

# **Report from the ASGT Ad Hoc Committee on Retroviral-mediated Gene Transfer to Hematopoietic Stem Cells**

## **Review of Large Animal Model Data on Retroviral and Lentiviral Gene Transfer to Hematopoietic Cells**

**Cynthia E. Dunbar and Hans-Peter Kiem**

### I. Introduction

#### A. Methods

### II. Non-Human Primate Models

#### A. Toxicity of Replication-Competent Retroviruses

#### B. IL2-Receptor Gamma Gene Transfer

#### C. Long-Term Primate Follow-up

### III. Canine Models

#### A. IL2-Receptor Gamma Gene Transfer

#### B. Long-Term Primate Follow-up

### IV. Sheep Models

#### A. IL2-Receptor Gamma Gene Transfer

#### B. Gene Transfer of Other Genes in the Preimmune Sheep Model

### V. Miscellaneous Other Species

### VI. Conclusions

### VII: Text References

### VIII. Tables of All Published Experience

#### 1. Non-Human Primates

2. Dogs
3. Sheep
4. Miscellaneous Species

#### IX. Tables on Primates and Dogs with High-Level Marking (> 1%) and Extended Follow-Up

1. NIH data
2. FHCRC data

### **I. Introduction**

Large animal models have proven invaluable for preclinical optimization of gene transfer approaches, specifically those targeting hematopoietic stem and progenitor cells. Clinical trials performed in the early 1990s utilized transduction methodologies that had been successful in murine models and in human hematopoietic progenitor cells cultured *in vitro*. The disappointing results of these trials, with very low level or transient detection of vector-containing cells following autologous transplantation indicated a need for more predictive models. (Deisseroth *et al.*, 1994; Brenner *et al.*, 1993; Dunbar *et al.*, 1995) Safety monitoring is also more relevant in non-inbred animals with life-spans and physiology closer to humans.

Three major species groups of large animals have been employed, each with different strengths and limitations for gene therapy investigations. The cost and complexity of working with these animals in general limits experiments to a small number of animals per experimental group, favors a competitive repopulation design allowing testing of more than one vector or approach in a single animal, and has curtailed widespread long-term follow-up of animals due to prohibitive holding expenses.

Non-human primates are the closest phylogenetically to humans. Cytokines, monoclonal antibodies and other reagents utilized during *ex vivo* culture, target cell selection or follow-up generally cross-react, allowing direct translation of results in these models to clinical trials. The life-spans of the three most commonly utilized species, rhesus macaques, cynomologous macaques and baboons, are at least 15-20 years, allowing very long-term safety and efficacy follow-up. Parameters with relevance to gene transfer, such as stem cell cycling and frequency, retroviral receptor expression, and response to hematopoietic stress much more closely match those of humans than do murine models. (Abkowitz, Catlin & Guttrop, 1996) Results in non-human primate models have very closely predicted those in human clinical trials. (Van Beusechem & Valerio 1996 3815 /id) (Van Beusechem & Valerio, 1996; Donahue & Dunbar, 2001) The main drawbacks to these models are expense, difficulty of supporting these animals through ablative or semi-ablative conditioning therapy, limiting availability of the models to a handful of specialized centers, and the lack of disease models for congenital disorders and cancer, although SIV serves as a relevant model for anti-HIV strategies. (Donahue *et al.*, 1998)

Dogs have the advantage of being closer to humans in size, allowing scale-up strategies to be explored, less expensive than non-human primates, and most importantly there are a number of disease models available due to centuries of inbreeding, including pedigrees with Severe Combined Immunodeficiencies and with hemophilias. However, limitations include lack of cross-reactivity with many human reagents, often requiring use of canine

reagents such as cytokines that are not widely available, and therefore less direct translation of results to human trials.

The third large animal model utilized most frequently is the xeno-engraftment of human cells in pre-immune sheep.(Zanjani, Almeida-Porada & Flake, 1996) Currently, this model is only available in one institution. Advantages include the ability to directly test the ability of gene transfer to correct cells of humans with target diseases (such as SCID) and the potential for very long-term follow-up (compared to murine xenografts). However, the behavior of human cells in this model may not directly reflect normal physiologic conditions, and once in the sheep microenvironment, correction of defects and potential toxicity may be difficult to assess. Lastly, a handful of studies have been performed in cats, pigs and rabbits.

