta^A$ 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 $\beta^A$. No haemoglobin tetramers containing human $\beta^A$ were measurble in any of the mice bearing RNS1 (table 1 in Supplementary Information). The proportion of mature peripheral blood red cells expressing human $\beta^A$ was elevate in most TNS9 bone marrow chimaeras, as shown by dual staining of human $\beta^A$ and TER-119. In contrast, chimaeras engrafted with RNS1-transduced bone marrow showed highly variable fractions of weakly staining $\beta^A$ -positive erythrocytes. Normalized to the fraction of circulating $\beta^A$ -positive mature red cells, the levels of haemoglobin containing lentivirus-encoded $\beta^A$ 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 $\beta^0$ -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 $\beta^A$ 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 $\beta^A$-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 $\alpha_2$: 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 $\beta^A$ chains are expected to have an advantage over the $\beta^S$ 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 femurs 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 ($5 \times 10^5$) 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 $\beta^A$ ($Hbb^{hu}$, mu $\alpha_2$: hu$\beta^A{}_2$) or murine β-globin ($Hbb^{mu}$, mu$\alpha_2$:mu$\beta_2$), and immunofluorescence, to determine the fraction of mature RBCs that contain human $\beta^A$ 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 $\beta^A$ and 100% $\beta^A$+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 $\beta^A$ also remained elevated and stable (about 70% to 80%), as shown by dual staining of human $\beta^A$ and TER-119.

## Example 8

### Long-Term amelioration of anemia

The stability of TNS9-encoded $\beta^A$ 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.

**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:<br>021121<br>PATENT TRADEMARK OFFICE | DIRECT TELEPHONE CALLS TO:<br>OPPEDAHL & LARSON LLP<br>(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 Aug 23, 2002 | | SIGNATURE [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 ___.

SUBSCRIBED AND SWORN TO BEFORE ME
THIS 23 DAY OF August 20 02
[signature]
NOTARY PUBLIC
For Michel Sadelain

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

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

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

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

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

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

## 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<br>PATENT TRADEMARK OFFICE | OPPEDAHL & LARSON LLP<br>(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<br>RIVELLA | FIRST NAME<br>STEFANO | MIDDLE NAME |
|---|---|---|---|
| RESIDENCE & CITIZENSHIP | CITY OF RESIDENCE<br>New York | STATE OR COUNTRY OF RESIDENCE<br>New York | COUNTRY OF CITIZENSHIP<br>Italy |
| POST OFFICE ADDRESS<br>736 West End Avenue<br>Apt. 5B | | CITY<br>New York | STATE/COUNTRY ZIP CODE<br>New York 10025 |
| DATE | | SIGNATURE | |

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

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

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 (signature) | | |
|---|---|---|---|
| Name | James S. Quirk | | |
| Date | 3/20/06 | 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

PTO/SB/81 (04-05)
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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<br>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 (signature) | 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)

206306

PTO/SB/96 (09-04)
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## 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.

| | |
|---|---|
| [signature] | 3/2/06 |
| Signature | Date |
| James S. Quirk | 212 639 6181 |
| Printed or Typed Name | 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

Application No. (if known): Not Yet Assigned	Attorney Docket No.: 64836DIV(51590)

# Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with 37 CFR 1.6(a)(4):

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on April 30, 2009 .
Date

/Peter C. Lauro/
Signature

Peter C. Lauro, Esq.
Typed or printed name of person signing Certificate

32,360
Registration Number, if applicable

(617) 517-5509
Telephone Number

Note: Each paper must have its own certificate of mailing.

Utility Patent Application Transmittal (1 page)
Application Data Sheet (5 pages)
Copy of application serial no. 10/188,221 (17 pages description; 7 pages claims; 1 page abstract; 4 pages drawings))
Preliminary Amendment (13 pages)
Computer Readable Form of Sequence Listing
Copy of Executed Combined Declaration and Power of Attorney (6 pages)
Copy of Executed Revocation of Power of Attorney (3 pages)
Charge $748.00to deposit order account #04-1105

BOS2 734996.1

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Michel Sadelain, *et al.*

Application No.: Not Yet Assigned
(Rule 53(b) Division of Application Ser. No. 10/188,221, filed on July 1, 2002 )

Confirmation No.: N/A

Filed: April 30, 2009

Art Unit: 1633

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

Examiner: M. Marvich

**PRELIMINARY AMENDMENT**

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

**INTRODUCTORY COMMENTS**

Prior to examination on the merits, please amend the above-identified U.S. patent 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 11 of this paper.

## AMENDMENTS TO THE SPECIFICATION

**p. 1, ¶ after "Statement Concerning Related Applications":**

[0001] This application is a divisional application of U.S. Serial No. 10/188,221, filed July 1, 2002, Issuing, which claims the benefit of U.S. Provisional Application No. 60/301,861 filed Jun. 29, 2001 and U.S. Provisional Application No. 60/302,852 filed Jul. 2, 2001, ~~both~~ each of which ~~are~~is incorporated herein by reference.

**p. 1, ¶ after "Statement Concerning Government Funding :**

[0002] The invention disclosed in this application was ~~supported by~~ made with funds provided under NHLBI grant No. HL57612. The United States government ~~may have~~ has certain rights in the invention.

**P.3, ¶ starting on line 5**

[0013] 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.

**p. 6, ¶¶ starting on line 4**

[0026] 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.

[0027] 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~~ progenitors and 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~~ known in the art that efficient ~~genes~~ gene transfer into human embryonic stem cells can be achieved using lentiviral vectors.

**p.7-8, bridging ¶**

[0031] To investigate the tissue specificity, stage specificity and expression level of the vector-encoded human β-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).

**p. 9, first full ¶ after titles in Ex 3**

[0034] 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 $\beta^{maj}$, $\beta^{min}$, $\beta^{s}$ and $\beta^{t}$. Primers were annealed to 4 μg 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.

**p.13-14, bridging ¶:**

[0044] 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-1 α (IL-1α), 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-free~~ 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 ($5 \times 10^5$) 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.

**p. 15, ¶¶ starting at line 3**

[0046] The stability of TNS9-encoded $\beta^A$ 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

[0047] 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~~ 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~~ and CFUs-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~~ β-thalassemias.[29]

[0048] The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and ~~age-match~~ age-matched 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~~ only 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~~ eGFP-transduced $Hbb^{th3/+}$ bone marrow cells showed ~~seral~~ several small foci of intrasinusoidal EMH.

EXAMPLE 10

[0049] 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

Please cancel claims 1-41 without prejudice or disclaimer and please add claims 42-72. The following listing of claims will replace all prior versions, and listings, of claims in the application.

1-41. (Cancelled)

42. (New) A method for treating a hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy which comprises

(a) introducing to the mammalian individual a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

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

43. (New) The method of claim 42, wherein introducing the recombinant lentiviral vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and returning the transformed cells to the mammalian individual.

44. (New) The method of claim 42, wherein said vector further comprises a selectable marker.

45. (New) The method of claim 44, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to returning the

cells to the mammalian individual.

46. (New) The method of claim 45, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate.

47. (New) The method of claim 46, wherein the antifolate is methotrexate.

48. (New) The method of claim 44, wherein the transformed cells are subjected to a selection process *in vivo* after returning the cells to the mammalian individual.

49. (New) The method of claim 48, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase, and wherein the selection process comprises administering an antifolate to said individual.

50. (New) The method of claim 49, wherein the antifolate is methotrexate.

51. (New) The method of claim 42, wherein said functional globin is a mutant globin.

52. (New) The method of claim 42, wherein said functional globin is a wild-type globin.

53. (New) The method of claim 42, wherein said functional globin is a β-globin.

54. (New) The method of claim 53 wherein said functional globin is a human β-globin.

55. (New) The method of claim 42, wherein said functional globin is a γ-globin.

56. (New) The method of claim 42, wherein said functional globin is an α-globin.

57. (New) The method of claim 42, wherein said hemoglobinopathy is β-thalassemia, α- thalassemia or sickle cell anemia.

58. (New) Mammalian hematopoietic progenitor cells or stem cells transduced with a recombinant lentiviral vector which comprises a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*.

59. (New) The cells of claim 58, wherein the mammalian hematopoietic progenitor or stem cells are human cells.

60. (New) The cells of claim 58, wherein said vector further comprises a selectable marker.

61. (New) The cells of claim 60, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase.

62. (New) The cells of claim 58, wherein said functional globin is a mutant globin.

63. (New) The cells of claim 58, wherein said functional globin is a wild-type globin.

64. (New) The cells of claim 58, wherein said functional globin is a β-globin.

65. (New) The cells of claim 64, wherein said functional globin is a human β-globin.

66. (New) The cells of claim 58, wherein said functional globin is a γ-globin.

67. (New) The cells of claim 58, wherein said functional globin is an α-globin.

68. (New) A method for making a therapeutic composition for treatment of hemoglobinopathy in a mammalian individual which comprises

(a) preparing a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

(b) transducing hematopoietic progenitor or stem cells obtained from the mammalian individual with the recombinant vector.

69. (New) The method cell of claim 68, wherein said vector further comprises a selectable marker.

70. (New) The method of claim 69, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase.

71. (New) The method of claim 70 which comprises performing an *ex vivo* selection using an antifolate.

72. (New) The method of claim 68, wherein said functional globin is a human β-globin.

## REMARKS

This application is a divisional application of U.S. Serial No. 10/188,221, filed July 1, 2002 (the parent application), Issuing, which claims the benefit of U.S. Provisional Application No. 60/301,861 filed Jun. 29, 2001 and U.S. Provisional Application No. 60/302,852 filed Jul. 2, 2001. Page 1 of the specification has been amended to reflect this information.

In addition, page 1 of the specification has been amended to conform the statement of government interest to the requirements of 35 U.S.C. §202(c)(6). Various other pages of the specification have been amended to correct typographical errors, to insert sequence identifiers and otherwise to make amendments to the specification that parallel those made in the parent application.

A restriction requirement was issued in the parent application and the claims of Group I were elected for prosecution. The instant divisional application is being filed to pursue non-elected subject matter and other aspects/embodiments of the invention.

Thus, claims 1-41 have been cancelled without prejudice or disclaimer and claims 42-72 have been added. Support for the new claims is found at least, for example, at the citations listed in the table below.

| New Claim | Support |
|---|---|
| 42 | Original claim 19, ¶¶21 and 22 |
| 43 | Original claim 25, ¶9 |
| 44 | ¶24 |
| 45 | Original claim 26, ¶9 |
| 46 | Original claims 20-24, 27 |
| 47 | Original claim 28 |
| 48 | ¶9 |
| 49 | Original claims 20-24, 27 |
| 50 | Original claim 28 |
| 51 | ¶20 |
| 52 | ¶20 |
| 53 | ¶20 |
| 54 | Original claim 29, ¶20 |

| New Claim | Support |
|---|---|
| 55 | ¶20 |
| 56 | ¶20 |
| 57 | ¶¶3 and 9 |
| 58 | Original claim 30 |
| 59 | Original claim 31 |
| 60 | ¶24 |
| 61 | Original claims 32-37 |
| 62 | ¶20 |
| 63 | ¶20 |
| 64 | ¶20 |
| 65 | Original claim 38, ¶20 |
| 66 | ¶20 |
| 67 | ¶20 |
| 68 | Original claim 39 |
| 69 | ¶24 |
| 70 | Original claims 20-24, 27 |
| 71 | Original claim 40 |
| 72 | Original claim 41 |

No new matter has been added. Applicants hereby reserve the right to pursue the claims as originally filed in this or one or more subsequent patent applications.

Applicants respectfully request early examination on the merits and allowance of the application with all claims presented herein. If a telephone conversation with Applicants' attorney would be helpful in expediting prosecution of the application, Applicants invite the Examiner to contact the undersigned at the telephone number listed below.

Dated: April 30, 2009

Respectfully submitted,

Electronic signature: /Peter C. Lauro/
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Registration No.: 32,360
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Boston, Massachusetts 02205
(617) 517-5509
Attorneys/Agents For Applicants

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | |
| **Filing Date:** | |
| **Title of Invention:** | Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Filer:** | Peter C. Lauro |
| **Attorney Docket Number:** | 64836DIV(51590) |

Filed as Small Entity

## Utility under 35 USC 111(a) Filing Fees

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| Utility filing Fee (Electronic filing) | 4011 | 1 | 82 | 82 |
| Utility Search Fee | 2111 | 1 | 270 | 270 |
| Utility Examination Fee | 2311 | 1 | 110 | 110 |
| **Pages:** | | | | |
| **Claims:** | | | | |
| Claims in excess of 20 | 2202 | 11 | 26 | 286 |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **748** |

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 5253610 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | Vector Encoding Human Globin Gene and Use Thereof in Treatment of Hemoglobinopathies |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Peter C. Lauro |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 30-APR-2009 |
| **Filing Date:** | |
| **Time Stamp:** | 16:38:54 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $748 |
| RAM confirmation Number | 2955 |
| Deposit Account | 041105 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Transmittal of New Application | 64836DVTransmittalLetter.pdf | 83408<br>6fd6d3e59c4d36bb557d057245ba11a80575906f | no | 1 |

**Warnings:**

**Information:**

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 2 | Application Data Sheet | 64836DIVADS.pdf | 1196928<br>d509403c4f59819dc0781f209d662b8303f650f6 | no | 5 |

**Warnings:**

**Information:**

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 3 | | 64836DIVApplication.pdf | 1085999<br>61452f7f2ba2e364f7a2a751d0406910738fd052 | yes | 29 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Specification | 1 | 17 |
| Claims | 18 | 24 |
| Abstract | 25 | 25 |
| Drawings-only black and white line drawings | 26 | 29 |

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The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing

**Information:**

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 4 | Oath or Declaration filed | 64836DIVDPOAS.pdf | 288893<br>2cdee422c106ca3a4204ed3faca3dc006311cd45 | no | 6 |

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| 5 | Power of Attorney | 64836DIVRevocation.pdf | 173588<br>468d987ca0309229aa78d4058c8614ab35b5a2c2 | no | 3 |

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| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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| 6 | Sequence Listing (Text File) | 64836DIVSequenceListing.txt | 732 | no | 0 |

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| Information: | | | | | |
| 8 | Preliminary Amendment | 64836DIVPreliminaryAmendment.pdf | 101791<br>fdd51d8aa71db6129ad6b91ff631a21ab20b9d80 | no | 13 |
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| Information: | | | | | |
| 9 | Fee Worksheet (PTO-875) | fee-info.pdf | 36456<br>99dd2138c03e522b444c407495572b54beb6c902 | no | 2 |
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| Information: | | | | | |
| | | **Total Files Size (in bytes):** | 3007072 | | |

