

**Report from the ASGT Ad Hoc Committee on Retroviral-mediated Gene
Transfer to Hematopoietic Stem Cells
April, 2003**

**Review of Data from Human Clinical Trials Using Retroviral-
Mediated Gene Transfer to Hematopoietic Stem Cells (HSC)**

Sections:

- A. Introduction**
- B. Gene marking studies**
- C. Chemotherapy Drug resistance Genes**
- D. Genetic Diseases**
- E. HIV-1**
- F. Summary/Conclusions**

A. Introduction

The first clinical trials of retroviral-mediated gene transfer to human hematopoietic stem cells (HSC) were begun in 1992. Since that time, at least 40 trials have been performed in which retroviral vectors were used to introduce genes into human HSC (Table 1)*¹.

Table 1. **Summary of Clinical Trials Performed Using Retroviral-Mediated Gene Transfer to Human Hematopoietic Stem Cells**

- 40 clinical trials with at least 232 subjects
- 34 U.S., 6 non-US.
 - 7 gene marking in auto BMT
 - CML (2), AML, Acute Leukemia, NHL, NB, Breast Ca,
 - 8 chemotherapy resistance genes
 - MDR-1 (6), MGMT (2)
 - breast (2), NHL, Germ cell, Carcinoma, Breast/Ovarian, CNS tumors, Sarcoma
 - 7 HIV/AIDS

*¹ The data collected here represent an ongoing effort by members of ASGT to obtain information on all of the clinical trials that have been done, world-wide. Some studies that were reviewed and received some or full regulatory approval were never inaugurated. Some trials that enrolled a small number of subjects or that had negative results have not been published in the scientific literature. Other trials are more recent and data have not yet been presented publicly or published. Any missing information that can be provided by involved investigators is welcome at any time and will be entered into this collection. Additionally, we would welcome notice of any errors or misstatements that are detected.

- 18 Genetic diseases
 - SCID: ADA (5), XSCID (2) Jak3 (1)
 - CGD (4), LAD (1), Gaucher (3), Fanconi (2)
-

No serious adverse events attributable to the gene transfer procedure or vectors were seen over more than a decade of such trials, until T cell leukemia developed in two subjects in a trial for X-linked form of severe combined immune deficiency (SCID). The reason why two cases of leukemia developed in a single trial, but not in any of the other trials, remains the subject of investigation. As will be shown in the accumulated data, the majority of clinical trials targeting HSC with retroviral vectors failed to achieve any detectable long-term gene transfer to hematopoietic stem cells or did so at only a very low level. With minimal gene transfer to HSC, the risks of insertional oncogenesis would be low, but no therapeutic effect would be realized.

Potentially novel factors of the XSCID study that may have predisposed to this complication may be speculated to include: the specific transgene (gammaC a component of multiple lymphoid cytokine receptors), the state of the hematopoietic stem/progenitor cells in XSCID patients, the young age of the subjects (1 and 3 months at time of the procedure), the relatively high numbers of CD34+ cells obtained for the small size of the subjects, the high levels of gene marking obtained, the very high numbers of gene-containing lymphoid cells produced in the course of immune reconstitution, the immune deficiency of the subjects at the time of treatment. While it is not possible to determine which of these factors are the relevant ones, it is important to collect the available data on all subjects of clinical trials of retroviral-mediated gene transfer who have undergone the procedure without complication, to provide a basis for contrasting the features to those in the XSCID study. As for any novel therapy, investigations of gene therapy must weigh the risks and benefits of the approach to those of the therapeutic alternatives.

Many of the early studies were so called “**gene marking**” studies in which a gene of no therapeutic intent was used to serve as a genetic tag of either hematopoietic stem cells or potentially contaminating tumor cells contained within bone marrow being used for autologous transplantation of patients with cancer or leukemia. Following on these gene marking studies in oncology patients were studies intending to introduce into normal HSC from patients with cancer, genes encoding **chemotherapy resistance**, to alleviate marrow myelosuppression from post-transplant chemotherapy.

At least 7 trials have been performed for patients infected with **HIV-1/AIDS**, with the goal of introducing into their HSC a synthetic gene that blocks replication of HIV -1 that could be expressed in T lymphocytes and other susceptible blood cells. No major benefit has been seen from these studies, although in several that have been suggestions that there was a modest protection of gene-containing cells from HIV -1 mediated cytopathicity.