### **A. Methods**

This report will summarize the long-term follow-up safety data in all identified large animal experiments involving the transfer of exogenous genetic material to hematopoietic cells utilizing integrating retroviral or lentiviral vectors. This includes *ex vivo* gene transfer to hematopoietic stem and progenitor cells or lymphocytes, followed by transplantation, or *in vivo* gene transfer of actual vector preparations to the marrow or peritoneal space, if gene transfer to hematopoietic cell populations was detected. It relies on published data identified through searches using the National Library of Medicine PubMed search engine ([www.ncbi.nlm.nih.gov/entrez](http://www.ncbi.nlm.nih.gov/entrez)) and review of compiled abstracts from the American and European Societies of Hematology and of Gene Therapy.

All investigators known to have current or previous experience with large animal models for gene transfer investigation, identified from publications and meetings, were surveyed regarding long-term follow-up on animals already described in publications and any relevant information on animals that had never been published. The follow-up information in most publications is likely too short to be relevant to the question of long-term risk of leukemogenesis, thus the need to contact investigators regarding the post-publication clinical status of their animals. More than 90% of surveyed investigators have provided the requested data. Tables 1-4 summarize all published information, with subsequent follow-up information received incorporated.

In this report, the text focuses on animals transplanted within the past 5-6 years. Prior to that time, long-term engraftment with marked cells at levels greater than 0.1-1% was rare. Insertional mutagenesis can only occur if there are significant numbers of insertions into repopulating cells, and our preliminary analysis indicates, as expected, that animals and patients with very low levels of vector containing cells have very few insertions. We have included detailed information (Tables 5 and 6) on dogs and monkeys followed for at least one year, with stable marking levels in peripheral blood leukocytes of at least 1%. We have also reported complete information on animals transplanted with vectors expressing the IL2Rgamma transgene, and on animals undergoing manipulations designed to result *in vivo* expansion of transduced cells, via drug-resistance genes or selective amplifier genes.

## **II. Non-Human Primate Models**

### ***A. Toxicity of Replication-Competent Retroviruses***

Since the initial preclinical development of retroviral gene transfer strategies, the major safety consideration has been the detection, prevention and assessment of consequences from exposure of primates to replication-competent retroviruses. Three healthy and two immunosuppressed rhesus macaques were administered amphotropic retroviral vectors intravenously or intraperitoneally and followed for up to ten years. (Cornetta *et al.*, 1991; Cornetta, Morgan & Anderson, 1991) (unpublished data, C.E. Dunbar) Viremia was transient and lymph nodes did not contain virus long-term. The investigators concluded that replication-competent viruses were likely inactivated rapidly by serum complement, or more slowly by specific humoral or cellular immunity, and did not pose a significant risk. However, immunosuppression given to two animals included only relatively low-dose cyclosporine, and was likely very mild.

Several years later the development of T cell lymphomas in rhesus macaques undergoing ablative autologous transplantation with CD34+ cells transduced with a retroviral vector contaminated by large amounts of replication-competent virus shook the gene therapy and regulatory communities, and resulted in much more stringent requirements for the testing of retroviral vector supernatants and producer cell lines for replication-competent recombinant virus before clinical trial utilization. A total of 8 rhesus macaques received CD34+ marrow cells (infused doses 10-40 million/kg) transduced with supernatant from the N263A2

retroviral producer clone. This cell line released up to  $10^9$  vector particles containing the neo gene/ml and up to  $10^4$  replication-competent virus particles/ml, arising from recombination events during producer clone generation via “ping-pong” amplification.(Bodine *et al.*, 1990) Three animals died of aggressive thymic T cell lymphomas 6-7 months following transplantation. These three animals had not developed serum antibodies to viral p30, and did have high titers of replication-competent virus in their serum. 100% of their T cells had helper virus sequences, and 1-10% had vector sequences.(Donahue *et al.*, 1992)

The replication-competent viruses were characterized. At least two different genomes were identified, arisen via recombination events between the packaging cell line helper genome and the vector, and/or endogenous murine retroviral sequences present in the 3T3 cells utilized to make the packaging clone.(Vanin *et al.*, 1994; Purcell *et al.*, 1996) Each tumor cell had at least 10-50 vector insertions. The insertion sites themselves were not at that time characterized, but the tumor tissue has been recovered, and the actual insertions are now being cloned. (J.F. Tisdale and E.F. Vanin, personal communications)

The other five animals remained healthy for almost ten years before they were sacrificed and had no detectable serum virus, but did have anti-p30 antibodies. It appears that there was a race between immune system recovery and progressive viremia: animals that were able to clear the virus rapidly did not run the risk of repeated vector insertions in T cells or T cell progenitors, and did not develop lymphoma . On autopsies performed almost ten years

post-transplantation, there were no abnormalities of the marrow, lymphoid organs or spleen.(C. Dunbar, unpublished data) These animals had stable vector marking levels of 0.1-1%. DNA has been recovered for clonal insertion site analysis and will be performed in the near future.