**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.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

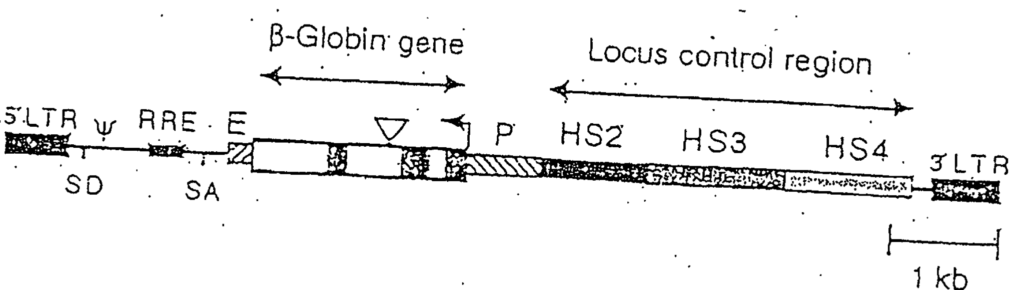



Fig. 1

# Clinical Use of Drug Resistance

## In Vivo Selection of Genetically Modified Stem Cells

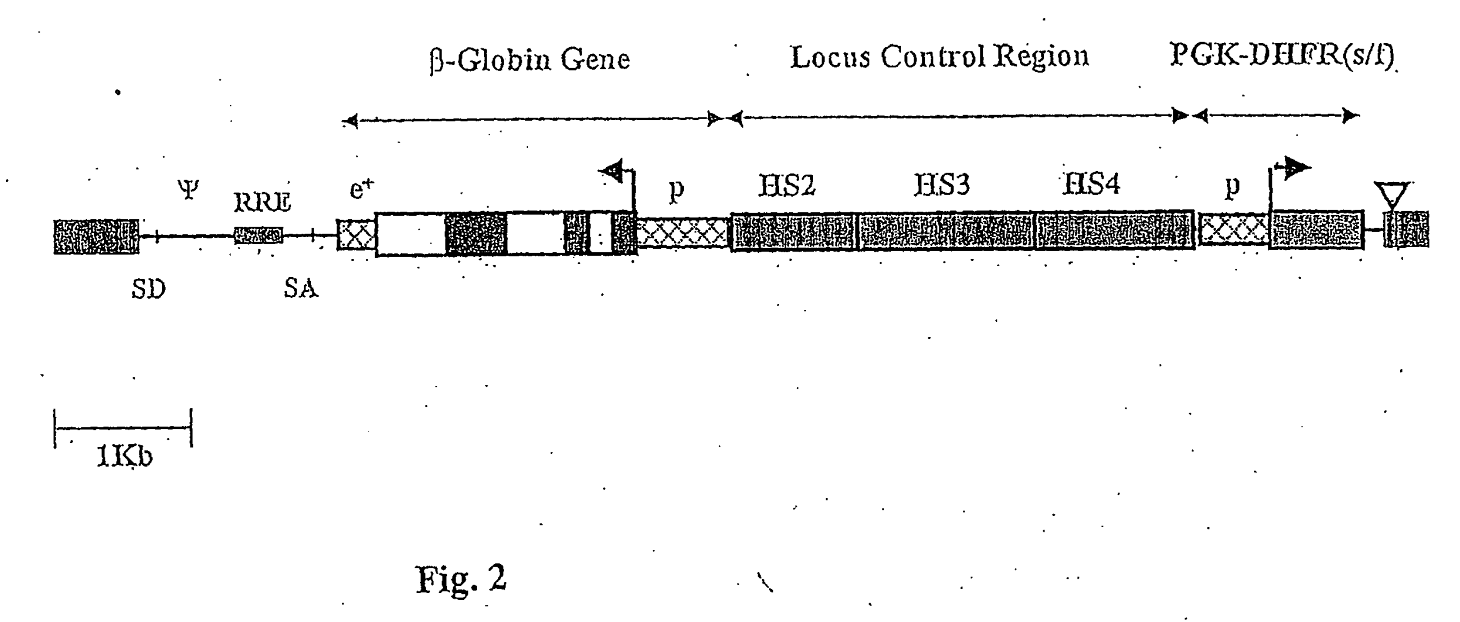



Fig. 2

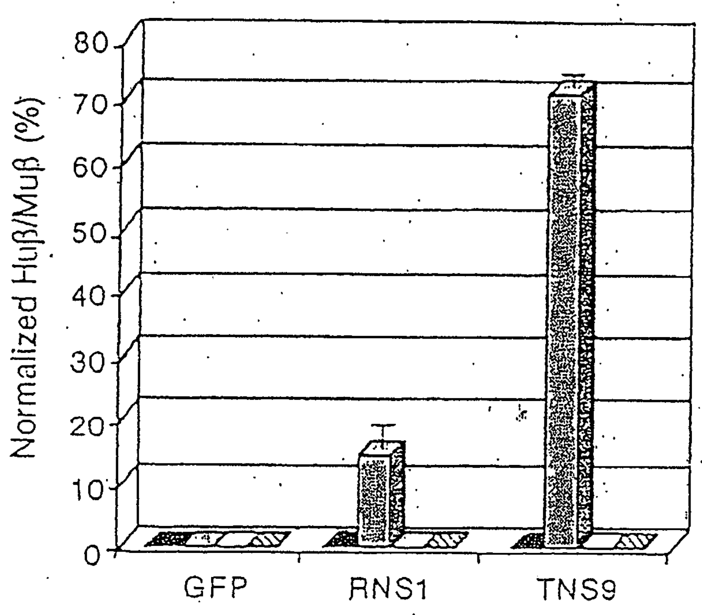



Fig. 3

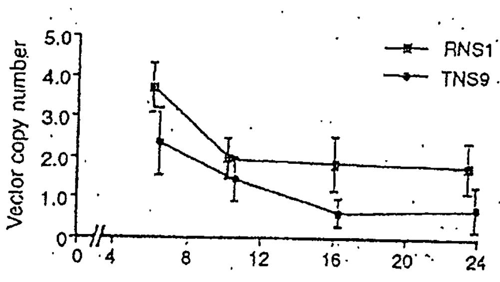



Fig. 4

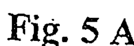
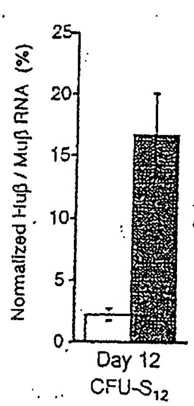


Fig. 5 A



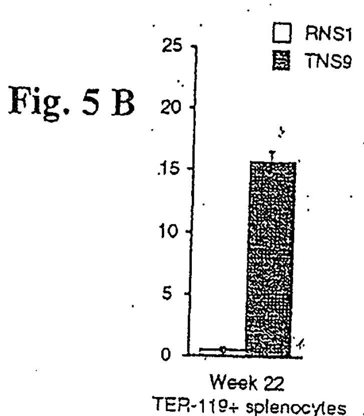




Fig. 5 B

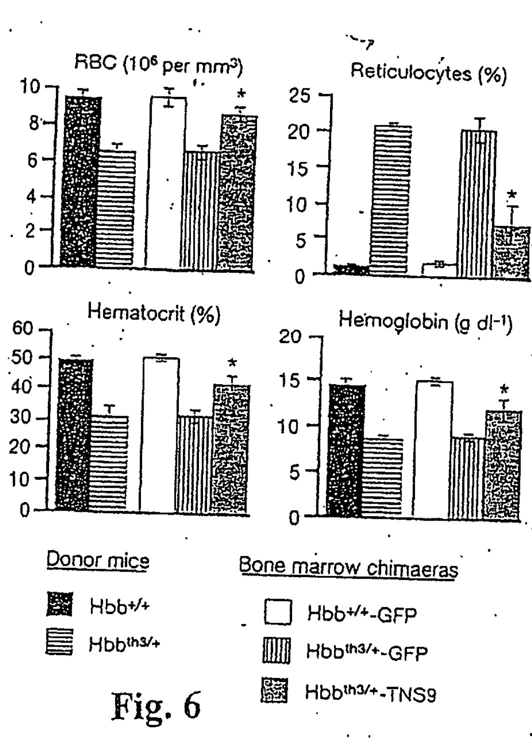



Fig. 6

PTO/SB/06 (12-04)

**Filing Date:** **04/30/09**

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: 12/433,412

### 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)) | N/A | N/A | N/A | 82 | | N/A | |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | 270 | | N/A | |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | 110 | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 31 minus 20 = | 11 | x$26 | 286 | OR | x$52 | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 3 minus 3 = | * | x$110 | | | x$220 | |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR | | | | | | |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | 195 | | | 390 | |
| | | | TOTAL | 748 | | TOTAL | |

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

### 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 ($) | ADDITIONAL FEE ($) | OR | OTHER THAN SMALL ENTITY RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|---|---|---|
| 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)) | | | | | N/A | | OR | N/A | |
| | | | | | TOTAL ADD'T FEE | | OR | TOTAL ADD'T FEE | |

**AMENDMENT B**

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | RATE ($) | ADDITIONAL FEE ($) | OR | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|---|---|---|
| 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)) | | | | | N/A | | OR | N/A | |
| | | | | | TOTAL ADD'T FEE | | OR | TOTAL ADD'T 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. Paten 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.*

============================================================
Sequence Listing could not be accepted due to errors.
See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Durreshwar Anjum
Timestamp: [year=2009; month=5; day=12; hr=13; min=11; sec=23; ms=229; ]
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**Validated By CRFValidator v 1.0.3**

Application No: 12433412 Version No: 1.0

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**Actual SeqID Count:** 4

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| E 250 | Structural Validation Error; Sequence listing may not be indexable |

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
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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | 1633 | 748 | 64836DIV(51590) | 31 | 3 |

**CONFIRMATION NO. 9026**

**FILING RECEIPT**

65488
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

*OC000000035954061*

Date Mailed: 05/21/2009

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Applicant(s)**

Michel Sadelain, New York, NY;
Stefano Rivella, New York, NY;
Chad May, New York, NY;
Joseph Bertino, Branford, CT;

**Assignment For Published Patent Application**

MEMORIAL SLOAN-KETTERING CANCER CENTER, New York City, NY

**Power of Attorney:** The patent practitioners associated with Customer Number 21874

**Domestic Priority data as claimed by applicant**

This application is a DIV of 10/188,221 07/01/2002 PAT 7,541,179
which claims benefit of 60/301,861 06/29/2001
and claims benefit of 60/302,852 07/02/2001

**Foreign Applications**

**If Required, Foreign Filing License Granted:** 05/13/2009

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/433,412**

**Projected Publication Date:** To Be Determined - pending completion of Corrected Papers

**Non-Publication Request:** No

**Early Publication Request:** No
**** SMALL ENTITY ****

**Title**

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

**Preliminary Class**

435

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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## Title 35, United States Code, Section 184

## Title 37, Code of Federal Regulations, 5.11 & 5.15

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

**NOT GRANTED**

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) |

**CONFIRMATION NO. 9026**

65488
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

**FORMALITIES LETTER**

*OC000000035954062*

Date Mailed: 05/21/2009

## NOTICE TO FILE CORRECTED APPLICATION PAPERS

***Filing Date Granted***

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. 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 required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings must be reasonably free from erasures and must be free from alterations, overwriting, interlineations, folds, and copy marks. See Figure(s) All.
- A substitute specification excluding claims in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125 is required. The substitute specification must be submitted with markings and be accompanied by a clean version (without markings) as set forth in 37 CFR 1.125(c) and a statement that the substitute specification contains no new matter (see 37 CFR 1.125(b)). Since a preliminary amendment was present on the filing date of the application and such amendment is part of the original disclosure of the application, the substitute specification must include all of the desired changes made in the preliminary amendment. See 37 CFR 1.115 and 1.215.
- A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing." Applicant must provide a substitute computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). Refer to attachment or PAIR document dated 05/12/09 .

**To Download Patentin Software, visit http://www.uspto.gov/web/patents/software.htm**
**For questions regarding compliance to these requirements, please contact:**

- **For Rules Interpretation, call (571) 272-0951**
- **For Patentin Software Program Help, call Patent EBC at 1-866-217-9197 or directly at 703-305-3028 / 703-308-6845 between the hours of 6 a.m. and 12 midnight, Monday through Friday, EST.**
- **Send e-mail correspondence for Patentin Software Program Help @ ebc@uspto.gov**

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

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If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/kgebremichael/

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Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) |

**CONFIRMATION NO. 9026**

**POA ACCEPTANCE LETTER**

*OC000000035961957*

65488
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

Date Mailed: 05/21/2009

## NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 04/30/2009.

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.

/kgebremichael/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Michel Sadelain et al.

Application No.: 12/433,412

Filed: April 30, 2009

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

Confirmation No.: 9026

Art Unit: 1633

Examiner: Not yet assigned

**RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS**

MS Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

In response to the Notice to File Corrected Application Papers – Filing Date Granted mailed May 21, 2009, Applicant respectfully submits Replacement Drawings (6 figures, 6 pages), a Substitute Specification, and a Sequence Listing.

Applicants submit concurrently herewith Replacement Drawings in compliance with 37 C.F.R. §1.84 and 37 C.F.R. §1.121(d). Six (6) replacement sheets are being submitted herewith. Specifically, the Figures have been formatted to be reasonably free from erasures and free from alterations, overwriting, interlineations, folds, and copy marks. The Replacement Drawings provided herewith are the best available copies of the Figures. No new matter is added.

Applicants submit concurrently herewith a Substitute Specification in compliance with 37 C.F.R. §1.52, 37 C.F.R. §1.121(b)(3), and 37 C.F.R. §1.125. The Substitute Specification is submitted with markings and is accompanied by a clean version (without markings) as set forth in 37 C.F.R. §1.125(c). A Substitute

Specification Statement Pursuant to 37 C.F.R. §1.125(b) is also submitted herewith stating that the substitute specification contains no new matter.

Pursuant to the requirements of 37 C.F.R. §1.822 and/or §1.823, Applicants submit concurrently herewith (1) a paper copy of the Sequence Listing and (2) a computer readable form (CRF) of the Sequence Listing. The Sequence Listing has been formatted according to the PAIR document dated May 12, 2009. A Statement Under 37 C.F.R. §1.821(f) and/or §1.821(g) is also included stating that the CRF version and the paper copy of the Sequence Listing being filed herewith are the same and that the Sequence Listing being filed herewith does not include new matter.

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. 64836DIV(51590).

Dated: July 1, 2009

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Elbert Chiang, Ph.D.
Registration No.: 60,325
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5502
Attorneys/Agents For Applicant

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Michel Sadelain et al.

Application No.: 12/433,412 | Confirmation No.: 9026

Filed: April 30, 2009 | Art Unit: 1633

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | Examiner: Not yet assigned

**SUBSTITUTE SPECIFICATION STATEMENT PURSUANT TO 37 C.F.R. §1.25(b)**

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the the Notice to File Corrected Application Papers – Filing Date Granted mailed May 21, 2009, Applicants submit a substitute specification with markings, accompanied by a clean version (without markings) in compliance with 37 C.F.R. §1.52, §1.121(b)(3) and §1.125.

Applicants hereby state that the substitute specification contains no new matter.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Applicants hereby authorize the Director 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.

Dated: July 1, 2009

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Elbert Chiang, Ph.D.
Registration No.: 32,360
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5502
Attorneys/Agents For Applicants

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

## Statement Concerning Government Funding

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

## Statement Concerning Related Applications

This application is a divisional application of U.S. Serial No. 10/188,221, filed July 1, 2002, Issuing, which 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~~ each of which ~~are~~is 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 $\alpha$- 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
5 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

10 (b) large portions of the β-globin locus control regions which include large portions of DNase 1 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
15 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
20 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).

25 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.
5 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
10 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.
15 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
20 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
25 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 by HS3 fragment (*Hin*dIII-*Bam*HI) and a 1069 by 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~~ 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~~ progenitors and 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~~ known in the art that efficient ~~genes~~ gene 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.

5 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 I Acad. Sci. (USA)*92: 6728-6732 (1995)) into lentiviral vector

10 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

15 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

20 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

25 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
5 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 ($\beta^A$) and mouse (β-globin transcripts were measured by quantitative primer extension. After normalization to vector copy number and
10 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
15 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
20 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 $\beta^A$ 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,
25 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
30 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.

5 **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
10 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 $\beta^{maj}$, $\beta^{min}$, $\beta^{s}$ and $\beta^{1}$. Primers were annealed to 4μg of RNA and reactions were run according to manufacturer's protocols. Radioactive bands were quantitated by
15 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-
20 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.

25 **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 $ml^{-1}$),
5 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 $m^{-I}$), 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
10 (8μg $m1^{-1}$), L-glutamine (200 mM), penicillin ( 100 IU $m1^{-1}$) and streptomycin (100 μg $m1^{-1}$), 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
15 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
20 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
25 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 $CFU\text{-}S_{12}$ 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 $\beta^A$ 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 $\beta^A$. No haemoglobin tetramers containing human $\beta^A$ were measurble in any of the mice bearing RNS1 (table 1 in Supplementary Information). The proportion of mature peripheral blood red cells expressing human $\beta^A$ was elevate in most TNS9 bone marrow chimaeras, as shown by dual staining of human $\beta^A$ and TER-119. In contrast, chimaeras engrafted with RNS1-transduced bone marrow showed highly variable fractions of weakly staining $\beta^A$ -positive erythrocytes. Normalized to the fraction of circulating $\beta^A$ -positive mature red cells, the levels of haemoglobin containing lentivirus-encoded $\beta^A$ 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
5 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
10 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
15 could correct the phenotype of thalassaemic cells using $\beta^0$ -thalassaemic heterozygote mice that lack a copy of their bl 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 $Hbb^{th3/+}$
20 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 =
25 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 $\beta^A$ 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 $\beta^A$ -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 $\alpha_2$: 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 $\beta^A$ chains are expected to have an advantage over the $\beta^S$ 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~~ 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, NJ). Bone marrow cells were resuspended in X-VIVO-15 serum-free medium and supplemented with 10 ng/mL interleukin-1 $\alpha$ (IL-1$\alpha$), 100 U/mL IL-3, 150 U/mL IL- 6, l0ng/mL, Kit ligand obtained from Genzyme (Cambridge, MA), 0.5mM $\beta$-mercaptoethanol obtained from Sigma (St. Louis, MO), 200-mM L-glutamine, 100 IU/mL penicillin, and 100 $\mu$g/mL streptomycin. Bone marrow cells were ~~ten~~ then pelleted and resuspended in ~~serrum-free~~ serum-free medium containing concentrated lentiviral supernatant and supplemented with 8 mg/mL polybrene (Sigma), 200mM L-glutamine, 100 U/mL penicillin, 100 $\mu$g/mL streptomycin and cytokines as above, and ~~cultureed~~ cultured for 8 hours. Transduced bone marrow cells (5 x$10^5$) 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.

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 $\beta^A$ ($Hbb^{hu}$, mu $\alpha_2$: hu$\beta^A{}_2$) or murine $\beta$-globin (Hbb $^{mu}$, mu$\alpha_2$:mu$\beta$2), and immunofluorescence, to determine the fraction of mature RBCs that contain human $\beta^A$ protein. Transgenic mice bearing one copy of a 230-kb yeast artificial chromosome encompassing the entire human $\beta$-globin-like gene cluster[28] served as reference, showing 14% of their total hemoglobin incorporating human $\beta^A$ and 100% $\beta^A$+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 $\beta^A$ also remained elevated and stable (about 70% to 80%), as shown by dual staining of human $\beta^A$ and TER-119.

**Example 8**

**Long-Term amelioration of anemia**

The stability of TNS9-encoded $\beta^{A}$ 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-mateched~~ 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~~ and CFUs-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 eGFPtrasduced $Hbb^{th3/+}$ bone marrow cells and in age-matched $Hbb^{th3/+}$ mice, as previously observed in another murine ~~modelof~~ model of ~~β-thalassemis~~ β-thalassemias.[29]

The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and ~~age-match~~ age-matched 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~~ only 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~~ eGFP-transduced $Hbb^{th3/+}$ 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]

**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.

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

5

## Statement Concerning Government Funding

The invention disclosed in this application was made with funds provided under NHLBI grant No. HL57612. The United States government has certain rights in the invention.

10 ## Statement Concerning Related Applications

This application is a divisional application of U.S. Serial No. 10/188,221, filed July 1, 2002, Issuing, which 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, each of which is incorporated herein by reference.