Clinical trials have been performed for subjects with at least 7 different **genetic diseases** affecting blood cell production or function (Table 1). The goal is to introduce a normal copy of the relevant gene into HSC, so that the function of the defective

inherited gene can be *trans*-complemented, to restore the specific blood cell activity. A significant therapeutic effect has only been achieved in recent studies for SCID, in the two trials for XSCID and in one for ADA-deficient SCID. For the other conditions, the levels of gene transfer to engrafting HSC has been too low to be of significant clinical benefit.

B. Gene marking studies

Hematopoietic stem cell transplantation (HSCT) have been performed as a method to deliver high dose chemotherapy and/or irradiation to kill high risk malignant diseases, with the HSCT used as rescue from the marrow ablative effects. HSCT using cells from another person (allogeneic) provides a source of new HSC that are free of the patient's malignant cells. However, immunologic reactions between cells of the donor and recipient can lead to rejection of the graft or an immunologic attack on the recipient (graft versus host disease), and limits donor selection to fully or closely HLA matched donors. Alternatively, a patient's own HSC could be used for "rescue" after the high dose chemotherapy/radiation, avoiding immunologic complications. However, the patient's marrow may be contaminated with their malignant cells and their re-infusion could be the source of relapse. In general relapse rates are higher in autologous transplants compared to allogeneic transplants, especially for leukemia that by its nature involves malignant cells in the marrow. A different explanation for the higher rate of relapse from autologous transplant may be the lack of immunologic reactivity of donor cells, which could play a role in the elimination of residual malignant cells (graft versus tumor or graft versus leukemia).

It is of great importance to determine the relative contribution of residual malignant cells in relapse after autologous HSCT, to know whether efforts need to be directed to "purging" contaminating malignant cells or to boosting of graft versus tumor types of immune reactivity. As techniques for gene transfer to mammalian cells with retroviral vectors were developed, the potential to use gene transfer to "tag" contaminating cells in bone marrow were conceived. Exposure of a portion of HSC to be used for autologous HSCT to a retroviral vector preparation could lead to gene transfer into contaminating tumor or leukemia cells; if the gene "tagged" malignant cells contribute to a subsequent relapse, the marker gene would be detected in malignant cells of the patient. An additional by-product of these gene marking studies would be that normal, non-malignant hematopoietic stem cells may become gene marked, allowing analysis of hematopoietic kinetics after transplant.

The first such gene marking studies were performed by Malcolm Brenner and co-workers at the St. Jude's Children's Cancer Research Hospital, starting in 1992 (1-3). They used a retroviral vector that carried a bacterial antibiotic resistance gene, the neomycin phosphotransferase gene (*neo*) encoding resistance to aminoglycoside antibiotics, to serve as a biologically inert marker that could be detected in patient samples using Southern blot analysis or PCR. Two trials were performed in children undergoing autologous BMT, one for acute myelogenous leukemia (AML) and one for neuroblastoma. In both studies, there was positive documentation that there were contaminating malignant cells in the autograft that subsequently contributed to relapses that occurred in a subset of the subjects. These findings affirmed the rationale for the

use of gene marking and support the contention that autologous HSCT inoculum may carry and return to the subjects malignant cells and would suggest that some type of purging, either depletion of tumor cells or enrichment of non-malignant HSC, may increase success rates from autologous transplants.

In addition, there was gene marking of non-malignant HSC in many of the subjects, with the marker neo gene seen in cells of multiple hematopoietic lineages, in some cases for many years (2). Indeed, the level of gene marking in the normal hematopoietic cells exceeded that achieved in subsequent trials targeting HSC for genetic diseases. It may be that the HSC of the pediatric oncology patients were of increased susceptibility to retroviral vector-mediated transduction if a high fraction were actively cycling during recovery from prior chemotherapy. Interestingly, this study represents one of the few among all trials of gene transfer to HSC that did not attempt to enrich for HSC by immuno-selection for CD34, a marker of human stem and progenitor cells. Whether the method led to gene transfer to a CD34- stem cell is unknown.