### ***B. IL2-Receptor Gamma Gene Transfer***

There is minimal experience in non-human primate models with transfer of growth-factor receptor or other genes that might be expected to impact on proliferation or survival of transduced hematopoietic cells. An et al transplanted 2 rhesus macaques with a self-inactivating HIV-based lentiviral vector carrying the human IL2RG driven from an internal murine leukemia virus LTR.(An et al., 2001) Gene transfer efficiency was extremely low, with vector present in less than 0.5% of PBMNCs even early after transplantation. At these levels, documentation of expression by flow cytometry was difficult, and not convincing. The animals were followed for 18 months without evidence for selective expansion of transduced cells or hematologic abnormalities, and were then sacrificed, and no tumors were detected at post-mortem.

### ***C. Long-Term Primate Follow-up***

A total of over 175 non-human primates are reported on in 42 papers as having received hematopoietic progenitor and stem cells or T lymphocytes transduced with retroviral or

lentiviral vectors.(Table 1) Median follow-up was one year or less, but very long-term follow-up clinical and molecular information is available on a significant minority. The most relevant cohorts of primates to consider regarding risk of insertional mutagenesis are those transplanted since approximately 1997. Both the Seattle and the NIH groups reported a marked increase in transduction efficiency at that time, utilizing standard retroviral vectors with a number of envelope pseudotypes when *flt3* ligand was added to cytokines utilized during transduction, and the carboxy fragment of fibronectin (Retronectin) was used to coat flasks or bags containing the target cells.(Tisdale *et al.*, 1998; Kiem *et al.*, 1998) Although not all animals with high level marking have been retained for prolonged follow-up due to expense, a substantial data set does exist, and the lack of any unexpected toxicity or hematologic disorders in these animals is reassuring.

NIH has gathered data on a total of 46 rhesus macaques with stable levels of vector-containing peripheral blood mononuclear cells of at least 1%, followed for at least 1 year post-transplantation. Table 5 includes details on each individual animal. 43 received CD34+ cells transduced with retroviral vectors, and 3 received cells transduced with lentiviral vectors. Transgenes included one or more of the following: neo (n=38), GFP (n=7), drug resistance genes (n=9), G6PD (n=1), or non-expressed DNA (n=6). All animals were conditioned with 500-1200 rads total body irradiation before transplantation. The median number of cells infused was 82 million (range 9-1400 million), or approximately 14 million per kg. All animals have remained clinically well, with a median follow-up in this group of 1077 days (range 343-2260). Complete blood counts have been performed at

least twice a year, and no animal has significant abnormalities in blood counts or the distribution of lymphoid or myeloid cells. All animals have had gene marking levels assessed periodically by flow cytometry, quantitative PCR and/or Southern blot analysis. Insertional mutagenesis would be expected to result in clonal expansion, but no animal to date has demonstrated a progressive increase in marking levels in peripheral blood granulocytes or lymphocytes. To date, 20 animals have had the number of individual transduced clones contributing to hematopoiesis assessed by LAM-PCR. All are highly polyclonal, with a median number of at least 30 contributing clones. There is no evidence for progressive development of oligo- or monoclonality. Sequence analysis of individual retroviral insertions has not yet identified direct interruption of known oncogenes, transcription factors or lmo-2 itself, but only 100 total clones have been sequenced thus far.

The FHCRC in Seattle has gathered data on 29 baboons with stable marking levels of greater than 1%. Data on individual animals is given in Table 6. 21 animals have been followed for greater than 1 year, with the longest follow-up 2007 days, and median follow-up of 827 days. 25 received cells transduced with retroviral vectors, and 4 received cells transduced with both lentiviral and retroviral vectors.. Transgenes included one or more of the following: neo (n=26), GFP or YFP (n=14), and CD18 (n=1). Median cell dose transplanted was 50 million, and this resulted in a median cell dose of approximately 7.5 million cells/kg. All animals have remained clinically well, and complete blood counts have been performed at least twice a year, and no animal has significant abnormalities in blood counts or in the distribution of lymphoid or myeloid cells. Animals were euthanized at the