15

## 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 $\alpha$- and β-thalessemia and sickle-cell disease.

20 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 25 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 30 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
5 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

10 (b) large portions of the β-globin locus control regions which include large portions of DNase 1 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
15 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
20 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).

25 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.

5 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 highly efficient gene transfer in cells transduced with the vector of the invention.

10 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.

15 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

20 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

25 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.
5 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
10 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.
15 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
20 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
25 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 by HS3 fragment (*Hin*dIII-*Bam*HI) and a 1069 by 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~~ 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 used in the specification and claims hereof, the term "hematopoietic progenitors and 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 known in the art that efficient gene 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.

5 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 I Acad. Sci. (USA)*92: 6728-6732 (1995)) into lentiviral vector

10 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

15 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

20 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

25 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).

To induce (β-globin transcription, transduced MEL cell pools were differentiated using hexamethylene bisacetamide HMBA). Human (β-globin ($\beta^A$) 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 $\beta^A$ 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.

5 **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
10 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 $\beta^{maj}$, $\beta^{min}$, $\beta^{s}$ and $\beta^{1}$. Primers were annealed to 4µg of RNA and reactions were run according to manufacturer's protocols. Radioactive bands were quantitated by
15 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-
20 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.

25 **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 $ml^{-1}$),
5 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
10 (8μg $m1^{-1}$), L-glutamine (200 mM), penicillin ( 100 IU $m1^{-1}$) and streptomycin (100 μg $m1^{-1}$), 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
15 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
20 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
25 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
5 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
10 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
15 incorporating vector-encoded human $\beta^A$ 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
20 human β-globin like gene cluster[20] showed 14% of their total haemoglobin incorporating human $\beta^A$. No haemoglobin tetramers containing human $\beta^A$ were measurble in any of the mice bearing RNS1 (table 1 in Supplementary Information). The proportion of mature peripheral blood red cells expressing human $\beta^A$ was elevate in most TNS9 bone marrow chimaeras, as shown by dual staining of human $\beta^A$ and TER-119. In contrast, chimaeras engrafted with RNS1-transduced
25 bone marrow showed highly variable fractions of weakly staining $\beta^A$ -positive erythrocytes. Normalized to the fraction of circulating $\beta^A$ -positive mature red cells, the levels of haemoglobin containing lentivirus-encoded $\beta^A$ 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
5 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
10 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
15 could correct the phenotype of thalassaemic cells using $\beta^0$ -thalassaemic heterozygote mice that lack a copy of their bl 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 $Hbb^{th3/+}$
20 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 =
25 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 $\beta^A$ 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 $\beta^A$ - 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 $\alpha_2$: 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 $\beta^A$ chains are expected to have an advantage over the $\beta^S$ 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 ~~femurs~~ 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, NJ). Bone marrow cells were resuspended in X-VIVO-15 serum-free medium and supplemented with 10 ng/mL interleukin-1 $\alpha$ (IL-1$\alpha$), 100 U/mL IL-3, 150 U/mL IL- 6, l0ng/mL, Kit ligand obtained from Genzyme (Cambridge, MA), 0.5mM $\beta$-mercaptoethanol obtained from Sigma (St. Louis, MO), 200-mM $_{L}$-glutamine, 100 IU/mL penicillin, and 100 $\mu$g/mL streptomycin. Bone marrow cells were ~~ten~~ then pelleted and resuspended in ~~serrum-free~~ serum-free medium containing concentrated lentiviral supernatant and supplemented with 8 mg/mL polybrene (Sigma), 200mM $_{L}$-glutamine, 100 U/mL penicillin, 100 $\mu$g/mL streptomycin and cytokines as above, and ~~cultureed~~ cultured for 8 hours. Transduced bone marrow cells (5 x$10^5$) 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.

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 $\beta^A$ ($Hbb^{hu}$, mu $\alpha_2$: hu$\beta^A{}_2$) or murine $\beta$-globin (Hbb $^{mu}$, mu$\alpha_2$:mu$\beta$2), and immunofluorescence, to determine the fraction of mature RBCs that contain human $\beta^A$ protein. Transgenic mice bearing one copy of a 230-kb yeast artificial chromosome encompassing the entire human $\beta$-globin-like gene cluster[28] served as reference, showing 14% of their total hemoglobin incorporating human $\beta^A$ and 100% $\beta^A$+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 $\beta^A$ also remained elevated and stable (about 70% to 80%), as shown by dual staining of human $\beta^A$ and TER-119.

**Example 8**

**Long-Term amelioration of anemia**

The stability of TNS9-encoded $\beta^{A}$ 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-mateched~~ 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~~ and CFUs-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 eGFPtrasduced Hbb$^{th3/+}$ bone marrow cells and in age-matched Hbb$^{th3/+}$ mice, as previously observed in another murine ~~modelof~~ model of ~~β-thalassemis~~ β-thalassemias.[29]

The regression of EMH was corroborated by morphologic examination of spleen and liver in long-term chimeras and ~~age-match~~ age-matched 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~~ only 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~~ eGFP-transduced $Hbb^{th3/+}$ 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]

**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.

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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michel Sadelain et al.

Application No.: 12/433,412 | Confirmation No.: 9026

Filed: April 30, 2009 | Art Unit: 1633

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | Examiner: Not yet assigned

## STATEMENT PURSUANT TO 37 C.F.R. §1.821(f) AND/OR §1.821(g)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

Submitted herewith for filing in connection with the above-referenced patent application is a computer readable copy of the Sequence Listing included in the application and a paper copy of the Sequence Listing.

I hereby state that I have reviewed the paper copy of the Sequence Listing contained on page 1 of said Sequence Listing, as required by 37 C.F.R. §1.821(c), and have reviewed the computer readable form of the Sequence Listing, as required by 37 C.F.R. 1..821(e), and that the content of the paper and computer readable copies being filed herewith for the above-referenced patent application are the same as required by 37 C.F.R. 37 CFR 1.8211.821(f) and/or 37 C.F.R. 37 CFR 1.821(g). No new matter is added.

Early favorable consideration of the patent application is respectfully solicited.

Dated: July 1, 2009

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Elbert Chiang, Ph.D.
Registration No.: 60,325
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5502
Attorneys/Agents For Applicant