Subsequently, gene marking studies were performed for adult patients undergoing autologous HSCT for breast cancer (4,5), chronic myelogenous leukemia (2 studies) (6), acute myelogenous leukemia, acute leukemia (ALL or AML), and non-Hodgkin's lymphoma (7). In some cases, gene marking was again seen in a subset of malignant cells at the time of subsequent relapses, demonstrating that they were present in the autologous transplant inoculum. But, gene marking of normal hematopoietic cells occurred only rarely and at low levels. The general protocols used in the gene marking studies involved either no attempt to activate HSC to cycle and take-up the retroviral vectors, or used combination of the cytokines that were available in that earlier time which are now known to be sub-optimal for augmenting gene transfer to HSC.

Few clinical gene marking studies with HSC have been proposed in recent years, but the approach of gene marking continues to be used in the non-human primate transplant studies to guide development of more effective gene transfer techniques for clinical trials with therapeutic intent.

Shi PA, Hematti P, von Kalle C, and Dunbar CE. Genetic marking as an approach to studying in vivo hematopoiesis: progress in the non-human primate model. *Oncogene* 21:3274-3283, 2002.

C. Chemotherapy Resistance Gene Transfer

At least eight trials have been performed transferring genes encoding resistance to chemotherapeutic agents into HSC of patients with cancer or leukemia during autologous HSCT (8-15). The goal of this approach is to confer increased relative resistance to the dose-limiting myelosuppressive effects of chemotherapy, so that additional anti-neoplastic treatment may be given to the patients prophylactically after transplant or if needed at relapse. In theory, the ability to deliver higher dosages of chemotherapy without severe suppression of bone marrow function could lead to a higher rate of cure of the underlying malignancy, especially if delivered at a time shortly after auto-BMT when the disease burden would be expected to be at a minimum.

In addition, if selection for gene-transduced stem cells can be achieved with this approach, it may be possible to use these drug resistance genes as co-selectable markers in vectors also carrying a therapeutic gene (e.g. β -globin). After transduced stem cells are transplanted, moderate doses of stem cell ablative chemotherapy could be given to reduce the fraction of non-transduced cells.

Initial clinical trials examined transfer of the multidrug resistance (MDR-1 or p-glycoprotein) cDNA into hematopoietic stem cells of cancer patients. MDR-1 acts as a drug pump, eliminating a variety of classes of chemical compounds from cells, including several clinically relevant chemotherapeutic agents.

These studies showed low but detectable numbers of cells containing the MDR-1 gene in some of the patients, although the benefits of expression of the exogenous MDR-1 gene during subsequent chemotherapy have not yet been demonstrated (8-14).

In a clinical trial performed more recently, Abonour et al. transferred the MDR-1 cDNA to HSC of patients with germ cell tumors undergoing tandem cycles of high-dose chemotherapy with autologous HSC rescue (15). The goal was to make some HSC relatively resistant to chemotherapy-induced myelosuppression by expression of MDR-1 to allow administration of post-transplant oral etoposide. CD34+ peripheral blood stem cells (PBSC) were obtained from G-CSF mobilized peripheral blood and used for support of two tandem transplants. In the first cycle, only non-transduced PBSC were given and in the second, only PBSC transduced with a retroviral vector carrying the human MDR-1 cDNA were given. This study was the first to use recombinant fibronectin as a support matrix for *ex vivo* transduction of CD34+ cells.

They achieved a relatively high level of gene-marking, with 6-12% of bone marrow CFU-C containing the vector as detected by polymerase chain reaction (PCR) up to a year after transplant. The level of marking was lower in peripheral blood cells, with 0.01% of granulocytes containing the gene. The level of gene marking increased modestly (to 0.1% of granulocytes) after the post-transplant etoposide, consistent with a relative resistance to chemotherapy for progenitor cells expressing the transferred MDR-1 gene.