end of the particular protocol they were part of, or due to clinical problems related to total body irradiation such as pulmonary fibrosis. None had evidence for lymphoma or leukemia. All animals have had gene marking levels assessed periodically by flow cytometry, quantitative PCR and/or Southern blot analysis, Insertional mutagenesis would be expected to result in clonal expansion, but no animal to date has demonstrated a progressive increase in marking levels in peripheral blood granulocytes or lymphocytes. To date, several animals have had the number of individual transduced clones contributing to hematopoiesis assessed by LAM-PCR. All are highly polyclonal. There is no evidence to date of progressive development of oligo- or monoclonality.

### **III. Canine Models**

#### ***A. IL2-Receptor Gamma Gene Transfer:***

Retroviral vectors carrying the human IL2R gene along with either MDR1 or Neo were utilized to transduce unstimulated or cytokine-stimulated autologous or allogeneic normal canine marrow, and reinfused following minimal myeloablation (200rads). (Whitwam *et al.*, 1998) 3 dogs had sustained presence of transgene-expressing cells, however, immunosuppression with cyclosporine and prednisone was required to again detect IL2RG-expressing cells more than 15 weeks post-transplantation. Levels of transgene expressing lymphocytes reached as high as 20-30% in three animals transplanted with cytokine-primed marrow target cells, however, B versus T cell expression was not assessed. There was no evidence for *in vivo* expansion of vector-containing and

potentially overexpressing cells, but these were normal dogs without immunodeficiency, so this is not surprising. The three animals with the highest marking levels were followed for over one year without development of hematologic abnormalities.

These three animals were treated with paclitaxel at approximately 2 years following transplantation to investigate whether drug selection could increase the level of IL2RG-MDR1 vector-containing cells.(Licht *et al.*, 2002) Two dogs did not survive the drug treatment, but the third dog survived and demonstrated a marked increase in the level of transgene-expressing cells following each cycle, with stabilization at levels of about 10% of lymphocytes. Clonal analysis has not yet been performed. This animal is still alive and healthy almost 4 years after the initial transplant, but has not been followed regarding gene transfer levels.

A breeding colony of dogs with X-SCID due to a frameshift mutation of the IL2RG have been transplanted with retrovirally-transduced cells and reported in the literature.(Felsburg *et al.*, 1998) The canine disorder is very similar clinically and phenotypically to human X-SCID. These animals can be reconstituted by allogeneic transplantation from histocompatible littermates.(Hartnett *et al.*, 2002) Only preliminary data is available to date on X-SCID animals transplanted with autologous marrow cells transduced with a retroviral vector expressing the human IL2R gene. Three neonatal dogs were transplanted without ablation, and at 6-11 weeks there was no evidence for immune reconstitution, but up to 10% of T cells expressed the human IL2RG transgene.(Ting *et al.*, 2002) Both animals

died of infections by 7 months post transplantation with little evidence for immune reconstitution or *in vivo* selection for transduced cells.(H. Malech, personal communication). These experiments will be repeated with a vector expressing canine IL2RG, given evidence that the human IL2RG was not able to correct function in the canine cells.

### ***B. Long-Term Canine Follow-up:***

The Seattle group has transplanted a large number of dogs over the last decade as they have developed and tested new methodologies for both gene transfer and non-ablative conditioning, in both autologous and allogeneic transplantation models. Table 6 gives detailed information on individual animals with stable *in vivo* marking levels of at least 1% after transplantation of CD34+ marrow cells transduced with integrating retroviral or lentiviral vectors. A total of 22 animals are included, with median follow-up of over 500 days. Twelve animals have been followed for more than one year. All received 920 rads TBI. Cell doses administered were a median of 130 million total cells, corresponding to a median of 13 (1.9-94) million/kg. 19 received cells transduced with retroviral vectors, and 3 received cells transduced with lentiviral vectors. Transgenes included one or more of the following: neo (n=10), GFP or YFP (n=18), drug resistance genes (n= 7), or a truncated mpl receptor fused to a dimerization domain (n= 2). The animals have been monitored with health checks, routine complete blood counts, and assessment of marking levels. Analysis of clonal insertion patterns is ongoing. No animal has developed any hematologic

abnormalities suggesting a lymphoproliferative or myeloproliferative condition. A Canadian group also reported on a relatively large number of dogs transplanted with marrow cells transduced with neo or alpha-L-iduronidase vectors in long-term marrow cultures, and reinfused generally without ablation.(Carter *et al.*, 1992; Bienzle *et al.*, 1994) These animals reportedly had detectable levels of neo-resistant CFU, but other quantitative analysis was lacking in these early studies, and this transduction technique did not result in efficient gene transfer in human marking trials.(Stewart *et al.*, 1999)