SEQUENCE LISTING

```
<110>   Sadelain, Michel
        May, Chad
        Bertino, Joseph
        Rivella, Stefano
<120>   Vector Encoding Human Globin Gene and Use Thereof in Treatment of
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<130>   MSK.P-050
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# Electronic Acknowledgement Receipt

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| **EFS ID:** | 5630009 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Elbert C. Chiang/Alyson Lucas |
| **Filer Authorized By:** | Elbert C. Chiang |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 01-JUL-2009 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 18:19:43 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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| 1 | Miscellaneous Incoming Letter | 64836DIV_Cert.pdf | 13989<br>381a2378088d8108072b566635a311fd39c1afd9 | no | 1 |

**Warnings:**

**Information:**

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| 3 | Amendment Copy Claims/Response to Suggested Claims | 64836DIV_Response.pdf | 16861<br>012f7b4dc3ad2527c5b71a34c7fd237ea87a5a9a | no | 2 |
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| **Information:** | | | | | |

| Total Files Size (in bytes): | 2569609 |
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**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.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Application No. (if known): 12/433,412 Attorney Docket No.: 64836DIV(51590)

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on July 1, 2009 .
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/Elbert Chiang, Ph.D./
Signature

Elbert Chiang, Ph.D.
Typed or printed name of person signing Certificate

60,325
Registration Number, if applicable

(617) 517-5502
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Note: Each paper must have its own certificate of mailing.

Substitute Specification (17 pages)
Marked-up Copy of Substitute Specification (17 pages)
Drawing(s) (6 figures, 6 pages)
Computer Readable form of Sequence Listing
Paper Copy of Sequence Listing (1 page)
Statement Pursuant to 37 C.F.R. §1.821(f) and or §1.821(g) (1 page)
Substitute Specification Statement Pursuant to 37 C.F.R. §1.25(b) (1 page)
Response to Notice to File Corrected Application Papers (2 pages)
Transmittal (1 page)

PTO/SB/21 (06-09)
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# TRANSMITTAL FORM

*(to be used for all correspondence after initial filing)*

| | |
|---|---|
| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | M. Marvich |
| Attorney Docket Number | 64836DIV(51590) |
| Total Number of Pages in This Submission | |

## 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)
- [x] Reply to Missing Parts/ Corrected Application Papers
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [x] 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):

Substitute Specification
Computer Readable form of Sequence Listing
Statement

Remarks

## SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

| | | | |
|---|---|---|---|
| Firm Name | EDWARDS ANGELL PALMER & DODGE LLP | | |
| Signature | /Elbert Chiang, Ph.D./ | | |
| Printed name | Elbert Chiang, Ph.D. | | |
| Date | June 26, 2009 | Reg. No. | 60,325 |

DocCode – SEQ.TXT

# SCORE Placeholder Sheet for IFW Content

Application Number: 12433412 Document Date: 07/01/2009

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Form Revision Date: February 8, 2006

DocCode – SCORE

# SCORE Placeholder Sheet for IFW Content

Application Number: 12433412 Document Date: 07/01/2009

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At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

# SCORE Placeholder Sheet for IFW Content

Application Number: 12433412 Document Date: 7/1/2009

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- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

**Replacement Sheet**



**Fig. 1**

# Replacement Sheet

**Clinical Use of Drug Resistance**

In Vivo Selection of Genetically Modified Stem Cells

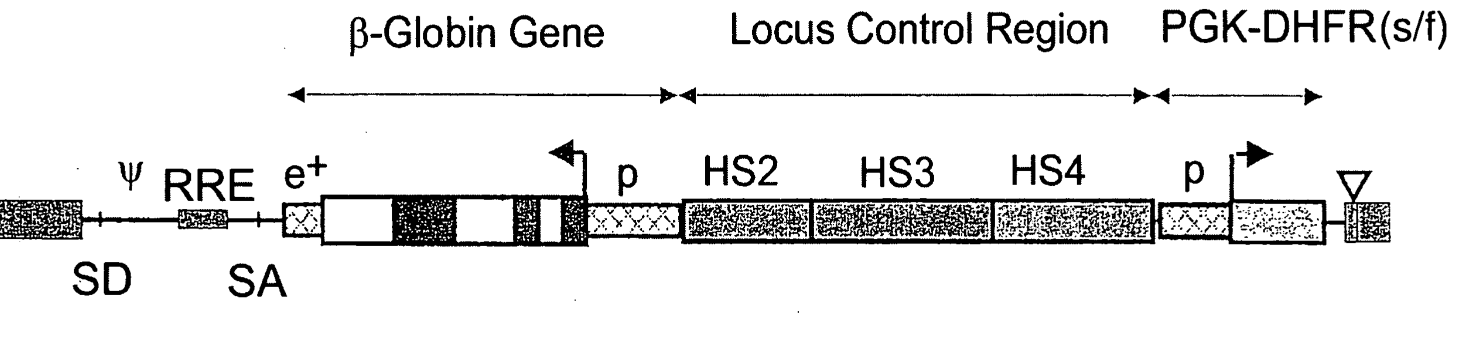


**Fig. 2**

# Replacement Sheet

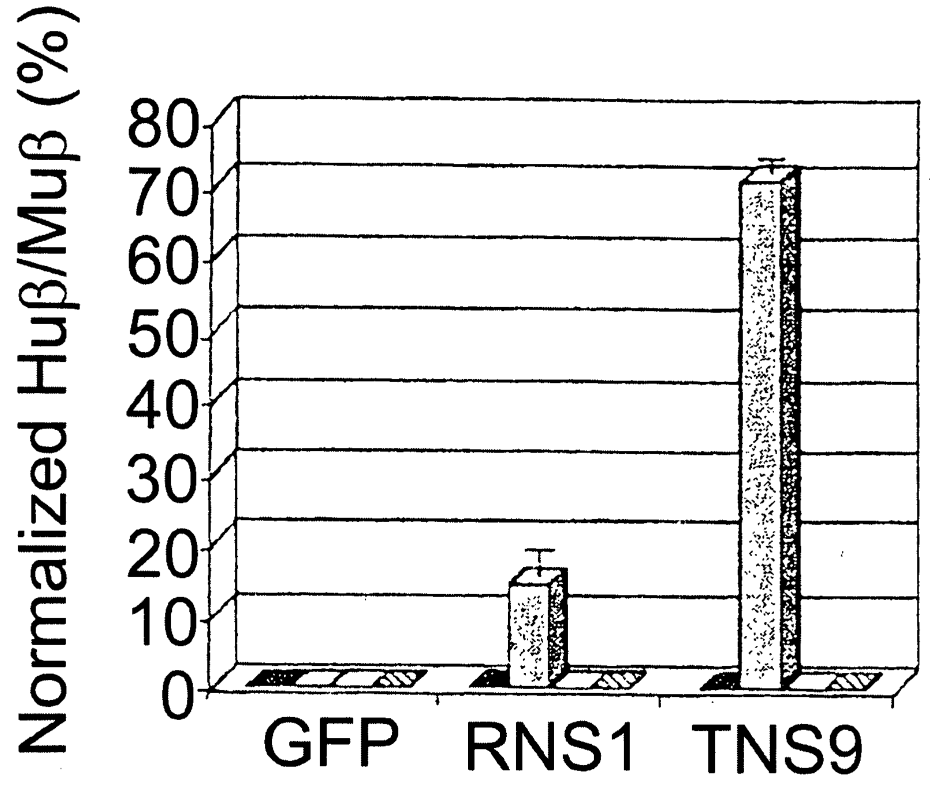


**Fig. 3**

# Replacement Sheet

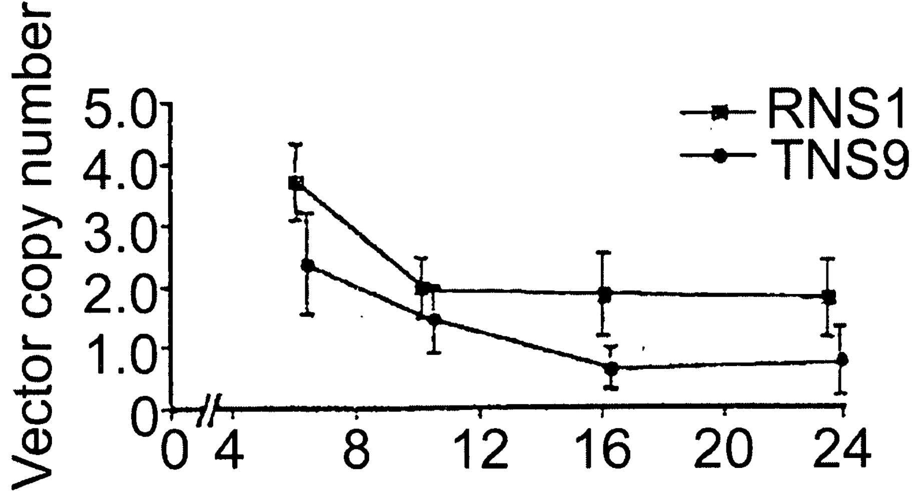


**Fig. 4**

## Replacement Sheet

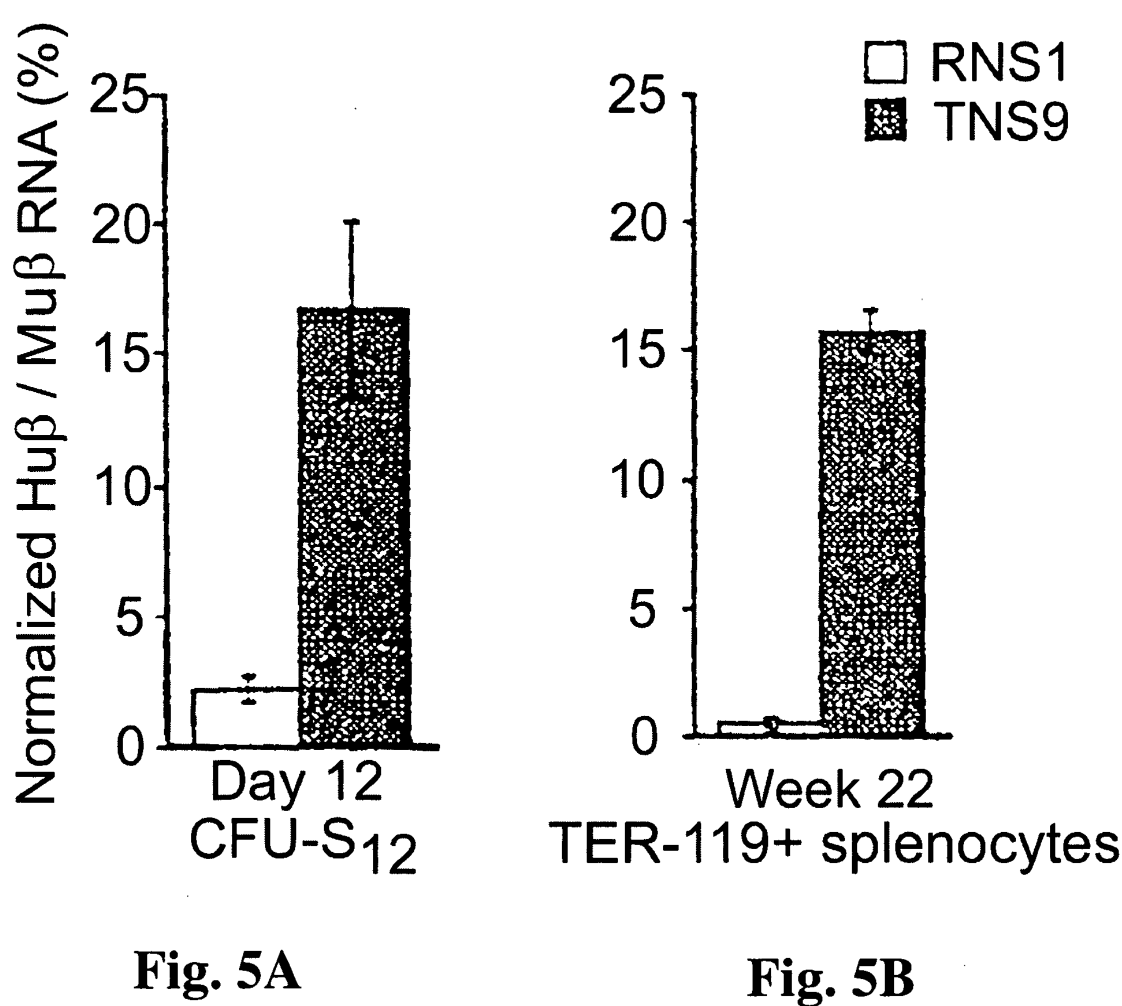


Fig. 5A

Fig. 5B

# Replacement Sheet

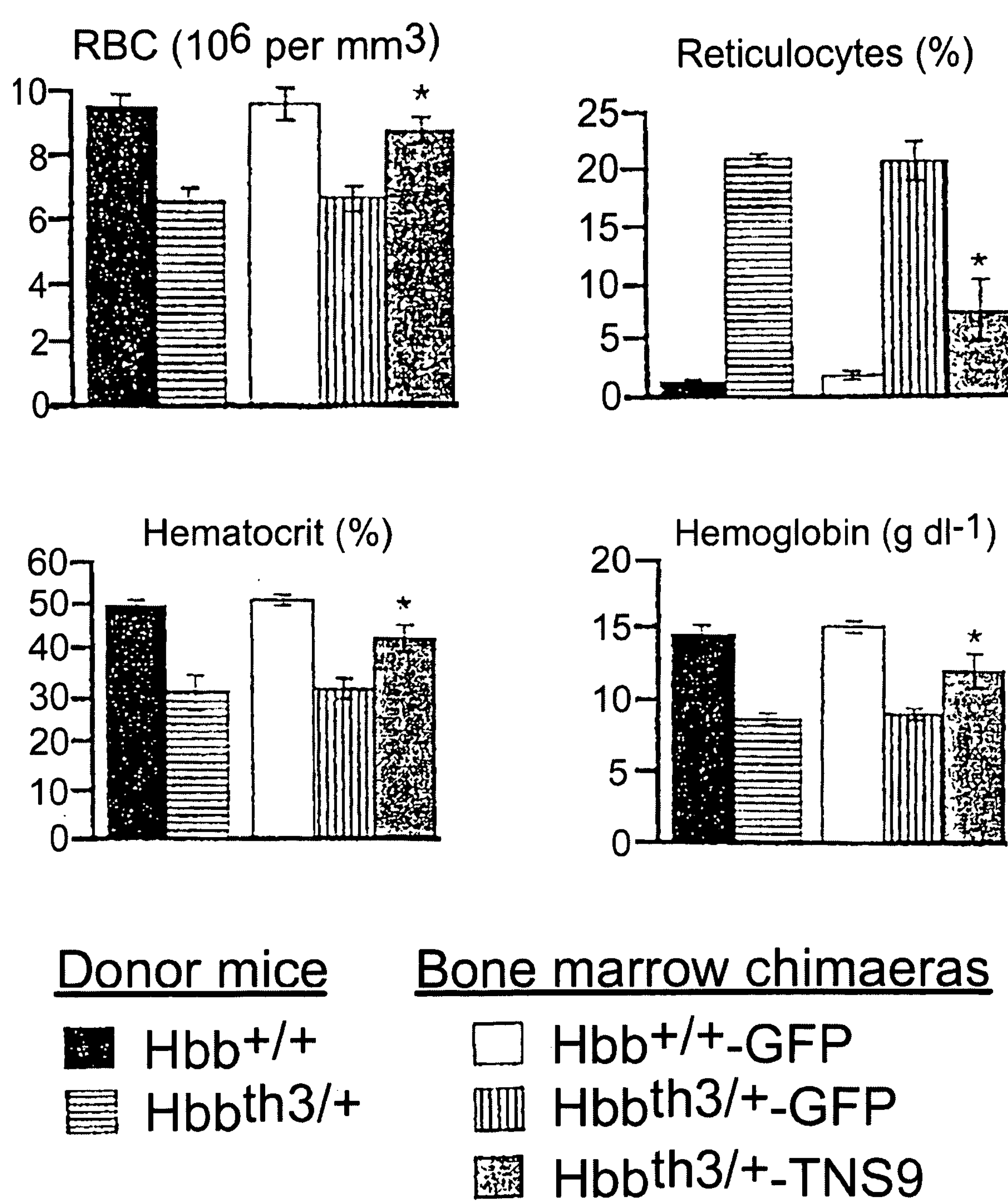


**Fig. 6**

PTO/SB/08a (06-09)
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# INFORMATION DISCLOSURE STATEMENT BY APPLICANT

*(Use as many sheets as necessary)*

| Sheet | 1 | of | 4 |
|---|---|---|---|

| Complete if Known | |
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| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | Not Yet Assigned |
| Attorney Docket Number | 64836DIV(51590) |

## U.S. PATENT DOCUMENTS

| Examiner Initials* | Cite No.[1] | Document Number<br>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 |
|---|---|---|---|---|---|
| | AA* | US-5,126,260 | 06-30-1992 | Tuan et al. | |
| | AB* | US-5,610,053 | 03-11-1997 | Chung et al. | |
| | AC* | US-5,631,162 | 05-20-1997 | LeBoulch et al. | |
| | AD* | US-5,834,256 | 11-10-1998 | Finer et al. | |
| | AE* | US-5,858,740 | 01-12-1999 | Finer et al. | |
| | AF* | US-5,981,276 | 11-09-1999 | Sodroski et al. | |
| | AG* | US-5,994,136 | 11-30-1999 | Naldini et al. | |
| | AH* | US-6,013,516 | 01-11-2000 | Verma et al. | |
| | AI* | US-6,090,608 | 07-18-2000 | Oppenheim et al. | |
| | AJ* | US-6,110,666 | 08-29-2000 | Grosveld et al. | |
| | AK* | US-6,218,187 | 04-17-2001 | Finer et al. | |
| | AL* | US-6,294,165 | 09-25-2001 | Lever et al. | |
| | AM* | US-6,312,682 | 11-06-2001 | Kingsman et al. | |
| | AN* | US-6,428,953 | 08-06-2002 | Naldini et al. | |
| | AO* | US-6,444,421 | 09-03-2002 | Chung | |
| | AP* | US-6,524,851 | 02-25-2003 | Ellis | |
| | AQ* | US-6,544,771 | 04-08-2003 | Riviere et al. | |
| | AR* | US-6,642,043 | 11-04-2003 | Bertino et al. | |
| | AS* | US-6,797,494 | 09-28-2004 | Antoniou et al. | |

## FOREIGN PATENT DOCUMENTS

| Examiner Initials* | Cite No.[1] | Foreign Patent Document<br>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] |
|---|---|---|---|---|---|---|
| | BA** | WO-97/33988-A1 | 09-18-1997 | Sloan Kettering Inst Cancer et al. | | |
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| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** | | | | Application Number | 12/433,412-Conf. #9026 |
| | | | | Filing Date | April 30, 2009 |
| | | | | First Named Inventor | Michel Sadelain |
| | | | | Art Unit | 1633 |
| *(Use as many sheets as necessary)* | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 4 | Attorney Docket Number | 64836DIV(51590) |

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|---|---|---|---|
| | CA** | 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. | |
| | CB** | 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 | |
| | CC** | Dull et al. (1998) J. Virol. 72:8463-8471, "A Third-Generation Lentivirus Vector with a Conditional Packaging System" | |
| | CD** | Dzierzak et al., Lineage-specific expression of a human B-globin gene in murine bone marrow transplant recipients reconstituded with retrovirus-transduced stem cells., 1988, Pages 35-41, Volume 331 | |
| | CE** | Ercikan et al., "Effect of codon 22 mutations on substrate in inhibitor binding for human dihydrofolate reductase", Chemistry and Biology of Pteridines and Folates, pp 515 - 519, 1993 | |
| | CF** | Gatlin et al. "In Vitro Selection of Lentivirus Vector-Transduced Human CD34+ Cells." Human Gene Therapy 11: 1949-1957 (2000) | |
| | CG** | Genbank NG-000007, priority date 6/19/2006, downloaded 7/24/06 | |
| | CH** | Huang. et al., "Nonadditivity of Mutational Effects at the Folate Binding Site of Escherichia coli Dihydrofolate Reductase", Biochemistry, Vol. 33, No. 38, pp. 11567-11585, 1994 | |
| | CI** | Kalberer et al., Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent extinction of expression of human B-globin in engrafted mice, PNAS, 2000, Pages 5411-5415, Volume 97, Number 10 | |
| | CJ** | May, et al., "Therapeutic haemoglobin synthesis in B-thalassaemic micc expressing lentivirus-encoded human b-globin", Nature, Vol. 406, pp. 82-86, July 6, 2000 | |

| Examiner Signature | | Date Considered | |
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Sheet 3 of 4

| Complete if Known | |
|---|---|
| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | Not Yet Assigned |
| Attorney Docket Number | 64836DIV(51590) |

## 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] |
|---|---|---|---|
| | CK** | May et al., Successful treatment of murine B-thalassemia intermedia by transfer of the human B-globin gene, Blood 2002, Pages 1902-1908, Volume 99, Number 6 | |
| | CL** | Melton et al., Stability of HPRT marker gene expression at different gene-targeted loci: observing and overcoming a position effect, Nucleic Acids Research, 1997, Vol. 25, No. 19 3937-3943. | |
| | CM** | 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. | |
| | CN** | Naldini et al. (1996) Science 272:263-267, :In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector" | |
| | CO** | NG_000007 (GI:13907843), Homo sapiens genomic beta globin region on chomosome 11, U.S. National Library of Medicine, Bethesda, MD, USA, May 2001, accessed by PTO on 3/2/07. | |
| | CP** | Raftopoulos et al., Long-Term Transfer and Expression of the Human B-Globin Gene in a Mouse Transplant Model, Blood, 1997, Pages 3414-3422, Volume 90, Number 9 | |
| | CQ** | Rivella et al., Genetic Treatment of Severe Hemoglobinopathies: The Combat Against Transgene Variegation and Transgene Silencing, Seminars in Hematology, 1998, Pages 112-125, Volume 35, Number 2 | |
| | CR** | Rivella et al. "Globin Gene Transfer: a Paradigm for Transgenic Regulation and Vector Safety." Gene Therapy and Regulation 00:0; 1-27 (2003) | |
| | CS** | Ryan et al., A single erythroid-specific DNAse I super-hypersensitive site activates high levels of human b-globin gene expression in transgenic mice, Genes and Development, Vol 3, pages 314-323, (see entire document). | |
| | CT** | Sabatino et al., Long-term expression of y-globin mRNA in mouse erythrocytes from retrovirus vectors containing the human y-globin gene.., PNAS, 2000, Pages 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. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (06-09)
Approved for use through 07/31/2009. 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 contains a valid OMB control number.

Substitute for form 1449/PTO

# INFORMATION DISCLOSURE STATEMENT BY APPLICANT

*(Use as many sheets as necessary)*

| Complete if Known | |
|---|---|
| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | Not Yet Assigned |
| Attorney Docket Number | 64836DIV(51590) |

Sheet 4 of 4

## 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] |
|---|---|---|---|
| | CU** | 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 B-globin gene" | |
| | CV** | Sadelain "Genetic Treatment of the Haemogloinopathies Recombinations and New Combinations." British Journal of Haematology 98: 247-253 (1997) | |
| | CW** | Sadelain et al. Issues in the Manufacture and Trsnplantation of Genetically Modified Hematopoietic Stem Cells." Current Opinion in Hematology 7: 364-377 (2000) | |
| | CX** | 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. | |
| | CY** | Tisdale et al. "Towards Gene Therapy for Disorders of Golbin Synthesis." Seminars in Hematology 38:4 382-392 (2001) | |
| | CZ** | Zufferey et al., "Self-Inactivating Lentibirus Vector for Safe and Efficient in Vivo Gene Delivery", Journal of Virology, Dec. 1998, Vol. 72, pp. 9873-9880. | |
| | | | |
| | | | |
| | | | |
| | | | |

| 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. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

# Electronic Acknowledgement Receipt

| | |
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| **EFS ID:** | 5671968 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Elbert C. Chiang/Alyson Lucas |
| **Filer Authorized By:** | Elbert C. Chiang |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 09-JUL-2009 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 14:46:51 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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**Warnings:**

**Information:**

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| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| | | | **Total Files Size (in bytes):** | 123931 | |

**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.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PTO/SB/21 (06-09)
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# TRANSMITTAL FORM

*(to be used for all correspondence after initial filing)*

| | |
|---|---|
| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | Not yet Assigned |
| Attorney Docket Number | 64836DIV(51590) |
| Total Number of Pages in This Submission | |

## 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
- [ ] Other Enclosure(s) (please Identify below):

Remarks

## SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

| | | | |
|---|---|---|---|
| Firm Name | EDWARDS ANGELL PALMER & DODGE LLP | | |
| Signature | /Elbert Chiang, Ph.D./ | | |
| Printed name | Elbert Chiang, Ph.D. | | |
| Date | July 9, 2009 | Reg. No. | 60,325 |

Application No. (if known): 12/433,412

Attorney Docket No.: 64836DIV(51590)

# Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with 37 CFR 1.6(a)(4):

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on July 9, 2009 .
Date

/Elbert Chiang, Ph.D./
Signature

Elbert Chiang, Ph.D.
Typed or printed name of person signing Certificate

60,325
Registration Number, if applicable

(617) 517-5502
Telephone Number

Note: Each paper must have its own certificate of mailing.

IDS (Citation) by Applicant (44 References) (4 pages)
Information Disclosure Statement (2 pages)
Transmittal (1 page)

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Michel Sadelain et al.

Application No.: 12/433,412 | Confirmation No.: 9026

Filed: April 30, 2009 | Art Unit: 1633

For: VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES | Examiner: Not Yet Assigned

**INFORMATION DISCLOSURE STATEMENT (IDS)**

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

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 within three months of the U.S. filing date (37 CFR 1.97(b)(1)).

Per 37 CFR 1.98(d), copies of the references cited in the Information Disclosure Statement are not provided herewith because such references were previously cited by or

submitted to the Patent and Trademark Office in patent application Serial No. 10/188,221 to which this application relies upon for an earlier filing date under 35 U.S.C. §120.

In accordance with 37 CFR 1.97(g), the filing of this Supplemental 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 Supplemental 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.

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. 64836DIV(51590).

Dated: July 9, 2009

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Elbert Chiang, Ph.D.
Registration No.: 60,325
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5502
Attorneys/Agents For Applicant

============================================================
Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Anne Corrigan
Timestamp: [year=2009; month=7; day=15; hr=14; min=46; sec=42; ms=71; ]
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**Validated By CRFValidator v 1.0.3**

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Bertino, Joseph
Rivella, Stefano

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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | 1633 | 748 | 64836DIV(51590) | 31 | 3 |

**CONFIRMATION NO. 9026**

65488
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

**UPDATED FILING RECEIPT**

*OC000000036977315*

Date Mailed: 07/24/2009

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Applicant(s)**

Michel Sadelain, New York, NY;
Stefano Rivella, New York, NY;
Chad May, New York, NY;
Joseph Bertino, Branford, CT;

**Assignment For Published Patent Application**

MEMORIAL SLOAN-KETTERING CANCER CENTER, New York City, NY

**Power of Attorney:** The patent practitioners associated with Customer Number 21874

**Domestic Priority data as claimed by applicant**

This application is a DIV of 10/188,221 07/01/2002 PAT 7,541,179
which claims benefit of 60/301,861 06/29/2001
and claims benefit of 60/302,852 07/02/2001

**Foreign Applications**

**If Required, Foreign Filing License Granted:** 05/13/2009

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/433,412**

**Projected Publication Date:** 11/05/2009

**Non-Publication Request:** No

**Early Publication Request:** No

**** SMALL ENTITY ****

**Title**

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

**Preliminary Class**

435

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

## LICENSE FOR FOREIGN FILING UNDER

## Title 35, United States Code, Section 184

## Title 37, Code of Federal Regulations, 5.11 & 5.15

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

**NOT GRANTED**

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
| --- | --- | --- | --- |
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) |

**CONFIRMATION NO. 9026**

65488
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

**PUBLICATION NOTICE**

*OC000000038628227*

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

**Publication No.**US-2009-0274671-A1
**Publication Date:**11/05/2009

## NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
| --- | --- | --- | --- | --- |
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

65488 7590 11/24/2010
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

| EXAMINER | |
| --- | --- |
| MARVICH, MARIA | |

| ART UNIT | PAPER NUMBER |
| --- | --- |
| 1633 | |

| MAIL DATE | DELIVERY MODE |
| --- | --- |
| 11/24/2010 | 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.

PTOL-90A (Rev. 04/07)

| *Office Action Summary* | Application No. | Applicant(s) |
|---|---|---|
| | 12/433,412 | SADELAIN ET AL. |
| | **Examiner** | **Art Unit** |
| | MARIA B. MARVICH | 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 *1* 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 _____.

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) *42-72* 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) *42-72* 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 _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

## DETAILED ACTION

Claims 42-72 are pending in this application and subject to restriction.

### *Election/Restrictions*

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

I. Claims 42-57, drawn to a method for treating a hemoglobinopathy using a Lentiviral vector comprising fragments from a human B-globin LCR operably linked to a functional globin coding sequence, classified in class 435, subclass 455.

II. Claims 58-67, drawn to a vector comprising fragments from a human B-globin LCR operably linked to a functional globin coding sequence, classified in class 435, subclass 320.1.

III. Claims 68-72, drawn to a method for making a therapeutic composition comprising a vector comprising fragments from a human B-globin LCR operably linked to a functional globin coding sequence, classified in class 514, subclass 44.

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

Inventions of Group II and Groups I or III 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 vector of Group II can be used in methods

other than those of Groups I or III. For example, the vector can be used to synthetically prepare globin.

Searching the inventions of either Groups I and III and Group II together would impose serious search burden. The inventions of Groups I and III and II have a separate status in the art as shown by their different classifications. Moreover, in the instant case, the search for the vectors and the method of using the vector are not coextensive. Prior art, which teaches a vector would not necessarily be applicable to the method of using the vector. Moreover, even if the product were known, the method of using the product may be novel and unobvious in view of the preamble or active steps.

Inventions II and III are related as combination and subcombination. Inventions in this relationship are distinct if it can be shown that (1) the combination as claimed does not require the particulars of the subcombination as claimed for patentability, and (2) that the subcombination has utility by itself or in other combinations (MPEP § 806.05(c)). In the instant case, the combination as claimed does not require the particulars of the subcombination as claimed because the method of Group I is directed towards methods of treating hemoglobinopathy which can be performed by direct administration of the vector to the cell. The subcombination has separate utility such as for expression of the globin for purposed of simply producing the globin.

