

Gene therapy for primary adaptive immune deficiencies

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Gene therapy has become an option for the treatment of 2 forms of severe combined immunodeficiency (SCID): X-linked SCID and adenosine deaminase deficiency. The results of clinical trials initiated more than 10 years ago testify to sustained and reproducible correction of the underlying T-cell immunodeficiency. Successful treatment is based on the selective advantage conferred on T-cell precursors through their expression of the therapeutic transgene. However, “first-generation” retroviral vectors also caused leukemia in some patients with X-linked SCID because of the constructs’ tendency to insert into active genes (eg, proto-oncogenes) in progenitor cells and transactivate an oncogene through a viral element in the long terminal repeat. These elements have been deleted from the vectors now in use. Together with the use of lentiviral vectors (which are more potent for transducing stem cells), these advances should provide a basis for the safe and effective extension of gene therapy’s indications in the field of primary immunodeficiencies. Nevertheless, this extension will have to be proved by examining the results of the ongoing clinical trials. (*J Allergy Clin Immunol* 2011;127:1356-9.)

Key words: Gene therapy, severe combined immune deficiency, retrovirus, lentivirus, Wiskott-Aldrich syndrome

There are many reasons that gene therapy has been developed in the field of primary immunodeficiencies (PIDs) over the last 20 years. Many PIDs are life-threatening conditions, notably severe combined immunodeficiencies (SCIDs) affecting T-cell development and function, Wiskott-Aldrich syndrome (WAS), hemophagocytic lymphohistiocytosis, innate immune deficiencies (eg, chronic granulomatous disease or Mendelian susceptibility to mycobacterial disease), and inherited autoimmune syndromes. The remarkable progress in treating PIDs has mostly been based on allogeneic hematopoietic stem cell transplantation (HSCT).¹ However, this approach is far from perfect, and serious adverse events (SAEs) can still occur (eg, graft-versus-host disease). In particular, graft-versus-host disease can damage the thymus and compromise the reconstitution of T-cell immunity. The limitations of HSCT are necessarily more pronounced in patients who lack HLA-compatible donors. Conversely, the success of HSCT

Abbreviations used

ADA:	Adenosine deaminase
HSCT:	Hematopoietic stem cell transplantation
LMO-2:	LIM domain only 2
LTR:	Long terminal repeat
NK:	Natural killer
PID:	Primary immunodeficiency
SAE:	Serious adverse event
SCID:	Severe combined immunodeficiency
SCID-X1:	X-linked severe combined immunodeficiency
SIN:	Self-inactivating
TCR:	T-cell receptor
WAS:	Wiskott-Aldrich syndrome
WASP:	Wiskott-Aldrich syndrome protein

provides a rational basis for the autotransplantation of transduced stem cells, the current approach in gene therapy for PIDs. Most PIDs display Mendelian inheritance, so that introduction of a normal copy of the mutated gene into the patient’s cells should (in principle) be effective. The fact that disease-related genes have now been found for most PIDs² makes gene therapy a feasible approach for many of these conditions.

For some PIDs (eg, T-cell immunodeficiencies), it has become clear that transduced precursor cells can have a selective growth advantage. In several T-cell PIDs, the occurrence of somatic mutations positively modifies the mutated genes and leads to the development of functional T cells; the observed attenuation of disease phenotypes strongly supports this concept. This growth advantage is based on (1) the tremendous ability of T-cell precursors in the thymus to divide in an IL-7–dependent manner and after expression of the pre-T-cell receptor (pre-TCR), (2) positive selection, and (3) the very long lifespan of mature T cells. One can thus expect a few transduced T-cell precursors to give rise to a full, stable T-cell pool in a given subject. Hence SCID is considered to be an optimal model for assessing the feasibility of gene therapy.