No dog in any study has spontaneously developed increasing levels of marked cells, although a number of animals transplanted with cells containing a drug-resistance gene or a dimerizable growth factor receptor have shown increases in marking levels following drug treatment.(Neff *et al.*, 2002b) (Neff *et al.*, 2002a) The increase in marking after treatment with the c-mpl dimerizer was transient and dependent on continued drug treatment.

Animals treated with BCNU after transplantation of CD34+ cells transduced with a vector expressing the drug resistance gene MGMT had more marked and sustained increases in marking levels. Clonal analysis in these groups of dogs that received cells placed under intense *in vivo* selection will be of great interest and may be relevant to the situation in the children that developed leukemia on the X-SCID trial. No other groups have data in dog models with follow-up for greater than one year.

#### **IV. Pre-Immune Sheep Models**

### **A. IL2-Receptor Gamma Gene Transfer**

A total of 6 preimmune sheep were injected intraperitoneally between days 55-60 of gestation with CD34+ cells from human X-SCID patients.(Tsai *et al.*, 2002) The cells were transduced with a standard retroviral vector expressing the human IL2RG gene. Five sheep received  $1.8 \times 10^5$  CD34+ cells each from one patient with X-SCID, and the sixth sheep received  $5 \times 10^6$  cells from a second patient with X-SCID. Sheep receiving the transduced cells developed human myeloid, B, and T cells in vivo, reported up to 10 months postnatally. Sheep receiving nontransduced cells from the same patients had only human myeloid cells detected in vivo. Thus this model seems very well suited for investigation of toxicity, clonality and other issues related to retroviral gene transfer of the IL2RG gene.(Zanjani, Almeida-Porada & Flake, 1996) Only one sheep has been maintained and followed now two years post-transplantation. The others were unfortunately euthanized for financial reasons.

### **B. Gene Transfer of Other Genes in the Preimmune Sheep Model**

There is limited additional experience with transplanting retrovirally-transduced human CD34+ cells into preimmune sheep. Six sheep received bone marrow CD34+ cells transduced with a neo/b-gal vector.(Porada *et al.*, 2002) No marked human cells were detectable after 8 months, despite persistence of unmarked human cells. However, only relatively insensitive methods (G418-resistant colony assays) were used to detect

transduced human cells.

Retroviral gene transfer in the preimmune sheep model has also involved direct injection of retroviral vectors intraperitoneally during fetal development. 14 sheep survived long-term following injection of 0.2-0.6 ml (2-6 million vector particles) of amphotropic MuLV-based retroviral supernatant. The vectors contained either the neo or B-galactosidase genes. 12/14 animals had consistent marking of peripheral blood or bone marrow cells. 9/12 were followed for greater than 40 months (the remaining three animals were sacrificed earlier for tissue harvesting or acquisition of tetanus). (Tran *et al.*, 2001) 1-5% of human PB and BM cells contained and expressed vector, and the animals were reported to be healthy at that time point. No insertion site analysis was yet performed.

Next, the same investigators first engrafted human CD34+ cells ( $6 \times 10^5$  per animal) in preimmune sheep, and then 19 days later injected retroviral vector supernatant intraperitoneally. (Porada *et al.*, 2002) Seven animals survived the procedures and were followed postnally. An average of 17% of human CFU contained the vector 3.5-15 months post-transplantation. Bone marrow cells were harvested from the primary sheep 8.5 months following transplantation and injected into secondary preimmune sheep. 6 months later the marrow from the secondary sheep (n=2) was harvested and used to transplant tertiary sheep (n=4). These sheep were also followed for 6 months. Therefore the total follow-up of the progeny cells derived from the original transduction was almost three years. Even the tertiary recipients still had high levels (mean 26%) of vector in human CFU. No

hematologic abnormalities were reported in any of these animals followed long-term.