This study is notable for being the first clinical trial to give only CD34+ cells that had been manipulated *ex vivo* for gene transfer after an essentially fully marrow cytoablative regimen. Prior trials had "hedged their bets" by giving mixtures of transduced and non-transduced cells, so that the latter cells at least would ensure hematopoietic reconstitution in case the gene transduction procedure damaged the stem cells engraftment capacity. The engraftment kinetics using solely the transduced PBSC were identical to those seen in the first of the tandem transplants that gave only non-transduced PBSC. The investigators attributed the rapid recovery of hematopoiesis in this trial as being due to preservation of stem cell engraftment capacity because of the use of the recombinant fibronectin. Nevertheless, there was minimal transduction and engraftment of long-term HSC, with no detectable gene-containing cells after one year in most subjects.

The use of MDR-1 as a drug resistance gene has recently come into question. In retrospect, the *mdr-1* cDNA is somewhat problematic for delivery by retroviral vectors.

It is a relatively large cDNA and contains cryptic splice sites, so that a significant portion of the vectors delivered truncated, inactive portions of the MDR-1 gene. Subsequently, these cryptic sites were eliminated in a second generation of vectors, but gene transfer still remained sub-optimal. Additionally, in the trials performed before 1998, combinations of cytokines and culture conditions that were used for gene transduction of HSC were sub-optimal, based upon current knowledge.

Of some concern, Sorrentino and co-workers observed that transduction of murine marrow with an MDR-1 gene vector conferred a relative proliferative advantage on cells leading to myeloproliferation. These proliferative effects of the MDR-1 gene were not observed in non-human primates transplanted with MDR-1-transduced CD34+ cells and so its clinical relevance is unclear. No evidence of myeloproliferation have been reported in any of the human subjects of these clinical trials.

Other genes being studied for the purpose of augmenting hematopoietic stem cells resistance to chemotherapy include: the dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate, the O⁶-methylguanine DNA methyltransferase (MGMT) gene conferring resistance to alkylating agents such as BCNU, and the aldehyde reductase (AR) gene conferring resistance to cyclophosphamide. Croop and co-workers have recently completed a trial using a retroviral vector carrying the MGMT gene into HSC of patients undergoing autologous transplant for brain tumors. The MGMT/BCNU system has been developed further to be especially robust for selection at the stem cell level by using a drug (O6-benzyl-guanine – O6-BG) to inactivate the wild-type MGMT gene in non-transduced HSC (and tumor cells) rendering them highly sensitive to BCNU-mediated cytotoxicity while over-expressing an O6-BG-resistant mutant of the MGMT gene in HSC to render them highly resistant to BCNU. Gerson and co-workers have a newly-initiated trial using this approach to transfer an O6-BG-resistant mutant of MGMT to CD34+ cells for autologous transplantation of patients with advanced malignancies to be followed by post-transplant anti-tumor treatment with BCNU and O6-BG.

It remains unknown whether selection for resistance to chemotherapy will be at the level of the stem cells, and thus long-lasting, or only at the progenitor cell level. Selection at the progenitor cell level would be expected to be transient and may revert to pre-selection levels after completion of a course of chemotherapy when new progenitor cells are made from non-selected stem cells. Additionally, it remains to be proven that decreased susceptibility to myelosuppression will allow sufficient dose escalations to the next limit of toxicity to truly result in increased clinical responses compared to, for example, the use of recombinant growth factors.

D. HIV-1/AIDS

A few studies have been performed using hematopoietic stem cells from patients infected by the AIDS virus, HIV-1. These studies are trying to introduce into the stem cells synthetic “anti-HIV-1 genes” that make cells incapable of supporting growth of HIV-1. Among the anti-HIV-1 genes that have been studied in clinical trials are ribozymes, RRE decoys and dominant-negative REV genes. Initial gene transfer studies in subjects infected by HIV-1 have been designed to determine whether hematopoietic

stem cells from patients with HIV -1 infection can be successfully transduced, whether they will engraft, and whether expression of these genes to inhibit HIV -1 will allow prolonged survival of transduced T-cells. Some of the clinical trials that have been performed sought to observe whether this selective advantage does occur, by using a competitive marking approach with half of each subject's CD34+ cells transduced with the anti-HIV-1 gene vector and half of the cells transduced by a neutral marker vector. Selective protection by the anti-HIV-1 gene would lead to higher levels of peripheral blood cells with the anti-HIV-1 gene than the neutral marker gene.