GENE TRANSFER TECHNOLOGY

In the meantime significant advances in viral vector technology have enabled the transduction of dividing cells and thus replication of the transgene in progeny cells. Replication-defective retroviral vectors have been based on murine oncoretroviruses (the γ retrovirus), simian and human lentiviral viruses, spumaviruses, and transposons.³ These vectors are generated in packaging cell lines transfected with (1) vector constructs containing sequences required for genome integration, the encapsidation sequence, the gene of interest, and various regulatory sequences and (2) constructs encoding the viral genes provided in trans to build replication-incompetent viral particles. A key advance was the creation of self-inactivating (SIN) viruses in which the absence of enhancer elements in their long terminal repeats (LTRs) makes them less able to transactivate endogenous genes after genome integration (see below).⁴ In the absence of enhancers,

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TABLE I. T-cell primary immunodeficiencies: gene therapy trials

	Trial period	No.	Results	Trial status
SCID-X1 (Paris)	1999-2002	10	Eight alive, 4 SAEs	Closed
SCID-X1 (London)	2002-2009	10	Ten alive, 1 SAE	Closed
SCID-X1 (France/ UK/US)	2010-present	3	Three alive	Open
SCID-ADA (Milan)	2000-2010	15	Fifteen alive, 13 off ERT	Open
SCID-ADA (London)	2004-2010	7	Seven alive, 3 off ERT	Open
SCID-ADA (US: 2 trials)	2007-2010	9	Nine alive, 5 off ERT	Open
WAS (Hanover)	2007-2010	10	Ten alive, 1 SAE	Closed
WAS (Milan)	2010-present	2	Alive	Open
WAS (France/ UK/US)	2010-present	2	Alive	Open

ERT, Enzyme replacement therapy; SCID-X1, X-linked severe combined immunodeficiency; UK, United Kingdom; US, United States.

several internal promoters can be used to drive transgene transcription. Culture conditions for the transduction of hematopoietic progenitor cells have been improved by selecting the best cytokine cocktails and promoting virus/cell interaction through the addition of fibronectin fragments.

GENE THERAPY IN PATIENTS WITH SCIDS

After the advent of this vector technology, clinical trials were successfully initiated for typical X-linked SCID (SCID-X1; γ c deficiency) in 1999 and then adenosine deaminase (ADA) deficiency. To date, gene therapy results are available for 20 patients with a typical SCID-X1, 5 patients with atypical SCIDs, and 31 patients with ADA deficiency (Table I).⁵⁻¹⁰

The SCID-X1 trials were associated with clinical events caused by vector genotoxicity and shall be discussed first.

Genotoxicity in SCID-X1 trials

Five of the 20 patients (4 in the Paris trial and 1 in the London trial) had T-cell leukemia 2 to 5.5 years after gene therapy.^{10,11} After chemotherapy, 4 patients survived and showed sustained remission and T-cell immunity (see below).⁷ One patient died from refractory leukemia.¹⁰ In all cases it was found that the abnormal clone had 1 or 2 provirus integrations within a proto-oncogene locus. Many other genomic abnormalities were found.^{10,11} Accordingly, the clinical trials were discontinued. Considerable effort was then devoted to investigating the mechanism underlying these SAEs. It was clearly shown that retroviruses do preferentially integrate within genes, especially actively transcribed genes. Epigenetic signatures that favor retroviral integration have been recently identified.^{12,13} It turned out that the LIM domain only 2 (*LMO2*) locus in hematopoietic progenitors contains several of the features that favor frequent local integration. In parallel it became clear that the viral LTRs' enhancer activity could permanently turn on transcription of the target gene and thus trigger the leukemic process.^{10,11} It is noteworthy that despite the use of a similar gene transfer technology in the ADA trials, none of the successfully treated patients (n = 21) had leukemia, a result that significantly differs from that of the SCID-X1 trials.¹⁴ These findings strongly suggest that 1 or more disease-associated factors

interfere with retroviral integration, such as the nature of progenitor cells in the bone marrow above the differentiation block, the possibly convergent effects of transgene and oncogene expression, and an inadequate *in vivo* milieu for cell growth (because of the inhibitory effects of purine accumulation in patients with ADA deficiency). The fact that a similar, *LMO2*-associated leukemic event was recently observed in a patient with WAS treated with *ex vivo* retrovirally mediated gene transfer into CD34 cells also indicates that the ADA deficiency setting should be regarded as unfavorable for the occurrence of leukemia.