## **V. Miscellaneous Other Species**

Several investigators have utilized alternative large animal models for very specific purposes. Table 4 summarizes the literature. Direct injection of retroviral vectors into the marrow space of rabbits resulted in low level gene transfer into hematopoietic cells.(Nelson *et al.*, 1997) Miniature swine had discordant MHC genes expressed from retroviral vectors in stem cells to try and induce tolerance to discordant solid organs later transplanted.(Sonntag *et al.*, 2001) Cats have been studied to investigate the utility of feline leukemia virus-based vector systems.(Josephson, Sabo & Abkowitz, 2000) No toxicities related to gene transfer have been noted in any of these reports.

## **VI Conclusions**

- Over 130 dogs, 175 non-human primates, 49 sheep, and small numbers of pigs, rabbits and cats have received hematopoietic cells transduced *ex vivo* or *in vivo* with integrating retroviral or lentiviral vectors. No animals exposed to helper-free vector preparations have developed leukemia, lymphoma, or any other evidence for toxicity related to the integration of gene transfer vectors.
- The only evidence for toxicity resulting from retroviral gene transfer in large animals occurred in rhesus macaques transplanted with cells contaminated with replication-competent retroviral vectors. These animals developed T cell lymphoma/leukemia

and tumor cells had multiple helper genome insertions. It is thus far unknown whether lmo-2 is involved.

- Follow-up of at least one year and a median of three years is available in a significant minority of animals that also have stable high-level long-term gene transfer levels in peripheral blood cells, including 46 rhesus macaques, 21 baboons, and 12 dogs, None have increasing levels of marked cells, monoclonality or oligoclonality, or any other worrisome features to date. At least 3000 different insertion events most likely occurred in these animals, based on an average of 30 clones per animal in those analyzed thus far.
- IL2RG gene transfer in dogs, primates, and sheep has been performed. Thus far, no leukemic or clonal events have occurred, although none of the models utilized closely matches the human parameters in the French trial.

## **References**

ABKOWITZ,J.L., CATLIN,S.N. & GUTTORP,P. (1996). Evidence that hematopoiesis may be a stochastic process in vivo. *Nat. Med.* **2**, 190-197.

AN,D.S., KUNG,S.K., BONIFACINO,A., WERSTO,R.P., METZGER,M.E., AGRICOLA,B.A., MAO,S.H., CHEN,I.S. & DONAHUE,R.E. (2001). Lentivirus vector-mediated hematopoietic stem cell gene transfer of common gamma-chain cytokine receptor in rhesus macaques. *J. Virol.* **75**, 3547-3555.

BIENZLE,D., ABRAMS-OGG,A.C.G., KRUTH,S.A., ACKLAND-SNOW,J., CATER,R.F., DICK,J.E., JACOBS,R.M., KAMEL-REID,S. & DUBÉ,I.D. (1994). Gene transfer into hematopoietic stem cells: long-

term maintenance of in vitro activated progenitors without marrow ablation. Proc. Natl. Acad. Sci. U. S. A. **91**, 350-354.

BODINE,D.M., MCDONAGH,K.T., BRANDT,S.J., NEY,P.A., AGRICOLA,B., BYRNE,E. & NIENHUIS,A.W. (1990). Development of a high-titer retrovirus producer cell line capable of gene transfer into rhesus monkey hematopoietic stem cells. Proc. Natl. Acad. Sci. U. S. A. **87**, 3738-3742.

BRENNER,M.K., RILL,D.R., HOLLADAY,M.S., HESLOP,H.E., MOEN,R.C., BUSCHLE,M., KRANCE,R.A., SANTANA,V.M., ANDERSON,W.F. & IHLE,J.N. (1993). Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. Lancet **342**, 1134-1137.

CARTER,R.F., ABRAMS-OGG,A.C.G., DICK,J.E., KRUTH,S.A., VALLI,V.E., KAMEL-REID,S. & DUBE,I.D. (1992). Autologous transplantation of canine long-term marrow culture cells genetically marked by retroviral vectors. Blood **79**, 356-364.

CORNETTA,K., MOEN,R.C., CULVER,K., MORGAN,R.A., MCLACHLIN,J.R., STURM,S., SELEGUE,J., LONDON,W., BLAESE,R.M. & ANDERSON,W.F. (1991). Amphotropic murine leukemia retrovirus is not an acute pathogen for primates. Hum. Gene Ther. **1**, 12-17.

CORNETTA,K., MORGAN,R.A. & ANDERSON,W.F. (1991). Safety issues related to retroviral-mediated gene transfer to humans. Hum. Gene Ther. **2**, 5-14.