Most of the initial clinical trials performed targeting anti-HIV -1 genes to HSC, found low levels of gene transfer with minimal levels of gene-containing cells seen *in vivo* after transplant (16-19). Thus, no detectable suppression of HIV -1 or selective survival advantage was seen. More recent studies have achieved somewhat higher levels of gene-containing leukocytes, and a modest preferential survival of cells with the active anti-HIV-1 gene have been seen (20).

E. Genetic Diseases

- 18 Clinical Trials for Genetic Diseases
 - SCID: ADA (5), XSCID (2) Jak3 (1),
 - CGD (4), LAD (1),
 - Gaucher (3),
 - Fanconi (2)

1. Severe Combined Immune Deficiency (SCID)

The original conception of gene therapy using HSC was for the correction of genetic disorders of blood cells, with initial focus on hemoglobinopathies such as thalassemia and sickle cell disease because the relevant beta-globin gene was the first human gene to be cloned. In the mid 1980's, the theoretical advantage of performing gene therapy for immune deficiencies diseases, such as Severe Combined Immune Deficiency (SCID) due to adenosine deaminase (ADA) deficiency became apparent. In SCID, donor-derived genetically normal T cells or their precursors were observed to have a potent selective survival and expansion advantage compared to the genetically defective cells of the SCID patients. Thus, even low levels of gene transfer to HSC could result in a clinical benefit; in contrast, a high percentage of HSC may need to be corrected for a benefit to be seen in hemoglobinopathies. Additionally, ADA is a ubiquitously-expressed housekeeping enzyme and a broad range of expression levels and locations may be beneficial and non-toxic; in contrast, it may be necessary to confine expression of beta-globin to erythroid cells and the levels would need to be fairly precise to match those of the endogenous alpha-globin chain.

Thus, ADA-deficient SCID represented an easier initial disease candidate and could serve as an initial stepping stone to approaching more common but more challenging diseases. In fact, when the first clinical trial of gene therapy was performed (at the National Institutes of Health, USA, by Michael Blaese and French Anderson, it was for ADA-deficient SCID, but targeted peripheral blood T cells rather than HSC because the former cell type was more readily transduced. Discussion of that trial is beyond the

scope of this review, but a long-term follow-up has recently been published (Muul et al Blood 2002 Nov 27;).

The first clinical trial of gene therapy targeting HSC was performed by Bordignon and co-workers in Milan Italy (21). They used a retroviral vector carrying the normal human ADA cDNA to target bone marrow cells from 2 subjects with ADA-deficient SCID. The trial design also involved the use of a second, similar but distinguishable retroviral vector to target peripheral blood T lymphocytes, as in the initial NIH study to directly compare the relative efficacy of the two approaches. They observed that initially, the peripheral blood T cells that contained vectors were derived from the transduced T cells, but over time, there was an increased contribution to peripheral blood T cells from the transduced bone marrow cells. These subjects were treated concomitantly with enzyme replacement therapy with polyethylene glycol-conjugated ADA, and thus no conclusions can be drawn about any effects on immune reconstitution from the gene therapy. No long-term follow-up has been published, so the longevity of gene marking is not known.

Shortly thereafter, Hoogerbrugge, Valerio and colleagues in the Netherlands performed a clinical trial for ADA-deficient SCID, targeting CD34+ cells from the bone marrow of three ADA-deficient SCID infants from France and England (22). They had poor recovery of cells following the in vitro transduction, and there was only minimal, short-term detection of gene-containing peripheral blood T cells.

In 1993, Kohn and colleagues at Childrens Hospital Los Angeles collaborated with Blaese and co-workers at the NIH to perform a trial of retroviral-mediated gene transfer to the umbilical cord blood CD34+ cells from ADA-deficient neonates (23,24). The same LASN retroviral vector that had been produced for the initial NIH trial targeting T cells was used. A moderate level of gene transfer was achieved, with gene-containing cell of myeloid and lymphoid lineages seen in all three subjects for more than 6-9 years. The level of gene-containing T cells was 10-1000-times higher than gene-containing myeloid cells, demonstrating the postulated selective survival advantage as the PEG-ADA enzyme replacement dosages were decreased. However, attempts to take one subject completely off PEG-ADA were unsuccessful, with a decline in numbers of B and NK cells and clinical signs of oral candidiasis. A recent analysis of one of the patients (with the highest levels of gene marking) using LAM-PCR revealed that the majority of gene-containing cells were derived from a single stem or progenitor cell, that gave rise to T cells with multiple T cell receptor gene rearrangements, indicating that it was transduced at a pre-thymic stage. Eight years after the neonatal gene transfer procedure, all CD4+ T cells in this subject (with or without the transferred ADA gene) have a memory (CD4+/CD45RO+) immunophenotype, suggesting that the transduced stem/progenitor cell was no longer actively giving rise to new T lymphocytes.