Researchers have made huge efforts to construct safer vectors with the development of enhancer-deleted LTR-SIN vectors containing an internal promoter. This type of vector has been shown to be less genotoxic in *in vitro* assays of the clonogenicity of myeloid precursors.^{4,15,16} Despite efforts to set up predictive *in vivo* assays in murine models, an absolute demonstration of safety can only be provided by the ongoing, recently initiated clinical trials. Furthermore, use of insulators (for functional isolation of the integrated provirus from the genomic environment) and addition of a suicide gene might be useful. Nevertheless, these measures will probably be only partially effective and have their own pitfalls. The use of HIV-derived lentiviral vectors might constitute an additional safeguard because this type of vector only integrates into genes (and not upstream of the transcription start site). This advantage might, however, be counterbalanced by greater transduction efficacy and thus more frequent vector integration into the patient's cells. Other potential improvements for the future include gene targeting to neutral ("safe harbor") genome regions and gene repair with target-specific nucleases.^{17,18}

Efficacy in the SCID-X1 trial

At present, 18 of 20 patients with SCID-X1 treated in the Paris/London trials are alive 3 to 11.5 years after treatment (median, 8.2 years). Seventeen patients show the sustained presence of transduced lymphocytes.⁵⁻⁷ Blood T-cell counts are in the normal or close-to-normal range, whereas phenotype and functional characteristics are also satisfactory. Remarkably, most patients (including the 4 who received chemotherapy) have some naive T cells, strongly suggesting the presence of ongoing, long-term thymopoiesis from transduced progenitor cells.

Gene therapy based on the development of T-cell immunity provided clear-cut clinical benefits to these patients because they can now deal normally with infections and are doing well in the absence of any therapy (apart from immunoglobulin substitution in some cases, see below). Long-term natural killer (NK) cell reconstitution is not as impressive, with only a few such cells in their blood (as is also observed after allogeneic HSCT in the absence of myeloablative conditioning). These results suggest that NK cell dynamics (precursor expansion, progeny lifespan, or both) differ significantly from T-cell dynamics. The patients' B-cell functions have been partially restored, despite very low (and decreasing) transduced B-lymphocyte counts. Accordingly, approximately half of the patients do not require immunoglobulin substitution. This observation might be due to (1) competition with normal B-cell development in the absence of γ c expansion/function and (2) B-cell dynamics. It might well be of value to establish whether plasma cells in the bone marrow express γ c. Thanks to the development of novel methods and technologies (eg, ligation-mediated PCR with multiple restriction enzymes and deep sequencing), a wealth of information has been provided through

the in-depth analysis of retroviral integration sites in the patients' cell populations. For example, it has been shown that the patients' T cells originate from as few as 1000 transduced progenitor cells. Given that the T cells display significant diversity in a TCR repertoire analysis, one can deduce that these few cells have divided extensively (thanks to the γ c receptor) before TCR rearrangement in the thymus. This finding validates the selective advantage concept on which gene therapy for SCID was launched.^{5-7,10,11}

It has also been noted that there is considerable variation over time in the abundance of clones, with no evidence for long-term selection. Furthermore, there are significant changes over time in the clonal composition of peripheral T cells; this could (as least in part) be explained by nonexhaustive detection of the more rarely represented clones at a certain time point, by unexpected variations in the immune system's use of T-cell progenitors, or both.

Lastly, although detection of the same integration sites in T cells and myeloid cells soon after treatment demonstrated that at least some multipotent hematopoietic progenitors had been transduced, only transduced T cells (including naive T cells) are found 8 to 11 years after gene therapy. The latter result suggests that some T-cell precursors do persist, perhaps in the thymus.