The examiner has required restriction between combination and subcombination inventions. Where applicant elects a subcombination, and claims thereto are subsequently found allowable, any claim(s) depending from or otherwise requiring all the limitations of the allowable subcombination will be examined for patentability in accordance with 37 CFR 1.104.

See MPEP § 821.04(a). Applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

In view of the fact that the combination of Group II does not require the particulars of the subcombination of Group III, examining the combination and subcombination together in a single application would impose a serious burden on the Office. Because the subcombination is narrower in scope, a search of the subcombination would not adequately encompass the combination. Therefore, even if the subcombination were found to be free of the art, an additional search would have to be conducted to determine patentability of the combination. Likewise, because the combination comprises additional process steps not comprised by the subcombination, a finding that the combination, as a whole, is free of the art does not evidence patentability of the subcombination. Furthermore, art reading on the combination might not be applicable to the subcombination because the subcombination is limited to comprising elements that are not recited in the combination. Therefore, patentability of the combination and subcombination must be determined separately.

Because these inventions are distinct for the reasons given above, have acquired a separate status in the art as shown by their different classification, and the search required for each group is not required for the other groups because each group requires a different non-patent literature search due to each group comprising different products and/or method steps, restriction for examination purposes as indicated is proper.

The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provision of MPEP 821.04. Process claims that depend for or otherwise include all the limitations of the patentable produce will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final rejection are governed by 37 CFR 1.116; amendment submitted after allowance are governed by 37 CFR 1.312.

In the event of rejoinder, the requirements for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 USC 101, 101, 103 and 112. Until an elected product claim is found allowable, an otherwise proper restriction between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowed product claim will not be rejoined. See "Guidance on Treatment of Product and Process Claim in light of *In re Ochiai, In re Brouwer* and 35 USC 103(b)," 1184 O.G. 86 (March 26, 1996). Additionally, in order to retain the right to rejoinder in accordance with the above policy, Applicant is advised that the process claims should be amended during prosecution either to maintain dependency on the product claims or to otherwise include the limitations of the product claims. **Failure to do so may result in loss of the right to rejoinder.**

Further, note that the prohibition against double patenting rejections of 35 USC 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP 804.01.

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 request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

## *Conclusion*

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). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Maria B Marvich, PhD
Examiner
Art Unit 1633

/Maria B Marvich/
Primary Examiner, Art Unit 1633

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of: Michel Sadelain, *et al.*

Application No.: 12/433,412

Confirmation No.: 9026

Filed: April 30, 2009

Art Unit: N/A

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

Examiner: Not Yet Assigned

## RESPONSE TO NON-FINAL OFFICE ACTION

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

Applicants submit this paper in response to the non-final Office Action dated November 24, 2010 issued in the above-referenced patent application. Applicants believe that no fees are required for consideration and entry of this paper. Nevertheless, Applicants authorize the Director 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 Deposit Account No. 04-1105, under Order No. 64836DIV(51590).

**The Listing of Claims** begins on page 2 of this paper.

**Remarks** begin on page 8 of this paper.

**LISTING OF THE CLAIMS**

1-41. (Cancelled)

42. (Previously Presented) A method for treating a hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy which comprises

(a) introducing to the mammalian individual a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

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

43. (Previously Presented) The method of claim 42, wherein introducing the recombinant lentiviral vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and returning the transformed cells to the mammalian individual.

44. (Previously Presented) The method of claim 42, wherein said vector further comprises a selectable marker.

45. (Previously Presented) The method of claim 44, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to returning the cells to the mammalian individual.

46. (Previously Presented) The method of claim 45, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase, and wherein the selection

process comprises exposure of the cells to an antifolate.

47. (Previously Presented) The method of claim 46, wherein the antifolate is methotrexate.

48. (Previously Presented) The method of claim 44, wherein the transformed cells are subjected to a selection process *in vivo* after returning the cells to the mammalian individual.

49. (Previously Presented) The method of claim 48, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase, and wherein the selection process comprises administering an antifolate to said individual.

50. (Previously Presented) The method of claim 49, wherein the antifolate is methotrexate.

51. (Previously Presented) The method of claim 42, wherein said functional globin is a mutant globin.

52. (Previously Presented) The method of claim 42, wherein said functional globin is a wild-type globin.

53. (Previously Presented) The method of claim 42, wherein said functional globin is a β-globin.

54. (Previously Presented) The method of claim 53 wherein said functional globin is a human β-globin.

55. (Previously Presented) The method of claim 42, wherein said functional globin is a γ-globin.

56. (Previously Presented) The method of claim 42, wherein said functional globin is an α-globin.

57. (Previously Presented) The method of claim 42, wherein said hemoglobinopathy is β-thalassemia, α- thalassemia or sickle cell anemia.

58. (Previously Presented) Mammalian hematopoietic progenitor cells or stem cells transduced with a recombinant lentiviral vector which comprises a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*.

59. (Previously Presented) The cells of claim 58, wherein the mammalian hematopoietic progenitor or stem cells are human cells.

60. (Previously Presented) The cells of claim 58, wherein said vector further comprises a selectable marker.

61. (Previously Presented) The cells of claim 60, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase.

62. (Previously Presented) The cells of claim 58, wherein said functional globin is a mutant globin.

63. (Previously Presented) The cells of claim 58, wherein said functional globin is a wild-type globin.

64. (Previously Presented) The cells of claim 58, wherein said functional globin is a β-globin.

65. (Previously Presented) The cells of claim 64, wherein said functional globin is a human β-globin.

66. (Previously Presented) The cells of claim 58, wherein said functional globin is a γ-globin.

67. (Previously Presented) The cells of claim 58, wherein said functional globin is an α-globin.

68. (Previously Presented) A method for making a therapeutic composition for treatment of hemoglobinopathy in a mammalian individual which comprises

(a) preparing a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

(b) transducing hematopoietic progenitor or stem cells obtained from the mammalian individual with the recombinant vector.

69. (Previously Presented) The method cell of claim 68, wherein said vector further comprises a selectable marker.

70. (Previously Presented) The method of claim 69, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase.

71. (Previously Presented) The method of claim 70 which comprises performing an *ex vivo* selection using an antifolate.

72. (Previously Presented) The method of claim 68, wherein said functional globin is a human β-globin.

## REMARKS

Claims 42-72 are pending in the application and are subject to restriction and election. No claim amendments are presented herein. Accordingly, claims 42-72 will remain pending in the application.

### *ELECTION/RESTRICTIONS*

The Examiner alleges that the inventions are distinct from each other because the inventions of Group II and Groups I or III are related as product and process of use. Accordingly, the Examiner requires restriction to one of the following inventions under 35 U.S.C. 121:

Group I, claims 42-57, drawn to a method for treating a hemoglobinopathy using a Lentiviral vector comprising fragments from a human β -globin LCR operably linked to a functional globin coding sequence, classified in class 435, subclass 455.

Group II, claims 58-67, drawn to a vector comprising fragments from a human β -globin LCR operably linked to a functional globin coding sequence, classified in class 435, subclass 320.1.

Group III, claims 68-72, drawn to a method for making a therapeutic composition comprising a vector comprising fragments from a human β -globin LCR operably linked to a functional globin coding sequence, classified in class 514, subclass 44.

Applicants provisionally elect, subject to the following traverse, Group II, 58-67, drawn to a vector comprising fragments from a human β -globin LCR operably linked to a functional globin coding sequence, classified in class 435, subclass 320.1.

Applicants understand that because they have elected claims directed to a composition of matter, upon allowance of the composition of matter claims, they will be entitled to rejoinder of method claims that have been amended to be commensurate in scope with the allowed composition of matter claims.

****************************************

Applicants respectfully traverse the requirement for restriction and election and submit that the requirement should be withdrawn.

First, Applicants assert that the various claims represent different embodiments of a single inventive concept for which a single patent should issue. The pending claims

represent an intricate web of knowledge, continuity of effort, and consequences of a single invention, which merit examination of all of the claims in a single application.

More particularly, a single, searchable, unifying aspect links all of the claims. This single, searchable, unifying aspect comprises fragments from a human β-globin LCR operably linked to a functional globin coding sequence.

Second, Applicants submit that a sufficient search and examination with respect to all the claims can be made without serious burden. As MPEP § 803 states:

> If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to independent or distinct inventions.

That is, even if the claims are patentably distinct, the Examiner must still examine the entire application on the merits because doing so will not result in a serious burden.

Applicants submit that the search and examination of all the claims will have substantial overlap, and no serious burden will result from searching and examining all claims in the same application. This is especially true given the single, searchable, unifying aspect that links all of the claims and given the robust and extensive computerized search engines and databases at the Examiner's disposal. At a minimum, this is particularly true with regard to Groups I and II inasmuch as these are both classified in Class 435.

Accordingly, in the interests of efficiency and cost savings to Applicants and the Patent Office, Applicants respectfully request that all the claims be rejoined and examined in the same application. At a minimum, Applicants request that Groups I and II be rejoined.

## CONCLUSION

If a telephone call with Applicants' representative would be helpful to resolve any issues regarding the restriction requirement and/or to otherwise expedite prosecution of the application, Applicants invite the Examiner to contact the undersigned at the telephone number shown below.

Dated: December 27, 2010

Respectfully submitted,

Electronic signature: /Peter C. Lauro/
Peter C. Lauro, Esq.
Registration No.: 32,360
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5509
Attorneys/Agents For Applicants

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 9115315 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Peter C. Lauro |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 27-DEC-2010 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 11:05:53 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Miscellaneous Incoming Letter | 64836DIVCertificateOfElectronicFiling.pdf | 37741<br>3ac0b5af7edc564663b42988e74f33f146f653fb | no | 1 |

**Warnings:**

**Information:**

| 2 | Response to Election / Restriction Filed | 64836DIVResponseToRestrictionRequirement.pdf | 85027<br>2e32f684dc621d771fe1632312d00da60529848e | no | 8 |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 122768 | | |

**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.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Application No. (if known): 12/433,412 Attorney Docket No.: 64836DIV(51590)

# Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with 37 CFR 1.6(a)(4):

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on December 27, 2010 .
Date

/Peter C. Lauro/
Signature

Peter C. Lauro, Esq.
Typed or printed name of person signing Certificate

32,360
Registration Number, if applicable

(617) 517-5509
Telephone Number

Note: Each paper must have its own certificate of mailing or must be listed below.

Response to Restriction Requirement (8 pages)

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. 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: 12/433,412
Filing Date: 04/30/2009
☐ To be Mailed

## APPLICATION AS FILED – PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | SMALL ENTITY ☒ RATE ($) | FEE ($) | OR | OTHER THAN SMALL ENTITY RATE ($) | FEE ($) |
|---|---|---|---|---|---|---|---|
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X $ = | | OR | X $ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 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)) | | | | | | | |
| | | | TOTAL | | | TOTAL | |

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

## APPLICATION AS AMENDED – PART II

| AMENDMENT | 12/27/2010 | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE ($) | ADDITIONAL FEE ($) | OR | OTHER THAN SMALL ENTITY RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total (37 CFR 1.16(i)) | * 31 | Minus | ** 31 | = 0 | X $26 = | 0 | OR | X $ = | |
| | Independent (37 CFR 1.16(h)) | * 3 | Minus | ***3 | = 0 | X $110 = | 0 | 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 | 0 | OR | TOTAL ADD'L FEE | |

| AMENDMENT | | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) | OR | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 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.

Legal Instrument Examiner:
/LAJUAN HICKSON/

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.*

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

65488 7590 01/12/2011

EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

| EXAMINER | |
|---|---|
| MARVICH, MARIA | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1633 | |

| MAIL DATE | DELIVERY MODE |
|---|---|
| 01/12/2011 | 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.

PTOL-90A (Rev. 04/07)

| Office Action Summary | Application No. | Applicant(s) |
|---|---|---|
| | 12/433,412 | SADELAIN ET AL. |
| | Examiner | Art Unit |
| | MARIA B. MARVICH | 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 *27 December 2010*.

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) *42-72* is/are pending in the application.

4a) Of the above claim(s) *42-57 and 68-72* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *58-67* 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 *30 April 2009* 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 *7/9/09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

This office action is in response to an amendment filed 12/27/10. Claims 42-72 are pending.

### Election/Restrictions

Applicant's election with traverse of Group I (claims 58-67) in the reply filed on 12/27/10 is acknowledged. The traversal is on the ground(s) that the claims comprise a single searchable unifying aspect. As well, applicants argue that a search and examination of the claims can be made without a serious search burden especially as Groups I and II are classified in class 435. This is not found persuasive because as to the relatedness of the instant invention by a single unifying aspect, restriction practice based upon a single unifying aspect serves as guidance for lack of unity practice which follows for cases filed under 35 USC 371. The instant case was not filed under 35 USC 371. However, the relatedness of the inventions of has been addressed by in re Ochia. Briefly, Groups I and III are related as product and process of use and in the event that Group II is found allowable, Groups I and III in as much as they read on the elected invention will be rejoined.

As to search burden, searching the inventions of Groups I-III would impose a serious burden. The MPEP teaches, “For purposes of the initial requirement, a serious burden on the examiner may be prima facie shown if the examiner shows by appropriate explanation of separate classification, or separate status in the art, or a different field of search as defined in MPEP § 808.02. That prima facie showing may be rebutted by appropriate showings or evidence by the applicant.” The inventions were determined to

be distinct and to comprise a serious search burden. Specifically, Groups I-III are separately classified. While Groups I and II are in the same class, separate classification relates to the subclass to which the invention falls. Secondly, Group II and III and I have a separate status in the art in that Groups II and I and III are related as product and process of use but are distinct as the vector encompassed by Group II can be used in distinct methods from those of Groups I and III. Inventions I and III (it is noted that the restriction incorrectly lists Groups II and III) are distinct as the combination of Group I is not required for the particulars of the subcombination of Group III. Therefore, the claims represent 3 distinct inventions that have a separate status in the art and are separately classified.

The requirement is still deemed proper and is therefore made FINAL. Claims 42-57 and 68-72 are withdrawn.

**Priority**

In the reference to the prior application inserted, as the first sentence of the specification of this application, the current status of all nonprovisional parent applications referenced should be updated. Specifically, U.S. Serial No. 10/188,221, is now U.S. Patent No. 7,705,503.

**Claim Objections**

Claim 58 is objected to because of the following informalities: claims should commence with an article. Claim 58 as recited is drawn to cells. However, the scope o the claim is not narrowed by reciting --A mammalian hematopoietic progenitor cell or a

stem cell-- as it is clear that by reciting one cell a group of such cells are also encompassed.

In claim 60, the vector does not "comprise" the marker but rather --encodes-- the marker. Appropriate correction is required.

## Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 58-67 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. The term "cells" defined by the specification at the abstract states that the cell can be in vivo. The scope of the claims, therefore encompasses a human being, which is non-statutory subject matter. As such, the recitation of the limitation "non-human" or "isolated" would be remedial. See 1077 O.G. 24, April 21, 1987.