In the more recent era, there have been three clinical trials for SCID (2 for XSCID, one for ADA-deficient SCID) that have shown unequivocal evidence for beneficial effects.

Fischer, Cavazzano-Calvo and co-workers in Paris performed the first trial of gene therapy for the X-linked form of SCID (26,27). Following extensive pre-clinical studies that showed efficacy and safety, a retroviral vector carrying the relevant normal human

gammaC cDNA was used to transduce CD34+ cells from the bone marrow of a total of 11 subjects, four infants with typical presentation and 1 teenage subject who had uncharacteristically long survival for an untreated SCID patient, but with a history of several significant infections. Rapid and robust immune reconstitution was achieved in all of the subjects, except the teenage subject described above and a child with a pre-existing opportunistic infection that resulted in massive splenomegaly that may have led to loss of the graft. The kinetics of T cell reconstitution were as rapid as with HLA-matched bone sibling bone marrow, with better B cell function than that seen in haplo-identical, T cell depleted BMT.

Tragically, after approximately 2.5 years, two of the subjects presented with clonal T cell proliferation consistent with T cell leukemia. These events are still under intensive investigation by Fischer and a cadre of collaborators. In both cases, the clonally proliferating T cell contain a single copy of intact retroviral vector present in or near the LMO2 gene. LMO2 is a transcriptional factor expressed in early HSC and progenitor cells and can be an oncogene when activated by chromosomal translocation or when expressed constitutively in transgenic mice. Thus, these two cases represent insertional oncogenesis, a complication that was thought to be theoretically possible, but highly unlikely in the absence of replication-competent retroviruses that could cause massive numbers of chromosomal insertions.

It remains unclear why these two cases of T cell leukemia associated with vector insertion at the LMO2 locus occurred in these patients, but not in any of the more than 200 subjects under review here. Speculations center around the possible role of the gammaC transgene as a co-factor augmenting LMO2-mediated transformation, unusual properties of the bone marrow HSC or progenitors from these subjects due to their underlying disease or young age that make them more susceptible to vector integration at the LMO2 locus or simply the high numbers of CD34+ cells that were transduced, increasing the stochastic probability of insertions near LMO2.

Thrasher and co-workers at Great Ormond Street Childrens Hospital in London have performed a similar trial of retroviral-mediated gene transfer for four XSCID subjects. They have observed similar extents of immune reconstitution as seen in the Paris study, without adverse events. However, it must be cautioned that the follow-up time on these subjects in London is shorter than the time at which the leukemia became apparent in the Paris study and therefore these subjects must be followed carefully.

The Milan group of Bordignon et al have performed a more recent study of retroviral-mediated gene transfer for ADA-deficient SCID (25). They enrolled subjects who were not receiving PEG-ADA, since enzyme replacement therapy may be responsible for blunting the selective advantage of gene correction, as occurred in the XSCID studies. Additionally, they gave the subjects moderate dosages of non-myeloablative cytoreductive chemotherapy (busulfan 4 mg/kg – full ablative dosages are generally 16 mg/kg) prior to re-infusion of the transduced CD34+ cells to “make space” and allow a higher level of engraftment of transduced cells. This was thought to be important for ADA-deficiency SCID to allow better correction of non-T cell lineages, such as RBC, granulocytes, etc which may be necessary for generalized metabolic detoxification of non-hematopoietic organs (e.g. liver, CNS). These subjects have achieved excellent

reconstitution of T and B cell function up to two years of follow-up. The levels of myeloid cells that contain the transferred gene are significantly higher than in other studies for SCID that did not give cytoreductive chemotherapy, supporting the use of non-myeloablative cytoreduction to attain higher levels of gene-containing cells. No adverse events were seen, except for the expected transient myelosuppression from the busulfan.