In addition to these 20 patients treated soon after diagnosis of a typical SCID-X1 early in life, 5 other patients were treated later in life (at between 10 and 20 years of age) because of either an atypical SCID-X1 caused by hypomorphic mutation or poorly reconstituted T-cell immunity years after HSCT. Despite technically efficient gene transfer, the results have been disappointing, with little or no improvement in T-cell immunity.¹⁹ Defective residual thymic function at a later age in patients with SCID very probably accounts for these failures and raises the question of how long the thymus remains potentially functional in a patient lacking effective thymopoiesis.

GENE THERAPY IN PATIENTS WITH ADA DEFICIENCY

Early attempts at ADA deficiency correction by means of gene transfer into hematopoietic progenitor cells did not succeed because (1) the technology at that time (eg, vector titers and transduction conditions) was not optimal; (2) patients were receiving enzyme replacement therapy, which suppressed the advantage conferred on transduced cells; and (3) no myeloablation was used.²⁰

ADA deficiency has now been treated with modern gene therapy techniques after the inclusion of 31 patients in 3 trials (performed in Italy, the United Kingdom, and the United States).^{9,14} The technology is essentially similar to that used to treat SCID-X1. An important difference relates to the use of a mild conditioning regimen (4 mg/kg busulfan for most patients) to improve transduced stem cell engraftment. This choice was motivated by the fact that ADA deficiency is a metabolic disease in which increasing the number of transduced cells within the different cell lineages could be advantageous. This chemotherapy has been well tolerated, and (as mentioned above) none of the patients has experienced treatment-related genotoxic complications.

Efficacy (judged in terms of T-cell development and the absence of clinical indications for supplementing patients with pegylated ADA) has been seen in 21 of the 31 patients, whereas the 10 others are alive and receiving enzyme replacement therapy. The median follow-up is 3.5 years (range, 1-9.5 years). The quality of T-cell reconstitution has been worse than in patients with SCID-X1,⁹ probably because of the unfavorable setting of ADA

deficiency in nonhematopoietic tissues, such as thymic epithelial cells. Nevertheless, T-cell reconstitution has been good enough to enable the patients to thrive. The provirus integration profile and characteristics are strikingly similar to those seen in the SCID-X1 trial. Furthermore, significant transduced B-cell, NK lymphocyte, and myeloid cell counts have been detected as a consequence of the mild myeloablation and the transduced stem cells' good engraftment. These results are very encouraging and suggest that gene therapy is a coherent therapeutic option for patients with ADA deficiency. These results also stress the potential value of using myeloablation to favor the engraftment of transduced stem cells, promote the development of transduced NK and B cells, and thus perhaps avoid the need for long-term immunoglobulin replacement therapy. Conversely, myeloablation (as discussed in the HSCT setting in patients with SCID) might produce adverse effects in sick children.

GENE THERAPY IN PATIENTS WITH WAS

WAS is a life-threatening immunodeficiency. Because lymphocyte development is not perturbed, transduced cells are not expected to have the full selective advantage observed in patients with SCID. Nevertheless, this might still be partially the case, given the WAS protein (WASP)'s functional involvement in migration of CD34 cells to the bone marrow. Myeloablation might favor the engraftment of transduced cells.

Lentiviral-mediated transfer of the *WASP* has now been recently initiated to achieve optimal transduction of stem cells. This was observed in the adrenoleukodystrophy trial,²¹ in which up to 10% of all hematopoietic lineages were found to be stably transduced 3 years after therapy. In the meantime a retrovirus-based trial for WAS was set up. It is also based on *ex vivo* gene transfer into CD34⁺ cells after myeloablation. The recently published preliminary results suggest that many aspects of the disease (eg, T- plus B-cell immunodeficiencies and thrombocytopenia) had been corrected in at least 2 patients after 2 years of follow-up, with encouraging results also observed in 7 of the other 8 treated patients.²² Although longer follow-up is obviously needed, these results are sufficiently encouraging to justify the development of clinical trials. It is noteworthy that a leukemic event occurred in one of the treated children, with LTR-mediated *LMO2* gene transactivation as a consequence of insertion in that locus. The vector used in the Wiskott Aldrich syndrome trial is a conventional retrovirus in which the *WASP* gene is under the control of the viral LTR (as in the SCID-X1 trials). These data further stress the need to use SIN vectors, as has now been initiated in ongoing trials (Table I).