## Claim Rejections - 35 USC § 112, first paragraph

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.

Claim 61 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a vector comprising a mutant dihydrofolate reductase marker having increased resistance to antifolates as compared to wild-type

dhfr, does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

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:

The instant claims are drawn to a mammalian hematopoietic progenitor cell or a stem cell comprising a vector wherein the vector comprises sequences that encode a functional globin by an operably linked 3.2 kb fragment that comprise three regions of the b-globin LCR. Claim 61 recites that the vector further encodes a marker that is a mutant dihydrofolate reductase. By referring to a mutant dhfr, a number of sequences are encompassed. However, the specification is clear that the functional properties of the mutant is an increase in resistance to antifolates. Absent such an indication, the scope of the invention is extremely broad. Given the unpredictability of the art, the poorly developed state of the art with regard to predicting the structural/ functional characteristics of mutants, the lack of adequate working examples and the lack of guidance provided by applicants, the skilled artisan would have to have conducted undue, unpredictable experimentation to practice the claimed invention.

**Double Patenting**

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process ... may obtain a patent therefor ..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See Miller v. Eagle Mfg. Co., 151 U.S. 186 (1894); In re Ockert, 245 F.2d 467, 114 USPQ 330 (CCPA 1957); and In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970).

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 58-67 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-24 of U.S. Patent 7,541,179.

An obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but an examined application claim is not patentably distinct from the reference claims because the examined claim is either anticipated by, or would have been obvious over, the reference claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the cited claims of the instant invention are generic to all that is recited in claims 1-24 of U.S. Patent 7,541,179. That is, the cited claims of U.S. Patent 7,541,179 anticipate and fall entirely within the scope of the rejected claims of the instant application. Specifically, the instant

claims are drawn to cells encompassing the vector of claims 1-24 of U.S. Patent 7,541,179.

Additionally, if a patent resulting from the instant claims was issued and transferred to an assignee different from the assignee holding the U.S. Patent 7,541,179, then two different assignees would hold a patent to the claimed invention of U.S. Patent 7,541,179, and thus improperly there would be possible harassment by multiple assignees.

Claims 58-67 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3, 5, 9-18, 20-28 and 30-31 of U.S. application 12/209913.

An obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but an examined application claim is not patentably distinct from the reference claims because the examined claim is either anticipated by, or would have been obvious over, the reference claims. Although the conflicting claims are not identical, they are not patentably distinct from each other because the cited claims of the instant invention are generic to all that is recited in claims 1-3, 5, 9-18, 20-28 and 30-31 of U.S. application 12/209913. That is, the cited claims of U.S. application 12/209913 anticipate and fall entirely within the scope of the rejected claims of the instant application. Specifically, U.S. application 12/209913 and the instant claims encompass vectors comprising the HS2, 3 and 4 from a human b-globin LCR

Additionally, if a patent resulting from the instant claims was issued and transferred to an assignee different from the assignee holding the U.S. application

12/209913 application, then two different assignees would hold a patent to the claimed invention of U.S. application 12/209913, and thus improperly there would be possible harassment by multiple assignees.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

## Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA B. MARVICH 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). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Maria B Marvich, PhD
Primary Examiner
Art Unit 1633

/Maria B Marvich/
Primary Examiner, Art Unit 1633

| *Notice of References Cited* | Application/Control No.<br>12/433,412 | Applicant(s)/Patent Under Reexamination<br>SADELAIN ET AL. | |
|---|---|---|---|
| | Examiner<br>MARIA B. MARVICH | Art Unit<br>1633 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| | A | US- | | | |
| | B | US- | | | |
| | C | US- | | | |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
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| | J | US- | | | |
| | K | US- | | | |
| | L | US- | | | |
| | M | US- | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Country | Name | Classification |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
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| | U | |
| | V | |
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| | 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 |
|---|---|---|
| | 12433412 | SADELAIN ET AL. |
| | **Examiner** | **Art Unit** |
| | MARIA B MARVICH | 1633 |

## SEARCHED

| Class | Subclass | Date | Examiner |
|---|---|---|---|
| | | | |

## SEARCH NOTES

| Search Notes | Date | Examiner |
|---|---|---|
| East, PALM inventor search | 1/11/11 | MM |
| East databases- USPAT, PGPUB, EPO, JPO, Derwent | 1/11/11 | MM |

## INTERFERENCE SEARCH

| Class | Subclass | Date | Examiner |
|---|---|---|---|
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## EAST Search History

## EAST Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
| --- | --- | --- | --- | --- | --- | --- |
| S127 | 4 | S126 and locus adj control.clm. | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 14:39 |
| S126 | 36 | S122 or S123 or S124 or S125 | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 14:38 |
| S125 | 27 | ((JOSEPH) near2 (BERTINO)).INV. | US-PGPUB; USPAT | OR | ON | 2011/01/10 14:26 |
| S124 | 4 | ((CHAD) near2 (MAY)).INV. | US-PGPUB; USPAT | OR | ON | 2011/01/10 14:25 |
| S123 | 3 | ((STEFANO) near2 (RIVELLA)).INV. | US-PGPUB; USPAT | OR | ON | 2011/01/10 14:07 |
| S122 | 13 | ((MICHEL) near2 (SADELAIN)).INV. | US-PGPUB; USPAT | OR | ON | 2011/01/10 13:53 |
| S121 | 3 | 10/188221 and resistance | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 13:47 |
| S120 | 3 | 10/188221 and mutant | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 13:47 |
| S119 | 3 | 10/188221 and marker | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 13:42 |
| S118 | 1 | 12/433412 and vivo | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 12:09 |

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| S117 | 1 | 12/433412 and cell | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 12:08 |
| S116 | 3 | 10/188221 and functional | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/01/10 11:50 |

**EAST Search History (Interference)**

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# INFORMATION DISCLOSURE STATEMENT BY APPLICANT

*(Use as many sheets as necessary)*

Sheet 1 of 4

| Complete if Known | |
|---|---|
| Application Number | 12/433,412-Conf. #9026 |
| Filing Date | April 30, 2009 |
| First Named Inventor | Michel Sadelain |
| Art Unit | 1633 |
| Examiner Name | Not Yet Assigned |
| Attorney Docket Number | 64836DIV(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 |
|---|---|---|---|---|---|
| | AA* | US-5,126,260 | 06-30-1992 | Tuan et al. | |
| | AB* | US-5,610,053 | 03-11-1997 | Chung et al. | |
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| | AD* | US-5,834,256 | 11-10-1998 | Finer et al. | |
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| 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] |
|---|---|---|---|---|---|---|
| | BA** | WO-97/33988-A1 | 09-18-1997 | Sloan Kettering Inst Cancer et al. | | |
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| Examiner Signature | /Maria Marvich/ | Date Considered | 01/10/2011 |
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*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. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120. [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.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M./

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| Substitute for form 1449/PTO | | | | Complete if Known | |
|---|---|---|---|---|---|
| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** | | | | Application Number | 12/433,412-Conf. #9026 |
| | | | | Filing Date | April 30, 2009 |
| | | | | First Named Inventor | Michel Sadelain |
| | | | | Art Unit | 1633 |
| *(Use as many sheets as necessary)* | | | | Examiner Name | Not Yet Assigned |
| Sheet | 2 | of | 4 | Attorney Docket Number | 64836DIV(51590) |

**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** | 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. | |
| | CB** | 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 | |
| | CC** | Dull et al. (1998) J. Virol. 72:8463-8471, "A Third-Generation Lentivirus Vector with a Conditional Packaging System" | |
| | CD** | Dzierzak et al., Lineage-specific expression of a human B-globin gene in murine bone marrow transplant recipients reconstituded with retrovirus-transduced stem cells., 1988, Pages 35-41, Volume 331 | |
| | CE** | Ercikan et al., "Effect of codon 22 mutations on substrate in inhibitor binding for human dihydrofolate reductase", Chemistry and Biology of Pteridines and Folates, pp 515 - 519, 1993 | |
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| | CH** | Huang. et al., "Nonadditivity of Mutational Effects at the Folate Binding Site of Escherichia coli Dihydrofolate Reductase", Biochemistry, Vol. 33, No. 38, pp. 11567-11585, 1994 | |
| | CI** | Kalberer et al., Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent extinction of expression of human B-globin in engrafted mice, PNAS, 2000, Pages 5411-5415, Volume 97, Number 10 | |
| | CJ** | May, et al., "Therapeutic haemoglobin synthesis in B-thalassaemic micc expressing lentivirus-encoded human b-globin", Nature, Vol. 406, pp. 82-86, July 6, 2000 | |

| Examiner Signature | /Maria Marvich/ | Date Considered | 01/10/2011 |
|---|---|---|---|

*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. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M./

PTO/SB/08b (06-09)
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|---|---|---|---|---|---|
| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** | | | | Application Number | 12/433,412-Conf. #9026 |
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| ***(Use as many sheets as necessary)*** | | | | Examiner Name | Not Yet Assigned |
| Sheet | 3 | of | 4 | Attorney Docket Number | 64836DIV(51590) |

**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] |
|---|---|---|---|
| | CK** | May et al., Successful treatment of murine B-thalassemia intermedia by transfer of the human B-globin gene, Blood 2002, Pages 1902-1908, Volume 99, Number 6 | |
| | CL** | Melton et al., Stability of HPRT marker gene expression at different gene-targeted loci: observing and overcoming a position effect, Nucleic Acids Research, 1997, Vol. 25, No. 19 3937-3943. | |
| | CM** | 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. | |
| | CN** | Naldini et al. (1996) Science 272:263-267, :In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral Vector" | |
| | CO** | NG_000007 (GI:13907843), Homo sapiens genomic beta globin region on chomosome 11, U.S. National Library of Medicine, Bethesda, MD, USA, May 2001, accessed by PTO on 3/2/07. | |
| | CP** | Raftopoulos et al., Long-Term Transfer and Expression of the Human B-Globin Gene in a Mouse Transplant Model, Blood, 1997, Pages 3414-3422, Volume 90, Number 9 | |
| | CQ** | Rivella et al., Genetic Treatment of Severe Hemoglobinopathies: The Combat Against Transgene Variegation and Transgene Silencing, Seminars in Hematology, 1998, Pages 112-125, Volume 35, Number 2 | |
| | CR** | Rivella et al. "Globin Gene Transfer: a Paradigm for Transgenic Regulation and Vector Safety." Gene Therapy and Regulation 00:0; 1-27 (2003) | |
| | CS** | Ryan et al., A single erythroid-specific DNAse I super-hypersensitive site activates high levels of human b-globin gene expression in transgenic mice, Genes and Development, Vol 3, pages 314-323, (see entire document). | |
| | CT** | Sabatino et al., Long-term expression of y-globin mRNA in mouse erythrocytes from retrovirus vectors containing the human y-globin gene.., PNAS, 2000, Pages 13294-13299, Volume 97, Number 24 | |

| Examiner Signature | /Maria Marvich/ | Date Considered | 01/10/2011 |
|---|---|---|---|

*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. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M./

PTO/SB/08b (06-09)
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| Substitute for form 1449/PTO | | | | *Complete if Known* | |
|---|---|---|---|---|---|
| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** | | | | Application Number | 12/433,412-Conf. #9026 |
| | | | | Filing Date | April 30, 2009 |
| | | | | First Named Inventor | Michel Sadelain |
| | | | | Art Unit | 1633 |
| *(Use as many sheets as necessary)* | | | | Examiner Name | Not Yet Assigned |
| Sheet | 4 | of | 4 | Attorney Docket Number | 64836DIV(51590) |

**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] |
|---|---|---|---|
| | CU** | 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 B-globin gene" | |
| | CV** | Sadelain "Genetic Treatment of the Haemogloinopathies Recombinations and New Combinations." British Journal of Haematology 98: 247-253 (1997) | |
| | CW** | Sadelain et al. Issues in the Manufacture and Trsnplantation of Genetically Modified Hematopoietic Stem Cells." Current Opinion in Hematology 7: 364-377 (2000) | |
| | CX** | 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. | |
| | CY** | Tisdale et al. "Towards Gene Therapy for Disorders of Golbin Synthesis." Seminars in Hematology 38:4 382-392 (2001) | |
| | CZ** | Zufferey et al., "Self-Inactivating Lentibirus Vector for Safe and Efficient in Vivo Gene Delivery", Journal of Virology, Dec. 1998, Vol. 72, pp. 9873-9880. | |
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| | | | |
| | | | |

| Examiner Signature | /Maria Marvich/ | Date Considered | 01/10/2011 |
|---|---|---|---|

*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. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /M.M./

Docket No.: 64836DIV(51590)
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of: Michel Sadelain, *et al.*

Application No.: 12/433,412 — Confirmation No.: 9026

Filed: April 30, 2009 — Art Unit: 1633

For: *VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES* — Examiner: Marvich, Maria

## AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

Applicants submit this paper in response to the non-final Office Action dated January 12, 2011 issued in the above-referenced patent application. Applicants believe that no fees are required for consideration and entry of this paper. Nevertheless, Applicants authorize the Director 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 Deposit Account No. 04-1105, under Order No. 64836DIV(51590).

Please amend the application as follows.

**Amendments to the Specification** begin at page 2 of the paper.

**Amendments to the Claims** are reflected in the listing of claims beginning at page 3 of this paper.

**Remarks** begin at page 8 of this paper.

## AMENDMENTS TO THE SPECIFICATION

At page 1, please replace paragraph [0001] following the heading "Statement Concerning Related Applications," with the following amended paragraph:

[0001] This application is a divisional application of U.S. Serial No. 10/188,221, filed July 1, 2002, ~~Issuing~~now U.S. Patent No. 7,541,179, which claims the benefit of U.S. Provisional Application No. 60/301,861 filed Jun. 29, 2001 and U.S. Provisional Application No. 60/302,852 filed Jul. 2, 2001, each of which is incorporated herein by reference.

**AMENDMENTS TO THE CLAIMS**

Please amend claims 44, 46. 49, 58-67, 69 and 70 without prejudice or disclaimer. The following listing of claims will replace all prior versions, and listings, of claims in the application.

1-41. (Cancelled)

42. (Withdrawn) A method for treating a hemoglobinopathy in a mammalian individual suffering from a hemoglobinopathy which comprises

(a) introducing to the mammalian individual a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

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

43. (Withdrawn) The method of claim 42, wherein introducing the recombinant vector is performed by transforming hematopoietic progenitor cells or stem cells with the recombinant vector *ex vivo* and returning the transformed cells to the mammalian individual.

44. (Withdrawn; Currently Amended) The method of claim 42, wherein said vector further comprises a nucleic acid encoding a selectable marker.

45. (Withdrawn) The method of claim 44, wherein the transformed cells are subjected to a selection process to increase the percentage of transformed cells prior to returning the

cells to the mammalian individual.

46. (Withdrawn; Currently Amended) The method of claim 45, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase having increased resistance to antifolates as compared to a wild-type dihydrofolate reductase, and wherein the selection process comprises exposure of the cells to an antifolate.

47. (Withdrawn) The method of claim 46, wherein the antifolate is methotrexate.

48. (Withdrawn) The method of claim 44, wherein the transformed cells are subjected to a selection process *in vivo* after returning the cells to the mammalian individual.

49. (Withdrawn; Currently Amended) The method of claim 48, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase having increased resistance to antifolates as compared to a wild-type dihydrofolate reductase, and wherein the selection process comprises administering an antifolate to said individual.

50. (Withdrawn) The method of claim 49, wherein the antifolate is methotrexate.

51. (Withdrawn) The method of claim 42, wherein said functional globin is a mutant globin.

52. (Withdrawn) The method of claim 42, wherein said functional globin is a wild-type globin.

53. (Withdrawn) The method of claim 42, wherein said functional globin is a β-globin.

54. (Withdrawn) The method of claim 53 wherein said functional globin is a human β-globin.

55. (Withdrawn) The method of claim 42, wherein said functional globin is a γ-globin.

56. (Withdrawn) The method of claim 42, wherein said functional globin is an α-globin.

57. (Withdrawn) The method of claim 42, wherein said hemoglobinopathy is β-thalassemia, α- thalassemia or sickle cell anemia.

58. (Currently Amended) An isolated mammalian~~Mammalian~~ hematopoietic progenitor ~~cells~~ or stem cell~~cells~~ transduced with a recombinant lentivector which comprises a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*.

59. (Currently Amended) The cell~~cells~~ of claim 58, wherein the mammalian hematopoietic progenitor or stem cell~~cells are~~ is a human cell~~cells~~.

60. (Currently Amended) The cell~~cells~~ of claim 58, wherein said vector further comprises a nucleic acid encoding a selectable marker.

61. (Currently Amended) The cell~~cells~~ of claim 60, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase having increased resistance to antifolates as compared to a wild-type dihydrofolate reductase.

62. (Currently Amended) The cell~~cells~~ of claim 58, wherein said functional globin is a mutant globin.

63. (Currently Amended) The cell~~cells~~ of claim 58, wherein said functional globin is a wild-type globin.

64. (Currently Amended) The cell~~cells~~ of claim 58, wherein said functional globin is a β-globin.

65. (Currently Amended) The cell~~cells~~ of claim 64, wherein said functional globin is a human β-globin.

66. (Currently Amended) The cell~~cells~~ of claim 58, wherein said functional globin is a γ-globin.

67. (Currently Amended) The cell~~cells~~ of claim 58, wherein said functional globin is an α-globin.

68 (Withdrawn) A method for making a therapeutic composition for treatment of hemoglobinopathy in a mammalian individual which comprises

(a) preparing a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

(b) obtaining hematopoietic progenitor or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