In 2001 the CHLA and NIH groups began a second collaborative trial for ADA-deficient SCID, using improved vectors and gene transduction conditions, compared to their earlier effort. A total of four subjects were treated, significantly older (4-20 years) than the infant subjects in the other studies, with subjects on PEG-ADA for 4-10 years. The patients were kept on PEG-ADA throughout and did not receive any cytoreductive conditioning. In the 1-1.5 years of follow-up to date, the levels of gene-containing peripheral blood cells have been low, but detectable in the two younger subjects. The gene transfer methods were similar to those of the Milan study, highlighting the benefits from the novel elimination of PEG-ADA and use of chemotherapy in that trial. No adverse events related to the gene transfer have been observed.

In summary, recent trials for SCID in XSCID or ADA-deficiency where the selective advantage was not blunted have proven the principle of gene therapy, but have been seriously marred by the development of leukemia in two patients as a direct complication of the approach. Careful assessments of the risks and benefits for gene therapy compared to alternatives (such as haplo-identical BMT, unrelated cord blood transplant) must be performed in considering this approach.

2. Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is an inherited immune deficiency that has been the subject of clinical gene therapy trials. CGD is characterized by the inability of granulocytes to kill bacteria they have ingested due to genetic absence of one of the oxidase proteins that generates toxic anti-microbial oxygen moieties. CGD patients have recurrent pyogenic infections, like Staphylococcal skin infections, and can have deep-seated abscesses with fungi, such as Aspergillus.

Malech and co-workers at the National Institutes of Health, Bethesda MD, have used G-CSF mobilized peripheral blood stem cells as the target for a retroviral vector carrying the normal oxidase gene (*p47phox*) that is lacking in some CGD subjects (29,30). They also performed similar studies with similar outcome using another normal oxidase gene (*gp91phox*) to correct X-linked CGD (31,32). Using a sensitive FACS-based assay for oxidase function, they demonstrated the presence of genetically-corrected neutrophils in the peripheral circulation from 2 - 12 weeks after treatment, although at a frequency of only 0.1 percent. After this period, the corrected neutrophils were no longer detected. Similar results have been seen in a study by Croop, Dinauer and colleagues at Indiana University for the X-linked form of CGD, with a retroviral vector carrying the *gp91 phox* cDNA. No selective survival advantage is expected for gene corrected granulocytes (only better function), so there was no amplification of the presumably low numbers of stem cells that were corrected. The findings are consistent with transduction and

engraftment of only relatively short-lived, committed progenitors; after the transduced progenitor cells had completed their time of contribution to mature cell pools, new progenitors derived from non-transduced stem cells produced neutrophils that lacked the transferred oxidase gene. It is possible that this transient production of gene-corrected neutrophils could confer some clinical benefits for a CGD patient suffering an acute infection.

3. Leukocyte Adherence Deficiency

Bauer and Hickstein have performed pre-clinical studies and a clinical trial for two subjects with Leukocyte Adhesion Deficiency, due to genetic defects in the gene for CD18, a component of a family of leukocyte hetero-dimeric adhesion molecules (28). They had shown that transfer of a normal CD18 gene into patient cells restores expression and function of the adhesion molecule. In the clinical trial targeting G-CSF mobilized PBSC, the levels of gene transfer achieved were too low and short-lived for an appreciable effect, as in the studies for CGD.

4. Gaucher Disease

Three studies were performed in the mid-1990's targeting stem cells from patients with Gaucher disease. These studies yielded relatively poor transduction of the marrow or peripheral blood CD34+ cells and minimal detectable gene-containing cells in the circulation of patients after treatment (33-35). In the absence of a selective survival advantage or significant cross-correction of host cells by gene-containing cells, Gaucher disease is likely to need relatively high levels of gene transfer to stem cells with at least partial cytoreduction for a therapeutic effect to be reached.