EXTENDING OUR PRESENT EXPERIENCE

The results achieved to date have provided proof of concept for gene therapy of SCIDs and WAS. It will be critical to see whether the SIN vectors are indeed as safe as expected in the ongoing SCID-X1 and WAS trials. Even from a cautious standpoint, extension of gene therapy to other SCID diseases is logical. Encouraging preclinical results have been reported for Artemis and Rag-2 deficiencies and, to a lesser extent, Rag-1 deficiency.²³⁻²⁵ Further development in the treatment of other primary T-cell immunodeficiencies (eg, hemophagocytic lymphohistiocytosis, immunodysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome) is underway. Two strategies can be considered in

such cases: the transduction of hematopoietic stem cells or that of diseased, mature T cells.²⁶

The fact that the sustained detection of transduced blood cells has been observed in the treatment of one disease (adrenoleukodystrophy) in which expression of the therapeutic transgene does not provide a competitive advantage suggests that a similar gene therapy strategy can be applied to PIDs characterized by the similar absence of growth/survival activity for the defective protein. This might open up the way to the safe and efficient treatment of PIDs of the myeloid lineages, in which consistent transduction of HSCs will be needed to ensure the daily renewal of neutrophils. Chronic granulomatous diseases and leukocyte adhesion deficiency are obvious target diseases.^{27,28} One limitation consists in the treatment of diseases for which the gene of interest is tightly regulated. For instance, CD40 ligand (the gene mutated in patients with X-linked hyper-IgM syndrome) is only expressed by activated CD4 T cells. It has been shown that the constitutive expression of CD40 ligand in mice induces lymphoma.²⁹ Thus there is a need for appropriate regulators that reproduce normal physiological conditions. Other concerns stem from the potential toxicity of overexpressing gene products involved in cell activation (eg, kinases).

Advances in gene therapy for PIDs will undoubtedly stem from technological progress, such as the above-mentioned safe harbor and gene-repair strategies. Technology based on engineered, site-specific nucleases could enable targeted insertion into the genome (ie, sufficiently far away from proto-oncogenes).¹⁷ Nuclease technology could also be used to repair of gene mutations by providing an adequate template for homologous recombination. A fair level of efficacy has already been shown in mature cells.^{30,31} However, use of these techniques is currently limited by the poor correction rate in hematopoietic progenitor cells and possible occurrence of off-target recombination events. Furthermore, *ex vivo* stem cell expansion³² could increase the number of treated cells and hence boost efficacy and (if clone selection can be performed) safety. Lastly, the production of HSCs from other cell sources (as recently achieved with human fibroblasts³³) opens up further development pathways.