DEISSEROTH,A.B., ZU,Z., CLAXTON,D., HANANIA,E.G., FU,S., ELLERSON,D., GOLDBERG,L., THOMAS,M., JANICEK,K., ANDERSON,W.F., HESTER,J., KORBLING,M., DURETT,A., MOEN,R., BERENSON,R., HEIMFELD,S., HAMER,J., CALVERT,L., TIBBITS,P., TALPAZ,M., KANTARJIAN,H., CHAMPLIN,R. & READING,C. (1994). Genetic marking shows that Ph<sup>+</sup> cell present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow transplantation in CML. Blood **83**, 3068-3076.

DONAHUE,R.E., BUNNELL,B.A., ZINK,M.C., METZGER,M.E., WESTRO,R.P., KIRBY,M., UNANGST,T., CLEMENTS,J.E. & MORGAN,R.A. (1998). Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes. Nat. Med. **4**, 181-186.

DONAHUE,R.E. & DUNBAR,C.E. (2001). An update on the use of non-human primate models for

preclinical testing of gene therapy approaches targeting hematopoietic cells. *Hum. Gene Ther.* **12**, 607-617.

DONAHUE,R.E., KESSLER,S.W., BODINE,D., MCDONAGH,K., DUNBAR,C., GOODMAN,S., AGRICOLA,B., BYRNE,E., RAFFELD,M., MOEN,R. & NIENHUIS,A.W. (1992). Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. *J. Exp. Med.* **176**, 1125-1135.

DUNBAR,C.E., COTTLER-FOX,M., O'SHAUGHNESSY,J., DOREN,S., CARTER,C.S., BERENSON,R., BROWN,S., MOEN,R.C., GREENBLATT,J., STEWART,F.M., LEITMAN,S.F., WILSON,W., COWAN,K.H. , YOUNG,N.S. & NIENHUIS,A.W. (1995). Retrovirally-marked CD34-enriched peripheral blood and bone marrow and cells contribute to long-term engraftment after autologous transplantation. *Blood* **85**, 3048-3057.

FELSBURG,P.J., SOMBERG,R.L., HARTNETT,B.J., HENTHORN,P.S. & CARDING,S.R. (1998). Canine X-linked severe combined immunodeficiency. A model for investigating the requirement for the common gamma chain (gamma c) in human lymphocyte development and function. *Immunol. Res* **17**, 63-73.

HARTNETT,B.J., YAO,D., SUTER,S.E., ELLINWOOD,N.M., HENTHORN,P.S., MOORE,P.E., MCSWEENEY,P.A., NASH,R.A., BROWN,J.D., WEINBERG,K.I. & FELSBURG,P.J. (2002).

Transplantation of X-linked severe combined immunodeficient dogs with CD34+ bone marrow cells. *Biol. Blood Marrow Transplant.* **8**, 188-197.

JOSEPHSON,N.C., SABO,K.M. & ABKOWITZ,J.L. (2000). Transduction of feline hematopoietic cells by oncoretroviral vectors pseudotyped with the subgroup A feline leukemia virus (FeLV-A). *Mol. Ther.* **2**, 56-62.

KIEM,H.P., ANDREWS,R.G., MORRIS,J., PETERSON,L., HEYWARD,S., ALLEN,J.M., RASKO,J.E.J., POTTER,J. & MILLER,A.D. (1998). Improved gene transfer into baboon marrow repopulating cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor. *Blood* **92**, 1878-1886.

LICHT,T., HASKINS,M., HENTHORN,P., KLEIMAN,S.E., BODINE,D.M., WHITWAM,T., PUCK,J.M., GOTTESMAN,M.M. & MELNICZEK,J.R. (2002). Drug selection with paclitaxel restores expression of linked IL-2 receptor gamma -chain and multidrug resistance (MDR1) transgenes in canine bone marrow.

Proc. Natl. Acad. Sci. U. S. A **99**, 3123-3128.

NEFF,T., HORN,P.A., PETERSON,L.J., THOMASSON,B.M., WILLIAMS,D.A., GEORGES,G.E., VON KALLE,C. & KIEM,H.-P. (2002a) BCNU-mediated in vivo selection of MGMT-transduced allogeneic hematopoietic cells in a large animal model. *Blood* **100**, 689a. (Abstract)

NEFF,T., HORN,P.A., VALLI,V.E., GOWN,A.M., WARDWELL,S., WOOD,B.L., VON KALLE,C., SCHMIDT,M., PETERSON,L.J. , MORRIS,J.C., RICHARD,R.E., CLACKSON,T., KIEM,H.P. & BLAU,C.A. (2002b). Pharmacologically regulated in vivo selection in a large animal. *Blood* **100**, 2026-2031.