69. (Withdrawn; Currently Amended) The method ~~cell~~of claim 68, wherein said vector further comprises a nucleic acid encoding a selectable marker.

70. (Withdrawn; Currently Amended) The method of claim 69, wherein the selectable marker is a dihydrofolate reductase or a mutant dihydrofolate reductase having increased resistance to antifolates as compared to a wild-type dihydrofolate reductase.

71. (Withdrawn) The method of claim 70 which comprises performing an *ex vivo* selection using an antifolate.

72. (Withdrawn) The method of claim 68, wherein said functional globin is a human β-globin.

**REMARKS**

Claims 42-72 are pending in the application with claims 42-57 and 68-72 withdrawn from consideration as directed to non-elected subject matter. Claims 44, 46, 49, 58-67, 69 and 70 have been amended without prejudice or disclaimer. Accordingly, claims 42-72 will remain pending in the application.

***Priority***

Page 1 of the specification has been amended to update the priority claim to include the patent number of the now issued parent application. Specifically, the recitation of related application data has been amended to recite that the parent application U.S. Serial No. 10/188,221 is now U.S. Patent No. 7,541,179. Applicants respectfully note that the patent number requested for inclusion by the Examiner as that of the issued parent application was incorrect.

***Claim Objections***

Claim 58 has been objected to because claims should begin with an article. Accordingly, Claim 58 has been amended to recite “an isolated hematopoietic progenitor or stem cell.” The dependent claims have been amended as necessary to provide the proper antecedent basis. Applicants have made these amendments with the understanding that such amendments do not narrow the scope of the claims, as acknowledged by the Examiner at pages 2-3 of the Office Action, inasmuch as “it is clear that by reciting one call a group of cells are also encompassed”. (See Office Action, page 2, last paragraph through page 3, top)

The Examiner has suggested that Claim 60 be amended to state that the vector encodes the selectable marker rather than comprises the selectable marker. To maintain parallel form with Claim 58, which recites that the “vector comprises a nucleic acid encoding . . .”, Applicants have amended Claim 60 to recite that the “vector further comprises a nucleic acid encoding a selectable marker.” Applicants believe this amendment satisfies the Examiner’s objection. Withdrawn Claims 44 and 69 have been similarly amended to facilitate rejoinder upon allowance of the elected composition of matter claims.

***The §101 Rejection***

Claims 58-67 have been rejected under 35 U.S.C. § 101 as allegedly directed to non-statutory subject matter for potentially encompassing a human being when the cells of the invention are *in vivo*. Applicants have amended Claim 58 to recite that the cells are isolated as suggested by the Examiner. Accordingly, this rejection is deemed overcome and withdrawal thereof is respectfully requested.

***The §112, First Paragraph Rejection***

Claim 61 has been rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement with respect to the scope of mutant dihydrofolate reductases (mutant DHFRs) embraced by the claim. In this regard, the Examiner has indicated that the specification is enabling for a mutant DHFR having increased resistance to antifolates as compared to a wild-type DHFR. Applicants respectfully disagree and traverse the rejection.

However, without acquiescing to the rejection and in order to expedite prosecution of the application, claim 61 been amended so it is commensurate in scope with the subject matter which the Examiners finds enabled. Accordingly, Applicants deem this rejection overcome and respectfully request withdrawal thereof. Withdrawn Claims 46, 49 and 70 have been similarly amended to facilitate rejoinder upon allowance of the elected composition of matter claims.

***The First Double Patenting Rejection***

Claims 58-67 have been rejected under the judicially-created doctrine of obviousness-type double patenting over Claims 1-24 of U.S. Patent No. 7,541,179 (hereafter "the '179 patent"). Applicants respectfully disagree and traverse the rejection.

Applicants respectfully submit that this rejection has been made in error because the present application is a divisional application of the '179 patent in which a restriction requirement was made that included a group with claims drawn to the presently elected subject matter. Pursuant to 35 U.S.C. § 121, a "patent issuing on an application with respect to which a requirement for restriction has been made . . . shall not be used as a

reference" in the PTO or in the courts against a properly-filed divisional application of the patent. MPEP § 804.01(B) further provides that the claims in the divisional application must be in consonance with the independent and distinct inventions identified in the parent application.

First, this application was filed on April 30, 2009 before the June 2, 2009 issue date of the '179 patent and is thus a properly-filed divisional application. Second, a restriction requirement in the '179 patent was made on May 5, 2004 and included four groups of patentably distinct subject matter.

Although Group I (Claims 1-18, drawn to a recombinant lentiviral vector) was elected and prosecuted in the '179 patent, the subject matter of non-elected Group IV is being prosecuted in the present divisional application. In this regard, the Group IV claims of the '179 patent were drawn to (a) mammalian hematopoietic progenitor or stem cells transduced with a lentiviral vector of the invention (Claims 30-38) and (b) a method for making a therapeutic composition for treatment of hemoglobinopathy by preparing a lentiviral vector of the invention, obtaining mammalian hematopoietic progenitor or stem cells and transducing those cells with the vector (Claims 39-41). A copy of the restriction requirement in the '179 patent, the claims subject to restriction and Applicants response thereto is attached.

Because the claims presently being examined (Claims 58-57) are directed to mammalian hematopoietic progenitor or stem cells transduced with a lentiviral vector of the invention, the subject matter of the claims is consonant with that of Group IV in the original restriction requirement of the '179 patent. In fact, this divisional application also includes the remaining subject matter of Group IV identified as (b) in the preceding paragraph, namely present Claims 68-72. Hence, the '179 patent cannot be cited against the currently prosecuted claims of the present application.

For these reasons, this first double patenting rejection is improper and should be withdrawn.

***The Second Double Patenting Rejection***

Claims 58-67 have been provisionally rejected under the judicially-created doctrine of obviousness-type double patenting over Claims 1-3, 5, 9-18, 20-28 and 30-31 of U.S. Serial No. 12/209,913 (hereafter “the ‘913 application”).

Applicants ask this provisional rejection be held in abeyance until this case is otherwise in condition for allowance. If the Examiner determines that all other conditions for patentability are satisfied in the present application, Applicants respectfully request that this provisional obviousness-type rejection be withdrawn and the present case allowed to issue, given that the ‘913 application is not yet in condition for allowance and was filed much later than the present application.

## CONCLUSION

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of all rejection and objections and allowance of the application with all the claims presented herein. If a telephone call with Applicants’ representative would be helpful to resolve any remaining issues and/or to otherwise expedite prosecution of the application, Applicants invite the Examiner to contact the undersigned at the telephone number shown below.

Dated: April 12, 2011

Respectfully submitted,

Electronic signature: /Peter C. Lauro/
Peter C. Lauro, Esq.
Registration No.: 32,360
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5509
Attorneys/Agents For Applicants

# Certificate of Electronic Filing Under 37 CFR 1.8

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with 37 CFR 1.6(a)(4):

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on April 12, 2011 .
Date

/Peter C. Lauro/
Signature

Peter C. Lauro, Esq.
Typed or printed name of person signing Certificate

| 32,360 | (617) 517-5509 |
|---|---|
| Registration Number, if applicable | Telephone Number |

Note: Each paper must have its own certificate of mailing or must be listed below.

Amendment and Response to Non-Final Office Action (11 pages)
Copy of Restriction Requirement dated May 5, 2004 issued in parent application and Response Thereto (13 pages)

## 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 | |

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Commissioner for Patents
PO Box 1450
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

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 | 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

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.

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 9865052 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Peter C. Lauro |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 12-APR-2011 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 19:19:26 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Amendment/Req. Reconsideration-After Non-Final Reject | 64836DIV51590_-_Amendment.pdf | 102292<br>a707e9ae385861de0c543561315009da8a3e270c | no | 11 |

**Warnings:**

**Information:**

| 2 | Miscellaneous Incoming Letter | 64836DIV51590_-_CertifElectronFiling.pdf | 37852<br>4d5fb894d717c80705e4779a39f9261519909a0a | no | 1 |
| --- | --- | --- | --- | --- | --- |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Miscellaneous Incoming Letter | 64836DIV51590_-_RespRRFromParent.pdf | 415247<br>92ee182d0d96a9d742ed2e3b987490bc512e8dc1 | no | 13 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 555391 | | |

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**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.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. 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 | Filing Date | |
|---|---|---|
| 12/433,412 | 04/30/2009 | ☐ To be Mailed |

## APPLICATION AS FILED – PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | 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)) | N/A | N/A | N/A | | | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X $ = | | OR | X $ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 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)) | | | | | | | |
| | | | TOTAL | | | TOTAL | |

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

## APPLICATION AS AMENDED – PART II

AMENDMENT

| 04/12/2011 | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | 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)) | * 31 | Minus | ** 31 | = 0 | X $26 = | 0 | OR | X $ = | |
| Independent (37 CFR 1.16(h)) | * 3 | Minus | ***3 | = 0 | X $110 = | 0 | 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 | 0 | OR | TOTAL ADD'L FEE | |

AMENDMENT

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) | OR | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|---|---|---|
| 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.

Legal Instrument Examiner:
/PAUL STANBACK/

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.*

PTO/SB/08b (06-09)
Approved for use through 07/31/2009. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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| Substitute for form 1449/PTO | | | | Complete if Known | |
|---|---|---|---|---|---|
| **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** | | | | Application Number | 12/433,412-Conf. #9026 |
| | | | | Filing Date | April 30, 2009 |
| | | | | First Named Inventor | Michel Sadelain |
| | | | | Art Unit | 1633 |
| *(Use as many sheets as necessary)* | | | | Examiner Name | Maria Marvich |
| Sheet | 1 | of | 1 | Attorney Docket Number | 64836DIV(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] |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |

**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 | Walsh, C., et al., "Regulated High Level Expression of a Human Upsilon-Globin Gene Introduced into Erythroid Cells By an Adeno-Associated Virus Vector" Proceedings of the National Academy of Science (PNAS) 89(15): 7257-7261 (August 1, 1992) | |
| | | | |
| | | | |

| 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. * CITE NO.: Those application(s) which are marked with an single asterisk (*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. ** CITE NO.: Those document(s) which are marked with an double asterisk (**) next to the Cite No. are not supplied because they were previously cited by or submitted to the Office in a prior application relied upon in this application for an earlier filing date under 35 U.S.C. 120. [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.

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 12433412 |
| **Filing Date:** | 30-Apr-2009 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Filer:** | Peter C. Lauro |
| **Attorney Docket Number:** | 64836DIV(51590) |

Filed as Small Entity

## Utility under 35 USC 111(a) 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-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Miscellaneous:** | | | | |
| Submission- Information Disclosure Stmt | 1806 | 1 | 180 | 180 |
| | | **Total in USD ($)** | | **180** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 10358860 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Peter C. Lauro |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 22-JUN-2011 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 10:58:39 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| Submitted with Payment | yes |
|---|---|
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $180 |
| RAM confirmation Number | 8707 |
| Deposit Account | 041105 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Miscellaneous Incoming Letter | 64836DIVCertificateOfElectronicFiling.pdf | 63873<br>a530c87866dc42878468b38df8ab6ba32a058723 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Transmittal Letter | 64836DIVInformationDisclosureStatement.pdf | 205371<br>31c37800868e577a309c528451db5b9027b08c6d | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Information Disclosure Statement (IDS) Form (SB08) | 64836DIVCitationList.pdf | 135030<br>62633fc7035b50b4f0b80d1a78288e6d37f1e399 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 4 | Non Patent Literature | 64836DIVWalshEtAl.pdf | 382682<br>08da59a9f7b94335d7b757e0d55bfe63fbe90e95 | no | 5 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Foreign Reference | 64836DIVSearchReport.pdf | 775096<br>e0f64cc221b31ec56fd51e3796a0d4c1ef06e0e2 | no | 8 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Fee Worksheet (SB06) | fee-info.pdf | 30470<br>765e08c8a25b4e9aa0e05dcfb1584257563b67d5 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 1592522 | | |

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**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.**

**New International Application Filed with the USPTO as a Receiving Office**

**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Application No. (if known): 12/433,412 Attorney Docket No.: 64836DIV(51590)

# Certificate of Electronic Filing Under 37 CFR 1.8

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MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on June 22, 2011
Date

/Peter C. Lauro/
Signature

Peter C. Lauro, Esq.
Typed or printed name of person signing Certificate

32,360
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Information Disclosure Statement (2 pages)
Citation List (1 page)
Copy of Walsh et al. Reference (5 pages)
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Charge $180.00 to Deposit Account No. 04-1105

Docket No.: 64836DIV(51590)
(PATENT)

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Michel Sadelain, *et al.*

Application No.: 12/433,412

Confirmation No.: 9026

Filed: April 30, 2009

Art Unit: 1633

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

Examiner: M. Marvich

## INFORMATION DISCLOSURE STATEMENT (IDS)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir/Madam:

Pursuant to 37 C.F.R. §§1.56, 1.97 and 1.98, Applicants invite the attention of the Patent and Trademark Office to the references listed on the attached PTO/SB/08. Applicants respectfully request 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.

The references listed on the attached PTO/Sb/08 were cited in an European Search Report issued in European patent application no. 10185464.4 that corresponds to the above-referenced patent application.

In accordance with 37 C.F.R. §1.98(a)(2)(ii), Applicants submit herewith a copy of the Walsh et al. non-patent literature reference as well as a copy of the European Search Report. Applicants note that the other three references cited on the Supplementary European Search Report are already of record in the application.

In accordance with 37 C.F.R. §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 C.F.R. §1.56(a) exists.

In accordance with 37 C.F.R. §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.

Applicants submit that the Information Disclosure Statement is in compliance with 37 C.F.R. §1.98 and respectfully request the Examiner to consider the references listed on the attached PTO SB/08.

Applicants submit this Information Disclosure Statement after issuance of a first substantive Office Action on the merits but before the mailing date of any of a final Office Action, a Notice of Allowance or an action that otherwise closes prosecution in the application (37 C.F.R. §1.97(c)). Accordingly, please charge the fee under 35 C.F.R. § 1.17(p) to Deposit Account No. 04-1105.

Applicants believe that no additional fees are required for consideration and entry of this Information Disclosure Statement. Nevertheless, Applicants authorize the Director 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 Deposit Account No. 04-1105, under Order No.64836DIV(51590).

Dated: June 22, 2011

Respectfully submitted,

Electronic signature: /Elbert Chiang, Ph.D./
Peter C. Lauro, Esq.
Registration No.: 32,360
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5509
Attorneys/Agents For Applicants

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

# NOTICE OF ALLOWANCE AND FEE(S) DUE

65488 7590 06/30/2011
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

| EXAMINER | |
|---|---|
| MARVICH, MARIA | |
| ART UNIT | PAPER NUMBER |
| 1633 | |

DATE MAILED: 06/30/2011

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

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

| APPLN. TYPE | SMALL ENTITY | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | YES | $755 | $300 | $0 | $1055 | 09/30/2011 |

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.**

**THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.**

**HOW TO REPLY TO THIS NOTICE:**

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.**

## PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail** **Mail Stop ISSUE FEE**
**Commissioner for Patents**
**P.O. Box 1450**
**Alexandria, Virginia 22313-1450**
**or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

65488 7590 06/30/2011
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

**Certificate of Mailing or Transmission**

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

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

| APPLN. TYPE | SMALL ENTITY | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | YES | $755 | $300 | $0 | $1055 | 09/30/2011 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| MARVICH, MARIA | 1633 | 435-325000 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 ____________________

2 ____________________

3 ____________________

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee
❑ Publication Fee (No small entity discount permitted)
❑ Advance Order - # of Copies ____________________

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.
❑ Payment by credit card. Form PTO-2038 is attached.
❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number ____________ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
❑ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature ____________________ Date ____________________

Typed or printed name ____________________ Registration No. ____________________

This collection of information is required by 37 CFR 1.311. 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, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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.