REFERENCES

- Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? *J Allergy Clin Immunol* 2010;126:602-10, e1-11.
- Notarangelo LD, Fischer A, Geha RS, Casanova JL, Chapel H, Conley ME, et al. Primary immunodeficiencies: 2009 update. *J Allergy Clin Immunol* 2009;124:1161-78.
- Verma IM, Weitzman MD. Gene therapy: twenty-first century medicine. *Annu Rev Biochem* 2005;74:711-38.
- Yu SF, von Ruden T, Kantoff PW, Garber C, Seiberg M, Ruther U, et al. Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. *Proc Natl Acad Sci U S A* 1986;83:3194-8.
- Cavazzana-Calvo M, Hacein-Bey S, De Saint Basile G, Gross F, Yvon E, Nussbaum P, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;288:669-72.
- Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010;363:355-64.
- Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet* 2004;364:2181-7.
- Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 2002;296:2410-3.
- Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009;360:447-58.
- Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008;118:3132-42.
- Howe SJ, Mansour MR, Schwarzmael K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008;118:3143-50.
- Dave UP, Akagi K, Tripathi R, Cleveland SM, Thompson MA, Yi M, et al. Murine leukemias with retroviral insertions at Lmo2 are predictive of the leukemias induced in SCID-X1 patients following retroviral gene therapy. *PLoS Genet* 2009;5:e1000491.
- Santoni FA, Hartley O, Luban J. Deciphering the code for retroviral integration target site selection. *PLoS Comput Biol* 2010;6:e1001008.
- Ferrua F, Brigida I, Aiuti A. Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency. *Curr Opin Allergy Clin Immunol* 2010;10:551-6.
- Cattoglio C, Pellin D, Rizzi E, Maruggi G, Corti G, Miselli F, et al. High-definition mapping of retroviral integration sites identifies active regulatory elements in human multipotent hematopoietic progenitors. *Blood* 2010;116:5507-17.
- Kustikova O, Brugman M, Baum C. The genomic risk of somatic gene therapy. *Semin Cancer Biol* 2010;20:269-78.
- Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, et al. Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. *Nat Biotechnol* 2011;29:73-8.
- Lombardo A, Genovese P, Beausejour CM, Colleoni S, Lee YL, Kim KA, et al. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nat Biotechnol* 2007;25:1298-306.
- Thrasher AJ, Hacein-Bey-Abina S, Gaspar HB, Blanche S, Davies EG, Parsley K, et al. Failure of SCID-X1 gene therapy in older patients. *Blood* 2005;105:4255-7.
- Gaspar HB, Aiuti A, Porta F, Candotti F, Hershfield MS, Notarangelo LD. How I treat ADA deficiency. *Blood* 2009;114:3524-32.
- Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science* 2009;326:818-23.
- Boztug K, Schmidt M, Schwarzer A, Banerjee PP, Diez IA, Dewey RA, et al. Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *N Engl J Med* 2010;363:1918-27.
- Benjelloun F, Garrigue A, Demerens-de Chappedelaine C, Soulas-Sprauel P, Malassis-Seris M, Stockholm D, et al. Stable and functional lymphoid reconstitution in Artemis-deficient mice following lentiviral Artemis gene transfer into hematopoietic stem cells. *Mol Ther* 2008;16:1490-9.
- Mostoslavsky G, Fabian AJ, Rooney S, Alt FW, Mulligan RC. Complete correction of murine Artemis immunodeficiency by lentiviral vector-mediated gene transfer. *Proc Natl Acad Sci U S A* 2006;103:16406-11.
- Lagresle-Peyrou C, Yates F, Malassis-Seris M, Hue C, Morillon E, Garrigue A, et al. Long-term immune reconstitution in RAG-1-deficient mice treated by retroviral gene therapy: a balance between efficiency and toxicity. *Blood* 2006;107:63-72.
- Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. Gene therapy for primary immunodeficiencies. *Immunol Allergy Clin North Am* 2010;30:237-48.
- Grez M, Reichenbach J, Schwable J, Seger R, Dinanier MC, Thrasher AJ. Gene therapy of chronic granulomatous disease: the engraftment dilemma. *Mol Ther* 2011;19:28-35.
- Hunter MJ, Tuschoff LM, Fowler CJ, Bauer TR Jr, Burkholder TH, Hickstein DD. Gene therapy of canine leukocyte adhesion deficiency using lentiviral vectors with human CD11b and CD18 promoters driving canine CD18 expression. *Mol Ther* 2011;19:28-35.
- Brown MP, Topham DJ, Sangster MY, Zhao J, Flynn KJ, Surman SL, et al. Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice. *Nat Med* 1998;4:1253-60.
- Zhang F, Cong L, Lodato S, Kosuri S, Church GM, Arlotta P. Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. *Nat Biotechnol* 2011;29:149-53.
- Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. *Nat Rev Genet* 2010;11:636-46.
- Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 2010;329:1345-8.
- Szabo E, Rampalli S, Risueno RM, Schnerch A, Mitchell R, Fiebig-Comyn A, et al. Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* 2010;468:521-6.