5. Fanconi's Anemia

Johnson Liu and Christopher Walsh have performed trials of retroviral-mediated transfer of the Fanconi anemia complementation group A (FAC) and C (FAC) gene (36). CD34+ cells from autologous mobilized peripheral blood were transduced and then infused without prior cytoablation. Corrected peripheral blood cells were seen in the first months after treatment but were not present after 1 year. One subject of this trial who subsequently underwent localized radiation therapy for a malignancy had the reappearance of gene-containing leukocytes, although long-term results in this subject have not been reported. This observation suggests that gene-containing cells were present at a level too low to detect until the radiation therapy caused preferential suppression of hematopoiesis from the endogenous radiation hypersensitive non-corrected marrow with a selective proliferation of the gene-corrected cells. Further incremental increases in the efficiency of gene transfer may lead to sufficient numbers of corrected cells to restore hematopoiesis in patients with Fanconi anemia.

F. Summary/Conclusions:

From this review of the information from all of these clinical trials, it can be seen that no SAE related to retroviral vectors were observed, except for the two cases in the XSCID

trial. But, in contrast to the very high level of gene-containing cells seen in the XSCID studies (and the Italian trial for ADA-deficiency), *most of the other studies have been characterized by very low levels of gene-containing cells in circulation (<1-2 years) and a lack of long-term persistence.* Many subjects in the gene marking studies were patients with advanced malignant disease (undergoing therapeutic HSCT) to which they succumbed within months to a few years of participation in the gene transfer trial. Most for genetic diseases were done without any cytoreductive therapy, and thus engraftment of gene-containing cells was at very low levels. **Thus, the lack of SAE in these studies cannot be used to support the general safety of retroviral-mediated gene transfer to HSC. One cannot make conclusions about the safety of gene therapy when there is not significant gene transfer to the relevant target cells.** It is possible that the level of gene transfer to HSC in these studies was higher than detected by gene marking of peripheral blood cells and the absence of lymphoproliferation indicates that this is not obligatorily a problem. The few exceptional studies that did show more long-term persistence of gene marked cells (the gene marking studies in pediatric subjects at St. Judes, the ADA gene transfer into umbilical cord blood cells of infants) did not have any cases of leukemia or other complications.

Inspection of the available data on cell dosages reveals that the numbers of cells/kg administered in other studies were much lower than in the XSCID studies. It is not known if this finding of uniquely high numbers of cells/kg in the French trial has any significance. If insertional oncogenesis is a purely stochastic event, then risk would be proportionate to absolute number of integrants, and would not be expected to be a function of #transduced cells/kg. The absolute numbers of cells that were given to the XSCID subjects were not excessive, compared to many studies, e.g. those giving transduced PBSC to adult oncology patients.

The high #CD34+ cells/kg that were obtained from the bone marrow harvests of these XSCID infants are atypical, compared to essentially all of the other trials that have been reported. It may be speculated that there were increased numbers of progenitors present in the marrow of the XSCID patients because of their expansion and accumulation as a result of a maturation block imposed by absence of gammaC and the inability to differentiate by lymphopoiesis in response to IL -7 and IL-2. Their young age may also contribute to the unusually high numbers of proliferating stem/progenitor cells present. There are scant data on the numbers and proliferation status of bone marrow cells in normal infants, due to the ethical limitations on performing non-therapeutic marrow aspirations. Certainly, umbilical cord blood is known to have greater numbers of proliferating primitive progenitor cells than adult bone marrow; the marrow of young infants may similarly have increased numbers of cycling stem/progenitor cells that are susceptible to retroviral transduction. The absence of this complication in the three ADA-deficient SCID infants treated with retroviral-transduced umbilical cord blood in 1993 may reflect less effective gene transfer with the vectors and conditions used then. In any case, with the relatively long latency period seen before the two cases of leukemia became clinically apparent in two subjects (more than 30 months), other subjects in the XSCID and SADA trials can be considered to still be at risk and close observation is merited.

Observations:

In performing this data collection effort, it is apparent that there is not a standard format used among the different trials for quantification of cell numbers, measurement of gene transfer in the grafted cells, assessment of presence of gene-containing cells, duration of follow-up, etc. It would greatly facilitate future efforts to analyze trial results if some uniformity could be adopted. The International Bone Marrow Transplant Registry (IBMTR) has formed a working group to begin the prospective collection of data from trials involving gene transfer to HSC. It may be recommended that similar activities be performed in other areas of gene therapy, although patient confidentiality and proprietary interests would need to be respected.