NELSON,D.M., METZGER,M.E., DONAHUE,R.E. & MORGAN,R.A. (1997). In vivo retrovirus-mediated gene transfer into multiple hematopoietic lineages in rabbits without preconditioning. *Hum. Gene Ther.* **8**, 747-754.

PORADA,C.D., TRAN,N.D., ALMEIDA-PORADA,G., GLIMP,H.A., PIXLEY,J.S., ZHAO,Y., ANDERSON,W.F. & ZANJANI,E.D. (2002). Transduction of long-term-engrafting human hematopoietic stem cells by retroviral vectors. *Hum. Gene Ther.* **13**, 867-879.

PURCELL,D.F., BROSCIUS,C.M., VANIN,E.F., BUCKLER,C.E., NIENHUIS,A.W. & MARTIN,M.A. (1996). An array of murine leukemia virus-related elements is transmitted and expressed in a primate recipient of retroviral gene transfer. *J. Virol.* **70**, 887-897.

SONNTAG,K.C., EMERY,D.W., YASUMOTO,A., HALLER,G., GERMANA,S., SABLINSKI,T., SHIMIZU,A., YAMADA,K., SHIMADA,H., ARN,S., SACHS,D.H. & LEGUERN,C. (2001). Tolerance to solid organ transplants through transfer of MHC class II genes. *J Clin Invest* **107**, 65-71.

STEWART,A.K., SUTHERLAND,D.R., NANJI,S., ZHAO,Y., LUTZKO,C., NAYAR,R., PECK,B., RUEDY,C., MCGARRITY,G., TISDALE,J. & DUBE,I.D. (1999). Engraftment of gene-marked hematopoietic progenitors in myeloma patients after transplant of autologous long-term marrow cultures. *Hum. Gene Ther.* **10**, 1953-1964.

TING,S.S., HARTNETT,B.J., LINTON,G.F., MALECH,H.L. & FELSBURG,P.J. (2002) Gene therapy in canine X-linked severe combined immunodeficiency by RD114 pseudotyped oncoretroviral vector. *Blood* **100**, 427a. (Abstract)

TISDALE,J.F., HANAZONO,Y., SELLERS,S.E., AGRICOLA,B.A., METZGER,M.E., DONAHUE,R.E. & DUNBAR,C.E. (1998). Ex vivo expansion of genetically marked rhesus peripheral blood progenitor cells results in diminished long-term repopulating ability. *Blood* **92**, 1131-1141.

TRAN,N.D., PORADA,C.D., ALMEIDA-PORADA,G., GLIMP,H.A., ANDERSON,W.F. & ZANJANI,E.D. (2001). Induction of stable prenatal tolerance to beta-galactosidase by in utero gene transfer into preimmune sheep fetuses. *Blood* **97**, 3417-3423.

TSAI,E.J., MALECH,H.L., KIRBY,M.R., HSU,A.P., SEIDEL,N.E., PORADA,C.D., ZANJANI,E.D., BODINE,D.M. & PUCK,J.M. (2002). Retroviral transduction of IL2RG into CD34(+) cells from X-linked severe combined immunodeficiency patients permits human T- and B-cell development in sheep chimeras. *Blood* **100**, 72-79.

VAN BEUSECHEM,V.W. & VALERIO,D. (1996). Gene transfer into hematopoietic stem cells of nonhuman primates. *Hum. Gene Ther.* **7**, 1649-1668.

VANIN,E.F., KALOSS,M., BROCIUS,C. & NIENHUIS,A.W. (1994). Characterization of replication-competent retrovirus from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. *J. Virol.* **68**, 4241-4250.

WHITWAM,T., HASKINS,M.E., HENTHORN,P.S., KRASZEWSKI,J.N., KLEIMAN,S.E., SEIDEL,N.E., BODINE,D.M. & PUCK,J.M. (1998). Retroviral marking of canine bone marrow: long-term, high-level expression of human interleukin-2 receptor common gamma chain in canine lymphocytes. *Blood* **92**, 1565-1575.

ZANJANI,E.D., ALMEIDA-PORADA,G. & FLAKE,A.W. (1996). The human/sheep xenograft model: a large animal model of human hematopoiesis. *Int. J. Hematol.* **63**, 179-192.