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. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

65488 7590 06/30/2011
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. BOX 55874
BOSTON, MA 02205

| EXAMINER | |
|---|---|
| MARVICH, MARIA | |
| ART UNIT | PAPER NUMBER |
| 1633 | |

DATE MAILED: 06/30/2011

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 147 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 147 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.



PTOL-85 (Rev. 02/11)

## Privacy Act Statement

**The Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| *Notice of Allowability* | **Application No.**<br>12/433,412 | **Applicant(s)**<br>SADELAIN ET AL. |
|---|---|---|
| | **Examiner**<br>MARIA MARVICH | **Art Unit**<br>1633 |

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to _an amendment filed 4/12/11_.
2. ☒ The allowed claim(s) is/are _58-72_.
3. ☐ 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 the:
   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)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review ( PTO-948) attached
   1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

| | |
|---|---|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☐ Notice of Informal Patent Application |
| 2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948) | 6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ . |
| 3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _6/22/11_ | 7. ☒ Examiner's Amendment/Comment |
| 4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material | 8. ☐ Examiner's Statement of Reasons for Allowance |
| | 9. ☐ Other _____. |

| *Search Notes* | Application/Control No.<br>12433412 | Applicant(s)/Patent Under Reexamination<br>SADELAIN ET AL. |
|---|---|---|
| | Examiner<br>MARIA B MARVICH | Art Unit<br>1633 |

## SEARCHED

| Class | Subclass | Date | Examiner |
|---|---|---|---|
| | | | |

## SEARCH NOTES

| Search Notes | Date | Examiner |
|---|---|---|
| East, PALM inventor search | 1/11/11 | MM |
| East databases- USPAT, PGPUB, EPO, JPO, Derwent | 1/11/11 | MM |
| search updated, sarch notes attached | 6/18/11 | MM |
| merits of claims 68-72 discussed with R. Kelly | 6/18/11 | MM |
| formailities discussed with J. Woitach | 6/18/11 | MM |

## INTERFERENCE SEARCH

| Class | Subclass | Date | Examiner |
|---|---|---|---|
| na | | 6/18/11 | MM |

| *Issue Classification* | Application/Control No. | Applicant(s)/Patent under Reexamination |
|---|---|---|
| | 12/433,412 | SADELAIN ET AL. |
| | **Examiner** | **Art Unit** |
| | MARIA MARVICH | 1633 |

# ISSUE CLASSIFICATION

| ORIGINAL | | INTERNATIONAL CLASSIFICATION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CLASS | SUBCLASS | CLAIMED | | | | | NON-CLAIMED | | | |
| 435 | 325 | C | 12 | N | 5 | /00 | | | | / |
| | | C | 12 | N | 15 | /00 | | | | / |
| | | C | 12 | N | 15 | /00 | | | | / |
| | | C | 12 | N | 15 | /67 | | | | / |
| | | C | 12 | N | 15 | /11 | | | | / |
| | | | | | | / | | | | / |
| | | | | | | / | | | | / |

**CROSS REFERENCES**

| CLASS | SUBCLASS (ONE SUBCLASS PER BLOCK) | | | |
|---|---|---|---|---|
| 435 | 326 | 320.1 | 69.1 | |
| 536 | 24.1 | 24.2 | | |
| | | | | |
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| | | |
|---|---|---|
| (Assistant Examiner) (Date) | /Maria Marvich/ 6/18/11 | **Total Claims Allowed: 15** |
| (Legal Instruments Examiner) (Date) | (Primary Examiner) (Date) | O.G. Print Claim(s): 58 — O.G. Print Fig.: no |

☒ **Claims renumbered in the same order as presented by applicant** ☐ CPA ☐ T.D. ☐ R.1.47

| Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 31 | | 61 | | 91 | | 121 | | 151 | | 181 |
| | 2 | | 32 | | 62 | | 92 | | 122 | | 152 | | 182 |
| | 3 | | 33 | | 63 | | 93 | | 123 | | 153 | | 183 |
| | 4 | | 34 | | 64 | | 94 | | 124 | | 154 | | 184 |
| | 5 | | 35 | | 65 | | 95 | | 125 | | 155 | | 185 |
| | 6 | | 36 | | 66 | | 96 | | 126 | | 156 | | 186 |
| | 7 | | 37 | | 67 | | 97 | | 127 | | 157 | | 187 |
| | 8 | | 38 | | 68 | | 98 | | 128 | | 158 | | 188 |
| | 9 | | 39 | | 69 | | 99 | | 129 | | 159 | | 189 |
| | 10 | | 40 | | 70 | | 100 | | 130 | | 160 | | 190 |
| | 11 | | 41 | | 71 | | 101 | | 131 | | 161 | | 191 |
| | 12 | | 42 | | 72 | | 102 | | 132 | | 162 | | 192 |
| | 13 | | 43 | | 73 | | 103 | | 133 | | 163 | | 193 |
| | 14 | | 44 | | 74 | | 104 | | 134 | | 164 | | 194 |
| | 15 | | 45 | | 75 | | 105 | | 135 | | 165 | | 195 |
| | 16 | | 46 | | 76 | | 106 | | 136 | | 166 | | 196 |
| | 17 | | 47 | | 77 | | 107 | | 137 | | 167 | | 197 |
| | 18 | | 48 | | 78 | | 108 | | 138 | | 168 | | 198 |
| | 19 | | 49 | | 79 | | 109 | | 139 | | 169 | | 199 |
| | 20 | | 50 | | 80 | | 110 | | 140 | | 170 | | 200 |
| | 21 | | 51 | | 81 | | 111 | | 141 | | 171 | | 201 |
| | 22 | | 52 | | 82 | | 112 | | 142 | | 172 | | 202 |
| | 23 | | 53 | | 83 | | 113 | | 143 | | 173 | | 203 |
| | 24 | | 54 | | 84 | | 114 | | 144 | | 174 | | 204 |
| | 25 | | 55 | | 85 | | 115 | | 145 | | 175 | | 205 |
| | 26 | | 56 | | 86 | | 116 | | 146 | | 176 | | 206 |
| | 27 | | 57 | | 87 | | 117 | | 147 | | 177 | | 207 |
| | 28 | | 58 | | 88 | | 118 | | 148 | | 178 | | 208 |
| | 29 | | 59 | | 89 | | 119 | | 149 | | 179 | | 209 |
| | 30 | | 60 | | 90 | | 120 | | 150 | | 180 | | 210 |

## DETAILED ACTION

Claims 42-72 are pending in this application.

It is noted that the clean copy of the specification filed 7/9/09 contains markings that should be removed. A clean copy without the markings is required in response to this notice.

### *Information Disclosure Statement*

An information disclosure statement filed 6/22/11 has been identified and the documents considered. The corresponding signed and initialed PTO Form 1449 has been mailed with this action.

## EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with.

The application has been amended as follows:

IN THE CLAIMS:

Claims 58-67 are directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(b), claims 68-72, directed to the process of making or using the allowable product, previously withdrawn from consideration as a result of a restriction requirement, is

hereby rejoined and fully examined for patentability under 37 CFR 1.104. Claims 42-57 directed to the invention(s) of unelected subject matter relating to a method for treating hemoglobinopathy do not require all the limitations of an allowable product claim, and have NOT been rejoined.

Because a claimed invention previously withdrawn from consideration under 37 CFR 1.142 has been rejoined, **the restriction requirement between groups II and III as set forth in the Office action mailed on 11/24/10 is hereby withdrawn**. In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Cancel claims 42-57.

Claims 58. (Currently Amended) An isolated mammalian hematopoietic progenitor cell or an isolated mammalian stem cell ~~transduced with~~ comprising a recombinant lentiviral vector ~~lentivector~~ which comprises a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide

fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*.

Claim 59. (Currently Amended) The cell of claim 58, wherein the mammalian hematopoietic progenitor cell or the stem cell is a human cell.

Claim 65. (Currently Amended) The cell of claim 64, wherein said ~~functional~~β-globin is a human β-globin.

Claim 68. (Currently Amended) A method for making a mammalian hematopoietic progenitor cell or a mammalian stem cell ~~therapeutic~~ composition ~~for treatment of hemoglobinopathy in a mammalian individual~~ which comprises

(a) preparing a recombinant lentiviral vector comprising a nucleic acid encoding a functional globin operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human ß-globin locus control region (LCR), the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR, a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR, and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR, said vector providing expression of the globin in a mammal *in vivo*; and

(b) obtaining hematopoietic progenitor cells or stem cells from the mammalian individual, and transducing the cells with the recombinant vector.

Claim 71. (Currently Amended) The method of claim 70, which further comprises performing ~~an *ex vivo*~~ selection using an antifolate on the transduced cell.

***Conclusion***

The above amendment has been made in claim 58, line 1 to provide adequate reference to the two cell cells. Secondly, the recitation that the cells are "transduced" implies a method step, to keep in line with the composition this has been amended to recite, "comprising". Thirdly, the term "lentivector" is not supported by the specification and hence the metes and bounds of this term are not clear. The article in claim 59 is consistent with the separate nature of the cells as set forth in the amendment to claim 58. For simplicity and more direct antecedent basis, claim 65 has been amended to refer to the limitation as recited in claim 64.

Claim 68 has been amended to delete reference to intended use to place the claim in condition for allowance. The composition has a number of uses such as for expression of globin. And, in line with this, claim 71 has been amended to delete reference to "ex vivo" as the methods do not involve *in vivo* method steps.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA MARVICH 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.

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Maria B Marvich, PhD
Primary Examiner
Art Unit 1633

/Maria B Marvich/
Primary Examiner, Art Unit 1633

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**CONFIRMATION NO. 9026**

| SERIAL NUMBER | FILING or 371(c) DATE | CLASS | GROUP ART UNIT | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009<br>RULE | 435 | 1633 | 64836DIV(51590) |

**APPLICANTS**

Michel Sadelain, New York, NY;
Stefano Rivella, New York, NY;
Chad May, New York, NY;
Joseph Bertino, Branford, CT;

** **CONTINUING DATA** *************************

This application is a DIV of 10/188,221 07/01/2002 PAT 7,541,179
which claims benefit of 60/301,861 06/29/2001
and claims benefit of 60/302,852 07/02/2001

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| Foreign Priority claimed ☐ Yes ☑ No<br>35 USC 119(a-d) conditions met ☐ Yes ☐ No<br>Verified and Acknowledged /MARIA MARVICH/ Examiner's Signature | ☐ Met after Allowance<br>Initials | STATE OR COUNTRY | SHEETS DRAWINGS | TOTAL CLAIMS | INDEPENDENT CLAIMS |
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| | | NY | 4 | 31 | 3 |

**ADDRESS**

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P.O. BOX 55874
BOSTON, MA 02205
UNITED STATES

**TITLE**

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

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| | | | | Art Unit | 1633 |
| *(Use as many sheets as necessary)* | | | | Examiner Name | Not Yet Assigned |
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| | | | | First Named Inventor | Michel Sadelain |
| | | | | Art Unit | 1633 |
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## EAST Search History

## EAST Search History (Prior Art)

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| S147 | 4 | hemoglobinopathy.clm. and globin.clm. | USPAT | OR | OFF | 2011/06/14 13:06 |
| S146 | 17 | hemoglobinopathy.clm. | USPAT | OR | OFF | 2011/06/14 13:02 |
| S145 | 103 | hemoglobinopathy | USPAT | OR | OFF | 2011/06/14 13:02 |
| S144 | 1 | 10/188221 and hemoglobinopathy | USPAT | OR | OFF | 2011/06/14 12:56 |
| S143 | 0 | 12/433412 and hemoglobinopathy | USPAT | OR | OFF | 2011/06/14 12:55 |
| S142 | 103 | 12/433412 hemoglobinopathy | USPAT | OR | OFF | 2011/06/14 12:55 |
| S141 | 0 | 12/433412 | USPAT | OR | OFF | 2011/06/14 12:54 |
| S140 | 1 | 12/433412 and lentivirus same vector | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/06/09 13:42 |
| S139 | 0 | 12/433412 and lentivirus adj vector | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/06/09 13:41 |
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| S137 | 1 | 12/433412 and vector | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/06/09 13:41 |
| S136 | 1 | 12/433412 and lentivirus | US-PGPUB; USPAT; EPO; JPO; DERWENT; IBM_TDB | OR | ON | 2011/06/09 13:41 |
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| S131 | 28 | ((JOSEPH) near2 (BERTINO)).INV. | US-PGPUB; USPAT | OR | ON | 2011/06/09 08:30 |
| S130 | 4 | ((CHAD) near2 (MAY)).INV. | US-PGPUB; USPAT | OR | ON | 2011/06/09 08:30 |
| S129 | 3 | ((STEFANO) near2 (RIVELLA)).INV. | US-PGPUB; USPAT | OR | ON | 2011/06/09 08:30 |
| S128 | 13 | ((MICHEL) near2 (SADELAIN)).INV. | US-PGPUB; USPAT | OR | ON | 2011/06/09 08:30 |

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**Alexandria, Virginia 22313-1450**
**or Fax (571) 273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

65488
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 12/433,412 | 04/30/2009 | Michel Sadelain | 64836DIV(51590) | 9026 |

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

| APPLN. TYPE | SMALL ENTITY | ISSUE FEE | PUBLICATION FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|
| nonprovisional | YES | $870.00 | $300.00 | $1,185.00 | 09/30/2011 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| MARVICH, MARIA | 1633 | 435-325000 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Edwards Angell Palmer & Dodge LLP
2 Peter C. Lauro, Esq.
3 Melissa Hunter-Ensor, Ph.D., Esq.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Memorial Sloan-Kettering Cancer Center

(B) RESIDENCE: (CITY and STATE OR COUNTRY): New York City, NY

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are enclosed:

☒ Issue Fee

☒ Publication Fee (No small entity discount permitted)

☒ Advance Order -# of Copies 5

4b. Payment of Fee(s):

☐ A check in the amount of the fee(s) is enclosed.

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5. **Change in Entity Status** (from status indicated above)

☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.
NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature /Peter C. Lauro/ Date September 30, 2011

Typed or printed name Peter C. Lauro, Esq. Registration No. 32,360

PTOL-85 (Rev. 08/08) Approved for use through 08/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 12433412 |
| **Filing Date:** | 30-Apr-2009 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Filer:** | Peter C. Lauro/Teresa Lauro |
| **Attorney Docket Number:** | 64836DIV(51590) |

Filed as Small Entity

## Utility under 35 USC 111(a) 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-Issuance:** | | | | |
| Utility Appl issue fee | 2501 | 1 | 870 | 870 |
| Publ. Fee- early, voluntary, or normal | 1504 | 1 | 300 | 300 |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| Printed copy of patent - no color | 8001 | 5 | 3 | 15 |
| | | **Total in USD ($)** | | **1185** |

## Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 11086142 |
| **Application Number:** | 12433412 |
| **International Application Number:** | |
| **Confirmation Number:** | 9026 |
| **Title of Invention:** | VECTOR ENCODING HUMAN GLOBIN GENE AND USE THEREOF IN TREATMENT OF HEMOGLOBINOPATHIES |
| **First Named Inventor/Applicant Name:** | Michel Sadelain |
| **Customer Number:** | 65488 |
| **Filer:** | Peter C. Lauro/Teresa Lauro |
| **Filer Authorized By:** | Peter C. Lauro |
| **Attorney Docket Number:** | 64836DIV(51590) |
| **Receipt Date:** | 30-SEP-2011 |
| **Filing Date:** | 30-APR-2009 |
| **Time Stamp:** | 12:55:34 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $1185 |
| RAM confirmation Number | 1989 |
| Deposit Account | 041105 |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Miscellaneous Incoming Letter | 64836DIVCertificateofElectronicFiling.pdf | 60782<br>2ed21e9cd873e8fd0d2b05d6f01ec8fa14f6c69b | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Issue Fee Payment (PTO-85B) | 64836DIVIssueFeeTransmittal.pdf | 164172<br>88992cd1bccf7fc1abb30ab5eca463ca573a7049 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 33623<br>cf999588f167b40c2714729d0e8075be8e4ca0ed | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 258577 | |

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**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.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Application No. (if known): 12/433,412 Attorney Docket No.: 64836DIV(51590)

# Certificate of Electronic Filing Under 37 C.F.R. § 1.8

I hereby certify that this correspondence is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4):

Commissioner for Patents
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Alexandria, VA 22313-1450

on September 30, 2011
Date

/Peter C. Lauro/
Signature

Peter C. Lauro, Esq.
Typed or printed name of person signing Certificate

32,360
Registration Number, if applicable

(617) 517-5509
Telephone Number

Note: Each paper must have its own certificate of mailing or must be listed below.

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Charge $1,185.00 to deposit account 04-1105

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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. | ISSUE DATE | PATENT NO. | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 12/433,412 | 11/15/2011 | 8058061 | 64836DIV(51590) | 9026 |

65488 7590 10/26/2011

EDWARDS WILDMAN PALMER LLP
P.O. BOX 55874
BOSTON, MA 02205

# ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 147 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Michel Sadelain, New York, NY;
Stefano Rivella, New York, NY;
Chad May, New York, NY;
Joseph Bertino, Branford, CT;

IR103 (Rev. 10/09)

AO 120 (Rev. 08/10)

| TO: **Mail Stop 8**<br>**Director of the U.S. Patent and Trademark Office**<br>**P.O. Box 1450**<br>**Alexandria, VA 22313-1450** | **REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK** |
|---|---|

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the Southern District of New York on the following

☐ Trademarks or ☒ Patents ( ☐ the patent action involves 35 U.S.C. § 292.):

| DOCKET NO | DATE FILED | U.S. DISTRICT COURT |
|---|---|---|
| 1:21-cv-08206-VSB | 10/5/2021 | for the Southern District of New York |

| PLAINTIFF | DEFENDANT |
|---|---|
| Errant Gene Therapeutics, LLC | Memorial Sloan-Kettering Cancer Center and Sloan Kettering Institute of Cancer Research |

| PATENT OR TRADEMARK NO. | DATE OF PATENT OR TRADEMARK | HOLDER OF PATENT OR TRADEMARK |
|---|---|---|
| 1 7,541,179 | 06/02/2009 | Memorial Sloan-Kettering Cancer Center |
| 2 8,058,061 | 11/15/2011 | Memorial Sloan-Kettering Cancer Center |
| 3 | | |
| 4 | | |
| 5 | | |

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

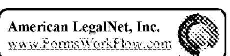
| DATE INCLUDED | INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading | |
|---|---|---|
| **PATENT OR TRADEMARK NO.** | **DATE OF PATENT OR TRADEMARK** | **HOLDER OF PATENT OR TRADEMARK** |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above—entitled case, the following decision has been rendered or judgement issued:

| DECISION/JUDGEMENT |
|---|
| |

| CLERK | (BY) DEPUTY CLERK | DATE |
|---|---|---|
| Ruby J. Krajick | /S/ S. James | 10/06/2021 |

**Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director**
**Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy**



AO 120 (Rev. 08/10)

| TO: **Mail Stop 8<br>Director of the U.S. Patent and Trademark Office<br>P.O. Box 1450<br>Alexandria, VA 22313-1450** | **REPORT ON THE<br>FILING OR DETERMINATION OF AN<br>ACTION REGARDING A PATENT OR<br>TRADEMARK** |
|---|---|

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. ( ☐ the patent action involves 35 U.S.C. § 292.):

| DOCKET NO. | DATE FILED<br>10.21.2021 | U.S. DISTRICT COURT<br>for the District of Delaware |
|---|---|---|
| PLAINTIFF<br>ERRANT GENE THERAPEUTICS, LLC | | DEFENDANT<br>BLUEBIRD BIO, INC. |

| PATENT OR TRADEMARK NO. | DATE OF PATENT OR TRADEMARK | HOLDER OF PATENT OR TRADEMARK |
|---|---|---|
| 1 7,541,179 B2 | 6/2/2009 | Memorial Sloan-Kettering Cancer Center |
| 2 8,058,061 B2 | 11/15/2011 | Memorial Sloan-Kettering Cancer Center |
| 3 | | |
| 4 | | |
| 5 | | |

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

| DATE INCLUDED | INCLUDED BY<br>☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading | |
|---|---|---|
| PATENT OR TRADEMARK NO. | DATE OF PATENT OR TRADEMARK | HOLDER OF PATENT OR TRADEMARK |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above—entitled case, the following decision has been rendered or judgement issued:

| DECISION/JUDGEMENT |
|---|
| |

| CLERK | (BY) DEPUTY CLERK | DATE |
|---|---|---|
| | | |

**Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director**
**Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy**