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Stem Cell

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Stem Cell Technology Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; technology; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Abe, K. (2008). "[Neuroprotective therapy for ischemic stroke with free radical scavenger and gene-stem cell therapy]." Rinsho Shinkeigaku **48**(11): 896-898.

A free radical scavenger Edaravone is the first clinical drug for neuroprotection in the world which has been used from 2001 in most ischemic stroke patients in Japan, and is especially useful in thrombolytic therapy with tissue plasminogen activator (tPA). Of great importance for regenerative therapy and gene therapy are the neural stem cells which are intrinsically activated or exogenously transplanted. Addition of NTFs greatly enhanced an intrinsic migration or invasion of stem cells into the scaffold, which could provide a future regenerative potential against ischemic brain damage at chronic stage.

Abe, K. (2009). "[Gene-stem Cell therapy for ischemic stroke]." Brain Nerve **61**(9): 1043-1049.

Besides blood flow restoration, neuroprotection is essential for treating strokes at an acute stage. Both neurotrophic factors (NTFs) and free radical scavengers can act as neuroprotective agents with abilities to inhibit cell death and facilitate cell survival under cerebral ischemia. For example, topical application of glial cell line-derived neurotrophic factor (GDNF) remarkably reduced infarct size and brain edema after middle cerebral artery (MCA) occlusion in rats. Reduction in the infarct size was not found to be related to a change in the cerebral blood flow (CBF), but was accompanied by marked reduction in BrdU-positive cells in the affected area after TdT-mediated dUTP-biotin nick end labeling (TUNEL) for caspses. Thus, GDNF elicited a direct protective effect against ischemic brain damage, but without improving CBF. Sendai virus vectors harboring the GDNF gene led to a remarkable reduction in infract volume without affecting regional CBF but reduced the translocation of apoptosis inducible factor (AIF) from the mitochondria to cytoplasm. Regenerative therapy involving neural stem cells which are intrinsically activated or exogenously transplanted, is an important treatment strategy. To facilitate stem cell migration, an artificial scaffold can be implanted into the injured brain for promoting ischemic brain repair. Addition of NTFs greatly enhanced an intrinsic migration or invasion of stem cells into the scaffold: this strategy could be used in the future for enhancing regenerative potential of

brain cells after chronic ischemia-induced brain damage.

Abkowitz, J. L., et al. (1997). "Strategies for hematopoietic stem cell gene therapy: insights from computer simulation studies." Blood **89**(9): 3192-3198.

We simulated gene therapy using parameters derived from the analysis of autologous transplantation studies in glucose-6-phosphate dehydrogenase heterozygous cats to determine how hematopoietic stem cell (HSC) biology might influence outcomes. Simulation illustrates that a successful experiment can result by chance and may not be the repeated outcome of a specific protocol design or technical approach. As importantly, in many simulated gene therapy experiments where 1, 2, or 6 of 30 transplanted HSC were labeled, there was significant variation in the contribution from marked clones over time. Variability was minimized in simulations in which large numbers of HSC were transplanted. Strategies that may permit consistent clinically successful results are presented. Taken together, these simulation studies demonstrate that the in vivo behavior of HSC must be considered when optimizing approaches to gene therapy in large animals, and perhaps by extension, in humans.

Aboody, K. S., et al. (2008). "Stem and progenitor cell-mediated tumor selective gene therapy." Gene Ther **15**(10): 739-752.

The poor prognosis for patients with aggressive or metastatic tumors and the toxic side effects of currently available treatments necessitate the development of more effective tumor-selective therapies. Stem/progenitor cells display inherent tumor-tropic properties that can be exploited for targeted delivery of anticancer genes to invasive and metastatic tumors. Therapeutic genes that have been inserted into stem cells and delivered to tumors with high selectivity include prodrug-activating enzymes (cytosine deaminase, carboxylesterase, thymidine kinase), interleukins (IL-2, IL-4, IL-12, IL-23), interferon-beta, apoptosis-promoting genes (tumor necrosis factor-related apoptosis-inducing ligand) and metalloproteinases (PEX). We and others have demonstrated that neural and mesenchymal stem cells can deliver therapeutic genes to elicit a significant antitumor response in animal models of intracranial glioma, medulloblastoma, melanoma brain metastasis, disseminated neuroblastoma and breast cancer lung metastasis. Most studies reported reduction in tumor volume (up to 90%) and increased survival of tumor-bearing animals. Complete cures have also been achieved (90% disease-free survival for >1 year of mice bearing disseminated neuroblastoma tumors). As we learn more about the biology of stem cells and the molecular mechanisms that mediate their tumor-tropism and we identify efficacious gene products for specific tumor types, the clinical utility of cell-based delivery strategies becomes increasingly evident.

Achberger, K., et al. (2021). "Human stem cell-based retina on chip as new translational model for validation of AAV retinal gene therapy vectors." Stem Cell Reports **16**(9): 2242-2256.

Gene therapies using adeno-associated viruses (AAVs) are among the most promising strategies to treat or even cure hereditary and acquired retinal diseases. However, the development of new efficient AAV vectors is slow and costly, largely because of the lack of suitable non-clinical models. By faithfully recreating structure and function of human tissues, human induced pluripotent stem cell (iPSC)-derived retinal organoids could become an essential part of the test cascade addressing translational aspects. Organ-on-chip (OoC) technology further provides the capability to recapitulate microphysiological tissue environments as well as a precise control over structural and temporal parameters. By employing our recently developed retina on chip that merges organoid and OoC technology, we analyzed the efficacy, kinetics, and cell tropism of seven first- and second-generation AAV vectors. The presented data demonstrate the potential of iPSC-based OoC models as the next generation of screening platforms for future gene therapeutic studies.

Ackermann, M., et al. (2014). "Promoter and lineage independent anti-silencing activity of the A2 ubiquitous chromatin opening element for optimized human pluripotent stem cell-based gene therapy." Biomaterials **35**(5): 1531-1542.

Epigenetic silencing of retroviral transgene expression in pluripotent stem cells (PSC) and their differentiated progeny constitutes a major roadblock for PSC-based gene therapy. As ubiquitous chromatin opening elements (UCOEs) have been successfully employed to stabilize transgene expression in murine hematopoietic and pluripotent stem cells as well as their differentiated progeny, we here investigated UCOE activity in their human counterparts to establish a basis for future clinical application of the element. To this end, we demonstrate profound anti-silencing activity of the A2UCOE in several human iPS and ES cell lines including their progeny obtained upon directed cardiac or hematopoietic differentiation. We also provide evidence for A2UCOE activity in murine iPSC-derived hepatocyte-like cells, thus establishing efficacy of the element in cells of different germ layers. Finally, we investigated combinations of the A2UCOE with viral promoter/enhancer elements again demonstrating profound stabilization of transgene expression. In all these settings the effect of the A2UCOE was associated with strongly reduced promoter DNA-methylation. Thus, our data clearly support the concept of the A2UCOE as a generalized

strategy to prevent epigenetic silencing in PSC and their differentiated progeny and strongly favors its application to stabilize transgene expression in PSC-based cell and gene therapy approaches.

Alderuccio, F., et al. (2011). "Hematopoietic stem cell gene therapy as a treatment for autoimmune diseases." Mol Pharm **8**(5): 1488-1494.

A key function of the immune system is to protect us from foreign pathogens such as viruses, bacteria, fungi and multicellular parasites. However, it is also important in many other aspects of human health such as cancer surveillance, tissue transplantation, allergy and autoimmune disease. Autoimmunity can be defined as a chronic immune response that targets self-antigens leading to tissue pathology and clinical disease. Autoimmune diseases, as a group of diseases that include type 1 diabetes, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus, have no effective cures, and treatment is often based on long-term broad-spectrum immunosuppressive regimes. While a number of strategies aimed at providing disease specific treatments are being explored, one avenue of study involves the use of hematopoietic stem cells to promote tolerance. In this manuscript, we will review the literature in this area but in particular examine the relatively new experimental field of gene therapy and hematopoietic stem cell transplantation as a molecular therapeutic strategy to combat autoimmune disease.

Alderuccio, F., et al. (2006). "Haematopoietic stem cell gene therapy to treat autoimmune disease." Curr Stem Cell Res Ther **1**(3): 279-287.

Autoimmune diseases affect approximately 6% of the population and are characterised by a pathogenic immune response that targets self-antigens. Well known diseases of this nature include type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. Treatment is often restricted to replacement therapy or immunosuppressive regimes and to date there are no cures. The strategy of utilising autologous or allogeneic haematopoietic stem cell transplantation to treat autoimmunity and induce immunological tolerance has been trailed with various levels of success. A major issue is disease relapse as the autoimmune response is reinitiated. Cells of the immune system originate from bone marrow and have a central role in the induction of immunological tolerance. The ability to isolate and genetically manipulate bone marrow haematopoietic stem cells therefore makes these cells a suitable vehicle for driving ectopic expression of defined autoantigens and induction of immunological tolerance.

Almeida-Porada, G., et al. (2016). "In utero stem cell transplantation and gene therapy: rationale, history, and recent advances toward clinical application." Mol Ther Methods Clin Dev **5**: 16020.

Recent advances in high-throughput molecular testing have made it possible to diagnose most genetic disorders relatively early in gestation with minimal risk to the fetus. These advances should soon allow widespread prenatal screening for the majority of human genetic diseases, opening the door to the possibility of treatment/correction prior to birth. In addition to the obvious psychological and financial benefits of curing a disease in utero, and thereby enabling the birth of a healthy infant, there are multiple biological advantages unique to fetal development, which provide compelling rationale for performing potentially curative treatments, such as stem cell transplantation or gene therapy, prior to birth. Herein, we briefly review the fields of in utero transplantation (IUTx) and in utero gene therapy and discuss the biological hurdles that have thus far restricted success of IUTx to patients with immunodeficiencies. We then highlight several recent experimental breakthroughs in immunology, hematopoietic/marrow ontogeny, and in utero cell delivery, which have collectively provided means of overcoming these barriers, thus setting the stage for clinical application of these highly promising therapies in the near future.

Altaner, C. and U. Altanerova (2019). "Mesenchymal Stem Cell Exosome-Mediated Prodrug Gene Therapy for Cancer." Methods Mol Biol **1895**: 75-85.

Exosomes derived from human mesenchymal stem cells (MSCs) engineered to express the suicide gene yeast cytosine deaminase::uracil phosphoribosyl transferase (yCD::UPRT) represent a new therapeutic approach for tumor-targeted innovative therapy. The yCD::UPRT-MSC-exosomes carry mRNA of the suicide gene in their cargo. Upon internalization by tumor cells, the exosomes inhibit the growth of broad types of cancer cells in vitro, in the presence of a prodrug. Here we describe the method leading to the production and testing of these therapeutic exosomes. The described steps include the preparation of replication-deficient retrovirus possessing the yCD::UPRT suicide gene, and the preparation and selection of MSCs transduced with yCD::UPRT suicide gene. We present procedures to obtain exosomes possessing the ability to induce the death of tumor cells. In addition, we highlight methods for the evaluation of the suicide gene activity of yCD::UPRT-MSC-exosomes.

Altaner, C. and V. Altanerova (2012). "Stem cell based glioblastoma gene therapy." Neoplasma **59**(6): 756-760.

There is no curative therapy for glioblastoma multiforme (GBM) thus far. Combined therapies including surgery, followed by concomitant irradiation and chemotherapy with the DNA alkylating agent

temozolomide (TMZ), slightly improves patients' survival but the prognosis remains poor. The fatal nature of glioblastoma is caused by tumor-initiating glioblastoma cells. The tumor tropic ability of adult mesenchymal stem cells offers the attractive possibility to use these cells as a vehicle to deliver therapeutic agents to the site of the tumor. In preclinical studies using animal models, mesenchymal stem cells engineered to express suicide genes were shown to elicit a significant antitumor response against various tumors including glioblastoma. This review summarizes the current state of knowledge about stem cell directed glioblastoma therapy. Results obtained in a preclinical study using mesenchymal stem cells engineered to express cytosine deaminase provided evidence that stem cell based gene therapy might also attack glioblastoma stem cells and therefore be curative. In addition to stem cell directed prodrug gene therapies, other immunotherapeutic modalities using mesenchymal stem cells are discussed as well. Encouraging results of preclinical studies of stem cell based gene therapy for glioblastoma support the argument to begin clinical studies.

Altanerova, U., et al. (2019). "Prodrug suicide gene therapy for cancer targeted intracellular by mesenchymal stem cell exosomes." Int J Cancer **144**(4): 897-908.

The natural behavior of mesenchymal stem cells (MSCs) and their exosomes in targeting tumors is a promising approach for curative therapy. Human tumor tropic mesenchymal stem cells (MSCs) isolated from various tissues and MSCs engineered to express the yeast cytosine deaminase::uracil phosphoribosyl transferase suicide fusion gene (yCD::UPRT-MSCs) released exosomes in conditional medium (CM). Exosomes from all tissue specific yCD::UPRT-MSCs contained mRNA of the suicide gene in the exosome's cargo. When the CM was applied to tumor cells, the exosomes were internalized by recipient tumor cells and in the presence of the prodrug 5-fluorocytosine (5-FC) effectively triggered dose-dependent tumor cell death by endocytosed exosomes via an intracellular conversion of the prodrug 5-FC to 5-fluorouracil. Exosomes were found to be responsible for the tumor inhibitory activity. The presence of microRNAs in exosomes produced from naive MSCs and from suicide gene transduced MSCs did not differ significantly. MicroRNAs from yCD::UPRT-MSCs were not associated with therapeutic effect. MSC suicide gene exosomes represent a new class of tumor cell targeting drug acting intracellular with curative potential.

Andre, I., et al. (2019). "Ex vivo generated human T-lymphoid progenitors as a tool to accelerate immune reconstitution after partially HLA compatible hematopoietic stem cell transplantation or after gene therapy." Bone Marrow Transplant **54**(Suppl 2): 749-755.

Prolonged T-cell immunodeficiency following HLA- incompatible hematopoietic stem cell transplantation (HSCT) represents a major obstacle hampering the more widespread use of this approach. Strategies to fasten T-cell reconstitution in this setting are highly warranted as opportunistic infections and an increased risk of relapse account for high rates of morbidity and mortality especially during early month following this type of HSCT. We have implemented a feeder free cell system based on the use of the notch ligand DL4 and cytokines allowing for the in vitro differentiation of human T-Lymphoid Progenitor cells (HTLPs) from various sources of CD34+ hematopoietic stem and precursor cells (HSPCs). Co-transplantion of human T-lymphoid progenitors (HTLPs) and non- manipulated HSPCs into immunodeficient mice successfully accelerated the reconstitution of a polyclonal T-cell repertoire. This review summarizes preclinical data on the use of T-cell progenitors for treatment of post- transplantation immunodeficiency and gives insights into the development of GMP based protocols for potential clinical applications including gene therapy approaches. Future clinical trials implementing this protocol will aim at the acceleration of immune reconstitution in different clinical settings such as SCID and leukemia patients undergoing allogeneic transplantation. Apart from pure cell-therapy approaches, the combination of DL-4 culture with gene transduction protocols will open new perspectives in terms of gene therapy applications for primary immunodeficiencies.

Avedillo Diez, I., et al. (2011). "Development of novel efficient SIN vectors with improved safety features for Wiskott-Aldrich syndrome stem cell based gene therapy." Mol Pharm **8**(5): 1525-1537.

Gene therapy is a promising therapeutic approach to treat primary immunodeficiencies. Indeed, the clinical trial for the Wiskott-Aldrich Syndrome (WAS) that is currently ongoing at the Hannover Medical School (Germany) has recently reported the correction of all affected cell lineages of the hematopoietic system in the first treated patients. However, an extensive study of the clonal inventory of those patients reveals that LMO2, CCND2 and MDS1/EVI1 were preferentially prevalent. Moreover, a first leukemia case was observed in this study, thus reinforcing the need of developing safer vectors for gene transfer into HSC in general. Here we present a novel self-inactivating (SIN) vector for the gene therapy of WAS that combines improved safety features. We used the elongation factor 1 alpha (EFS) promoter, which has been extensively evaluated in

terms of safety profile, to drive a codon-optimized human WASP cDNA. To test vector performance in a more clinically relevant setting, we transduced murine HSPC as well as human CD34+ cells and also analyzed vector efficacy in their differentiated myeloid progeny. Our results show that our novel vector generates comparable WAS protein levels and is as effective as the clinically used LTR-driven vector. Therefore, the described SIN vectors appear to be good candidates for potential use in a safer new gene therapy protocol for WAS, with decreased risk of insertional mutagenesis.

Azhagiri, M. K. K., et al. (2021). "Homology-directed gene-editing approaches for hematopoietic stem and progenitor cell gene therapy." Stem Cell Res Ther **12**(1): 500.

The advent of next-generation genome engineering tools like CRISPR-Cas9 has transformed the field of gene therapy, rendering targeted treatment for several incurable diseases. Hematopoietic stem and progenitor cells (HSPCs) continue to be the ideal target cells for gene manipulation due to their long-term repopulation potential. Among the gene manipulation strategies such as lentiviral gene augmentation, non-homologous end joining (NHEJ)-mediated gene editing, base editing and prime editing, only the homology-directed repair (HDR)-mediated gene editing provides the option of inserting a large transgene under its endogenous promoter or any desired locus. In addition, HDR-mediated gene editing can be applied for the gene knock-out, correction of point mutations and introduction of beneficial mutations. HSPC gene therapy studies involving lentiviral vectors and NHEJ-based gene-editing studies have exhibited substantial clinical progress. However, studies involving HDR-mediated HSPC gene editing have not yet progressed to the clinical testing. This suggests the existence of unique challenges in exploiting HDR pathway for HSPC gene therapy. Our review summarizes the mechanism, recent progresses, challenges, and the scope of HDR-based gene editing for the HSPC gene therapy.

Badawy, S. M., et al. (2021). "A systematic review of quality of life in sickle cell disease and thalassemia after stem cell transplant or gene therapy." Blood Adv **5**(2): 570-583.

Patients with sickle cell disease (SCD) and thalassemia experience several complications across their lifespan that lead to impairment in different health-related quality of life (HRQOL) domains. There is increasing interest in curative therapies for patients with SCD and thalassemia, including hematopoietic stem cell transplant (HSCT) and gene therapy; however, the effect of these therapies on various HRQOL domains remains unclear. Our objective was to systematically evaluate the most recent evidence for the effect of HSCT and gene therapy on HRQOL in patients with SCD and thalassemia. A systematic search of medical literature databases was conducted. A total of 16 studies (thalassemia, n = 9; SCD, n = 6; both, n = 1) involving 517 participants met inclusion criteria (thalassemia, n = 416; SCD, n = 101). HSCT was associated with a small to large positive effects in most HRQOL domains (Cohen's d; mean = 0.47; median = 0.37; range, 0.27-2.05). In thalassemia, HSCT was frequently associated with large positive effects in physical and emotional HRQOL domains (median d = 0.79 and d = 0.57, respectively). In SCD, HSCT was associated with large positive effects in all HRQOL domains. Emerging data suggest improvement in HRQOL outcomes across different domains following gene therapy in thalassemia and SCD. The quality of evidence was moderate in 13 studies (81%). HSCT has a positive impact on several HRQOL domains in patients with SCD and thalassemia; however, more longitudinal studies are warranted to assess the sustainability of these effects. Reporting HRQOL outcomes from ongoing gene therapy or gene-editing trials in SCD and thalassemia is key to better understand the benefits of such therapies.

Bagnis, C. and P. Mannoni (1997). "Stem Cell-Based Gene Therapy." Oncologist **2**(3): 196-202.

Many researchers and clinicians wonder if gene therapy remains a way to treat genetic or acquired life-threatening diseases. For the last few years, many experimental, pre-clinical, and clinical data have been published showing that it is possible to transfer with relatively high efficiency new genetic information (transgene) in many cells or tissues including both hematopoietic progenitor cells and differentiated cells. Based on experimental works, addition of the normal gene to cells with deletions, mutations, or alterations of the corresponding endogenous one has been shown to reverse the phenotype and to restore (in some case) the functional defect. In spite of very attractive preliminary results, however, suggesting the feasibility and safety of this process, therapeutically efficient gene transfer and expression in targeted cells or tissues must be proven. In this review, we will focus primarily on the attempts to use gene transfer in hematopoietic stem cells as a model for more general genetic manipulations of stem cells. Hematopoietic stem cells are included in a subset of bone marrow, cord blood, or peripheral blood cells identified by the expression of the CD34 antigen on their membrane.

Bai, J., et al. (2001). "Multivalent anti-CCR ribozymes for stem cell-based HIV type 1 gene therapy." AIDS Res Hum Retroviruses **17**(5): 385-399.

HIV-1 infection of susceptible cells is mediated by the specific interaction of viral envelope glycoproteins with the cell surface CD4 receptor and a chemokine coreceptor, CCR5 or CXCR4. Individuals with a CCR5 genetic defect show resistance to HIV-1 infection, indicating that downregulation of CCR5 expression on target cells can prevent viral infection. In previous studies we demonstrated the utility of an anti-CCR5 ribozyme targeted to a single cleavage site in downregulating CCR5 expression and consequently providing resistance to viral infection. To improve on the level of downregulation we designed a construct containing an anti-CCR5 ribozyme heterotrimer (R5RbzTM) targeted to three different cleavage sites in CCR5 mRNA. In vitro tests showed that the anti-CCR5 ribozyme heterotrimer could effectively cleave the CCR5 RNA substrates to yield products of the expected sizes. This construct was introduced into various retroviral vectors for stable gene transduction. HOS.CD4/R5 cells stably transduced with this anti-CCR5 heterotrimer showed a marked reduction in the surface expression of CCR5 and a concomitant 70% reduction in macrophage-tropic viral infection. In addition, a retroviral vector containing the anti-CCR5 ribozyme heterotrimer and an anti-HIV-1 tat-rev ribozyme heterodimer was constructed. This construct also showed a similar inhibition of CCR5 surface expression and reduced infectability by the macrophage-tropic HIV-1 vector in HOS.CD4/R5 cells. The trimeric and multimeric ribozyme constructs were transduced into CD34+ hematopoietic progenitor cells to determine their effects on lineage-specific differentiation. We show that multivalent ribozyme gene-transduced hematopoietic progenitors differentiated normally into mature macrophages that bear CD14 and CD4 surface markers. Macrophages containing the transgenes expressed ribozymes, and showed resistance to M-tropic HIV-1 infection. These results provide strong support for the use of the trimeric anti-CCR5 ribozyme approach in a gene therapy setting for the treatment of HIV infection.

Baillou, C., et al. (2003). "Highly active antiretroviral therapy corrects hematopoiesis in HIV-1 infected patients: interest for peripheral blood stem cell-based gene therapy." AIDS **17**(4): 563-574.

OBJECTIVES: To study, in asymptomatic HIV-1-infected (HIV+) patients, whether peripheral blood hematopoietic progenitor/stem cells (PBPC) mobilized by granulocyte colony stimulating factor (G-CSF), can be used as a source of cells for retroviral gene therapy. DESIGN: PBPC from two groups of HIV+ patients (treated or untreated by highly active antiretroviral therapy) and from seronegative donors were mobilized with G-CSF. METHODS: PBPC collected by leukapheresis were enriched for CD34 cells, immunophenotypically and functionally characterized, cultured and infected with retroviral vectors. HIV proviral integration was studied on fresh and cultured cells. RESULTS: G-CSF moderately and transiently increased the viral load in untreated patients only, and induced in both groups of HIV+ patients mobilization of percentages and numbers of CD34 cells comparable to those of seronegative volunteers. The most immature CD34 cell subset, the clonogenic progenitor and long-term culture initiating cells were significantly decreased in leukapheresis products and CD34-enriched fractions from untreated HIV+ patients but not in those from treated HIV+ patients. Cell cycle activation and growth factor responses of CD34 cells from both groups of HIV+ patients were not different from those of the control group. Culture and retroviral infection of CD34 cells from HIV+ patients did not enhance HIV replication, and yielded transduction levels similar to those obtained using CD34 cells from seronegative donors. CONCLUSIONS: G-CSF-mobilized PBPC can be safely used for HIV retroviral gene therapy in asymptomatic treated patients while highly active antiretroviral therapy would control the G-CSF-induced increase in viral load and correct the defective hematopoiesis observed in untreated patients, without inhibiting the retroviral transduction of PBPC.

Bak, X. Y., et al. (2011). "Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma." Hum Gene Ther **22**(11): 1365-1377.

Mesenchymal stem cells (MSCs) possess tumor-tropic properties and consequently have been used to deliver therapeutic agents for cancer treatment. Their potential in cancer therapy highlights the need for a consistent and renewable source for the production of uniform human MSCs suitable for clinical applications. In this study, we seek to investigate whether human embryonic stem cells can be used as a cell source to fulfill this goal. We generated MSC-like cells from two human embryonic stem cell lines, HuES9 and H1, and observed that MSC-like cells derived from human embryonic stem cells were able to migrate into human glioma intracranial xenografts after being injected into the cerebral hemisphere contralateral to the tumor inoculation site. We engineered these cells with baculoviral and lentiviral vectors, respectively, for transient and stable expression of the herpes simplex virus thymidine kinase gene. In tumor-bearing mice the engineered MSC-like cells were capable of inhibiting tumor growth and prolonging survival in the presence of ganciclovir after they were injected either directly into the xenografts or into the opposite hemisphere. Our findings suggest that human embryonic stem cell-derived MSCs may be a viable and attractive

alternative for large-scale derivation of targeting vehicles for cancer therapy.

Baksh, D., et al. (2004). "Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy." J Cell Mol Med **8**(3): 301-316.

A considerable amount of retrospective data is available that describes putative mesenchymal stem cells (MSCs). However, there is still very little knowledge available that documents the properties of a MSC in its native environment. Although the precise identity of MSCs remains a challenge, further understanding of their biological properties will be greatly advanced by analyzing the mechanisms that govern their self-renewal and differentiation potential. This review begins with the current state of knowledge on the biology of MSCs, specifically with respect to their existence in the adult organism and postulation of their biological niche. While MSCs are considered suitable candidates for cell-based strategies owing to their intrinsic capacity to self-renew and differentiate, there is currently little information available regarding the molecular mechanisms that govern their stem cell potential. We propose here a model for the regulation of MSC differentiation, and recent findings regarding the regulation of MSC differentiation are discussed. Current research efforts focused on elucidating the mechanisms regulating MSC differentiation should facilitate the design of optimal in vitro culture conditions to enhance their clinical utility cell and gene therapy.

Baldwin, K., et al. (2015). "Enrichment of human hematopoietic stem/progenitor cells facilitates transduction for stem cell gene therapy." Stem Cells **33**(5): 1532-1542.

Autologous hematopoietic stem cell (HSC) gene therapy for sickle cell disease has the potential to treat this illness without the major immunological complications associated with allogeneic transplantation. However, transduction efficiency by beta-globin lentiviral vectors using CD34-enriched cell populations is suboptimal and large vector production batches may be needed for clinical trials. Transducing a cell population more enriched for HSC could greatly reduce vector needs and, potentially, increase transduction efficiency. CD34(+) /CD38(-) cells, comprising approximately 1%-3% of all CD34(+) cells, were isolated from healthy cord blood CD34(+) cells by fluorescence-activated cell sorting and transduced with a lentiviral vector expressing an antisickling form of beta-globin (CCL-beta(AS3) -FB). Isolated CD34(+) /CD38(-) cells were able to generate progeny over an extended period of long-term culture (LTC) compared to the CD34(+) cells and required up to 40-fold less vector for transduction compared to bulk CD34(+) preparations containing an equivalent number of CD34(+) /CD38(-) cells. Transduction of isolated CD34(+) /CD38(-) cells was comparable to CD34(+) cells measured by quantitative PCR at day 14 with reduced vector needs, and average vector copy/cell remained higher over time for LTC initiated from CD34(+) /38(-) cells. Following in vitro erythroid differentiation, HBBAS3 mRNA expression was similar in cultures derived from CD34(+) /CD38(-) cells or unfractionated CD34(+) cells. In vivo studies showed equivalent engraftment of transduced CD34(+) /CD38(-) cells when transplanted in competition with 100-fold more CD34(+) /CD38(+) cells. This work provides initial evidence for the beneficial effects from isolating human CD34(+) /CD38(-) cells to use significantly less vector and potentially improve transduction for HSC gene therapy.

Bank, A. (2003). "Hematopoietic stem cell gene therapy: selecting only the best." J Clin Invest **112**(10): 1478-1480.

Hematopoietic stem cell (HSC) gene therapy can potentially cure a variety of human hematopoietic diseases, such as sickle cell disease. Selection and expansion of gene-corrected HSCs has now been accomplished for the first time using HSC from large animals - dogs and humans - with a novel drug-resistance gene, MGMT, which is not expressed in normal HSCs (see the related articles beginning on pages 1561 and 1581). Highly efficient lentiviral transfer and expression of MGMT into relatively few HSCs led to repopulation of most of the hematopoietic compartment with gene-corrected cells following suitable drug treatment. This selection system may be useful in human clinical trials to permit gene therapy in autologous and allogeneic bone marrow transplantation settings.

Bao, Q., et al. (2012). "Mesenchymal stem cell-based tumor-targeted gene therapy in gastrointestinal cancer." Stem Cells Dev **21**(13): 2355-2363.

Mesenchymal stem (or stromal) cells (MSCs) are nonhematopoietic progenitor cells that can be obtained from bone marrow aspirates or adipose tissue, expanded and genetically modified in vitro, and then used for cancer therapeutic strategies in vivo. Here, we review available data regarding the application of MSC-based tumor-targeted therapy in gastrointestinal cancer, provide an overview of the general history of MSC-based gene therapy in cancer research, and discuss potential problems associated with the utility of MSC-based therapy such as biosafety, immunoprivilege, transfection methods, and distribution in the host.

Barquinero, J. (1998). "Stem cell gene therapy--second conference. Biology and technology. 31 May - 3 June 1998, Orcas Island, WA, USA." IDrugs **1**(3): 278-280.

This conference, which was organized by George Stamatoyanno-poulos (University of Washington, Seattle, WA, USA), comprised 44 oral and 55 poster presentations. It started immediately after the First Annual Meeting of the American Society of Gene Therapy in Seattle, and took place at the Rosario Resort, located in the beautiful and quiet Orcas Island, in the Pacific Northwest. Several issues including stem cell biology, viral vectors, gene transfer into hematopoietic stem cells (HSCs) and a variety of clinical trials were covered. There were about 150 attendees including students, postdoctoral fellows and prominent experts in different aspects of the field. Most of these were from academia including research institutes, universities and hospitals, with a very small proportion from industry. Some of the information had already been presented a few days before at the Seattle meeting.

Barquinero, J. and M. Garcia Escarp (2001). "[Stem cell gene therapy: myths an realities]." Med Clin (Barc) **117**(20): 778-780.

Bauer, T. R., Jr., et al. (2006). "Correction of the disease phenotype in canine leukocyte adhesion deficiency using ex vivo hematopoietic stem cell gene therapy." Blood **108**(10): 3313-3320.

Canine leukocyte adhesion deficiency (CLAD) represents the canine counter-part of the human disease leukocyte adhesion deficiency (LAD). Defects in the leukocyte integrin CD18 adhesion molecule in both CLAD and LAD lead to recurrent, life-threatening bacterial infections. We evaluated ex vivo retroviral-mediated gene therapy in CLAD using 2 nonmyeloablative conditioning regimens--200 cGy total body irradiation (TBI) or 10 mg/kg busulfan--with or without posttransplantation immunosuppression. In 6 of 11 treated CLAD dogs, therapeutic levels of CD18(+) leukocytes were achieved. Conditioning with either TBI or busulfan allowed long-term engraftment, and immunosuppression was not required for efficacy. The percentage of CD18(+) leukocytes in the peripheral blood progressively increased over 6 to 8 months after infusion to levels ranging from 1.26% to 8.37% at 1-year follow-up in the 6 dogs. These levels resulted in reversal or moderation of the severe CLAD phenotype. Linear amplification-mediated polymerase chain reaction assays indicated polyclonality of insertion sites. These results describe ex vivo hematopoietic stem cell gene transfer in a disease-specific, large animal model using 2 clinically applicable conditioning regimens, and they provide support for the use of nonmyeloablative conditioning regimens in preclinical protocols of retroviral-mediated gene transfer for nonmalignant hematopoietic diseases such as LAD.

Baum, C. (2007). "Insertional mutagenesis in gene therapy and stem cell biology." Curr Opin Hematol **14**(4): 337-342.

PURPOSE OF REVIEW: Recent preclinical and clinical studies revealed that the semirandom insertion of transgenes into chromosomal DNA of hematopoietic cells may induce clonal competition, which potentially may even trigger leukemia or sarcoma. Insertional mutagenesis caused by gene vectors has thus led to major uncertainty among those developing advanced hematopoietic cell therapies. This review summarizes novel studies of underlying mechanisms; these studies have demonstrated the possibility of improved gene vector biosafety and generated new insights into stem cell biology. RECENT FINDINGS: The characteristic insertion pattern of various retroviral gene vector systems may be explained by properties of the viral integrase and associated cellular cofactors. Cell culture assays and animal models, including disease-specific and cancer-prone mouse models, are emerging that reveal the contributions of vector features and systemic factors to induction of clonal imbalance. Databases summarizing vector insertion sites in dominant hematopoietic clones are evolving as new tools to identify genes that regulate clonal homeostasis. SUMMARY: Mechanistic studies of insertional mutagenesis by random gene vector insertion will lead to improved tools for advanced hematopoietic cell therapy. Simultaneously, fascinating insights into gene networks that regulate cell fitness will be generated, with important consequences for the fields of hematology, oncology and regenerative medicine.

Becker, P. S. (2002). "Hematopoietic stem cell gene therapy for inherited bone marrow disorders: past accomplishments and continued challenges." J Cell Biochem Suppl **38**: 55-64.

From the time that the genes encoding the defective proteins were cloned for a number of inherited diseases, it became a goal to correct those conditions by restoring the normal gene and thereby, its product. For the inherited disorders affecting the blood and its progenitor cells, the hematopoietic stem cells were the ideal target cells for gene transfer, because the normal gene would then be transferred to all of the progeny cells, theoretically for the lifetime of the recipient. However, the tasks of isolating the hematopoietic stem cells, introducing the new genes in such a manner as to preserve engraftment of the manipulated cells, and achieving long-term gene expression, have not been straightforward in the

clinical trial setting, although there has been moderate success for cells in vitro, and in murine studies. With the report of clinical efficacy of gene transfer in children with X-linked severe combined immunodeficiency disease, the dream of clinical gene transfer to hematopoietic cells has become a reality. But there are still significant impediments remaining for a number of diseases. The innovations of introduction of synthetic receptors that confer growth advantage, the use of lentiviral vectors with increased stem cell transduction efficiency, and the addition of modified promoter/enhancer sequences to augment and preserve gene expression may bring wider success to gene therapy clinical trials for bone marrow disorders in the near future.

Bernardo, M. E. and A. Aiuti (2016). "The Role of Conditioning in Hematopoietic Stem-Cell Gene Therapy." Hum Gene Ther **27**(10): 741-748.

Gene therapy (GT) approaches based on autologous hematopoietic stem cells (HSC) corrected ex vivo have shown therapeutic benefit in a number of inherited disorders. GT bares the advantage of allowing each patient to be her/his own donor while reducing the risks of immune-mediated complications as compared with allogeneic hematopoietic stem-cell transplantation (HSCT). In order to achieve stable engraftment of HSC, patients undergoing transplantation of allogeneic or autologous HSC receive a chemotherapy- and/or radiotherapy-based preparation. With regard to HSC-GT for inherited genetic disorders, the ideal conditioning regimen should aim to contain toxicity by reducing the dosage and/or the number of chemotherapeutic agents administered, in comparison to fully myeloablative preparations employed in conventional allogeneic HSCT. To meet this aim, a profound knowledge of the disease-specific biological background and of the therapeutic transgene levels, as well as of the key principles of transplantation, are required. While low-dose conditioning is sufficient to create a mixed chimerism when gene-corrected cells are endowed with a natural selective advantage, such as in the case of immune deficiencies, myeloablative doses are necessary when high levels of engraftment are required in disease such as lysosomal storage disorders and beta thalassemia. Therefore, the intensity and type of conditioning regimen administered to patients undergoing HSC-GT should be tailored to reach a minimal efficacious therapeutic target level while sparing toxicity. Novel strategies based on monoclonal antibodies selectively depleting blood cells and associated with limited extramedullary toxicity might be successfully employed in the context of HSC-GT in the near future. This review focuses on the role of the conditioning regimen in HSC-GT, and in particular, it highlights the importance of modulating the preparative chemotherapy based on disease biology and transgene expression in order to optimize outcome.

Biffi, A. (2017). "Hematopoietic Stem Cell Gene Therapy for Storage Disease: Current and New Indications." Mol Ther **25**(5): 1155-1162.

Lysosomal storage disorders (LSDs) are a broad class of monogenic diseases with an overall incidence of 1:7,000 newborns, due to the defective activity of one or more lysosomal hydrolases or related proteins resulting in storage of un-degraded substrates in the lysosomes. The over 40 different known LSDs share a life-threatening nature, but they are present with extremely variable clinical manifestations, determined by the characteristics and tissue distribution of the material accumulating due to the lysosomal dysfunction. The majority of LSDs lack a curative treatment. This is particularly true for LSDs severely affecting the CNS. Based on current preclinical and clinical evidences, among other treatment modalities, hematopoietic stem cell gene therapy could potentially result in robust therapeutic benefit for LSD patients, with particular indication for those characterized by severe brain damage. Optimization of current approaches and technology, as well as implementation of clinical trials for novel indications, and prolonged and more extensive follow-up of the already treated patients will allow translating this promise into new medicinal products.

Biffi, A., et al. (2013). "Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy." Science **341**(6148): 1233158.

Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Patients with MLD exhibit progressive motor and cognitive impairment and die within a few years of symptom onset. We used a lentiviral vector to transfer a functional ARSA gene into hematopoietic stem cells (HSCs) from three presymptomatic patients who showed genetic, biochemical, and neurophysiological evidence of late infantile MLD. After reinfusion of the gene-corrected HSCs, the patients showed extensive and stable ARSA gene replacement, which led to high enzyme expression throughout hematopoietic lineages and in cerebrospinal fluid. Analyses of vector integrations revealed no evidence of aberrant clonal behavior. The disease did not manifest or progress in the three patients 7 to 21 months beyond the predicted age of symptom onset. These findings indicate that extensive genetic engineering of human hematopoiesis can be achieved with lentiviral vectors and that this approach may offer therapeutic benefit for MLD patients.

Bigger, B. W. and R. F. Wynn (2014). "Novel approaches and mechanisms in hematopoietic stem cell gene therapy." Discov Med **17**(94): 207-215.

Hematopoietic stem cell gene therapy is one of the most exciting clinical tools to emerge from the gene therapy stable. This technology combines the expansion capability of hematopoietic stem cells, capable of replacing the entire blood and immune system of an individual, with the capacity for long-term replacement of one or more gene copies using integrating gene therapy vectors. Hematopoietic stem cell gene therapy benefits significantly from the pre-existing experience of standard blood and marrow transplantation, whilst at the same time having the capacity to deliver a safer and more effective therapy to a wider range of diseases. In this review we summarize the potential of hematopoietic stem cell gene therapy to expand the scope of hematopoietic stem cell transplantation, including the evolution of vector delivery systems and the success and failures of current clinical experience with this treatment. In particular we deal with the incidence of vector mediated transformation in patients and the steps that have been taken to minimize this risk. Finally we discuss the innovations in preclinical development that are likely to drive the future of this field, including the expansion to many more genetic diseases, particularly those affecting the brain.

Burtner, C. R., et al. (2015). "(211)Astatine-Conjugated Monoclonal CD45 Antibody-Based Nonmyeloablative Conditioning for Stem Cell Gene Therapy." Hum Gene Ther **26**(6): 399-406.

Most hematopoietic stem cell gene therapy studies require host conditioning to allow for efficient engraftment of gene-modified cells. Conditioning regimens with lower treatment-related toxicities are especially relevant for the treatment of nonmalignant blood disorders, such as hemoglobinopathies and immunodeficiencies, and for patients who are otherwise ineligible for conventional high-dose conditioning. Radioimmunotherapy, which employs an alpha- or a beta-emitting radionuclide conjugated to a targeting antibody, is effective for delivering cytotoxic doses of radiation to a cell type of interest while minimizing off-target toxicity. Here, we demonstrate the feasibility of using a nonmyeloablative dose of a monoclonal anti-CD45 antibody conjugated to the alpha-emitter Astatine-211 ((211)At) to promote engraftment of an autologous gene-modified stem cell graft in the canine model. The doses used provided myelosuppression with rapid autologous recovery and minimal off-target toxicity. Engraftment levels were low in all dogs and reflected the low numbers of gene-modified cells infused. Our data suggest that a cell dose exceeding 1x10(6) cells/kg be used with nonmyeloablative doses of (211)At-anti-CD45 monoclonal antibodies for sustained engraftment in the dog model.

Busuttil, F., et al. (2017). "Combining Gene and Stem Cell Therapy for Peripheral Nerve Tissue Engineering." Stem Cells Dev **26**(4): 231-238.

Despite a substantially increased understanding of neuropathophysiology, insufficient functional recovery after peripheral nerve injury remains a significant clinical challenge. Nerve regeneration following injury is dependent on Schwann cells, the supporting cells in the peripheral nervous system. Following nerve injury, Schwann cells adopt a proregenerative phenotype, which supports and guides regenerating nerves. However, this phenotype may not persist long enough to ensure functional recovery. Tissue-engineered nerve repair devices containing therapeutic cells that maintain the appropriate phenotype may help enhance nerve regeneration. The combination of gene and cell therapy is an emerging experimental strategy that seeks to provide the optimal environment for axonal regeneration and reestablishment of functional circuits. This review aims to summarize current preclinical evidence with potential for future translation from bench to bedside.

Cai, L., et al. (2018). "A Universal Approach to Correct Various HBB Gene Mutations in Human Stem Cells for Gene Therapy of Beta-Thalassemia and Sickle Cell Disease." Stem Cells Transl Med **7**(1): 87-97.

Beta-thalassemia is one of the most common recessive genetic diseases, caused by mutations in the HBB gene. Over 200 different types of mutations in the HBB gene containing three exons have been identified in patients with beta-thalassemia (beta-thal) whereas a homozygous mutation in exon 1 causes sickle cell disease (SCD). Novel therapeutic strategies to permanently correct the HBB mutation in stem cells that are able to expand and differentiate into erythrocytes producing corrected HBB proteins are highly desirable. Genome editing aided by CRISPR/Cas9 and other site-specific engineered nucleases offers promise to precisely correct a genetic mutation in the native genome without alterations in other parts of the human genome. Although making a sequence-specific nuclease to enhance correction of a specific HBB mutation by homology-directed repair (HDR) is becoming straightforward, targeting various HBB mutations of beta-thal is still challenging because individual guide RNA as well as a donor DNA template for HDR of each type of HBB gene mutation have to be selected and validated. Using human induced pluripotent stem cells (iPSCs) from two beta-thal patients with different HBB gene mutations, we

devised and tested a universal strategy to achieve targeted insertion of the HBB cDNA in exon 1 of HBB gene using Cas9 and two validated guide RNAs. We observed that HBB protein production was restored in erythrocytes derived from iPSCs of two patients. This strategy of restoring functional HBB gene expression will be able to correct most types of HBB gene mutations in beta-thal and SCD. Stem Cells Translational Medicine 2018;7:87-97.

Calbi, V., et al. (2018). "Use of Defibrotide to help prevent post-transplant endothelial injury in a genetically predisposed infant with metachromatic leukodystrophy undergoing hematopoietic stem cell gene therapy." Bone Marrow Transplant **53**(7): 913-917.

Calne, R. Y., et al. (2013). "Gene and stem cell therapy for diabetes." Clin Transpl: 111-112.

Gene and stem cell therapy has been on the scientific agenda in many laboratories for more than 20 years. The literature is enormous, but practical applications have been few. Recently advances in stem cell biology and gene therapy are clarifying some of the issues. I have made a few observations concerning our own studies on bone marrow mesenchymal stem cells cultured to produce a small percentage of insulin-producing cells and human insulin gene engineered into Lenti and AA viruses. The aim of clinical application would still seem to be several years away, if all goes well. The first step will be to produce enough insulin-secreting cells to be of potential value to patients. The next crucial question will be how to persuade the cells to respond to blood glucose levels swiftly and appropriately. With both stem cell and gene therapy, another important factor will be to ensure that any positive results will continue long enough to be preferable to insulin injections.

Canarutto, D., et al. (2021). "Peripheral blood stem and progenitor cell collection in pediatric candidates for ex vivo gene therapy: a 10-year series." Mol Ther Methods Clin Dev **22**: 76-83.

Hematopoietic stem and progenitor cell (HSPC)-based gene therapy (GT) requires the collection of a large number of cells. While bone marrow (BM) is the most common source of HSPCs in pediatric donors, the collection of autologous peripheral blood stem cells (PBSCs) is an attractive alternative for GT. We present safety and efficacy data of a 10-year cohort of 45 pediatric patients who underwent PBSC collection for backup and/or purification of CD34(+) cells for ex vivo gene transfer. Median age was 3.7 years and median weight 15.8 kg. After mobilization with lenograstim/plerixafor (n = 41) or lenograstim alone (n = 4) and 1-3 cycles of leukapheresis, median collection was 37 x 10(6) CD34(+) cells/kg. The procedures were well tolerated. Patients who collected >/=7 and >/=13 x 10(6) CD34(+) cells/kg in the first cycle had pre-apheresis circulating counts of at >/=42 and >/=86 CD34(+) cells/muL, respectively. Weight-adjusted CD34(+) cell yield was positively correlated with peripheral CD34(+) cell counts and influenced by female gender, disease, and drug dosage. All patients received a GT product above the minimum target, ranging from 4 to 30.9 x 10(6) CD34(+) cells/kg. Pediatric PBSC collection compares well to BM harvest in terms of CD34(+) cell yields for the purpose of GT, with a favorable safety profile.

Cao, X., et al. (2000). "Enhanced antitumoral effect of adenovirus-mediated cytosine deaminase gene therapy by induction of antigen-presenting cells through stem cell factor/granulocyte-macrophage colony-stimulating factor gene transfer." Cancer Gene Ther **7**(2): 177-186.

Suicide gene therapy has been studied intensively for the treatment of cancer. A limited antitumoral effect was obtained by intratumoral injection of adenovirus harboring Escherichia coli cytosine deaminase gene (AdCD) in tumor-bearing mice followed by continuous administration of 5-fluorocytosine (5FC). To address the drawbacks of the limited potential for the induction of antitumoral immunity by CD suicide gene therapy, we hypothesized that antigen-presenting cells (APCs) might contribute to the efficient induction of an antitumoral immune response in tumor-bearing mice undergoing suicide gene therapy. We preinjected the mice with murine stem cell factor (SCF)-encoding adenovirus (AdSCF) and murine granulocyte-macrophage colony-stimulating factor (GM-CSF)-encoding adenovirus (AdGM-CSF); after 7 days, the mice were inoculated with CT26 colon adenocarcinoma. AdCD was injected intratumorally into tumor-bearing mice followed by 5FC administration. The results showed that AdSCF/AdGM-CSF treatment could increase the number, surface molecule expression, and function of APCs efficiently. A more significant growth inhibition of established tumors and a prolongation of the survival period were observed in tumor-bearing mice after AdSCF/AdGM-CSF pretreatment in combination with AdCD/5FC therapy when compared with mice treated with AdSCF or AdGM-CSF in combination with AdCD/5FC, or AdCD/5FC alone ($P < .01$). Cytotoxic T-lymphocyte activity was induced efficiently after the combined therapy, and mRNA of tumor necrosis factor-alpha, interleukin-4, interferon-gamma, and interleukin-2 was present in the tumor mass after combined therapy, suggesting that a more potent antitumoral response was induced by enhanced APCs. Our results demonstrated that AdSCF/AdGM-CSF

pretreatment could activate APCs, and that these APCs could present the tumor antigens released from AdCD/5FC-killed tumor cells and activate the antitumoral response of the host, thus increasing the therapeutic efficiency of suicide gene therapy.

Cartier, N. and P. Aubourg (2008). "Hematopoietic stem cell gene therapy in Hurler syndrome, globoid cell leukodystrophy, metachromatic leukodystrophy and X-adrenoleukodystrophy." Curr Opin Mol Ther **10**(5): 471-478.

Hurler syndrome, metachromatic leukodystrophy, globoid-cell leukodystrophy (Krabbe's disease) and X-linked adrenoleukodystrophy are inherited diseases of the CNS that can be cured or arrested by allogeneic hematopoietic stem-cell transplantation (HSCT). Despite significant progress in medical procedures and the availability of banked umbilical cord blood, HSCT is still associated with significant risks of graft failure or GVHD that can lead to death. Transplantation of autologous hematopoietic stem cells genetically modified to express the missing protein may circumvent the majority of the problems associated with allogeneic HSCT. Promising in concept, these strategies are now at a stage to be tested in phase I/II clinical trials to assess safety and potential efficacy.

Cartier, N. and P. Aubourg (2010). "Hematopoietic stem cell transplantation and hematopoietic stem cell gene therapy in X-linked adrenoleukodystrophy." Brain Pathol **20**(4): 857-862.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only therapeutic approach that can arrest cerebral demyelination of X-linked adrenoleukodystrophy (ALD) in boys and results in long-term in a good quality of life, provided the procedure is performed at an early stage of disease. Similar benefits of allogeneic HSCT have been demonstrated in adults with cerebral ALD. However, it is not yet known whether allogeneic HSCT can prevent or rescue adrenomyeloneuropathy. Allogeneic HSCT remains associated with significant morbidity and mortality risks, particularly in adults, and not all ALD patients have donors despite the availability of cord blood. The absence of biological markers that can predict the evolutivity of cerebral disease is a major limitation to propose in due time allogeneic HSCT to ALD patients. Recently, HSC gene therapy using lentiviral vector was shown to have comparable efficacy than allogeneic HSCT in two boys with cerebral ALD who had no Human-leukocyte-antigen (HLA)-matched donor. If these results are confirmed in an extended series of patients, HSC gene therapy may become the first therapeutic option for all ALD male patients who develop cerebral demyelination.

Cartier, N., et al. (2009). "Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy." Science **326**(5954): 818-823.

X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in ALD protein, an adenosine triphosphate-binding cassette transporter encoded by the ABCD1 gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT). We initiated a gene therapy trial in two ALD patients for whom there were no matched donors. Autologous CD34+ cells were removed from the patients, genetically corrected ex vivo with a lentiviral vector encoding wild-type ABCD1, and then re-infused into the patients after they had received myeloablative treatment. Over a span of 24 to 30 months of follow-up, we detected polyclonal reconstitution, with 9 to 14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein. These results strongly suggest that hematopoietic stem cells were transduced in the patients. Beginning 14 to 16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients stopped, a clinical outcome comparable to that achieved by allogeneic HCT. Thus, lentiviral-mediated gene therapy of hematopoietic stem cells can provide clinical benefits in ALD.

Chen, W., et al. (2015). "PDGFB-based stem cell gene therapy increases bone strength in the mouse." Proc Natl Acad Sci U S A **112**(29): E3893-3900.

Substantial advances have been made in the past two decades in the management of osteoporosis. However, none of the current medications can eliminate the risk of fracture and rejuvenate the skeleton. To this end, we recently reported that transplantation of hematopoietic stem/progenitor cells (HSCs) or Sca1(+) cells engineered to overexpress FGF2 results in a significant increase in lamellar bone matrix formation at the endosteum; but this increase was attended by the development of secondary hyperparathyroidism and severe osteomalacia. Here we switch the therapeutic gene to PDGFB, another potent mitogen for mesenchymal stem cells (MSCs) but potentially safer than FGF2. We found that modest overexpression of PDGFB using a relatively weak phosphoglycerate kinase (PGK) promoter completely avoided osteomalacia and secondary hyperparathyroidism, and simultaneously increased trabecular bone formation and trabecular connectivity, and decreased cortical porosity. These effects led to a 45% increase in the bone strength. Transplantation of PGK-PDGFB-transduced Sca1(+) cells increased MSC proliferation, raising the possibility that PDGF-BB enhances expansion of MSC in the vicinity of the hematopoietic niche where the osteogenic milieu

propels the differentiation of MSCs toward an osteogenic destination. Our therapy should have potential clinical applications for patients undergoing HSC transplantation, who are at high risk for osteoporosis and bone fractures after total body irradiation preconditioning. It could eventually have wider application once the therapy can be applied without the preconditioning.

Chen, W., et al. (2020). "Unique anabolic action of stem cell gene therapy overexpressing PDGFB-DSS6 fusion protein in OVX osteoporosis mouse model." Bone Rep **12**: 100236.

In the present study we sought to improve the efficacy and safety of our Sca1(+) PDGFB stem cell gene therapy for osteoporosis in ovariectomized (OVX) mouse model. This therapy is administered by marrow transplantation. We established the promise of this approach by previously showing that this therapy in normal mice increase bone density, increased endosteal cortical and trabecular bone formation, caused de novo trabecular bone formation, increased cortical thickness and improve bone strength. In the current study we produced a fusion gene, PDGFB-DSS6. We reasoned that the DSS6, calcium binding protein would trap the PDGFB at the bone surface and thereby limit the amount of PDGFB required to produce an optimal bone formation response, i.e. efficacy with a lower engraftment. The result shows that indeed with a very low level of engraftment we achieved a large increase in bone formation in the OVX model of bone loss. Serum analysis for biochemical marker of new bone formation showed an approximate 75% increase in alkaline phosphatase levels in Sca1(+)PDGFB-DSS6 group as compared to other groups. Quantitative analysis of bone by microCT showed a massive increase in trabecular bone density and trabecular connectivity of the femur in the metaphysis in Sca1(+) PDGFB-DSS6 group. The increased cortical porosity produced by OVX was replaced by the Sca1(+) PDGFB-DSS6 therapy but not by the positive control Sca1(+) PDGFB. Additionally, an increase in the femur bone strength was also observed specifically in Sca1(+) PDGFB-DSS6 as compared to other treatment groups, emphasizing the functional significance of the observed anabolic action is on bone formation. In future work we will focus on nontoxic preconditioning of our marrow transplantation procedure and also on transcriptional control of therapeutic gene expression to avoid excess bone formation.

Cherqui, S. (2021). "Hematopoietic Stem Cell Gene Therapy for Cystinosis: From Bench-to-Bedside." Cells **10**(12).

Cystinosis is an autosomal recessive metabolic disease that belongs to the family of lysosomal storage disorders. The gene involved is the CTNS gene that encodes cystinosin, a seven-transmembrane domain lysosomal protein, which is a proton-driven cystine transporter. Cystinosis is characterized by the lysosomal accumulation of cystine, a dimer of cysteine, in all the cells of the body leading to multi-organ failure, including the failure of the kidney, eye, thyroid, muscle, and pancreas, and eventually causing premature death in early adulthood. The current treatment is the drug cysteamine, which is onerous and expensive, and only delays the progression of the disease. Employing the mouse model of cystinosis, using Ctns(-/-) mice, we first showed that the transplantation of syngeneic wild-type murine hematopoietic stem and progenitor cells (HSPCs) led to abundant tissue integration of bone marrow-derived cells, a significant decrease in tissue cystine accumulation, and long-term kidney, eye and thyroid preservation. To translate this result to a potential human therapeutic treatment, given the risks of mortality and morbidity associated with allogeneic HSPC transplantation, we developed an autologous transplantation approach of HSPCs modified ex vivo using a self-inactivated lentiviral vector to introduce a functional version of the CTNS cDNA, pCCL-CTNS, and showed its efficacy in Ctns(-/-) mice. Based on these promising results, we held a pre-IND meeting with the Food and Drug Administration (FDA) to carry out the FDA agreed-upon pharmacological and toxicological studies for our therapeutic candidate, manufacturing development, production of the GMP lentiviral vector, design Phase 1/2 of the clinical trial, and filing of an IND application. Our IND was cleared by the FDA on 19 December 2018, to proceed to the clinical trial using CD34(+) HSPCs from the G-CSF/plerixafor-mobilized peripheral blood stem cells of patients with cystinosis, modified by ex vivo transduction using the pCCL-CTNS vector (investigational product name: CTNS-RD-04). The clinical trial evaluated the safety and efficacy of CTNS-RD-04 and takes place at the University of California, San Diego (UCSD) and will include up to six patients affected with cystinosis. Following leukapheresis and cell manufacturing, the subjects undergo myeloablation before HSPC infusion. Patients also undergo comprehensive assessments before and after treatment to evaluate the impact of CTNS-RD-04 on the clinical outcomes and cystine and cystine crystal levels in the blood and tissues for 2 years. If successful, this treatment could be a one-time therapy that may eliminate or reduce renal deterioration as well as the long-term complications associated with cystinosis. In this review, we will describe the long path from bench-to-bedside for autologous HSPC gene therapy used to treat cystinosis.

Chien, Y., et al. (2015). "Synergistic effects of carboxymethyl-hexanoyl chitosan, cationic polyurethane-short branch PEI in miR122 gene delivery: accelerated differentiation of iPSCs into mature hepatocyte-like cells and improved stem cell therapy in a hepatic failure model." Acta Biomater **13**: 228-244.

MicroRNA122 (miR122), a liver-specific microRNA, plays critical roles in homeostatic regulation and hepatic-specific differentiation. Induced pluripotent stem cells (iPSCs) have promising potential in regenerative medicine, but it remains unknown whether non-viral vector-mediated miR122 delivery can enhance the differentiation of iPSCs into hepatocyte-like cells (iPSC-Heps) and rescue thioacetamide-induced acute hepatic failure (AHF) in vivo. In this study, we demonstrated that embedment of miR122 complexed with polyurethane-graft-short-branch polyethylenimine copolymer (PU-PEI) in nanostructured amphiphatic carboxymethyl-hexanoyl chitosan (CHC) led to dramatically enhanced miR122 delivery into human dental pulp-derived iPSCs (DP-iPSCs) and facilitated these DP-iPSCs to differentiate into iPSC-Heps (miR122-iPSC-Heps) with mature hepatocyte functions. Microarray and bioinformatics analysis further indicated that CHC/PU-PEI-miR122 promoted the gene-signature pattern of DP-iPSCs to shift into a liver-specific pattern. Furthermore, intrahepatic delivery of miR122-iPSC-Heps, but not miR-Scr-iPSC-Heps, improved liver functions and rescued recipient survival, and CHC-mediated delivery showed a better efficacy than that using phosphate buffered saline as a delivery vehicle. In addition, these transplanted miR122-iPSC-Heps remained viable and could produce circulatory albumin for 4 months. Taken together, our findings demonstrate that non-viral delivery of miR122 shortens the time of iPSC differentiation into hepatocytes and the delivery of miR122-iPSC-Heps using CHC as a vehicle exhibited promising hepatoprotective efficacy in vivo. miR122-iPSC-Heps may represent a feasible cell source and provide an efficient and alternative strategy for hepatic regeneration in AHF.

Chiesa, R. and M. E. Bernardo (2022). "Haematopoietic stem cell gene therapy in inborn errors of metabolism." Br J Haematol **198**(2): 227-243.

Over the last 30 years, allogeneic haematopoietic stem cell transplantation (allo-HSCT) has been adopted as a therapeutic strategy for many inborn errors of metabolism (IEM), due to the ability of donor-derived cells to provide life-long enzyme delivery to deficient tissues and organs. However, (a) the clinical benefit of allo-HSCT is limited to a small number of IEM, (b) patients are left with a substantial residual disease burden and (c) allo-HSCT is still associated with significant short- and long-term toxicities and transplant-related mortality. Haematopoietic stem/progenitor cell gene therapy (HSPC-GT) was established in the 1990s for the treatment of selected monogenic primary immunodeficiencies and over the past few years, its use has been extended to a number of IEM. HSPC-GT is particularly attractive in neurodegenerative IEM, as gene corrected haematopoietic progenitors can deliver supra-physiological enzyme levels to difficult-to-reach areas, such as the brain and the skeleton, with potential increased clinical benefit. Moreover, HSPC-GT is associated with reduced morbidity and mortality compared to allo-HSCT, although this needs to be balanced against the potential risk of insertional mutagenesis. The number of clinical trials in the IEM field is rapidly increasing and some HSPC-GT products recently received market approval. This review describes the development of ex vivo HSPC-GT in a number of IEM, with a focus on recent results from GT clinical trials and risks versus benefits considerations, when compared to established therapeutic strategies, such as allo-HSCT.

Chung, J. Y., et al. (2014). "Gene therapy delivery of myelin oligodendrocyte glycoprotein (MOG) via hematopoietic stem cell transfer induces MOG-specific B cell deletion." J Immunol **192**(6): 2593-2601.

The various mechanisms that have been described for immune tolerance govern our ability to control self-reactivity and minimize autoimmunity. However, the capacity to genetically manipulate the immune system provides a powerful avenue to supplement this natural tolerance in an Ag-specific manner. We have previously shown in the mouse model of experimental autoimmune encephalomyelitis that transfer of bone marrow (BM) transduced with retrovirus encoding myelin oligodendrocyte glycoprotein (MOG) promotes disease resistance and CD4(+) T cell deletion within the thymus. However, the consequence of this strategy on B cell tolerance is not known. Using BM from IgH(MOG) mice that develop MOG-specific B cell receptors, we generated mixed chimeras together with BM-encoding MOG. In these animals, the development of MOG-specific B cells was abrogated, resulting in a lack of MOG-specific B cells in all B cell compartments examined. This finding adds a further dimension to our understanding of the mechanisms of tolerance that are associated with this gene therapy approach to treating autoimmunity and may have important implications for Ab-mediated autoimmune disorders.

Chung, T., et al. (2016). "Dihydropyrimidine Dehydrogenase Is a Prognostic Marker for Mesenchymal Stem Cell-Mediated Cytosine

Deaminase Gene and 5-Fluorocytosine Prodrug Therapy for the Treatment of Recurrent Gliomas." Theranostics **6**(10): 1477-1490.

We investigated a therapeutic strategy for recurrent malignant gliomas using mesenchymal stem cells (MSC), expressing cytosine deaminase (CD), and prodrug 5-Fluorocytosine (5-FC) as a more specific and less toxic option. MSCs are emerging as a novel cell therapeutic agent with a cancer-targeting property, and CD is considered a promising enzyme in cancer gene therapy which can convert non-toxic 5-FC to toxic 5-Fluorouracil (5-FU). Therefore, use of prodrug 5-FC can minimize normal cell toxicity. Analyses of microarrays revealed that targeting DNA damage and its repair is a selectable option for gliomas after the standard chemo/radio-therapy. 5-FU is the most frequently used anti-cancer drug, which induces DNA breaks. Because dihydropyrimidine dehydrogenase (DPD) was reported to be involved in 5-FU metabolism to block DNA damage, we compared the survival rate with 5-FU treatment and the level of DPD expression in 15 different glioma cell lines. DPD-deficient cells showed higher sensitivity to 5-FU, and the regulation of DPD level by either siRNA or overexpression was directly related to the 5-FU sensitivity. For MSC/CD with 5-FC therapy, DPD-deficient cells such as U87MG, GBM28, and GBM37 showed higher sensitivity compared to DPD-high U373 cells. Effective inhibition of tumor growth was also observed in an orthotopic mouse model using DPD- deficient U87MG, indicating that DPD gene expression is indeed closely related to the efficacy of MSC/CD-mediated 5-FC therapy. Our results suggested that DPD can be used as a biomarker for selecting glioma patients who may possibly benefit from this therapy.

Cihova, M., et al. (2011). "Stem cell based cancer gene therapy." Mol Pharm **8**(5): 1480-1487.

The attractiveness of prodrug cancer gene therapy by stem cells targeted to tumors lies in activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. Suicide gene therapy using genetically engineered mesenchymal stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. This review provides an explanation of the stem cell-targeted prodrug cancer gene therapy principle, with focus on the choice of prodrug, properties of bone marrow and adipose tissue-derived mesenchymal stem and neural stem cells as well as the mechanisms of their tumor homing ability. Therapeutic achievements of the cytosine deaminase/5-fluorocytosine prodrug system and Herpes simplex virus thymidine kinase/ganciclovir are discussed. In addition, delivery of immunostimulatory cytokines, apoptosis inducing genes, nanoparticles and antiangiogenic proteins by stem cells to tumors and metastases is discussed as a promising approach for antitumor therapy. Combinations of traditional, targeted and stem cell-directed gene therapy could significantly advance the treatment of cancer.

Clay, T. M., et al. (1999). "Potential use of T cell receptor genes to modify hematopoietic stem cells for the gene therapy of cancer." Pathol Oncol Res **5**(1): 3-15.

The purpose of this review is to illustrate some of the technical and biological hurdles that need to be addressed when developing new gene therapy based clinical trials. Gene transfer approaches can be used to "mark" cells to monitor their persistence in vivo in patients, to protect cells from toxic chemotherapeutic agents, correct a genetic defect within the target cell, or to confer a novel function on the target cell. Selection of the most suitable vector for gene transfer depends upon a number of factors such as the target cell itself and whether gene expression needs to be sustained or transient. The TCR gene transfer approach described here represents one innovative strategy being pursued as a potential therapy for metastatic melanoma. Tumor reactive T cells can be isolated from the tumor infiltrating lymphocytes (TIL) of melanoma patients. A retroviral vector has been constructed containing the T cell receptor (TCR) alpha and beta chain genes from a MART-1-specific T cell clone (TIL 5). Jurkat cells transduced with this virus specifically release cytokine in response to MART-1 peptide pulsed T2 cells, showing that the virus can mediate expression of a functional TCR. HLA-A2 transgenic mice are being used to examine whether transduced bone marrow progenitor cells will differentiate in vivo into mature CD8+ T cells expressing the MART-1-specific TCR. Expression of the human TCR alpha and beta chain genes has been detected by RT-PCR in the peripheral blood of HLA-A2 transgenic mice reconstituted with transduced mouse bone marrow. Expression of the TIL 5 TCR genes in the peripheral blood of these mice was maintained for greater than 40 weeks after bone marrow reconstitution. TIL 5 TCR gene expression was also maintained following transfer of bone marrow from mice previously reconstituted with transduced bone marrow to secondary mouse recipients, suggesting that a pluripotent progenitor or lymphocyte progenitor cell has been transduced.

Clement, F., et al. (2017). "Stem cell manipulation, gene therapy and the risk of cancer stem cell emergence." Stem Cell Investig **4**: 67.

Stem cells (SCs) have been extensively studied in the context of regenerative medicine. Human

hematopoietic stem cell (HSC)-based therapies have been applied to treat leukemic patients for decades. Handling of mesenchymal stem cells (MSCs) has also raised hopes and concerns in the field of tissue engineering. Lately, discovery of cell reprogramming by Yamanaka's team has profoundly modified research strategies and approaches in this domain. As we gain further insight into cell fate mechanisms and identification of key actors and parameters, this also raises issues as to the manipulation of SCs. These include the engraftment of manipulated cells and the potential predisposition of those cells to develop cancer. As a unique and pioneer model, the use of HSCs to provide new perspectives in the field of regenerative and curative medicine will be reviewed. We will also discuss the potential use of various SCs from embryonic to adult stem cells (ASCs), including induced pluripotent stem cells (iPSCs) as well as MSCs. Furthermore, to sensitize clinicians and researchers to unresolved issues in these new therapeutic approaches, we will highlight the risks associated with the manipulation of human SCs from embryonic or adult origins for each strategy presented.

Daley, G. Q. (2007). "Towards the generation of patient-specific pluripotent stem cells for combined gene and cell therapy of hematologic disorders." Hematology Am Soc Hematol Educ Program: 17-22.

Hematopoietic stem cell transplantation (HSCT) has proven successful for the treatment of a host of genetic and malignant diseases of the blood, but immune barriers to allogeneic tissue transplantation have hindered wider application. Likewise, gene therapy now appears effective in the treatment of various forms of immune deficiency, and yet insertional mutagenesis from viral gene transfer has raised safety concerns. One strategy for addressing the limitations of both gene therapy and allogeneic transplantation entails the creation of pluripotent stem cells from a patient's own somatic cells, thereby enabling precise in situ gene repair via homologous recombination in cultured cells, followed by autologous tissue transplantation. In murine model systems, the methods of somatic cell nuclear transfer, parthenogenesis, and direct somatic cell reprogramming with defined genetic factors have been used to generate pluripotent stem cells, and initial efforts at therapeutic gene repair and tissue transplantation suggest that the technology is feasible. Generating patient-specific autologous pluripotent stem cells provides an opportunity to combine gene therapy with autologous cell therapy to treat a host of human conditions. However, a number of technical hurdles must be overcome before therapies based on pluripotent human stem cells will appear in the clinic.

Dang, Z., et al. (2019). "Nerve growth factor gene therapy improves bone marrow sensory innervation and nociceptor-mediated stem cell release in a mouse model of type 1 diabetes with limb ischaemia." Diabetologia **62**(7): 1297-1311.

AIMS/HYPOTHESIS: Sensory neuropathy is common in people with diabetes; neuropathy can also affect the bone marrow of individuals with type 2 diabetes. However, no information exists on the state of bone marrow sensory innervation in type 1 diabetes. Sensory neurons are trophically dependent on nerve growth factor (NGF) for their survival. The aim of this investigation was twofold: (1) to determine if sensory neuropathy affects the bone marrow in a mouse model of type 1 diabetes, with consequences for stem cell liberation after tissue injury; and (2) to verify if a single systemic injection of the NGF gene exerts long-term beneficial effects on these phenomena. METHODS: A mouse model of type 1 diabetes was generated in CD1 mice by administration of streptozotocin; vehicle was administered to non-diabetic control animals. Diabetic animals were randomised to receive systemic gene therapy with either human NGF or beta-galactosidase. After 13 weeks, limb ischaemia was induced in both groups to study the recovery post injury. When the animals were killed, samples of tissue and peripheral blood were taken to assess stem cell mobilisation and homing, levels of substance P and muscle vascularisation. An in vitro cellular model was adopted to verify signalling downstream to human NGF and related neurotrophic or pro-apoptotic effects. Normally distributed variables were compared between groups using the unpaired Student's t test and non-normally distributed variables were assessed by the Wilcoxon-Mann-Whitney test. The Fisher's exact test was employed for categorical variables. RESULTS: Immunohistochemistry indicated a 3.3-fold reduction in the number of substance P-positive nociceptive fibres in the bone marrow of type 1 diabetic mice ($p < 0.001$ vs non-diabetic). Moreover, diabetes abrogated the creation of a neurokinin gradient which, in non-diabetic mice, favoured the mobilisation and homing of bone-marrow-derived stem cells expressing the substance P receptor neurokinin 1 receptor (NK1R). Pre-emptive gene therapy with NGF prevented bone marrow denervation, contrasting with the inhibitory effect of diabetes on the mobilisation of NK1R-expressing stem cells, and restored blood flow recovery from limb ischaemia. In vitro hNGF induced neurite outgrowth and exerted anti-apoptotic actions on rat PC12 cells exposed to high glucose via activation of the canonical neurotrophic tyrosine kinase receptor type 1 (TrkA) signalling pathway. CONCLUSIONS/INTERPRETATION: This study shows, for the first time, the occurrence of sensory neuropathy in the bone marrow of type 1 diabetic mice,

which translates into an altered modulation of substance P and depressed release of substance P-responsive stem cells following ischaemia. NGF therapy improves bone marrow sensory innervation, with benefits for healing on the occurrence of peripheral ischaemia. Nociceptors may represent a new target for the treatment of ischaemic complications in diabetes.

Davis, B. R., et al. (2000). "Micro-injection-mediated hematopoietic stem cell gene therapy." Curr Opin Mol Ther **2**(4): 412-419.

Over the past decade, significant attention has been devoted to the development of viral vectors (i.e., retrovirus, lentivirus, adeno-associated virus) and conditions capable of transducing hematopoietic stem cells. After several years of disappointing results, recent reports in humans and other primates, most particularly the French report of successful treatment of X-linked severe combined immune deficiency (SCID) [1.], indicate that viral approaches will be successful in treating specific hematopoietic diseases. However, it is clear that alternate non-viral methods of gene delivery and genetic modification offer significant advantages, and may in fact be the only effective approach for treating certain blood diseases. In this review, we focus on glass needle-mediated micro-injection as a method for the delivery of genetic material into blood stem cells, with an emphasis on molecules capable of either compensating gene deletions/mutations or directly repairing gene mutations.

De Ravin, S. S., et al. (2016). "Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency." Sci Transl Med **8**(335): 335ra357.

X-linked severe combined immunodeficiency (SCID-X1) is a profound deficiency of T, B, and natural killer (NK) cell immunity caused by mutations inIL2RGencoding the common chain (gammac) of several interleukin receptors. Gamma-retroviral (gammaRV) gene therapy of SCID-X1 infants without conditioning restores T cell immunity without B or NK cell correction, but similar treatment fails in older SCID-X1 children. We used a lentiviral gene therapy approach to treat five SCID-X1 patients with persistent immune dysfunction despite haploidentical hematopoietic stem cell (HSC) transplant in infancy. Follow-up data from two older patients demonstrate that lentiviral vector gammac transduced autologous HSC gene therapy after nonmyeloablative busulfan conditioning achieves selective expansion of gene-marked T, NK, and B cells, which is associated with sustained restoration of humoral responses to immunization and clinical improvement at 2 to 3 years after treatment. Similar gene marking levels have been achieved in three younger patients, albeit with only 6 to 9 months of follow-up. Lentiviral gene therapy with reduced-intensity conditioning appears safe and can restore humoral immune function to posthaploidentical transplant older patients with SCID-X1.

Doering, C. B., et al. (2018). "Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A." Hum Gene Ther **29**(10): 1183-1201.

Genetically modified, autologous hematopoietic stem and progenitor cells (HSPCs) represent a new class of genetic medicine. Following this therapeutic paradigm, we are developing a product candidate, designated CD68-ET3-LV CD34(+), for the treatment of the severe bleeding disorder, hemophilia A. The product consists of autologous CD34(+) cells transduced with a human immunodeficiency virus 1-based, monocyte lineage-restricted, self-inactivating lentiviral vector (LV), termed CD68-ET3-LV, encoding a bioengineered coagulation factor VIII (fVIII) transgene, termed ET3, designed for enhanced expression. This vector was shown capable of high-titer manufacture under clinical scale and Good Manufacturing Practice. Biochemical and immunogenicity testing of recombinant ET3, as well as safety and efficacy testing of CD68-ET3-LV HSPCs, were utilized to demonstrate overall safety and efficacy in murine models. In the first model, administration of CD68-ET3-LV-transduced stem-cell antigen-1(+) cells to hemophilia A mice resulted in sustained plasma fVIII production and hemostatic correction without signs of toxicity. Patient-derived, autologous mobilized peripheral blood (mPB) CD34(+) cells are the clinical target cells for ex vivo transduction using CD68-ET3-LV, and the resulting genetically modified cells represent the investigational drug candidate. In the second model, CD68-ET3-LV gene transfer into mPB CD34(+) cells isolated from normal human donors was utilized to obtain in vitro and in vivo pharmacology, pharmacokinetic, and toxicology assessment. CD68-ET3-LV demonstrated reproducible and efficient gene transfer into mPB CD34(+) cells, with vector copy numbers in the range of 1 copy per diploid genome equivalent without affecting clonogenic potential. Differentiation of human CD34(+) cells into monocytes was associated with increased fVIII production, supporting the designed function of the CD68 promoter. To assess in vivo pharmacodynamics, CD68-ET3-LV CD34(+) cell product was administered to immunodeficient mice. Treated mice displayed sustained plasma fVIII levels and no signs of product related toxicity. Collectively, the findings of the current study support the preclinical safety and efficacy of CD68-ET3-LV CD34(+).

Dogan, Y., et al. (2022). "Screening chimeric GAA variants in preclinical study results in hematopoietic stem cell gene therapy candidate vectors for Pompe disease." Mol Ther Methods Clin Dev **27**: 464-487.

Pompe disease is a rare genetic neuromuscular disorder caused by acid alpha-glucosidase (GAA) deficiency resulting in lysosomal glycogen accumulation and progressive myopathy. Enzyme replacement therapy, the current standard of care, penetrates poorly into the skeletal muscles and the peripheral and central nervous system (CNS), risks recombinant enzyme immunogenicity, and requires high doses and frequent infusions. Lentiviral vector-mediated hematopoietic stem and progenitor cell (HSPC) gene therapy was investigated in a Pompe mouse model using a clinically relevant promoter driving nine engineered GAA coding sequences incorporating distinct peptide tags and codon optimizations. Vectors solely including glycosylation-independent lysosomal targeting tags enhanced secretion and improved reduction of glycogen, myofiber, and CNS vacuolation in key tissues, although GAA enzyme activity and protein was consistently lower compared with native GAA. Genetically modified microglial cells in brains were detected at low levels but provided robust phenotypic correction. Furthermore, an amino acid substitution introduced in the tag reduced insulin receptor-mediated signaling with no evidence of an effect on blood glucose levels in Pompe mice. This study demonstrated the therapeutic potential of lentiviral HSPC gene therapy exploiting optimized GAA tagged coding sequences to reverse Pompe disease pathology in a preclinical mouse model, providing promising vector candidates for further investigation.

Dong, W. J., et al. (2003). "[Development of gene therapy for hematopoietic stem cell using viral vectors]." Yi Chuan Xue Bao **30**(4): 382-388.

Hematopoietic stem cells (HSC) are attractive targets for gene therapy of inherited and acquired disorders in hematopoietic system in that they possess the properties of self-renewal, proliferation, and multi-lineage differentiation. For successful gene therapy, the viral vector-mediated gene addition strategy has two essential prerequisites: 1) the efficient transfer of therapeutic gene into HSC; 2) the long-term and stable expression of the transgene at therapeutic levels. The oncoretrovirus-derived vectors are best understood and most widely investigated. Recent successful cases of gene therapy for severe combined immunodeficiency due to adenosine deaminase or gamma c chain deficiencies have provided strong evidences that retrovirus-mediated gene transfer into HSC will work in clinical treatment. While these results are encouraging, some obstacles remain to be circumvented including low efficiency of gene transfer and gene silencing in retroviral vector system. The therapeutic gene can be efficiently introduced into HSC by HIV-1-based lentiviral vector due to its capability to infect the quiescent cells. A variety of preclinical studies are now conducted and a number of valuable results highlight the efficacy of lentiviral-mediated gene transfer into HSC. However, the potential value of lentiviral vectors in human gene therapy remains to be demonstrated. Adeno-associated virus vector is an alternative to retroviral and lentiviral vectors. This review summarizes the characteristics of integrating vectors, the improved HSC transduction protocols, and the optimized gene expression strategies and outlines the important advances of preclinical and clinical trials in hematopoietic stem cell gene therapy.

Donnelly, E. M. and N. M. Boulis (2012). "Update on gene and stem cell therapy approaches for spinal muscular atrophy." Expert Opin Biol Ther **12**(11): 1463-1471.

INTRODUCTION: Spinal muscular atrophy (SMA) is the leading genetic cause of pediatric death to which at present there is no effective therapeutic. The genetic defect is well characterized as a mutation in exon 7 of the survival of motor neuron (SMN) gene. The current gene therapy approach focuses on two main methodologies, the replacement of SMN1 or augmentation of SMN2 readthrough. The most promising of the current work focuses on the delivery of SMN via AAV9 vectors via intravenous delivery. AREAS COVERED: In the review the authors examine the current research in the field of stem cell and gene therapy approaches for SMA. Also focusing on delivery methods, timing of administration and general caveats that must be considered with translational work for SMA. EXPERT OPINION: Gene therapy currently offers the most promising avenue of research for a successful therapeutic for SMA. There are many important practical and ethical considerations which must be carefully considered when dealing with clinical trial in infants such as the invasiveness of the surgery, the correct patient cohort and the potential risks.

Douillard-Guilloux, G., et al. (2009). "Partial phenotypic correction and immune tolerance induction to enzyme replacement therapy after hematopoietic stem cell gene transfer of alpha-glucosidase in Pompe disease." J Gene Med **11**(4): 279-287.

BACKGROUND: Glycogen storage disease type II (GSDII) or Pompe disease is an inherited disease of glycogen metabolism caused by a lack of functional lysosomal acid alpha-glucosidase (GAA). Affected individuals store glycogen in lysosomes resulting in fatal hypertrophic cardiomyopathy and

respiratory failure in the most severe form. Even if enzyme replacement therapy (ERT) has already proven some efficacy, its results remain heterogeneous in skeletal muscle, especially in cross reactive immunological material (CRIM)-negative patients. We investigated for the first time the use of hematopoietic stem cell (HSC) gene therapy in a murine model of GSDII. METHODS: Deficient HSC were transduced with a lentiviral vector expressing human GAA or enhanced green fluorescent protein (GFP) under the control of the retroviral MND promoter and transplanted into lethally irradiated GSDII mice. Animals were then subjected to an ERT protocol for 5 weeks and monitored for metabolic correction and GAA-induced immune reaction. RESULTS: GAA was expressed as a correctly processed protein, allowing a complete enzymatic correction in transduced deficient cells without toxicity. Seventeen weeks after transplantation, a partial restoration of the GAA enzymatic activity was observed in bone marrow and peripheral blood cells of GSDII mice, allowing a significant glycogen clearance in skeletal muscle. ERT induced a robust antibody response in GFP-transplanted mice, whereas no immune reaction could be detected in GAA-transplanted mice. CONCLUSIONS: Lentiviral vector-mediated HSC gene therapy leads to a partial metabolic correction and induces a tolerance to ERT in GSDII mice. This strategy could enhance the efficacy of ERT in CRIM-negative Pompe patients.

Drakopoulou, E., et al. (2011). "The Ongoing Challenge of Hematopoietic Stem Cell-Based Gene Therapy for beta-Thalassemia." Stem Cells Int **2011**: 987980.

beta-thalassemia is characterized by reduced or absence of beta-globin production, resulting in anemia. Current therapies include blood transfusion combined with iron chelation. BM transplantation, although curative, is restricted by the matched donor limitation. Gene therapy, on the other hand, is promising, and its success lies primarily on designing efficient globin vectors that can effectively and stably transduce HSCs. The major breakthrough in beta-thalassemia gene therapy occurred a decade ago with the development of globin LVs. Since then, researchers focused on designing efficient and safe vectors, which can successfully deliver the therapeutic transgene, demonstrating no insertional mutagenesis. Furthermore, as human HSCs have intrinsic barriers to HIV-1 infection, attention is drawn towards their ex vivo manipulation, aiming to achieve higher yield of genetically modified HSCs. This paper presents the current status of gene therapy for beta-thalassemia, its success and limitations, and the novel promising strategies available involving the therapeutic role of HSCs.

Droz-Georget Lathion, S., et al. (2015). "A single epidermal stem cell strategy for safe ex vivo gene therapy." EMBO Mol Med **7**(4): 380-393.

There is a widespread agreement from patient and professional organisations alike that the safety of stem cell therapeutics is of paramount importance, particularly for ex vivo autologous gene therapy. Yet current technology makes it difficult to thoroughly evaluate the behaviour of genetically corrected stem cells before they are transplanted. To address this, we have developed a strategy that permits transplantation of a clonal population of genetically corrected autologous stem cells that meet stringent selection criteria and the principle of precaution. As a proof of concept, we have stably transduced epidermal stem cells (holoclones) obtained from a patient suffering from recessive dystrophic epidermolysis bullosa. Holoclones were infected with self-inactivating retroviruses bearing a COL7A1 cDNA and cloned before the progeny of individual stem cells were characterised using a number of criteria. Clonal analysis revealed a great deal of heterogeneity among transduced stem cells in their capacity to produce functional type VII collagen (COLVII). Selected transduced stem cells transplanted onto immunodeficient mice regenerated a non-blistering epidermis for months and produced a functional COLVII. Safety was assessed by determining the sites of proviral integration, rearrangements and hit genes and by whole-genome sequencing. The progeny of the selected stem cells also had a diploid karyotype, was not tumorigenic and did not disseminate after long-term transplantation onto immunodeficient mice. In conclusion, a clonal strategy is a powerful and efficient means of by-passing the heterogeneity of a transduced stem cell population. It guarantees a safe and homogenous medicinal product, fulfilling the principle of precaution and the requirements of regulatory affairs. Furthermore, a clonal strategy makes it possible to envision exciting gene-editing technologies like zinc finger nucleases, TALENs and homologous recombination for next-generation gene therapy.

Duan, M. L., et al. (2000). "[Future cure of hearing disorders? Gene therapy and stem cell implantation are possible new therapeutic alternatives]." Lakartidningen **97**(10): 1106-1108, 1111-1102.

Hearing loss is a very common disorder; nearly 10 per cent of the population is affected. Recently, a few findings such as the roles of neurotrophins, nitric oxide, reactive oxygen species and glutamate receptors in the peripheral hearing system have been highlighted. In this review, focus is set on

possible mechanisms of peripheral hearing disorders, and on recent advances to prevent and treat hearing loss. Clinically useful treatment strategies, especially gene therapy and the use of embryonic stem cells, are particularly stressed.

Dunbar, C. E. (2007). "The yin and yang of stem cell gene therapy: insights into hematopoiesis, leukemogenesis, and gene therapy safety." Hematology Am Soc Hematol Educ Program: 460-465.

Over the past decade, success in the treatment of serious genetic disorders via gene therapy was finally achieved. However, this progress was tempered by the occurrence of serious adverse events related to vector integration into the genome and activation of adjacent proto-oncogenes. Investigators are now focused on retaining the clinical potential of integrating vectors while decreasing the risk of insertional mutagenesis.

Dwyer, R. M., et al. (2010). "Advances in mesenchymal stem cell-mediated gene therapy for cancer." Stem Cell Res Ther **1**(3): 25.

Mesenchymal stem cells have a natural tropism for tumours and their metastases, and are also considered immunoprivileged. This remarkable combination of properties has formed the basis for many studies investigating their potential as tumour-specific delivery vehicles for suicide genes, oncolytic viruses and secreted therapeutic proteins. The aim of the present review is to discuss the range of approaches that have been used to exploit the tumour-homing capacity of mesenchymal stem cells for gene delivery, and to highlight advances required to realize the full potential of this promising approach.

Eddleman, K. A., et al. (1996). "Circulating hematopoietic stem cell populations in human fetuses: implications for fetal gene therapy and alterations with in utero red cell transfusion." Fetal Diagn Ther **11**(4): 231-240.

Circulating progenitor cell populations in normal human fetuses and fetuses with various hematological problems were evaluated. Thirty blood samples from 21 human fetuses (17-36 weeks of gestation) were assayed for erythroid, myeloid, and mixed-cell progenitor cells. The mean number of progenitor cells/10(4) blood mononuclear cells in the normal fetal population was 103 +/- 47. Granulomonocytic and mixed progenitor cells (capable of giving rise to both erythroid and myeloid progeny) were the predominant progenitor types in these samples, with pure erythroid progenitors barely detectable. The frequency of progenitor cells in the samples from fetuses with hematological disorders was within the range of normal in all but 1 fetus infected with parvovirus in whom very few progenitor cells were detected. The frequency of progenitor cells in the blood did not change after intravascular red cell transfusion for alloimmunization despite the large volumes transfused, indicating that transfusion may have triggered a release of progenitor cells into the circulation. Progenitor cells in human fetal blood are present in distributions similar to those commonly detected in cord blood. Their total number in the circulating blood is in the same order used for pediatric and adult bone marrow transplantation. These results can be used to calculate the number of colony-forming cells which could be obtained from a fetus by in utero apheresis and which could be made available for autologous fetal gene therapy.

Emery, D. W. and G. Stamatoyannopoulos (1999). "Stem cell gene therapy for the beta-chain hemoglobinopathies. Problems and progress." Ann N Y Acad Sci **872**: 94-107; discussion 107-108.

Virus vectors hold great promise for the stem cell gene therapy of beta-chain hemoglobinopathies. However, conventional vectors suffer from low gene transfer rates, low expression levels, and inconsistent or short-lived expression in vivo. In this review we summarize the current status of vector systems for the transduction of hematopoietic stem cells, including the development of novel vector systems and methods for selection of transduced stem cells in vivo. We also summarize efforts to achieve therapeutic expression levels of transferred globin genes with retrovirus vectors, including the manipulation of transcription cassettes, the use of globin gene enhancers, and advances in the use of chromatin insulators for improving the frequency of gene expression following hematopoietic stem cell transduction.

Engel, B. C. and D. B. Kohn (1999). "Stem cell directed gene therapy." Front Biosci **4**: e26-33.

A potential therapeutic approach to HIV-1 infection is the genetic modification of cells of a patient to make them resistant to HIV-1. Hematopoietic stem cells are an attractive target for gene therapy of AIDS because of their ability to generate a broad repertoire of mature T lymphocytes, as well as the monocytic cells (macrophages, dendritic cells and microglia) which are also involved in HIV-1 pathogenesis. A number of synthetic "anti-HIV-1 genes" have been developed which inhibit HIV-1 replication. However, current methods for gene transfer into human hematopoietic stem cells, using retroviral vectors derived from the Moloney murine leukemia virus, have been minimally effective. Clinical trials performed to date in which hematopoietic cells from HIV-1-positive patients have been transduced with retroviral vectors and then reinfused have produced

low to undetectable levels of gene-containing peripheral blood leukocytes. New vector delivery systems, such as lentiviral vectors, need to be developed to ensure efficient gene transfer and persistent transgene expression to provide life-long resistance to the cells targeted by HIV-1.

Erlich, S., et al. (1999). "Fluorescence-based selection of gene-corrected hematopoietic stem and progenitor cells from acid sphingomyelinase-deficient mice: implications for Niemann-Pick disease gene therapy and the development of improved stem cell gene transfer procedures." Blood **93**(1): 80-86.

The general utility of a novel, fluorescence-based procedure for assessing gene transfer and expression has been demonstrated using hematopoietic stem and progenitor cells. Lineage-depleted hematopoietic cells were isolated from the bone marrow or fetal livers of acid sphingomyelinase-deficient mice, and retrovirally transduced with amphotropic or ecotropic vectors encoding a normal acid sphingomyelinase (ASM) cDNA. Anti-c-Kit antibodies were then used to label stem- and progenitor-enriched cell populations, and the Bodipy fluorescence was analyzed in each group after incubation with a Bodipy-conjugated sphingomyelin. Only cells expressing the functional ASM (ie, transduced) could degrade the sphingomyelin, thereby reducing their Bodipy fluorescence as compared with nontransduced cells. The usefulness of this procedure for the in vitro assessment of gene transfer into hematopoietic stem cells was evaluated, as well as its ability to provide an enrichment of transduced stem cells in vivo. To show the value of this method for in vitro analysis, the effects of retroviral transduction using ecotropic versus amphotropic vectors, various growth factor combinations, and adult bone marrow versus fetal liver stem cells were assessed. The results of these studies confirmed the fact that ecotropic vectors were much more efficient at transducing murine stem cells than amphotropic vectors, and that among the three most commonly used growth factors (stem cell factor [SCF] and interleukins 3 and 6 [IL-3 and IL-6]), SCF had the most significant effect on the transduction of stem cells, whereas IL-6 had the most significant effect on progenitor cells. In addition, it was determined that fetal liver stem cells were only approximately twofold more "transducible" than stem cells from adult bone marrow. Transplantation of Bodipy-selected bone marrow cells into lethally irradiated mice showed that the number of spleen colony-forming units that were positive for the retroviral vector (as determined by polymerase chain reaction) was 76%, as compared with 32% in animals that were transplanted with cells that were nonselected. The methods described within this manuscript are particularly useful for evaluating hematopoietic stem cell gene transfer in vivo because the marker gene used in the procedure (ASM) encodes a naturally occurring mammalian enzyme that has no known adverse effects, and the fluorescent compound used for selection (Bodipy sphingomyelin) is removed from the cells before transplantation.

Fang, Q., et al. (2019). "Adipocyte-derived stem cell-based gene therapy upon adipogenic differentiation on microcarriers attenuates type 1 diabetes in mice." Stem Cell Res Ther **10**(1): 36.

BACKGROUND: Insulin replenishment is critical for patients with type 1 diabetes; however, current treatments such as pancreatic islet transplantation and insulin injection are not ideal. In addition to stem cell or gene therapy alone, stem cell combined with gene therapy may provide a new route for insulin replenishment, which could avoid an autoimmune reaction against differentiated beta cells or systematic viral vector injection. METHODS: In this study, human adipocyte-derived stem cells (ADSCs) were transducted with lentiviral vectors expressing a furin-cleavable insulin gene. The expression levels of insulin were measured before and after adipogenic differentiation in the presence or absence of an adipocyte-specific promoter AP2. In vitro proliferation and in vivo survival of cells were examined on cytodex and cytopore microcarriers. The effect of ADSC-based gene therapy upon adipogenic differentiation on microcarriers was evaluated in the streptozotocin-induced type 1 diabetic mouse model. RESULTS: We found that differentiation of ADSCs into adipocytes increased insulin expression under the EF1 promoter, while adipocyte-specific AP2 promoter further increased insulin expression upon differentiation. The microcarriers supported cell attachment and proliferation during in vitro culture and facilitate cell survival after transplantation. Functional cells on the cytopore 1 microcarrier formed tissue-like structures and alleviated hyperglycemia in the type 1 diabetic mice after subcutaneous injection. CONCLUSIONS: Our results indicated that differentiation of ADSC and tissue-specific promotors may enhance the expression of therapeutic genes. The use of microcarriers may facilitate cell survival after transplantation and hold potential for long-term cell therapy.

Fathi, E., et al. (2023). "Adipose Tissue-Mesenchymal Stem Cells Caused to Change the Methylation Status of hTERT Gene Promoter CpG Islands of Molt-4 Leukemia Cells as Cell-based Therapy." Curr Mol Med **23**(3): 266-274.

BACKGROUND: DNA methylation was considered as prognostic information in some hematological malignancies. Previous studies have

reported the in vitro and in vivo biology role of mesenchymal stem cells (MSCs) on leukemic cells. The aim of this study was to investigate the effect of MSCs on the promoter methylation status of hTERT as a catalytic subunit of telomerase enzyme. METHODS: In the experimental study, the Molt-4 leukemic cells were co-cultured with MSCs for 7 days. At the end of the co-culture period, the Molt-4 cells were collected, DNA and protein were extracted. Then methylation specific-PCR and western blotting were done for evaluating the hTERT gene promoter methylation status and cyclin D1 and hTERT protein expression, respectively. In the following, the flow cytometry was done for cell cycle distribution assay. RESULTS: It was found that MSCs resulted in a significant decrease in the cyclin D1 and hTERT protein expression levels. Also, MSCs caused changes in the methylation status of the CpG islands in the hTERT gene promoter region. The following results showed that MSCs caused a significant increase in the number of cells at G0/G1 phase and arrest the G0/G1 phase as well as decrease in the cell proliferation of Molt-4 cells. CONCLUSION: It is concluded that co-culture of MSCs with Molt-4 cells could be involved in changing the methylation status of hTERT gene promoter, cell cycle and hTERT protein expression; it could be potentially beneficial for further investigations regarding the cell transplantation and cell-based therapy.

Ferguson, C., et al. (2005). "Hematopoietic stem cell gene therapy: dead or alive?" Trends Biotechnol **23**(12): 589-597.

Despite some reports of toxicity in recent clinical trials, many scientists believe that the use of gene therapy in the treatment of congenital genetic defects and acquired disorders has too much potential to abandon. Hematopoietic stem cells (HSCs) have been primary targets for gene therapy owing to their capacity for differentiation and self-renewal, whereby multiple cell lineages can potentially be corrected for the lifetime of an individual. These efforts represent a long-term investment towards broadening physicians' treatment options for patients whose diseases, in particular certain immunodeficiencies, are fatal and where no other therapy is available. We review the recent progress and clinical triumphs as well as the reported toxicity related to insertional mutagenesis. We also discuss the current risk-to-benefit estimates and future strategies to reduce the risks and allow full realization of clinical potential. Scientists are continually revising protocols: going both from "bench to bedside" and, as strikingly demonstrated by HSC gene therapy, from "bedside to bench."

Fernandes, A. R. and D. M. Chari (2016). "Part I: Minicircle vector technology limits DNA size restrictions on ex vivo gene delivery using nanoparticle vectors: Overcoming a translational barrier in neural stem cell therapy." J Control Release **238**: 289-299.

Genetically engineered neural stem cell (NSC) transplant populations offer key benefits in regenerative neurology, for release of therapeutic biomolecules in ex vivo gene therapy. NSCs are 'hard-to-transfect' but amenable to 'magnetofection'. Despite the high clinical potential of this approach, the low and transient transfection associated with the large size of therapeutic DNA constructs is a critical barrier to translation. We demonstrate for the first time that DNA minicircles (small DNA vectors encoding essential gene expression components but devoid of a bacterial backbone, thereby reducing construct size versus conventional plasmids) deployed with magnetofection achieve the highest, safe non-viral DNA transfection levels (up to 54%) reported so far for primary NSCs. Minicircle-functionalized magnetic nanoparticle (MNP)-mediated gene delivery also resulted in sustained gene expression for up to four weeks. All daughter cell types of engineered NSCs (neurons, astrocytes and oligodendrocytes) were transfected (in contrast to conventional plasmids which usually yield transfected astrocytes only), offering advantages for targeted cell engineering. In addition to enhancing MNP functionality as gene delivery vectors, minicircle technology provides key benefits from safety/scale up perspectives. Therefore, we consider the proof-of-concept of fusion of technologies used here offers high potential as a clinically translatable genetic modification strategy for cell therapy.

Ferrua, F., et al. (2019). "Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study." Lancet Haematol **6**(5): e239-e253.

BACKGROUND: Wiskott-Aldrich syndrome is a rare, life-threatening, X-linked primary immunodeficiency characterised by microthrombocytopenia, infections, eczema, autoimmunity, and malignant disease. Lentiviral vector-mediated haemopoietic stem/progenitor cell (HSPC) gene therapy is a potentially curative treatment that represents an alternative to allogeneic HSPC transplantation. Here, we report safety and efficacy data from an interim analysis of patients with severe Wiskott-Aldrich syndrome who received lentiviral vector-derived gene therapy. METHODS: We did a non-randomised, open-label, phase 1/2 clinical study in paediatric patients with severe Wiskott-Aldrich syndrome, defined by either WAS gene mutation or absent Wiskott-Aldrich syndrome protein (WASP) expression or a Zhu clinical score of 3 or higher. We included patients who had no HLA-identical sibling

donor available or, for children younger than 5 years of age, no suitable 10/10 matched unrelated donor or 6/6 unrelated cord blood donor. After treatment with rituximab and a reduced-intensity conditioning regimen of busulfan and fludarabine, patients received one intravenous infusion of autologous CD34+ cells genetically modified with a lentiviral vector encoding for human WAS cDNA. The primary safety endpoints were safety of the conditioning regimen and safety of lentiviral gene transfer into HSPCs. The primary efficacy endpoints were overall survival, sustained engraftment of genetically corrected HSPCs, expression of vector-derived WASP, improved T-cell function, antigen-specific responses to vaccinations, and improved platelet count and mean platelet volume normalisation. This interim analysis was done when the first six patients treated had completed at least 3 years of follow-up. The planned analyses are presented for the intention-to-treat population. This trial is registered with ClinicalTrials.gov (number NCT01515462) and EudraCT (number 2009-017346-32). FINDINGS: Between April 20, 2010, and Feb 26, 2015, nine patients (all male) were enrolled of whom one was excluded after screening; the age range of the eight treated children was 1.1-12.4 years. At the time of the interim analysis (data cutoff April 29, 2016), median follow-up was 3.6 years (range 0.5-5.6). Overall survival was 100%. Engraftment of genetically corrected HSPCs was successful and sustained in all patients. The fraction of WASP-positive lymphocytes increased from a median of 3.9% (range 1.8-35.6) before gene therapy to 66.7% (55.7-98.6) at 12 months after gene therapy, whereas WASP-positive platelets increased from 19.1% (range 4.1-31.0) to 76.6% (53.1-98.4). Improvement of immune function was shown by normalisation of in-vitro T-cell function and successful discontinuation of immunoglobulin supplementation in seven patients with follow-up longer than 1 year, followed by positive antigen-specific response to vaccination. Severe infections fell from 2.38 (95% CI 1.44-3.72) per patient-year of observation (PYO) in the year before gene therapy to 0.31 (0.04-1.11) per PYO in the second year after gene therapy and 0.17 (0.00-0.93) per PYO in the third year after gene therapy. Before gene therapy, platelet counts were lower than 20 x 10(9) per L in seven of eight patients. At the last follow-up visit, the platelet count had increased to 20-50 x 10(9) per L in one patient, 50-100 x 10(9) per L in five patients, and more than 100 x 10(9) per L in two patients, which resulted in independence from platelet transfusions and absence of severe bleeding events. 27 serious adverse events in six patients occurred after gene therapy, 23 (85%) of which were infectious (pyrexia [five events in three patients], device-related infections, including one case of sepsis [four events in three patients], and gastroenteritis, including one case due to rotavirus [three events in two patients]); these occurred mainly in the first 6 months of follow-up. No adverse reactions to the investigational drug product and no abnormal clonal proliferation or leukaemia were reported after gene therapy. INTERPRETATION: Data from this study show that gene therapy provides a valuable treatment option for patients with severe Wiskott-Aldrich syndrome, particularly for those who do not have a suitable HSPC donor available. FUNDING: Italian Telethon Foundation, GlaxoSmithKline, and Orchard Therapeutics.

Fumagalli, F., et al. (2022). "Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access." Lancet **399**(10322): 372-383.

BACKGROUND: Effective treatment for metachromatic leukodystrophy (MLD) remains a substantial unmet medical need. In this study we investigated the safety and efficacy of atidarsagene autotemcel (arsa-cel) in patients with MLD. METHODS: This study is an integrated analysis of results from a prospective, non-randomised, phase 1/2 clinical study and expanded-access frameworks. 29 paediatric patients with pre-symptomatic or early-symptomatic early-onset MLD with biochemical and molecular confirmation of diagnosis were treated with arsa-cel, a gene therapy containing an autologous haematopoietic stem and progenitor cell (HSPC) population transduced ex vivo with a lentiviral vector encoding human arylsulfatase A (ARSA) cDNA, and compared with an untreated natural history (NHx) cohort of 31 patients with early-onset MLD, matched by age and disease subtype. Patients were treated and followed up at Ospedale San Raffaele, Milan, Italy. The coprimary efficacy endpoints were an improvement of more than 10% in total gross motor function measure score at 2 years after treatment in treated patients compared with controls, and change from baseline of total peripheral blood mononuclear cell (PBMC) ARSA activity at 2 years after treatment compared with values before treatment. This phase 1/2 study is registered with ClinicalTrials.gov, NCT01560182. FINDINGS: At the time of analyses, 26 patients treated with arsa-cel were alive with median follow-up of 3.16 years (range 0.64-7.51). Two patients died due to disease progression and one due to a sudden event deemed unlikely to be related to treatment. After busulfan conditioning, all arsa-cel treated patients showed sustained multilineage engraftment of genetically modified HSPCs. ARSA activity in PBMCs was significantly increased above baseline 2 years after treatment by a mean 18.7-fold (95% CI 8.3-42.2; p<0.0001) in patients with the late-infantile variant and 5.7-fold (2.6-12.4; p<0.0001) in

patients with the early-juvenile variant. Mean differences in total scores for gross motor function measure between treated patients and age-matched and disease subtype-matched NHx patients 2 years after treatment were significant for both patients with late-infantile MLD (66% [95% CI 48.9-82.3]) and early-juvenile MLD (42% [12.3-71.8]). Most treated patients progressively acquired motor skills within the predicted range of healthy children or had stabilised motor performance (maintaining the ability to walk). Further, most displayed normal cognitive development and prevention or delay of central and peripheral demyelination and brain atrophy throughout follow-up; treatment benefits were particularly apparent in patients treated before symptom onset. The infusion was well tolerated and there was no evidence of abnormal clonal proliferation or replication-competent lentivirus. All patients had at least one grade 3 or higher adverse event; most were related to conditioning or to background disease. The only adverse event related to arsa-cel was the transient development of anti-ARSA antibodies in four patients, which did not affect clinical outcomes. INTERPRETATION: Treatment with arsa-cel resulted in sustained, clinically relevant benefits in children with early-onset MLD by preserving cognitive function and motor development in most patients, and slowing demyelination and brain atrophy. FUNDING: Orchard Therapeutics, Fondazione Telethon, and GlaxoSmithKline.

Fuster, V. and J. Sanz (2007). "Gene therapy and stem cell therapy for cardiovascular diseases today: a model for translational research." Nat Clin Pract Cardiovasc Med **4 Suppl 1**: S1-8.

Clinical trials looking at ways to promote myocardial regeneration have reported that the administered therapies have either neutral effects or modest benefits of questionable impact. These somewhat disappointing results should emphasize the need for translational research, with bidirectional feedback between the basic research laboratory and the clinical arena. Such a translational pathway is illustrated by the quest to find an effective therapy for restenosis, which culminated in the development of sirolimus. At this point a move away from the bedside and a return to the bench seems necessary to better understand the mechanisms of action of progenitor cells and stimulating factors. Without such basic knowledge research might be prematurely discouraged and the opportunity to fully understand the true potential of cardiovascular regenerative therapy might be missed.

Gleitz, H. F., et al. (2018). "Brain-targeted stem cell gene therapy corrects mucopolysaccharidosis type II via multiple mechanisms." EMBO Mol Med **10**(7).

The pediatric lysosomal storage disorder mucopolysaccharidosis type II is caused by mutations in IDS, resulting in accumulation of heparan and dermatan sulfate, causing severe neurodegeneration, skeletal disease, and cardiorespiratory disease. Most patients manifest with cognitive symptoms, which cannot be treated with enzyme replacement therapy, as native IDS does not cross the blood-brain barrier. We tested a brain-targeted hematopoietic stem cell gene therapy approach using lentiviral IDS fused to ApoEII (IDS.ApoEII) compared to a lentivirus expressing normal IDS or a normal bone marrow transplant. In mucopolysaccharidosis II mice, all treatments corrected peripheral disease, but only IDS.ApoEII mediated complete normalization of brain pathology and behavior, providing significantly enhanced correction compared to IDS. A normal bone marrow transplant achieved no brain correction. Whilst corrected macrophages traffic to the brain, secreting IDS/IDS.ApoEII enzyme for cross-correction, IDS.ApoEII was additionally more active in plasma and was taken up and transcytosed across brain endothelia significantly better than IDS via both heparan sulfate/ApoE-dependent receptors and mannose-6-phosphate receptors. Brain-targeted hematopoietic stem cell gene therapy provides a promising therapy for MPS II patients.

Goebel, W. S., et al. (2002). "Donor chimerism and stem cell function in a murine congenic transplantation model after low-dose radiation conditioning: effects of a retroviral-mediated gene transfer protocol and implications for gene therapy." Exp Hematol **30**(11): 1324-1332.

OBJECTIVE: We investigated low-dose radiation conditioning for the transplantation of retrovirus-transduced cells in a C57Bl6/J murine model. MATERIALS AND METHODS: The effect of low-dose radiation on stem cell function was investigated using a competitive repopulation assay. Stem cell function of marrow cells that underwent a retroviral-mediated gene transfer (RMGT) protocol was examined by this assay, and donor chimerism of these cells when transplanted into 160-cGy conditioned syngeneic hosts was compared to fresh marrow. RESULTS: Irradiation with 300 or 160 cGy substantially decreased stem cell function as measured by competitive repopulation. Animals conditioned with 160 cGy and transplanted with 20 x 10(6) fresh marrow cells permitted donor cell engraftment of 53.6% +/- 11.4% 6 months after transplant compared to 100% donor cell engraftment after 1100 cGy irradiation. Lymphoid and myeloid engraftment did not significantly differ from total engraftment in submyeloablated hosts. When transplanted into lethally irradiated hosts, the competitive repopulating activity

of marrow treated with a single dose of 5-fluorouracil followed by ex vivo culture according to a standard RMGT protocol was equal to 5-fluorouracil-only treated marrow. However, cells treated with 5-fluorouracil or 5-fluorouracil plus ex vivo culture for RMGT repopulated less well than fresh marrow cells in 160 cGy conditioned hosts. CONCLUSIONS: Low-dose irradiation decreases host stem cell function, allowing engraftment of both fresh and RMGT protocol-treated marrow, although the engraftment of 5-fluorouracil-treated cells was reduced at least two-fold, and 5-fluorouracil plus RMGT protocol-treated cells at least three-fold, compared to fresh marrow. Modification of current RMGT protocols may be important for optimizing engraftment under these conditions.

Goessler, U. R., et al. (2006). "Perspectives of gene therapy in stem cell tissue engineering." Cells Tissues Organs **183**(4): 169-179.

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function. It is hoped that forming tissue de novo will overcome many problems in plastic surgery associated with such areas as wound healing and the immunogenicity of transplanted tissue that lead to dysfunctional repair. Gene therapy is the science of the transfer of genetic material into individuals for therapeutic purposes by altering cellular function or structure at the molecular level. Recently, tissue engineering has been used in conjunction with gene therapy as a hybrid approach. This combination of stem-cell-based tissue engineering with gene therapy has the potential to provide regenerative tissue cells within an environment of optimal regulatory protein expression and would have many benefits in various areas such as the transplantation of skin, cartilage or bone. The aim of this review is to outline tissue engineering and possible applications of gene therapy in the field of biomedical engineering as well as basic principles of gene therapy, vectors and gene delivery.

Golchin, A., et al. (2018). "Promotion of Cell-Based Therapy: Special Focus on the Cooperation of Mesenchymal Stem Cell Therapy and Gene Therapy for Clinical Trial Studies." Adv Exp Med Biol **1119**: 103-118.

Regenerative medicine (RM) is a promising new field of medicine that has mobilized several new tools to repair or replace lost or damaged cells or tissues by stimulating natural regenerative mechanisms nearby cell and tissue-based therapy approaches. However, mesenchymal stem cell (MSC) based therapy has been shown to be safe and effective to a certain degree in multiple clinical trial studies (CTSs) of several diseases, in most MSC CTSs the efficacy of treatment has been reported low. Therefore, researchers have focused on efficacy enhancing of MSC to improve migratory and homing, survival, stemness, differentiation and other therapeutic applicable properties by using different approaches. Gene therapy is one of the experimental technique tools that uses genes to change cells for therapeutic and investigation purposes. In this study has been focused on genetically modified MSCs for use in RM with an emphasis on CTSs. We highlight the basic concept of genetic modifications and also discuss recent clinical studies aspects. Recently reviewed studies show that MSC therapy with assistant gene therapy can be used in cancer therapy, heart diseases, Fanconi anemia and several other diseases.

Goldman, S. A. (2004). "Directed mobilization of endogenous neural progenitor cells: the intersection of stem cell biology and gene therapy." Curr Opin Mol Ther **6**(5): 466-472.

Multipotential neural stem cells, which are capable of giving rise to both neurons and glia, line the cerebral ventricles of all adult animals, including humans. These cells may be mobilized and induced to undergo neuronal differentiation in vivo, by stimulating resident progenitor cells with both delivered and virally expressed growth factors. This strategy may be particularly efficacious in striatal neurodegenerative conditions such as Huntington's disease, in which lost medium spiny striatal neurons may be replenished through directed induction of progenitor cells lining the striatal ventricular wall. More broadly, our increasing understanding of the molecular control of progenitor cell mobilization and differentiation will likely afford many new opportunities for using induced neuronal replacement as a therapeutic strategy for neurodegenerative diseases.

Greco, S. J. and P. Rameshwar (2012). "Mesenchymal stem cells in drug/gene delivery: implications for cell therapy." Ther Deliv **3**(8): 997-1004.

Stem cells have been therapeutically utilized in replacement of hematopoetic cells for decades. This is in contrast to the recent emergence of adult stem cells as, perhaps, safe and beneficial therapeutics for multiple diseases and disorders. In particular, mesenchymal stem cells (MSCs) are currently used in multiple human clinical trials. Although MSCs are ubiquitous, bone marrow, umbilical cord and adipose tissue are the sources where MSCs are isolated for research and clinical application. MSCs were thought to be mesodermal due to the initial reports showing their differentiation into specialized mesodermal cells such as chondrocytes. However, it now appears that

MSCs might be neuroectodermal in origin. Thus far, there is no evidence of in vivo transformation of MSCs. However, it is too early to prove or disprove that MSCs can be transformed in vivo in clinical trials. MSCs display immunosuppressive properties when placed in a milieu of inflammatory mediators. This phenotype makes MSCs easily available for therapies as 'off-the-shelf cells. Additionally, MSCs express chemotactic receptors, thereby allowing them to migrate to sites of tissue injury. This latter property has proven useful in the embodiment of MSCs as cellular vehicles to deliver targeted therapeutics to precise regions. The MSCs would typically harbor a prodrug or ectopically express a therapeutic gene to be delivered at a targeted site. This approach has been utilized in a number of different indications requiring precise therapeutic delivery, specifically cancer, cardiovascular disorders and neurodegenerative diseases. Combined with their immune-privileged status, safe clinical profile and low tumorigenicity, MSCs offer vast potential to benefit patients with serious diseases, for which limited treatment options exist.

Greenberger, J. S. (2008). "Gene therapy approaches for stem cell protection." Gene Ther **15**(2): 100-108.

Cytotoxic exposure of bone marrow and other non-hematopoietic organs containing self-renewing stem cell populations is associated with damage to the supportive microenvironment. Recent evidence indicates that radical oxygen species resulting from the initial oxidative stress persist for months after ionizing irradiation exposure of tissues including oral cavity, esophagus, lung and bone marrow. Antioxidant gene therapy using manganese superoxide dismutase plasmid liposomes has provided organ-specific radiation protection associated with delay or prevention of acute and late toxicity. Recent evidence has suggested that manganese superoxide dismutase transgene expression in cells of the organ microenvironment contributes significantly to the mechanism of protection. Incorporating this knowledge into designs of novel approaches for stem cell protection is addressed in the present review.

Guo, L., et al. (2021). "The use of gene-modified bone marrow mesenchymal stem cells for cochlear cell therapy." Transpl Immunol **68**: 101433.

BACKGROUND: The aim of this study was to investigate the potential of using bone marrow mesenchymal stem cells (BMSCs) for treatment of inflammation and autoimmune sensorineural hearing loss. METHODS: Fifty-five immunized guinea pigs were divided into five groups. Group A received BMSCs expressing IL-4, group B received BMSCs expressing an empty carrier vector, group C received recombinant lentivirus expressing IL-4, group D received recombinant lentivirus expressing an empty carrier vector, and group E received phosphate-buffered saline. Auditory function was monitored using brain stem responses (ABRs) to evaluate the auditory changes. The distribution of implanted BMSCs in the inner ear was estimated using fluorescence microscopy. The distribution and expression of IL-4 gene products in the inner ear were detected via immunohistochemistry. RESULTS: After transplantation, the ABR III wave threshold decreased significantly in BMSCs expressing exogenous IL-4 group (group A), BMSCs expressing empty carrier vector group (group B), and recombinant lentivirus expressing IL-4 group (group C) ($P < 0.001$), which means the auditory functions of the experimental guinea pigs were improved. Further statistical analysis revealed that BMSCs expressing exogenous IL-4 group (group A) and BMSCs expressing empty carrier vector group (group B) were able to improve the auditory function more obviously ($P < 0.05$). Lentivirus-infected BMSCs were able to migrate to the inner ear. Fluorescence-positive BMSCs were scattered in the scala tympani and vestibule. CONCLUSIONS: These results demonstrated that BMSCs expressing exogenous IL-4 successfully migrated into the inner ear in an in vitro study. BMSCs expressing exogenous IL-4 and BMSCs can be used to treat inflammatory injury in autoimmune inner ear diseases.

Holley, R. J., et al. (2018). "Macrophage enzyme and reduced inflammation drive brain correction of mucopolysaccharidosis IIIB by stem cell gene therapy." Brain **141**(1): 99-116.

Mucopolysaccharidosis IIIB is a paediatric lysosomal storage disease caused by deficiency of the enzyme alpha-N-acetylglucosaminidase (NAGLU), involved in the degradation of the glycosaminoglycan heparan sulphate. Absence of NAGLU leads to accumulation of partially degraded heparan sulphate within lysosomes and the extracellular matrix, giving rise to severe CNS degeneration with progressive cognitive impairment and behavioural problems. There are no therapies. Haematopoietic stem cell transplant shows great efficacy in the related disease mucopolysaccharidosis I, where donor-derived monocytes can transmigrate into the brain following bone marrow engraftment, secrete the missing enzyme and cross-correct neighbouring cells. However, little neurological correction is achieved in patients with mucopolysaccharidosis IIIB. We have therefore developed an ex vivo haematopoietic stem cell gene therapy approach in a mouse model of mucopolysaccharidosis IIIB, using a high-titre lentiviral vector and the myeloid-specific CD11b promoter, driving the expression of NAGLU (LV.NAGLU). To understand the mechanism of

correction we also compared this with a poorly secreted version of NAGLU containing a C-terminal fusion to IGFII (LV.NAGLU-IGFII). Mucopolysaccharidosis IIIB haematopoietic stem cells were transduced with vector, transplanted into myeloablated mucopolysaccharidosis IIIB mice and compared at 8 months of age with mice receiving a wild-type transplant. As the disease is characterized by increased inflammation, we also tested the anti-inflammatory steroidal agent prednisolone alone, or in combination with LV.NAGLU, to understand the importance of inflammation on behaviour. NAGLU enzyme was substantially increased in the brain of LV.NAGLU and LV.NAGLU-IGFII-treated mice, with little expression in wild-type bone marrow transplanted mice. LV.NAGLU treatment led to behavioural correction, normalization of heparan sulphate and sulphation patterning, reduced inflammatory cytokine expression and correction of astrocytosis, microgliosis and lysosomal compartment size throughout the brain. The addition of prednisolone improved inflammatory aspects further. Substantial correction of lysosomal storage in neurons and astrocytes was also achieved in LV.NAGLU-IGFII-treated mice, despite limited enzyme secretion from engrafted macrophages in the brain. Interestingly both wild-type bone marrow transplant and prednisolone treatment alone corrected behaviour, despite having little effect on brain neuropathology. This was attributed to a decrease in peripheral inflammatory cytokines. Here we show significant neurological disease correction is achieved using haematopoietic stem cell gene therapy, suggesting this therapy alone or in combination with anti-inflammatories may improve neurological function in patients.

Holley, R. J., et al. (2019). "Delivering Hematopoietic Stem Cell Gene Therapy Treatments for Neurological Lysosomal Diseases." ACS Chem Neurosci **10**(1): 18-20.

Neurological lysosomal storage diseases are rare, inherited conditions resulting mainly from lysosomal enzyme deficiencies. Current treatments, such as enzyme replacement therapy and hematopoietic stem cell transplantation, fail to effectively treat neurological disease due to insufficient brain delivery of the missing enzyme. Ex vivo gene therapy approaches to overexpress the missing enzyme in hematopoietic stem cells prior to transplant are an emerging technology that has the potential to offer a viable therapy for patients with these debilitating diseases.

Horgan, C., et al. (2022). "Current and Future Treatment of Mucopolysaccharidosis (MPS) Type II: Is Brain-Targeted Stem Cell Gene Therapy the Solution for This Devastating Disorder?" Int J Mol Sci **23**(9).

Mucopolysaccharidosis type II (Hunter Syndrome) is a rare, x-linked recessive, progressive, multi-system, lysosomal storage disease caused by the deficiency of iduronate-2-sulfatase (IDS), which leads to the pathological storage of glycosaminoglycans in nearly all cell types, tissues and organs. The condition is clinically heterogeneous, and most patients present with a progressive, multi-system disease in their early years. This article outlines the pathology of the disorder and current treatment strategies, including a detailed review of haematopoietic stem cell transplant outcomes for MPSII. We then discuss haematopoietic stem cell gene therapy and how this can be employed for treatment of the disorder. We consider how preclinical innovations, including novel brain-targeted techniques, can be incorporated into stem cell gene therapy approaches to mitigate the neuropathological consequences of the condition.

Horiuchi, Y., et al. (2009). "Kinetics and effect of integrin expression on human CD34(+) cells during murine leukemia virus-derived retroviral transduction with recombinant fibronectin for stem cell gene therapy." Hum Gene Ther **20**(7): 777-783.

The CH-296 recombinant fragment of human fibronectin is essential for murine leukemia virus (MLV)-derived retroviral transduction of CD34(+) cells for the purpose of stem cell gene therapy. Although the major effect of CH-296 is colocalization of the MLV-derived retrovirus and target cells at specific adhesion domains of CH-296 mediated by integrins expressed on CD34(+) cells, the precise roles of the integrins are unclear. We examined the kinetics of integrin expression on CD34(+) cells during the course of MLV-derived retrovirus-mediated gene transduction with CH-296. Flow cytometry revealed that the levels of both very late activation protein (VLA)-4 and VLA-5 on CD34(+) cells freshly isolated from cord blood were insufficient for effective MLV-derived retroviral transduction. However, increases were achieved during culture for preinduction and MLV-derived retrovirus-mediated gene transduction in the presence of a cocktail of cytokines. In addition, we confirmed by using specific antibodies that inhibition of the cell adhesion mediated by the integrins significantly reduced transduction efficiency, indicating that integrin expression is indeed important for CH-296-based MLV-derived retroviral transduction. Only a few cytokines are capable of inducing integrin expression, and stem cell factor plus thrombopoietin was found to be the minimal combination that was sufficient for effective transduction of an MLV-derived retrovirus based on CH-296. Our findings should be useful for improving the culture conditions for CH-

296-based MLV-derived retroviral transduction in stem cell gene therapy.

Hosseini, S. A., et al. (2018). "Stem cell- and gene-based therapies as potential candidates in Alzheimer's therapy." J Cell Biochem **119**(11): 8723-8736.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which is associated with impairments of memory, thinking, language, and reasoning. Despite extensive research aiming at the treatment of AD, durable and complete remissions are rare. Hence, new therapeutic approaches are required. Among various therapeutic approaches, stem cells (ie, neural stem cells, mesenchymal stem cells, and embryonic stem cells) and delivery of protective genes such as encoding nerve growth factor, APOE, and glial cell-derived neurotrophic factor have generated promise in AD therapy. Here, we summarized a variety of effective therapeutic approaches (ie, stem cells, and genes) in AD therapy.

Hsiao, F. S., et al. (2011). "Toward an ideal animal model to trace donor cell fates after stem cell therapy: production of stably labeled multipotent mesenchymal stem cells from bone marrow of transgenic pigs harboring enhanced green fluorescence protein gene." J Anim Sci **89**(11): 3460-3472.

The discovery of postnatal mesenchymal stem cells (MSC) with their general multipotentiality has fueled much interest in the development of cell-based therapies. Proper identification of transplanted MSC is crucial for evaluating donor cell distribution, differentiation, and migration. Lack of an efficient marker of transplanted MSC has precluded our understanding of MSC-related regenerative studies, especially in large animal models such as pigs. In the present study, we produced transgenic pigs harboring an enhanced green fluorescent protein (EGFP) gene. The pigs provide a reliable and reproducible source for obtaining stable EGFP-labeled MSC, which is very useful for donor cell tracking after transplantation. The undifferentiated EGFP-tagged MSC expressed a greater quantity of EGFP while maintaining MSC multipotentiality. These cells exhibited homogeneous surface epitopes and possessed classic trilineage differentiation potential into osteogenic, adipogenic, and chondrogenic lineages, with robust EGFP expression maintained in all differentiated progeny. Injection of donor MSC can dramatically increase the thickness of infarcted myocardium and improve cardiac function in mice. Moreover, the MSC, with their strong EGFP expression, can be easily distinguished from the background autofluorescence in myocardial infarcts. We demonstrated an efficient, effective, and easy way to identify MSC after long-term culture and transplantation. With the transgenic model, we were able to obtain stem or progenitor cells in earlier passages compared with the transfection of traceable markers into established MSC. Because the integration site of the transgene was the same for all cells, we lessened the potential for positional effects and the heterogeneity of the stem cells. The EGFP-transgenic pigs may serve as useful biomedical and agricultural models of somatic stem cell biology.

Hu, J., et al. (2019). "Coreceptor-Based Hematopoietic Stem Cell Gene Therapy for HIV Disease." Curr Stem Cell Res Ther **14**(7): 591-597.

Combination antiretroviral therapy (cART) has significantly reduced the mortality rate and morbidity, and has increased the life expectancy of the human immunodeficiency virus (HIV) infected patients. However, the current cART is incapable of eradicating viruses from the human body, and HIV remains one of the most notorious viruses mankind has ever faced. HIV-1 enters target cells through the binding of gp120 viral protein to a CD4 receptor and then to a coreceptor, C-C chemokine receptor 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4). Individuals homozygous for a 32-bp deletion in the CCR5 allele, CCR5Delta32, are almost completely resistant to HIV-1 acquisition. Moreover, several of natural CXCR4 mutants which have been identified can reduce HIV-1 entry without impairing either ligand binding or signaling. In order to get rid of indefinite treatment for HIV patients, there is a growing interest in creating an HIV-resistant immune system through the use of CCR5 and CXCR4-modified hematopoietic stem cells (HSCs). Proof of concept for this approach has been provided in the instance of "Berlin patient" transplanted with allogeneic stem cells from a donor with homozygosity for the CCR5Delta32 deletion. Here, we review the progress of coreceptor-based HSC gene therapy for HIV disease and present new strategies.

Hu, P., et al. (2016). "Hematopoietic Stem cell transplantation and lentiviral vector-based gene therapy for Krabbe's disease: Present convictions and future prospects." J Neurosci Res **94**(11): 1152-1168.

Currently, presymtomatic hematopoietic stem and progenitor cell transplantation (HSPCT) is the only therapeutic modality that alleviates Krabbe's disease (KD)-induced central nervous system damage. However, all HSPCT-treated patients exhibit severe deterioration in peripheral nervous system function characterized by major motor and expressive language pathologies. We hypothesize that a combination of several mechanisms contribute to this phenomenon, including 1) nonoptimal conditioning protocols with consequent inefficient engraftment and biodistribution of donor-derived cells and 2) insufficient uptake of donor cell-secreted galactocerebrosidease (GALC)

secondary to a naturally low expression level of the cation-independent mannose 6-phosphate-receptor (CI-MPR). We have characterized the effects of a busulfan (Bu) based conditioning regimen on the efficacy of HSPCT in prolonging twi mouse average life span. There was no correlation between the efficiency of bone marrow engraftment of donor cells and twi mouse average life span. HSPCT prolonged the average life span of twi mice, which directly correlated with the aggressiveness of the Bu-mediated conditioning protocols. HSPC transduced with lentiviral vectors carrying the GALC cDNA under control of cell-specific promoters were efficiently engrafted in twi mouse bone marrow. To facilitate HSPCT-mediated correction of GALC deficiency in target cells expressing low levels of CI-MPR, a novel GALC fusion protein including the ApoE1 receptor was developed. Efficient cellular uptake of the novel fusion protein was mediated by a mannose-6-phosphate-independent mechanism. The novel findings described here elucidate some of the cellular mechanisms that impede the cure of KD patients by HSPCT and concomitantly open new directions to enhance the therapeutic efficacy of HSPCT protocols for KD. (c) 2016 The Authors. Journal of Neuroscience Research Published by Wiley Periodicals, Inc.

Hu, W., et al. (2011). "Human umbilical blood mononuclear cell-derived mesenchymal stem cells serve as interleukin-21 gene delivery vehicles for epithelial ovarian cancer therapy in nude mice." Biotechnol Appl Biochem **58**(6): 397-404.

Ovarian cancer causes more deaths than any other cancer of the female reproductive system, and its overall cure rate remains low. The present study investigated human umbilical blood mononuclear cell (UBMC)-derived mesenchymal stem cells (UBMC-MSCs) as interleukin-21 (IL-21) gene delivery vehicles for ovarian cancer therapy in nude mice. MSCs were isolated from UBMCs and the expanded cells were phenotyped by flow cytometry. Cultured UBMCs were differentiated into osteocytes and adipocytes using appropriate media and then the UBMC-MSCs were transfected with recombinant pIRES2-IL-21-enhancement green fluorescent protein. UBMC-MSCs expressing IL-21 were named as UBMC-MSC-IL-21. Mice with A2780 ovarian cancer were treated with UBMC-MSC-IL-21 intravenously, and the therapeutic efficacy was evaluated by the tumor volume and mouse survival. To address the mechanism of UBMC-MSC-IL-21 against ovarian cancer, the expression of IL-21, natural killer glucoprotein 2 domain and major histocompatibility complex class I chain-related molecules A/B were detected in UBMC-MSC-IL-21 and in the tumor sites. Interferon-gamma-secreting splenocyte numbers and natural killer cytotoxicity were significantly increased in the UBMC-MSC-IL-21-treated mice as compared with the UBMC-MSCs or the UBMC-MSC-mock plasmid-treated mice. Most notably, tumor growth was delayed and survival was prolonged in ovarian-cancer-bearing mice treated with UBMC-MSC-IL-21. Our data provide important evidence that UBMC-MSCs can serve as vehicles for IL-21 gene delivery and inhibit the established tumor.

Huang, M., et al. (2011). "Double knockdown of prolyl hydroxylase and factor-inhibiting hypoxia-inducible factor with nonviral minicircle gene therapy enhances stem cell mobilization and angiogenesis after myocardial infarction." Circulation **124**(11 Suppl): S46-54.

BACKGROUND: Under normoxic conditions, hypoxia-inducible factor (HIF)-1alpha is rapidly degraded by 2 hydroxylases: prolyl hydroxylase (PHD) and factor-inhibiting HIF-1 (FIH). Because HIF-1alpha mediates the cardioprotective response to ischemic injury, its upregulation may be an effective therapeutic option for ischemic heart failure. METHODS AND RESULTS: PHD and FIH were cloned from mouse embryonic stem cells. The best candidate short hairpin (sh) sequences for inhibiting PHD isoenzyme 2 and FIH were inserted into novel, nonviral, minicircle vectors. In vitro studies after cell transfection of mouse C2C12 myoblasts, HL-1 atrial myocytes, and c-kit(+) cardiac progenitor cells demonstrated higher expression of angiogenesis factors in the double-knockdown group compared with the single-knockdown and short hairpin scramble control groups. To confirm in vitro data, shRNA minicircle vectors were injected intramyocardially after left anterior descending coronary artery ligation in adult FVB mice (n=60). Functional studies using MRI, echocardiography, and pressure-volume loops showed greater improvement in cardiac function in the double-knockdown group. To assess mechanisms of this functional recovery, we performed a cell trafficking experiment, which demonstrated significantly greater recruitment of bone marrow cells to the ischemic myocardium in the double-knockdown group. Fluorescence-activated cell sorting showed significantly higher activation of endogenous c-kit(+) cardiac progenitor cells. Immunostaining showed increased neovascularization and decreased apoptosis in areas of injured myocardium. Finally, western blots and laser-capture microdissection analysis confirmed upregulation of HIF-1alpha protein and angiogenesis genes, respectively. CONCLUSIONS: We demonstrated that HIF-1alpha upregulation by double knockdown of PHD and FIH synergistically increases stem cell mobilization and myocardial angiogenesis, leading to improved cardiac function.

Hunter, M. J., et al. (2011). "Gene therapy for canine leukocyte adhesion deficiency with lentiviral vectors using the murine stem cell virus and human phosphoglycerate kinase promoters." Hum Gene Ther **22**(6): 689-696.

Children with leukocyte adhesion deficiency type 1 (LAD-1) and dogs with canine LAD (CLAD) develop life-threatening bacterial infections due to mutations in the leukocyte integrin CD18. Here, we compared the human phosphoglycerate kinase (hPGK) promoter to the murine stem cell virus (MSCV) promoter/enhancer in a self-inactivating HIV-1-derived lentiviral vector to treat animals with CLAD. Four CLAD dogs were infused with CD34(+) cells transduced with the hPGK vector, and two CLAD dogs received MSCV vector-transduced CD34(+) cells. Infusions were preceded by a nonmyeloablative dose of 200 cGy total body irradiation. Comparable numbers of transduced cells were infused in each group of animals. Only one of four CLAD animals treated with the hPGK-cCD18 vector had reversal of CLAD, whereas both MSCV-cCD18 vector-treated dogs had reversal of the phenotype. Correction of CLAD depends both upon the percentage of CD18(+) myeloid cells and the level of expression of CD18 on individual myeloid cells. In this regard, the hPGK promoter directed low levels of expression of CD18 on neutrophils compared to the MSCV promoter, likely contributing to the suboptimal clinical outcome with the hPGK vector.

Ide, L. M., et al. (2007). "Hematopoietic stem-cell gene therapy of hemophilia A incorporating a porcine factor VIII transgene and nonmyeloablative conditioning regimens." Blood **110**(8): 2855-2863.

Insufficient expression of factor VIII (fVIII) is a major hurdle in the development of successful nucleic acid treatments for hemophilia. However, we recently showed that under myeloablative and reduced-intensity total body irradiation (TBI) conditioning, transplantation of hematopoietic stem cells (HSCs) transduced with recombinant retroviruses containing B domain-deleted porcine fVIII (BDDpfVIII) sequences provides curative fVIII levels in a hemophilia A mouse model. In the current study, we tested BDDpfVIII activity after nonmyeloablative conditioning with busulfan, cyclophosphamide, or fludarabine and immunosuppressive agents CTLA4-Ig + anti-CD40L or anti-(murine)thymocyte serum (ATS). ATS is similar in action to anti-(human)thymocyte globulin (ATG), which is used clinically with busulfan in bone marrow transplantations to increase donor cell engraftment. Mice conditioned with busulfan + ATS and that received a transplant of BDDpfVIII-transduced stem-cell antigen 1-positive cells exhibited moderate levels of donor cell chimerism (between 20% and 60%) and achieved sustained fVIII levels more than 1 U/mL. Similar results were observed in mice preimmunized with human fVIII and conditioned with 5 Gy TBI + ATS or busulfan + ATS. These data demonstrate that it is possible to achieve sufficient fVIII expression after transplantation of BDDpfVIII-transduced HSCs following low-toxicity pretransplantation conditioning with targeted immunosuppression, potentially even in the context of preexisting inhibitors.

Igarashi, Y., et al. (2017). "Single Cell-Based Vector Tracing in Patients with ADA-SCID Treated with Stem Cell Gene Therapy." Mol Ther Methods Clin Dev **6**: 8-16.

Clinical improvement in stem cell gene therapy (SCGT) for primary immunodeficiencies depends on the engraftment levels of genetically corrected cells, and tracing the transgene in each hematopoietic lineage is therefore extremely important in evaluating the efficacy of SCGT. We established a single cell-based droplet digital PCR (sc-ddPCR) method consisting of the encapsulation of a single cell into each droplet, followed by emulsion PCR with primers and probes specific for the transgene. A fluorescent signal in a droplet indicates the presence of a single cell carrying the target gene in its genome, and this system can clearly determine the ratio of transgene-positive cells in the entire population at the genomic level. Using sc-ddPCR, we analyzed the engraftment of vector-transduced cells in two patients with severe combined immunodeficiency (SCID) who were treated with SCGT. Sufficient engraftment of the transduced cells was limited to the T cell lineage in peripheral blood (PB), and a small percentage of CD34(+) cells exhibited vector integration in bone marrow, indicating that the transgene-positive cells in PB might have differentiated from a small population of stem cells or lineage-restricted precursor cells. sc-ddPCR is a simplified and powerful tool for the detailed assessment of transgene-positive cell distribution in patients treated with SCGT.

Isgro, A., et al. (2010). "Progress in hematopoietic stem cell transplantation as allogeneic cellular gene therapy in thalassemia." Ann N Y Acad Sci **1202**: 149-154.

Allogeneic hemopoietic stem cell transplantation (HSCT) represents one of the best cures for thalassemia. Currently, HSCT for thalassemia consists of allogeneic stem cell gene therapy and still awaits autologous genetically modified stem cell transplantation. HSCT for thalassemia has substantially improved over the last two decades, due in large part to improvements in preventive strategies, the effective control of transplant-related complications, and the development of new preparative regimens. A risk classes-based approach to transplantation in thalassemia has led to disease-free survival probability

of 87, 85, and 80% in classes 1, 2, and 3 patients, respectively. Adult thalassemia patients, who are higher risk patients for transplant-related toxicity due to an advanced phase of the disease, have a cure rate of 65% with current treatment protocol. Patients who do not have matched family or unrelated donors could benefit from haploidentical mother-to-child transplantation. Overall, the results of this type of transplantation appear encouraging.

Ishii, M., et al. (2009). "Mesenchymal stem cell-based gene therapy with prostacyclin synthase enhanced neovascularization in hindlimb ischemia." Atherosclerosis **206**(1): 109-118.

OBJECTIVE: Bone marrow cell therapy contributes to collateral formation through the secretion of angiogenic factors by progenitor cells and muscle cells per se, thereby presenting a novel option for patients with critical limb ischemia. However, some cases are refractory to this therapy due to graft failure. Therefore, we used genetic modification of mesenchymal stem cells (MSCs) to overexpress a vasoregulatory protein, prostacyclin (PGI(2)), to examine whether it could enhance engraftment and neovascularization in hindlimb ischemia. METHODS AND RESULTS: We engineered the overexpression of PGI(2) synthase (PGIS) within MSCs, which resulted in higher expression levels of phosphorylated Akt and Bcl-2 than in control. Under hypoxic conditions, the overexpression of PGIS led to upregulated expression of cyclooxigenase-2 and peroxisome proliferator-activated receptor delta, following a 40% increased rate of proliferation in MSCs. We then produced unilateral hindlimb ischemia in C57BL6/J mice, which were injected either with MSCs transfected with GFP, with MSCs overexpressing PGIS, or with vehicle. Laser Doppler analyses demonstrated that the administration of MSCs effectively recovered blood perfusion, and that the peak blood flow was reached within 7 days of surgery in mice with MSCs overexpressing PGIS, which was earlier than that in mice with MSCs transfected with GFP. This beneficial effect was correlated to enhanced collateral formation and muscle bundle proliferation. CONCLUSION: Sustained release of PGI(2) enhanced the proangiogenic function of MSCs and subsequent muscle cell regrowth in the ischemic tissue suggesting potential therapeutic benefits of cell-based gene therapy for critical limb ischemia.

Iwai, M., et al. (2001). "Gene therapy with adenovirus-mediated glial cell line-derived neurotrophic factor and neural stem cells activation after ischemic brain injury." Hum Cell **14**(1): 27-38.

Recent advancements in molecular biology are made to expect the appearance of the new treatment of stroke patients. One is the administration of neurotrophic factors, and another is the use of neural stem cell. In this report, we performed two experiments. First experiment is administration of glial cell line-derived neurotrophic factor (GDNF) using an adenovirus vector into ischemic rat brain. A replication-defective adenoviral vector containing GDNF gene (Ad-GDNF) was directly injected into the cerebral cortex at 1 day before 90 min of transient middle cerebral artery occlusion (MCAO) in rats. Infarct volume of the Ad-GDNF injected group at 24 h after the transient MCAO was significantly smaller than that of vehicle or Ad-LacZ treated group. These results suggest that the successful exogenous GDNF gene transfer ameliorates the ischemic brain injury after transient MCAO in association with the reduction of apoptotic signals. Second one is the neural stem cell activation after transient ischemia. We investigated a possible expression of highly polysialylated neural cell adhesion molecule (PSA-NCAM) in gerbil hippocampus after 5 min of transient global ischemia in association to the proliferation of neural stem cell labeled with bromodeoxyuridine (BrdU). The number of PSA-NCAM positive cells increased in dentate gyrus (DG) at 10 and 20 days, and that of BrdU-labeled cells increased in DG at 5 and 10 days after the reperfusion. Immunofluorescence for PSA-NCAM and BrdU showed that a few cells per section were double labeled in DG only at 10 days after the reperfusion. These results suggest different chronological change of PSA-NCAM positive and BrdU-labeled cells in DG after transient ischemia.

Iwamoto, H., et al. (2013). "[Cancer vaccine therapy using genetically modified induced pluripotent stem cell-derived dendritic cells expressing the TAA gene]." Gan To Kagaku Ryoho **40**(12): 1575-1577.

It is generally accepted that the difficulty in obtaining a sufficient number of functional dendritic cells (DCs) poses a serious problem in DC-based immunotherapy. Therefore, we used induced pluripotent stem (iPS) cell-derived DCs (iPSDCs) instead. If the therapeutic efficacy of iPSDCs was equivalent to that of bone marrow-derived DCs(BMDCs), then the above-mentioned problems may be solved. In this study, we generated iPSDCs from iPS cells and compared their capacity to mature and migrate to the regional lymph nodes with that of BMDCs. We adenovirally transduced the hgp100 gene, which codes for a natural tumor antigen, into the DCs and immunized the mice with these genetically modified DCs. The cytotoxic activity of CD8(+) cytotoxic T lymphocytes(CTLs) was assayed using a 51Cr-release assay. The therapeutic efficacy of the vaccination was examined in a subcutaneous tumor model. Our results demonstrated that iPSDCs equaled

BMDCs in terms of their maturation and migration capacity. Furthermore, hgp100-specific CTLs were generated in mice that were immunized with the genetically modified iPSDCs. These CTLs exhibited a high level of cytotoxicity against B16 cells, which is similar to that exhibited by CTLs generated in BMDCs immunized mice. Moreover, vaccination with genetically modified iPSDCs elicited a high level of therapeutic efficacy equaling that of vaccination with BMDCs. This study clarified experimentally that genetically modified iPSDCs are equivalent to BMDCs in terms of tumor-associated antigen-specific therapeutic antitumor immunity. This vaccination strategy may therefore be useful for future clinical application as a cancer vaccine.

Kenmochi, H., et al. (2020). "Nicotine does not affect stem cell properties requisite for suicide gene therapy against glioma." Neurol Res **42**(10): 818-827.

Glioblastoma is one of the most lethal tumors in adult central nervous system with a median survival of a year and half and effective therapeutic strategy is urgently needed. For that reason, stem cell-based suicide gene therapies have attracted much interest because of potent tumor tropism of stem cells and bystander effect. In this current clinical situation, stem cells are promising delivery tool of suicide genes for glioma therapy. Since habitual cigarette smoking still prevails worldwide, we investigated the effect of nicotine on stem cell tropism toward glioma and gap junctional intercellular communication (GJIC) function between glioma and stem cells, both of which are important for suicide gene therapies. Methods: Mouse induced pluripotent stem cell-derived neural stem cells (iPS-NSCs) and human dental pulp mesenchymal stem cells (DPSCs) were used. The effect of nicotine on tumor tropism to glioma-conditioned medium (CM) at a non-cytotoxic concentration was assessed with Matrigel invasion assay. Nicotine effect on GJIC was assessed with the scrape loading/dye transfer (SL/DT) assay for co-culture of glioma and stem cells and the parachute assay among glioma cells using high-content analysis. Results: Tumor tropism of iPS-NSCs toward GL261-CM and DPSCs toward U251-CM was not affected by nicotine (0.1 and 1 microM). Nicotine at the concentrations equivalent to habitual smoking (1 microM) did not affect GJIC of iPS-NSC/GL261 and DPSC/U251 and GJIC among each glioma cells. Conclusions: The study demonstrated that non-cytotoxic concentrations of nicotine did not significantly change the stem cell properties requisite for stem cell-based suicide gene therapy.

Kermani, A. J., et al. (2008). "Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy." Rejuvenation Res **11**(2): 379-386.

Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. Despite the recent isolation of human umbilical cord vein mesenchymal stem cells (UVMSC), the self-renewal capacity and the potential clinical application of the cells are not well known. In the present study, we have successfully isolated and cultured human UVMSCs. Our data further revealed that the isolated cells express the self-renewal genes Oct-4, Nanog, ZFX, Bmi-1, and Nucleostemin; but not Zic-3, Hoxb-4, TCL-1, Tbx-3 and Esrrb. In addition, our immunocytochemistry results revealed the expression of SSEA-4, but not SSEA-3, TRA-1-60, and TRA-1-81 embryonic stem cell surface markers in the cells. Also, we were able to transfect the cells with a reporter, enhanced green fluorescent protein (EGFP), and a therapeutic human brain-derived neurotrophic factor (hBDNF) gene by means of electroporation and obtained a stable cell line, which could constantly express both transgenes. The latter data provide further evidence on the usefulness of umbilical cord vein mesenchymal stem cells as a readily available source of stem cells, which could be genetically manipulated and used in cell-based gene therapy applications.

Kesser, B. W. and A. K. Lalwani (2009). "Gene therapy and stem cell transplantation: strategies for hearing restoration." Adv Otorhinolaryngol **66**: 64-86.

Strategies to restore sensorineural hearing loss focus on the replacement of lost hair cells, the specialized mechanoreceptors in the organ of Corti that convert the mechanical energy of sound into electrical energy. Hair cells in mammalian systems do not have the capacity to regenerate, but two exciting lines of research hold promise in restoring inner ear function. Here we review basic principles of gene therapy and discuss its application in the inner ear. We survey the various viral vectors and routes of delivery into the inner ear. Applications of gene therapy in the inner include hair cell protection in the face of chemical or noise-induced ototoxicity, spiral ganglion cell survival following hair cell death or injury, and hair cell regeneration. More recently, the viability of gene therapy in human inner ear tissue has been reported. Transplantation of progenitor cells that can differentiate into functioning hair cells with the appropriate connections to their corresponding spiral ganglion cells is yet another strategy to restore

sensorineural hearing loss. Neonatal or embryonic stem cells, adult mouse inner ear stem cells, and stem cells from the central nervous system have been shown to differentiate into cells containing hair cell markers and proteins. Prospects for stem cell therapy in the inner ear, and its limitations, will also be examined.

Kiem, H. P., et al. (2012). "Hematopoietic-stem-cell-based gene therapy for HIV disease." Cell Stem Cell **10**(2): 137-147.

Although combination antiretroviral therapy can dramatically reduce the circulating viral load in those infected with HIV, replication-competent virus persists. To eliminate the need for indefinite treatment, there is growing interest in creating a functional HIV-resistant immune system through the use of gene-modified hematopoietic stem cells (HSCs). Proof of concept for this approach has been provided in the instance of an HIV-infected adult transplanted with allogeneic stem cells from a donor lacking the HIV coreceptor, CCR5. Here, we review this and other strategies for HSC-based gene therapy for HIV disease.

Kim, J. H., et al. (2014). "Stem cell based gene therapy in prostate cancer." Biomed Res Int **2014**: 549136.

Current prostate cancer treatment, especially hormone refractory cancer, may create profound iatrogenic outcomes because of the adverse effects of cytotoxic agents. Suicide gene therapy has been investigated for the substitute modality for current chemotherapy because it enables the treatment targeting the cancer cells. However the classic suicide gene therapy has several profound side effects, including immune-compromised due to viral vector. Recently, stem cells have been regarded as a new upgraded cellular vehicle or vector because of its homing effects. Suicide gene therapy using genetically engineered mesenchymal stem cells or neural stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. The attractiveness of prodrug cancer gene therapy by stem cells targeted to tumors lies in activating the prodrug directly within the tumor mass, thus avoiding systemic toxicity. Therapeutic achievements using stem cells in prostate cancer include the cytosine deaminase/5-fluorocytosine prodrug system, herpes simplex virus thymidine kinase/ganciclovir, carboxyl esterase/CPT11, and interferon-beta. The aim of this study is to review the stem cell therapy in prostate cancer including its proven mechanisms and also limitations.

Kim, J. H., et al. (2016). "Mesenchymal stem cell-based gene therapy for erectile dysfunction." Int J Impot Res **28**(3): 81-87.

Despite the overwhelming success of PDE5 inhibitor (PDE5I), the demand for novel pharmacotherapeutic and surgical options for ED continues to rise owing to the increased proportion of elderly individuals in the population, in addition to the growing percentage of ED patients who do not respond to PDE5I. Surgical treatment of ED is associated with many complications, thus warranting the need for nonsurgical therapies. Moreover, none of the above-mentioned treatments essentially corrects, cures or prevents ED. Although gene therapy is a promising option, many challenges and obstacles such as local inflammatory response and random transgene expression, in addition to other safety issues, limit its use at the clinical level. The use of stem cell therapy alone also has many shortcomings. To overcome these inadequacies, many scientists and clinicians are investigating new gene and stem cell therapies.

Kim, S. M., et al. (2014). "Potential application of temozolomide in mesenchymal stem cell-based TRAIL gene therapy against malignant glioma." Stem Cells Transl Med **3**(2): 172-182.

Because the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively kills tumor cells, it is one of the most promising candidates for cancer treatment. TRAIL-secreting human mesenchymal stem cells (MSC-TRAIL) provide targeted and prolonged delivery of TRAIL in glioma therapy. However, acquired resistance to TRAIL of glioma cells is a major problem to be overcome. We showed a potential therapy that used MSC-TRAIL combined with the chemotherapeutic agent temozolomide (TMZ). The antitumor effects of the combination with MSC-TRAIL and TMZ on human glioma cells were determined by using an in vitro coculture system and an in vivo experimental xenografted mouse model. Intracellular signaling events that are responsible for the TMZ-mediated sensitization to TRAIL-induced apoptosis were also evaluated. Treatment of either TRAIL-sensitive or -resistant human glioma cells with TMZ and MSC-TRAIL resulted in a significant enhancement of apoptosis compared with the administration of each agent alone. We demonstrated that TMZ effectively increased the sensitivity to TRAIL-induced apoptosis via extracellular signal-regulated kinase-mediated upregulation of the death receptor 5 and downregulation of antiapoptotic proteins, such as X-linked inhibitor of apoptosis protein and cellular FLICE-inhibitory protein. Subsequently, this combined treatment resulted in a substantial increase in caspase activation. Furthermore, in vivo survival experiments and bioluminescence imaging analyses showed that treatment using MSC-TRAIL combined with TMZ had greater therapeutic efficacy than did single-agent

treatments. These results suggest that the combination of clinically relevant TMZ and MSC-TRAIL is a potential therapeutic strategy for improving the treatment of malignant gliomas.

Kim, S. U. (2011). "Neural stem cell-based gene therapy for brain tumors." Stem Cell Rev Rep **7**(1): 130-140.

Advances in gene-based medicine since 1990s have ushered in new therapeutic strategy of gene therapy for inborn error genetic diseases and cancer. Malignant brain tumors such as glioblastoma multiforme and medulloblastoma remain virtually untreatable and lethal. Currently available treatment for brain tumors including radical surgical resection followed by radiation and chemotherapy, have substantially improved the survival rate in patients suffering from these brain tumors; however, it remains incurable in large proportion of patients. Therefore, there is substantial need for effective, low-toxicity therapies for patients with malignant brain tumors, and gene therapy targeting brain tumors should fulfill this requirement. Gene therapy for brain tumors includes many therapeutic strategies and these strategies can be grouped in two major categories: molecular and immunologic. The widely used molecular gene therapy approach is suicide gene therapy based on the conversion of non-toxic prodrugs into active anticancer agents via introduction of enzymes and genetic immunotherapy involves the gene transfer of immune-stimulating cytokines including IL-4, IL-12 and TRAIL. For both molecular and immune gene therapy, neural stem cells (NSCs) can be used as delivery vehicle of therapeutic genes. NSCs possess an inherent tumor tropism that supports their use as a reliable delivery vehicle to target therapeutic gene products to primary brain tumors and metastatic cancers throughout the brain. Significance of the NSC-based gene therapy for brain tumor is that it is possible to exploit the tumor-tropic property of NSCs to mediate effective, tumor-selective therapy for primary and metastatic cancers in the brain and outside, for which no tolerated curative treatments are currently available.

Kim, S. U. (2014). "Lysosomal storage diseases: Stem cell-based cell- and gene-therapy." Cell Transplant.

Lysosomal storage diseases (LSDs) are caused by inborn genetic defects and most affected babies show pathology in the CNS. LSDs are caused by a specific inherited enzyme deficiency that results in accumulation of substrates in the lysosomes, distension of the organelles and subsequent cellular malfunction. Currently, no effective treatment is available for most of the LSDs, because the blood?brain barrier bars entry of enzyme preparations into the brain. Treatment for LSDs can be divided into those address symptoms or those address cause. At present, successful treatments for the LSDs are enzyme replacement therapy (ERT) and cell therapy. ERT is most successful in Gaucher disease and has been approved for Fabry disease, and mucopolysaccharidosis I (MPS I). In addition, ERT for Pompe disease, MPS II, MPS VI and MPS VII has been planned and awaiting approval for treatment. Limitations in ERT include need for life-long treatment, development of antibodies, and inability to cross blood brain barrier (BBB) resulting in failure to halt disease progression in the brain. Transplantation of hematopoietic stem cells (HSCs), bone marrow stem cells (BMSCs) and umbilical cord blood-derived stem cells (UCBSCs) offer effective but limited efficacy for patients suffering from Krabbe disease, MPS VII and adrenal leukodystrophy but in other LSDs they are ineffective. Intracranial/intracerebral transplantation of genetically modified stem cells as enzyme delivery system could bypass the BBB effectively and ensure release of therapeutically beneficial amount of enzymes to affected CNS lesion sites. For this reason, stem cell-based gene therapy is the most effective treatment for LSDs. In mouse models of LSDs, genetically modified neural stem cells encoding enzyme genes effectively decreased lysosomal storage, reduced pathology and extended life span of animals. Cell-based gene therapies for LSDs bridge the application of ERT and gene therapy and are important direction to pursue in the future.

King, W. D., et al. (2010). "Pilot assessment of HIV gene therapy-hematopoietic stem cell clinical trial acceptability among minority patients and their advisors." J Natl Med Assoc **102**(12): 1123-1128.

Clinical trials involving technologically involved novel treatments such as gene therapy delivered through hematopoietic stem cells as human immunodeficiency virus (HIV) treatment will need to recruit ethnically diverse patients to ensure the acceptance among broad groups of individuals and generalizability of research findings. Five focus groups of 47 HIV-positive men and women, religious and community leaders and health providers, mostly from African American and low-income communities, were conducted to examine knowledge about gene therapy and stem cell research and to assess the moral and ethical beliefs that might influence participation in clinical trials. Three themes emerged from these groups: (1) the need for clarification of terminology and the ethics of understanding gene therapy-stem cell research, (2) strategies to avoid mistrust of medical procedures and provider mistrust, and (3) the conflict between science and religious beliefs as it pertains to gene therapy-stem cell research.

Kitchen, S. G., et al. (2011). "Stem cell-based anti-HIV gene therapy." Virology **411**(2): 260-272.

Human stem cell-based therapeutic intervention strategies for treating HIV infection have recently undergone a renaissance as a major focus of investigation. Unlike most conventional antiviral therapies, genetically engineered hematopoietic stem cells possess the capacity for prolonged self-renewal that would continuously produce protected immune cells to fight against HIV. A successful strategy therefore has the potential to stably control and ultimately eradicate HIV from patients by a single or minimal treatment. Recent progress in the development of new technologies and clinical trials sets the stage for the current generation of gene therapy approaches to combat HIV infection. In this review, we will discuss two major approaches that are currently underway in the development of stem cell-based gene therapy to target HIV: one that focuses on the protection of cells from productive infection with HIV, and the other that focuses on targeting immune cells to directly combat HIV infection.

Kitzberger, C., et al. (2023). "Mesenchymal Stem Cell-mediated Image-guided Sodium Iodide Symporter (NIS) Gene Therapy Improves Survival of Glioblastoma-bearing Mice." Clin Cancer Res **29**(5): 930-942.

PURPOSE: Mesenchymal stem cells (MSC) have emerged as cellular-based vehicles for the delivery of therapeutic genes in cancer therapy based on their inherent tumor-homing capability. As theranostic gene, the sodium iodide symporter (NIS) represents a successful target for noninvasive radionuclide-based imaging and therapy. In this study, we applied genetically engineered MSCs for tumor-targeted NIS gene transfer in experimental glioblastoma (GBM)-a tumor with an extremely poor prognosis. EXPERIMENTAL DESIGN: A syngeneic, immunocompetent GL261 GBM mouse model was established by subcutaneous and orthotopic implantation. Furthermore, a subcutaneous xenograft U87 model was used. Bone marrow-derived MSCs were stably transfected with a NIS-expressing plasmid driven by the constitutively active cytomegalovirus promoter (NIS-MSC). After multiple or single intravenous injection of NIS-MSCs, tumoral iodide uptake was monitored in vivo using 123I-scintigraphy or 124I-PET. Following validation of functional NIS expression, a therapy trial with 131I was performed on the basis of the most optimal application regime as seen by 124I-PET imaging in the orthotopic approach. RESULTS: A robust tumoral NIS-specific radionuclide accumulation was observed after NIS-MSC and radioiodide application by NIS-mediated in vivo imaging. NIS immunofluorescence staining of GBM and non-target tissues showed tumor-selective MSC homing along with NIS expression. Application of therapeutically effective 131I led to significantly delayed tumor growth and prolonged median survival after NIS-MSC treatment as compared with controls. CONCLUSIONS: A strong tumor-selective recruitment of systemically applied MSCs into GBM was found using NIS as reporter gene followed by successful therapeutic application of radioiodide demonstrating the potential use of NIS-based MSCs as therapy vehicles as a new GBM therapy approach.

Klein, C., et al. (2003). "Gene therapy for Wiskott-Aldrich syndrome: rescue of T-cell signaling and amelioration of colitis upon transplantation of retrovirally transduced hematopoietic stem cells in mice." Blood **101**(6): 2159-2166.

The Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency that is caused by mutations in the recently identified WASP gene. WASP plays an important role in T-cell receptor-mediated signaling to the actin cytoskeleton. In these studies we assessed the feasibility of using retroviral gene transfer into WASP-deficient hematopoietic stem cells (HSCs) to rescue the T-cell signaling defect that is characteristic of WAS. Upon transplantation of WASP-deficient (WKO) HSCs that have been transduced with WASP-expressing retroviruses, mature B and T cells developed in normal numbers. Most importantly, the defect in antigen receptor-induced proliferation was significantly improved in T cells. Moreover, the susceptibility of colitis by WKO HSCs was prevented or ameliorated in recipient bone marrow chimeras by retrovirus-mediated expression of WASP. A partial reversal of the T-cell signaling defect could also be achieved following transplantation of WASP-deficient HSCs expressing the WASP-homologous protein N-WASP. Furthermore, we have documented a selective advantage of WT over WKO cells in lymphoid tissue using competitive repopulation experiments and Southern blot analysis. Our results provide proof of principle that the WAS-associated T-cell signaling defects can be improved upon transplantation of retrovirally transduced HSCs without overt toxicity and may encourage clinical gene therapy trials.

Klein, O. R., et al. (2023). "Transplant for non-malignant disorders: an International Society for Cell & Gene Therapy Stem Cell Engineering Committee report on the role of alternative donors, stem cell sources and graft engineering." Cytotherapy.

Hematopoietic stem cell transplantation (HSCT) is curative for many non-malignant disorders. As HSCT and supportive care technologies improve, this life-saving treatment may be offered to more and more patients. With the development of new preparative regimens, expanded alternative donor

availability, and graft manipulation techniques, there are many options when choosing the best regimen for patients. Herein the authors review transplant considerations, transplant goals, conditioning regimens, donor choice, and graft manipulation strategies for patients with non-malignant disorders undergoing HSCT.

Knoop, K., et al. (2011). "Image-guided, tumor stroma-targeted 131I therapy of hepatocellular cancer after systemic mesenchymal stem cell-mediated NIS gene delivery." Mol Ther **19**(9): 1704-1713.

Due to its dual role as reporter and therapy gene, the sodium iodide symporter (NIS) allows noninvasive imaging of functional NIS expression by (123)I-scintigraphy or (124)I-PET imaging before the application of a therapeutic dose of (131)I. NIS expression provides a novel mechanism for the evaluation of mesenchymal stem cells (MSCs) as gene delivery vehicles for tumor therapy. In the current study, we stably transfected bone marrow-derived CD34(-) MSCs with NIS cDNA (NIS-MSC), which revealed high levels of functional NIS protein expression. In mixed populations of NIS-MSCs and hepatocellular cancer (HCC) cells, clonogenic assays showed a 55% reduction of HCC cell survival after (131)I application. We then investigated body distribution of NIS-MSCs by (123)I-scintigraphy and (124)I-PET imaging following intravenous (i.v.) injection of NIS-MSCs in a HCC xenograft mouse model demonstrating active MSC recruitment into the tumor stroma which was confirmed by immunohistochemistry and ex vivo gamma-counter analysis. Three cycles of systemic MSC-mediated NIS gene delivery followed by (131)I application resulted in a significant delay in tumor growth. Our results demonstrate tumor-specific accumulation and therapeutic efficacy of radioiodine after MSC-mediated NIS gene delivery in HCC tumors, opening the prospect of NIS-mediated radionuclide therapy of metastatic cancer using MSCs as gene delivery vehicles.

Knoop, K., et al. (2015). "Mesenchymal stem cell-mediated, tumor stroma-targeted radioiodine therapy of metastatic colon cancer using the sodium iodide symporter as theranostic gene." J Nucl Med **56**(4): 600-606.

The tumor-homing property of mesenchymal stem cells (MSCs) allows targeted delivery of therapeutic genes into the tumor microenvironment. The application of sodium iodide symporter (NIS) as a theranostic gene allows noninvasive imaging of MSC biodistribution and transgene expression before therapeutic radioiodine application. We have previously shown that linking therapeutic transgene expression to induction of the chemokine CCL5/RANTES allows a more focused expression within primary tumors, as the adoptively transferred MSC develop carcinoma-associated fibroblast-like characteristics. Although RANTES/CCL5-NIS targeting has shown efficacy in the treatment of primary tumors, it was not clear if it would also be effective in controlling the growth of metastatic disease. METHODS: To expand the potential range of tumor targets, we investigated the biodistribution and tumor recruitment of MSCs transfected with NIS under control of the RANTES/CCL5 promoter (RANTES-NIS-MSC) in a colon cancer liver metastasis mouse model established by intrasplenic injection of the human colon cancer cell line LS174t. RANTES-NIS-MSCs were injected intravenously, followed by (123)I scintigraphy, (124)I PET imaging, and (131)I therapy. RESULTS: Results show robust MSC recruitment with RANTES/CCL5-promoter activation within the stroma of liver metastases as evidenced by tumor-selective iodide accumulation, immunohistochemistry, and real-time polymerase chain reaction. Therapeutic application of (131)I in RANTES-NIS-MSC-treated mice resulted in a significant delay in tumor growth and improved overall survival. CONCLUSION: This novel gene therapy approach opens the prospect of NIS-mediated radionuclide therapy of metastatic cancer after MSC-mediated gene delivery.

Kohlscheen, S., et al. (2017). "Promises and Challenges in Hematopoietic Stem Cell Gene Therapy." Hum Gene Ther **28**(10): 782-799.

Hematopoietic stem cell-directed gene therapy (HSC-GT) provides an innovative treatment option for hematological disorders. Gene therapy promises to cure the disease "at the root" and is therefore exceptional in its potential, but also formidable in its challenges, as long-term side effects are hard to predict and clinical experience remains limited. Many excellent reviews on the topic by designated experts in the field of HSC-GT have come forth, elucidating the successes and pitfalls in the various clinical studies. This review attempts to discuss what we understand from those studies to represent current state of the art with respect to vectors, stem cell transduction, and pretransplant preparatory regimes, what limitations may remain, and which types of diseases may be more suited for HSC-GT than others (targets). We thus discuss the available vector platforms (tools) and preclinical/clinical and basic research (tricks) that contribute to our current understanding of HSC-GT, as well as some overarching principles we can conclude from these. The field has also learned from previous shortcomings, although some of the major concerns of the past, specifically insertional mutagenesis, may not be of relevance for future trials. This very positive

development in HSC-GT, however, has to compete with the improvements in hematopoietic stem cell transplantation or enzyme-replacement therapy, leaving a narrow margin for gene therapy.

Kohn, D. B. (2017). "Historical Perspective on the Current Renaissance for Hematopoietic Stem Cell Gene Therapy." Hematol Oncol Clin North Am **31**(5): 721-735.

Gene therapy using hematopoietic stem cells (HSC) has developed over the past 3 decades, with progressive improvements in the efficacy and safety. Autologous transplantation of HSC modified with murine gammaretroviral vectors first showed clinical benefits for patients with several primary immune deficiencies, but some of these patients suffered complications from vector-related genotoxicity. Lentiviral vectors have been used recently for gene addition to HSC and have yielded clinical benefits for primary immune deficiencies, metabolic diseases, and hemoglobinopathies, without vector-related complications. Gene editing using site-specific endonucleases is emerging as a promising technology for gene therapy and is moving into clinical trials.

Koike, K. and Y. Utsunomiya (2006). "[Gene therapy for kidney diseases: Inflamed site-specific transgenesis using a stem cell]." Nihon Rinsho **64 Suppl 2**: 667-671.

Koller, U. (2020). "[Ex vivo stem cell gene therapy of the skin : Ready for clinical use?]." Hautarzt **71**(2): 85-90.

BACKGROUND: Use of ex vivo stem cell gene therapy enables the correction of the genetic cause of a monogenetic skin disease. OBJECTIVES: The procedure and choice of gene therapy method in the course of ex vivo gene therapy of the skin are presented. MATERIALS AND METHODS: Current gene therapeutic applications focus on the addition or targeted correction of the respective gene within the genome. RESULTS: So far, gene replacement therapy has been successfully used in patients suffering from the blistering skin disease epidermolysis bullosa. Designer nuclease-based gene therapy approaches are at the preclinical stage. CONCLUSIONS: The selection of the gene therapy method depends on its safety profile, the target genodermatoses and the genetic mutation to correct.

Korbling, M. (1995). "Blood stem cell transplantation and gene therapy of cancer." Stem Cells **13 Suppl 3**: 106-113.

Based on the concept of circulating hematopoietic stem cells with indefinite self-renewal capacity that gives rise to all three cell lineages, peripheral blood progenitor cells (PBPCs) have widely replaced the use of bone marrow (BM) progenitors for autologous transplantation purposes in patients with malignant hematological disorders and selected solid tumors. Ex vivo purification of normal CD34+ cell subsets contained in the patient's apheresis product possibly eliminates clonogenic tumor cells, but also serves as a target cell population for gene transduction. Genetic tagging of PBPC autografts has proven that: 1) NEOR gene expression is sustained for more than 18 months and 2) clonogenic tumor cells contaminating the autograft contribute to relapse. A second generation of gene transduction studies includes new treatment strategies such as the induction of chemoprotection (multidrug resistance gene-1), chemotherapy sensitization (p53), cancer vaccination and genetic chemosensitization. Most recently allogeneic PBPC transplantation has successfully been introduced with the intention of improving the graft-versus-leukemia effect without inducing a higher incidence or more severe graft-versus-host disease (GVHD) than what is expected after BM transplantation. Introducing the herpes virus thymidine kinase cDNA into activated donor T cells makes them susceptible to gangciclovir, thus allowing the in vivo inactivation of GVHD-inducing T cells. With the close interaction of molecular genetics and clinical oncology/hematology, genetic engineering of stem cell grafts will lead into a new stage of stem cell transplantation technology.

Kramerov, A. A., et al. (2016). "Adenoviral Gene Therapy for Diabetic Keratopathy: Effects on Wound Healing and Stem Cell Marker Expression in Human Organ-cultured Corneas and Limbal Epithelial Cells." J Vis Exp(110): e54058.

The goal of this protocol is to describe molecular alterations in human diabetic corneas and demonstrate how they can be alleviated by adenoviral gene therapy in organ-cultured corneas. The diabetic corneal disease is a complication of diabetes with frequent abnormalities of corneal nerves and epithelial wound healing. We have also documented significantly altered expression of several putative epithelial stem cell markers in human diabetic corneas. To alleviate these changes, adenoviral gene therapy was successfully implemented using the upregulation of c-met proto-oncogene expression and/or the downregulation of proteinases matrix metalloproteinase-10 (MMP-10) and cathepsin F. This therapy accelerated wound healing in diabetic corneas even when only the limbal stem cell compartment was transduced. The best results were obtained with combined treatment. For possible patient transplantation of normalized stem cells, an example is also presented of the optimization of gene transduction in stem cell-enriched cultures using polycationic enhancers. This approach may be useful not only for

the selected genes but also for the other mediators of corneal epithelial wound healing and stem cell function.

Lee, S. Y. and S. K. Chung (2016). "Integrating Gene Correction in the Reprogramming and Transdifferentiation Processes: A One-Step Strategy to Overcome Stem Cell-Based Gene Therapy Limitations." Stem Cells Int **2016**: 2725670.

The recent advent of induced pluripotent stem cells (iPSCs) and gene therapy tools has raised the possibility of autologous cell therapy for rare genetic diseases. However, cellular reprogramming is inefficient in certain diseases such as ataxia telangiectasia, Fanconi anemia, LIG4 syndrome, and fibrodysplasia ossificans progressiva syndrome, owing to interference of the disease-related genes. To overcome these therapeutic limitations, it is necessary to fundamentally correct the abnormal gene during or prior to the reprogramming process. In addition, as genetic etiology of Parkinson's disease, it has been well known that induced neural stem cells (iNSCs) were progressively depleted by LRRK2 gene mutation, LRRK2 (G2019S). Thus, to maintain the induced NSCs directly derived from PD patient cells harboring LRRK2 (G2019S), it would be ideal to simultaneously treat the LRRK2 (G2019S) fibroblast during the process of TD. Therefore, simultaneous reprogramming (or TD) and gene therapy would provide the solution for therapeutic limitation caused by vulnerability of reprogramming or TD, in addition to being suitable for general application to the generation of autologous cell-therapy products for patients with genetic defects, thereby obviating the need for the arduous processes currently required.

Leibel, S. L., et al. (2019). "Reversal of Surfactant Protein B Deficiency in Patient Specific Human Induced Pluripotent Stem Cell Derived Lung Organoids by Gene Therapy." Sci Rep **9**(1): 13450.

Surfactant protein B (SFTPB) deficiency is a fatal disease affecting newborn infants. Surfactant is produced by alveolar type II cells which can be differentiated in vitro from patient specific induced pluripotent stem cell (iPSC)-derived lung organoids. Here we show the differentiation of patient specific iPSCs derived from a patient with SFTPB deficiency into lung organoids with mesenchymal and epithelial cell populations from both the proximal and distal portions of the human lung. We alter the deficiency by infecting the SFTPB deficient iPSCs with a lentivirus carrying the wild type SFTPB gene. After differentiating the mutant and corrected cells into lung organoids, we show expression of SFTPB mRNA during endodermal and organoid differentiation but the protein product only after organoid differentiation. We also show the presence of normal lamellar bodies and the secretion of surfactant into the cell culture medium in the organoids of lentiviral infected cells. These findings suggest that a lethal lung disease can be targeted and corrected in a human lung organoid model in vitro.

Leonard, A., et al. (2022). "Curative therapy for hemoglobinopathies: an International Society for Cell & Gene Therapy Stem Cell Engineering Committee review comparing outcomes, accessibility and cost of ex vivo stem cell gene therapy versus allogeneic hematopoietic stem cell transplantation." Cytotherapy **24**(3): 249-261.

Thalassemia and sickle cell disease (SCD) are the most common monogenic diseases in the world and represent a growing global health burden. Management is limited by a paucity of disease-modifying therapies; however, allogeneic hematopoietic stem cell transplantation (HSCT) and autologous HSCT after genetic modification offer patients a curative option. Allogeneic HSCT is limited by donor selection, morbidity and mortality from transplant conditioning, graft-versus-host disease and graft rejection, whereas significant concerns regarding long-term safety, efficacy and cost limit the broad applicability of gene therapy. Here the authors review current outcomes in allogeneic and autologous HSCT for transfusion-dependent thalassemia and SCD and provide our perspective on issues surrounding accessibility and costs as barriers to offering curative therapy to patients with hereditary hemoglobinopathies.

Leonard, A., et al. (2020). "Curative options for sickle cell disease: haploidentical stem cell transplantation or gene therapy?" Br J Haematol **189**(3): 408-423.

Haematopoietic stem cell transplantation (HSCT) is curative in sickle cell disease (SCD); however, the lack of available matched donors makes this therapy out of reach for the majority of patients with SCD. Alternative donor sources such as haploidentical HSCT expand the donor pool to nearly all patients with SCD, with recent data showing high overall survival, limited toxicities, and effective reduction in acute and chronic graft-versus-host disease (GVHD). Simultaneously, multiple gene therapy strategies are entering clinical trials with preliminary data showing their success, theoretically offering all patients yet another curative strategy without the morbidity and mortality of GVHD. As improvements are made for alternative donors in the allogeneic setting and as data emerge from gene therapy trials, the optimal curative strategy for any individual patient with SCD will be determined by many critical factors including efficacy, transplant morbidity and mortality, safety, patient disease status and preference, cost and applicability. Haploidentical may be the preferred

choice now based mostly on availability of data; however, gene therapy is closing the gap and may ultimately prove to be the better option. Progress in both strategies, however, makes cure more attainable for the individual with SCD.

Levy, A., et al. (2015). "Pluripotent stem cells as a cellular model for skin: relevance for physiopathology, cell/gene therapy and drug screening." Eur J Dermatol **25 Suppl 1**: 12-17.

The skin represents the largest tissue in the human body. Its external part, the epidermis, accomplishes vital functions such as barrier protection, thermoregulation and immune function. The mammalian skin epidermis has been for decades the paradigm for studying the molecular events that occur in tissue homeostasis and repair. Many genes and signaling pathways have been identified by the use of manipulated transgenic and KO mice. However, despite numerous elegant transgenic mice experiments, absence of an appropriate in vitro model system has hampered the molecular study of the early events responsible for epidermal and dermal commitments, stages at which congenital genetic alterations are responsible for hundreds of rare skin diseases. For most of them, etiology and treatment are still missing. Here we review the last decade of studies aimed at designing cellular models from pluripotent stem cells (PSC) that recapitulate in vitro the main molecular steps of skin formation. As described below, PSC-based models are powerful tools to (i) clarify early molecular events that occur during epithelial/mesenchymal interactions, (ii) produce in large amount skin cells that could become an alternative for cell/gene therapies and (iii) screen for therapeutic compounds to treat genodermatoses.

Li, C., et al. (2020). "Prophylactic In Vivo Hematopoietic Stem Cell Gene Therapy with an Immune Checkpoint Inhibitor Reverses Tumor Growth in Syngeneic Mouse Tumor Models." Cancer Res **80**(3): 549-560.

Population-wide testing for cancer-associated mutations has established that more than one-fifth of ovarian and breast carcinomas are associated with inherited risk. Salpingo-oophorectomy and/or mastectomy are currently the only effective options offered to women with high-risk germline mutations. Our goal here is to develop a long-lasting approach that provides immunoprophylaxis for mutation carriers. Our approach leverages the fact that at early stages, tumors recruit hematopoietic stem/progenitor cells (HSPC) from the bone marrow and differentiate them into tumor-supporting cells. We developed a technically simple technology to genetically modify HSPCs in vivo. The technology involves HSPC mobilization and intravenous injection of an integrating HDAd5/35++ vector. In vivo HSPC transduction with a GFP-expressing vector and subsequent implantation of syngeneic tumor cells showed >80% GFP marking in tumor-infiltrating leukocytes. To control expression of transgenes, we developed a miRNA regulation system that is activated only when HSPCs are recruited to and differentiated by the tumor. We tested our approach using the immune checkpoint inhibitor anti-PD-L1-gamma1 as an effector gene. In in vivo HSPC-transduced mice with implanted mouse mammary carcinoma (MMC) tumors, after initial tumor growth, tumors regressed and did not recur. Conventional treatment with an anti-PD-L1 mAb had no significant antitumor effect, indicating that early, self-activating expression of anti-PD-L1-gamma1 can overcome the immunosuppressive environment in MMC tumors. The efficacy and safety of this approach was further validated in an ovarian cancer model with typical germline mutations (ID8 p53(-/-) brca2(-/-)), both in a prophylactic and therapeutic setting. This HSPC gene therapy approach has potential for clinical translation. SIGNIFICANCE: Considering the limited prophylactic options that are currently offered to women with high-risk germ-line mutations, the in vivo HSPC gene therapy approach is a promising strategy that addresses a major medical problem.

Li, J., et al. (2022). "A Single Nucleotide Polymorphism (SNP) in the SLC22A3 Transporter Gene Is Associated With the Severity of Oral Mucositis in Multiple Myeloma Patients Receiving Autologous Stem Cell Transplant Followed by Melphalan Therapy." Anticancer Res **42**(1): 385-395.

BACKGROUND: It has been reported that expression of OCT3 enhanced the sensitivity to melphalan in cells, indicative of potential roles of OCT3 in melphalan transport. Herein we investigated the association of select single nucleotide polymorphisms in SLC22A3 (gene encoding OCT3) with clinical outcomes in multiple myeloma (MM) patients with hematopoietic autologous stem cell transplants followed by high-dose melphalan therapy. MATERIALS AND METHODS: Melphlan concentrations in blood samples from 108 MM patients were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MuS); genotypes of rs2048327, rs1810126, and rs3088442 in these patients were determined using quatitive RT-PCR assays. RESULTS: Rs3088442 A variant-carriers had a significantly increased risk of severe oral mucositis in comparison with homozygous rs3088442 G-carriers with adjusted odds ratio of 4.00 (95% CI=1.25-14.7; p=0.027). Rs3088442 A carriers tended to have lower creatinine clearance (p=0.10) and higher maximum plasma concentration of melphalan (p=0.07).

CONCLUSION: OCT3 might be involved in melphalan transport in MM patients.

Li, M., et al. (2019). "Exploiting tumor-intrinsic signals to induce mesenchymal stem cell-mediated suicide gene therapy to fight malignant glioma." Stem Cell Res Ther **10**(1): 88.

BACKGROUND: Human mesenchymal stem cell (MSC)-based tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene delivery is regarded as an effective treatment for glioblastoma (GBM). However, adverse-free target site homing of the delivery vehicles to the tumor microsatellite nests is challenging, leading to erroneously sustained released of this suicide protein into the normal brain parenchyma; therefore, limiting off-target cytotoxicity and controlled expression of the suicide inductor is a prerequisite for the safe use of therapeutic stem cells. METHODS: Utilizing the intrinsic expression profile of GBM and its elevated expression of TGF-beta relative to normal brain tissue, we sought to engineer human adipose-derived MSCs (hAMSC-SBE4-TRAIL) which augment the expression of TRAIL under the trigger of TGF-beta signaling. We validated our therapeutic technology in a series of functional in vitro and in vivo assays using primary patient-derived GBM models. RESULTS: Our current findings show that these biologic delivery vehicles have high tumor tropism efficacy and expression TRAIL gene under the trigger of TGF-beta-secreting GBMs, as well as avoid unspecific TRAIL secretion into normal brain tissue. hAMSC-SBE4-TRAIL inhibited the proliferation and induced apoptosis in experimental GBMs both in vitro and in vivo. In addition, our improved platform of engineered MSCs significantly decreased the tumor volume and prolonged survival time in a murine model of GBM. CONCLUSIONS: Our results on the controlled release of suicide inductor TRAIL by exploiting an endogenous tumor signaling pathway demonstrate a significant improvement for the clinical utility of stem cell-mediated gene delivery to treat brain cancers. Harvesting immune-compatible MSCs from patients' fat by minimally invasive procedures further highlights the clinical potential of this approach in the vision of applicability in a personalized manner. The hAMSC-SBE4-TRAIL exhibit great curative efficacy and are a promising cell-based treatment option for GBM to be validated in clinical exploration.

Li, X., et al. (2015). "[Application of Reporter Gene Labeling in Stem Cell Therapy for Acute Myocardial Infarction]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **37**(5): 612-617.

Stem cell therapy for acute myocardial infarction is drawing great attention. However,the biological behavior and function mechanism of implanted stem cells remain controversial, as well as their clinical benefits. With the development of imaging probes and devices, molecular imaging enables noninvasive, dynamic tracking of stem cells in vivo. In this review, we summarize the use of various markers,especially the technique of reporter gene labeling, in the field of stem cell therapy, and highlight some recent preclinical and clinical achievements.

Li, Y., et al. (2014). "Gene therapy in patient-specific stem cell lines and a preclinical model of retinitis pigmentosa with membrane frizzled-related protein defects." Mol Ther **22**(9): 1688-1697.

Defects in Membrane Frizzled-related Protein (MFRP) cause autosomal recessive retinitis pigmentosa (RP). MFRP codes for a retinal pigment epithelium (RPE)-specific membrane receptor of unknown function. In patient-specific induced pluripotent stem (iPS)-derived RPE cells, precise levels of MFRP, and its dicistronic partner CTRP5, are critical to the regulation of actin organization. Overexpression of CTRP5 in naive human RPE cells phenocopied behavior of MFRP-deficient patient RPE (iPS-RPE) cells. AAV8 (Y733F) vector expressing human MFRP rescued the actin disorganization phenotype and restored apical microvilli in patient-specific iPS-RPE cell lines. As a result, AAV-treated MFRP mutant iPS-RPE recovered pigmentation and transepithelial resistance. The efficacy of AAV-mediated gene therapy was also evaluated in Mfrp(rd6)/Mfrp(rd6) mice--an established preclinical model of RP--and long-term improvement in visual function was observed in AAV-Mfrp-treated mice. This report is the first to indicate the successful use of human iPS-RPE cells as a recipient for gene therapy. The observed favorable response to gene therapy in both patient-specific cell lines, and the Mfrp(rd6)/Mfrp(rd6) preclinical model suggests that this form of degeneration caused by MFRP mutations is a potential target for interventional trials.

Li, Z., et al. (2009). "Toward a stem cell gene therapy for breast cancer." Blood **113**(22): 5423-5433.

Current approaches for treatment of late-stage breast cancer rarely result in a long-term cure. In part this is due to tumor stroma that prevents access of systemically or intratumorally applied therapeutics. We propose a stem cell gene therapy approach for controlled tumor stroma degradation that uses the pathophysiologic process of recruitment of inflammatory cells into the tumor. This approach involves genetic modification of hematopoietic stem cells (HSCs) and their subsequent transplantation into tumor-bearing mice. We show that inducible, intratumoral expression of relaxin (Rlx) either by transplanting tumor cells that contained the Rlx gene or

by transplantation of mouse HSCs transduced with an Rlx-expressing lentivirus vector delays tumor growth in a mouse model of breast cancer. The antitumor effect of Rlx was mediated through degradation of tumor stroma, which provided increased access of infiltrating antitumor immune cells to their target tumor cells. Furthermore, we have shown in a human/mouse chimeric model that genetically modified HSCs expressing a transgene can access the tumor site. Our findings are relevant for cancer gene therapy and immunotherapy.

Liang, Q., et al. (2015). "Lentiviral Stem Cell Gene Therapy for Pompe Disease." J Neuromuscul Dis **2**(s1): S64.

Lieu, F. H., et al. (1997). "Transmissibility of murine stem cell virus-based retroviral vectors carrying both interleukin-12 cDNAs and a third gene: implications for immune gene therapy." Cancer Gene Ther **4**(3): 167-175.

The combination of immunotherapy with conventional treatments such as radio- and chemotherapy may be necessary to eradicate minimal residual disease. Interleukin 12 (IL-12) is a heterodimeric cytokine composed of two subunits, p40 and p35. Coordinate expression of the IL-12 p40 and p35 genes in several solid tumor models has been found to induce strong and specific antitumor immune responses. In the interest of obtaining high level IL-12 expression in leukemia/lymphoma cells for use as vaccines in cancer immunotherapy, we evaluated three IL-12 retroviral vector designs based on the murine stem cell virus (MSCV) vector which efficiently transduces functional genes into normal hematopoietic cells. MSCVpac-mlL-12 and MIPV-mIL-12 contain an encephalomyocarditis virus internal ribosome entry site for internal translation of bicistronic mRNA transcripts, while MDCVpac-mIL-12 carries an expression cassette in the U3 region of the 3' long terminal repeat. We found that the MSCVpac-mIL-12 vector directed robust expression of both p40 and p35 genes in several murine tumor cell lines of hematopoietic origin, including a T-cell lymphoma, a B-cell lymphoma, and a plasmacytoma/myeloma. In contrast, genomic instability or promoter interference hampered p40 gene expression in cells transduced with the MIPV-mIL-12 and MDCVpac-mIL-12 vectors, respectively. These findings provide the basis for the design of IL-12 retroviral vectors for the treatment of hematologic malignancies in humans.

Lin, H. T., et al. (2016). "Application of Droplet Digital PCR for Estimating Vector Copy Number States in Stem Cell Gene Therapy." Hum Gene Ther Methods **27**(5): 197-208.

Stable gene transfer into target cell populations via integrating viral vectors is widely used in stem cell gene therapy (SCGT). Accurate vector copy number (VCN) estimation has become increasingly important. However, existing methods of estimation such as real-time quantitative PCR are more restricted in practicality, especially during clinical trials, given the limited availability of sample materials from patients. This study demonstrates the application of an emerging technology called droplet digital PCR (ddPCR) in estimating VCN states in the context of SCGT. Induced pluripotent stem cells (iPSCs) derived from a patient with X-linked chronic granulomatous disease were used as clonable target cells for transduction with alpharetroviral vectors harboring codon-optimized CYBB cDNA. Precise primer-probe design followed by multiplex analysis conferred assay specificity. Accurate estimation of per-cell VCN values was possible without reliance on a reference standard curve. Sensitivity was high and the dynamic range of detection was wide. Assay reliability was validated by observation of consistent, reproducible, and distinct VCN clustering patterns for clones of transduced iPSCs with varying numbers of transgene copies. Taken together, use of ddPCR appears to offer a practical and robust approach to VCN estimation with a wide range of clinical and research applications.

Liu, H., et al. (2010). "Fiber-modified adenovirus can mediate human adipose tissue-derived mesenchymal stem cell-based anti-angiogenic gene therapy." Biotechnol Lett **32**(8): 1181-1188.

A fiber-modified adenovirus (rAd5F11B), loaded with the Kringle1-5 gene (rAd-K1-5) was used to infect human adipose tissue-derived mesenchymal stem cells (HAMSCs). At a multiplicity of infection of 20, the transfection efficiency in HAMSCs was 90% and the cell expansion and differentiation of infected HAMSCs were not significantly suppressed. HAMSCs infected with rAd-K1-5 expressed the exogenous Kringle1-5 protein, an angiogenic inhibitor, and conditioned media from HAMSCs expressing the Kringle1-5 protein blocked VEGF-induced neovascularization both in vitro and in vivo. rAd5F11B may therefore be a promising gene transfer vector in HAMSCs-based anti-angiogenic gene therapy because of its low toxicity and high transfection efficiency.

Liu, Y., et al. (2022). "Inducible caspase-9 suicide gene under control of endogenous oct4 to safeguard mouse and human pluripotent stem cell therapy." Mol Ther Methods Clin Dev **24**: 332-341.

Pluripotent stem cells (PSCs) are promising in regenerative medicine. A major challenge of PSC therapy is the risk of teratoma formation because of the contamination of undifferentiated stem cells.

Constitutive promoters or endogenous SOX2 promoters have been used to drive inducible caspase-9 (iCasp9) gene expression but cannot specifically eradicate undifferentiated PSCs. Here, we inserted iCasp9 gene into the endogenous OCT4 locus of human and mouse PSCs without affecting their pluripotency. A chemical inducer of dimerization (CID), AP1903, induced iCasp9 activation, which led to the apoptosis of specific undifferentiated PSCs in vitro and in vivo. Differentiated cell lineages survived because of the silence of the endogenous OCT4 gene. Human and mouse PSCs were controllable when CID was administrated within 2 weeks after PSC injection in immunodeficient mice. However, an interval longer than 2 weeks caused teratoma formation and mouse death because a mass of somatic cells already differentiated from the PSCs. In conclusion, we have developed a specific and efficient PSC suicide system that will be of value in the clinical applications of PSC-based therapy.

Lofvall, H., et al. (2019). "Hematopoietic Stem Cell-Targeted Neonatal Gene Therapy with a Clinically Applicable Lentiviral Vector Corrects Osteopetrosis in oc/oc Mice." Hum Gene Ther **30**(11): 1395-1404.

Infantile malignant osteopetrosis (IMO) is an autosomal recessive disorder characterized by nonfunctional osteoclasts. Approximately 50% of the patients have mutations in the TCIRG1 gene, encoding for a subunit of the osteoclast proton pump. Gene therapy represents a potential alternative treatment to allogeneic stem cell transplantation for IMO. The oc/oc mouse is a model of IMO characterized by a 1,500 bp deletion in the TCIRG1 gene, severe osteopetrosis, and a life span of only 3 weeks. Here we show that the osteopetrotic phenotype in oc/oc mice can be reversed by hematopoietic stem cell-targeted gene therapy with a clinically applicable lentiviral vector expressing a wild-type form of human TCIRG1 under the mammalian promoter elongation factor 1alpha short (EFS-hT). oc/oc c-kit(+) fetal liver cells transduced with EFS-hT were transplanted into sublethally irradiated oc/oc mice by temporal vein injection 1 day after birth. A total of 9 of 12 mice survived long term (19-25 weeks) with evidence of tooth eruption, uncharacteristic of oc/oc mice. Splenocytes were harvested 19-25 weeks after transplantation and differentiated into osteoclasts on bone slices to assess resorption and on plastic to assess TCIRG1 protein expression. Vector-corrected osteoclasts showed human TCIRG1 expression by Western blot. CTX-I release relative to that mediated by oc/oc-derived osteoclasts increased 8-239-fold. Resorption pits on bone slices were observed for osteoclasts derived from 7/9 surviving transplanted oc/oc mice. Histopathology of the bones of surviving animals showed varying degrees of rescued phenotype, the majority with almost full correction. The average vector copy number per cell in the bone marrow was 1.8 +/- 0.5. Overall, 75% of transplanted mice exhibited long-term survival and marked reversal of the osteopetrotic bone phenotype. These findings represent a significant step toward the clinical application of gene therapy for IMO.

Loukogeorgakis, S. P. and A. W. Flake (2014). "In utero stem cell and gene therapy: current status and future perspectives." Eur J Pediatr Surg **24**(3): 237-245.

Advances in prenatal diagnosis have led to the development of fetal therapies for congenital disorders. Although in utero surgical intervention has been used successfully for correction of anatomical defects that cause fetal demise or long-term disability, its clinical indications remain limited. In contrast, prenatal stem cell and gene therapy might have tremendous potential to treat multiple inherited disorders, and could dramatically expand the use of fetal intervention to a wide range of anticipated pediatric and adult diseases. Despite encouraging results from studies in animal models of disease, the clinical utility of such therapies has been restricted by poor efficacy and concerns about safety. The aim of this review is to summarize experimental progress toward clinical application of in utero stem cell transplantation and gene transfer for the treatment of congenital disease.

Ludwig, P. E., et al. (2019). "Novel stem cell and gene therapy in diabetic retinopathy, age related macular degeneration, and retinitis pigmentosa." Int J Retina Vitreous **5**: 7.

Degenerative retinal disease leads to significant visual morbidity worldwide. Diabetic retinopathy and macular degeneration are leading causes of blindness in the developed world. While current therapies for these diseases slow disease progression, stem cell and gene therapy may also reverse the effects of these, and other, degenerative retinal conditions. Novel therapies being investigated include the use of various types of stem cells in the regeneration of atrophic or damaged retinal tissue, the prolonged administration of neurotrophic factors and/or drug delivery, immunomodulation, as well as the replacement of mutant genes, and immunomodulation through viral vector delivery. This review will update the reader on aspects of stem cell and gene therapy in diabetic retinopathy, age-related macular degeneration, retinitis pigmentosa and other less common inherited retinal dystrophies. These therapies include the use of adeno-associated viral vector-based therapies for treatment of various types of retinitis pigmentosa and dry age-related macular degeneration. Other potential therapies reviewed include the use of mesenchymal stem cells in local immunomodulation, and the use of

stem cells in generating structures like three-dimensional retinal sheets for transplantation into degenerative retinas. Finally, aspects of stem cell and gene therapy in diabetic retinopathy, age-related macular degeneration, retinitis pigmentosa, and other less common inherited retinal dystrophies will be reviewed.

Luo, Y., et al. (2015). "A Double-Switch Cell Fusion-Inducible Transgene Expression System for Neural Stem Cell-Based Antiglioma Gene Therapy." Stem Cells Int **2015**: 649080.

Recent progress in neural stem cell- (NSC-) based tumor-targeted gene therapy showed that NSC vectors expressing an artificially engineered viral fusogenic protein, VSV-G H162R, could cause tumor cell death specifically under acidic tumor microenvironment by syncytia formation; however, the killing efficiency still had much room to improve. In the view that coexpression of another antitumoral gene with VSV-G can augment the bystander effect, a synthetic regulatory system that triggers transgene expression in a cell fusion-inducible manner has been proposed. Here we have developed a double-switch cell fusion-inducible transgene expression system (DoFIT) to drive transgene expression upon VSV-G-mediated NSC-glioma cell fusion. In this binary system, transgene expression is coregulated by a glioma-specific promoter and targeting sequences of a microRNA (miR) that is highly expressed in NSCs but lowly expressed in glioma cells. Thus, transgene expression is "switched off" by the miR in NSC vectors, but after cell fusion with glioma cells, the miR is diluted and loses its suppressive effect. Meanwhile, in the syncytia, transgene expression is "switched on" by the glioma-specific promoter. Our in vitro and in vivo experimental data show that DoFIT successfully abolishes luciferase reporter gene expression in NSC vectors but activates it specifically after VSV-G-mediated NSC-glioma cell fusion.

Lupo-Stanghellini, M. T., et al. (2010). "Clinical impact of suicide gene therapy in allogeneic hematopoietic stem cell transplantation." Hum Gene Ther **21**(3): 241-250.

Allogeneic hematopoietic stem cell transplantation (allo-SCT) from an HLA-matched related or unrelated donor is a curative option for patients with high-risk hematological diseases. In the absence of a matched donor, patients have been offered investigational transplantation strategies such as umbilical cord blood SCT or family haploidentical SCT. Besides the activity of the conditioning regimen, most of the antileukemic potential of allo-SCT relies on alloreactivity, promoted by donor lymphocytes reacting against patient-specific antigens, such as minor and major histocompatibility antigens, ultimately translating into cancer immunotherapy. Unfortunately, alloreactivity is also responsible for the most serious and frequent complication of allo-SCT: graft-versus-host-disease (GvHD). The risk of GvHD increases with the level of HLA disparity between host and donor, and leads to impaired quality of life and reduced survival expectancy, particularly among patients receiving transplants from HLA-mismatched donors. Gene transfer technologies are promising tools to manipulate donor T cell immunity to enforce the graft-versus-tumor effect, to promote functional immune reconstitution (graft vs. infection), and to prevent or control GvHD. To this purpose, several cell and gene transfer approaches have been investigated at the preclinical level, and are being implemented in clinical trials. Suicide gene therapy is to date the most extensive clinical application of T cell-based gene therapy. In several phase I-II clinical studies conducted worldwide this approach proved highly feasible, safe, and effective in promoting a dynamic and patient-specific modulation of alloreactivity. This review focuses on this approach.

Lytle, A. M., et al. (2016). "Effects of FVIII immunity on hepatocyte and hematopoietic stem cell-directed gene therapy of murine hemophilia A." Mol Ther Methods Clin Dev **3**: 15056.

Immune responses to coagulation factors VIII (FVIII) and IX (FIX) represent primary obstacles to hemophilia treatment. Previously, we showed that hematopoietic stem cell (HSC) retroviral gene therapy induces immune nonresponsiveness to FVIII in both naive and preimmunized murine hemophilia A settings. Liver-directed adeno-associated viral (AAV)-FIX vector gene transfer achieved similar results in preclinical hemophilia B models. However, as clinical immune responses to FVIII and FIX differ, we investigated the ability of liver-directed AAV-FVIII gene therapy to affect FVIII immunity in hemophilia A mice. Both FVIII naive and preimmunized mice were administered recombinant AAV8 encoding a liver-directed bioengineered FVIII expression cassette. Naive animals receiving high or mid-doses subsequently achieved near normal FVIII activity levels. However, challenge with adjuvant-free recombinant FVIII induced loss of FVIII activity and anti-FVIII antibodies in mid-dose, but not high-dose AAV or HSC lentiviral (LV) vector gene therapy cohorts. Furthermore, unlike what was shown previously for FIX gene transfer, AAV-FVIII administration to hemophilia A inhibitor mice conferred no effect on anti-FVIII antibody or inhibitory titers. These data suggest that functional differences exist in the immune modulation achieved to FVIII or FIX in hemophilia mice by gene therapy approaches

incorporating liver-directed AAV vectors or HSC-directed LV.

Magnani, A., et al. (2022). "Long-term safety and efficacy of lentiviral hematopoietic stem/progenitor cell gene therapy for Wiskott-Aldrich syndrome." Nat Med **28**(1): 71-80.

Patients with Wiskott-Aldrich syndrome (WAS) lacking a human leukocyte antigen-matched donor may benefit from gene therapy through the provision of gene-corrected, autologous hematopoietic stem/progenitor cells. Here, we present comprehensive, long-term follow-up results (median follow-up, 7.6 years) (phase I/II trial no. NCT02333760) for eight patients with WAS having undergone phase I/II lentiviral vector-based gene therapy trials (nos. NCT01347346 and NCT01347242), with a focus on thrombocytopenia and autoimmunity. Primary outcomes of the long-term study were to establish clinical and biological safety, efficacy and tolerability by evaluating the incidence and type of serious adverse events and clinical status and biological parameters including lentiviral genomic integration sites in different cell subpopulations from 3 years to 15 years after gene therapy. Secondary outcomes included monitoring the need for additional treatment and T cell repertoire diversity. An interim analysis shows that the study meets the primary outcome criteria tested given that the gene-corrected cells engrafted stably, and no serious treatment-associated adverse events occurred. Overall, severe infections and eczema resolved. Autoimmune disorders and bleeding episodes were significantly less frequent, despite only partial correction of the platelet compartment. The results suggest that lentiviral gene therapy provides sustained clinical benefits for patients with WAS.

Mallack, E. J., et al. (2019). "The Landscape of Hematopoietic Stem Cell Transplant and Gene Therapy for X-Linked Adrenoleukodystrophy." Curr Treat Options Neurol **21**(12): 61.

PURPOSE OF REVIEW: To present an updated appraisal of hematopoietic stem cell transplant (HSCT) and gene therapy for X-linked adrenoleukodystrophy (ALD) in the setting of a novel, presymptomatic approach to disease. RECENT FINDINGS: Outcomes in HSCT for ALD have been optimized over time due to early patient detection, improved myeloablative conditioning regimens, and adjunctive treatment for patients with advanced cerebral disease. Gene therapy has arrested disease progression in a cohort of boys with childhood cerebral ALD. New therapeutic strategies have provided the clinical basis for the implementation of Newborn Screening (NBS). With the help of advocacy groups, NBS has been implemented, allowing for MRI screening for the onset of cerebral ALD from birth. Gene therapy and optimized hematopoietic stem cell transplant for childhood CALD have changed the natural history of this previously devastating neurological disease.

Marini, F. C., et al. (1999). "Purging of contaminating breast cancer cells from hematopoietic stem cell grafts by adenoviral GAL-TEK gene therapy and magnetic antibody cell separation." Clin Cancer Res **5**(6): 1557-1568.

The presence of contaminating tumor cells in autologous bone marrow or peripheral blood stem cell (PB-SC) preparations increase the likelihood of relapse in women receiving transplants for metastatic breast cancer. We describe a new technique for purging breast cancer cells (BCCs) that combines two independent strategies: (a) the specific enrichment of CD34+ progenitor stem cells by magnetic antibody cell separation (MACS), and then (b) infection of the contaminating BCCs with a recombinant adGAL-TEK marker/suicide gene adenovirus (ad-v), followed by the addition of ganciclovir (GCV). Infection with this ad-v results in three to four times greater expression of ad-v-delivered reporter gene in BCCs than in CD34+ cells. In addition -2 h, -low multiplicity of infection (50:1) adGAL-TEK infections of BCC lines (MCF-7 and BT474) eradicated >99% of BCCs after 72 h of exposure to 20 microM GCV. However, exposure to both adenovirus and GCV at the MOIs and doses used had little effect on hematopoietic stem cells to form colonies in colony-forming unit assays. adGAL-TEK infection in our model system (10(3)-10(5) BCCs added into 10(7) HSCs) also resulted in the 3 to 5 log eradication of clonogenic BCCs after the addition of GCV. MACS enrichment/purification of CD34+ cells from PB-SC contaminated with 2 x 10(6) to 5 x 10(7) BCCs followed by adGAL-TEK infection and GCV addition resulted in 5-7-log depletion of clonogenic BCCs as well as enrichment of CD34+ progenitor cells to >98%, with the recovery of >70% of hematopoietic stem cells. This adenoviral purging system is so robust that poor MACS purification, resulting in 1.5-log depletion of BCCs, still permits excellent ad-v infection and BCC killing.

Marktel, S., et al. (2019). "Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ss-thalassemia." Nat Med **25**(2): 234-241.

ss-thalassemia is caused by ss-globin gene mutations resulting in reduced (beta(+)) or absent (beta(0)) hemoglobin production. Patient life expectancy has recently increased, but the need for chronic transfusions in transfusion-dependent thalassemia (TDT) and iron chelation impairs quality

of life(1). Allogeneic hematopoietic stem cell (HSC) transplantation represents the curative treatment, with thalassemia-free survival exceeding 80%. However, it is available to a minority of patients and is associated with morbidity, rejection and graft-versus-host disease(2). Gene therapy with autologous HSCs modified to express ss-globin represents a potential therapeutic option. We treated three adults and six children with ss(0) or severe ss(+) mutations in a phase 1/2 trial (NCT02453477) with an intrabone administration of HSCs transduced with the lentiviral vector GLOBE. Rapid hematopoietic recovery with polyclonal multilineage engraftment of vector-marked cells was achieved, with a median of 37.5% (range 12.6-76.4%) in hematopoietic progenitors and a vector copy number per cell (VCN) of 0.58 (range 0.10-1.97) in erythroid precursors at 1 year, in absence of clonal dominance. Transfusion requirement was reduced in the adults. Three out of four evaluable pediatric participants discontinued transfusions after gene therapy and were transfusion independent at the last follow-up. Younger age and persistence of higher VCN in the repopulating hematopoietic cells are associated with better outcome.

Marofi, F., et al. (2017). "Mesenchymal Stromal/Stem Cells: A New Era in the Cell-Based Targeted Gene Therapy of Cancer." Front Immunol **8**: 1770.

In recent years, in light of the promising potentials of mesenchymal stromal/stem cells (MSCs) for carrying therapeutic anticancer genes, a complete revisitation on old chemotherapy-based paradigms has been established. This review attempted to bring forward and introduce the novel therapeutic opportunities of using genetically engineered MSCs. The simplicities and advantages of MSCs for medical applications make them a unique and promising option in the case of cancer therapy. Some of the superiorities of using MSCs as therapeutic gene micro-carriers are the easy cell-extraction procedures and their abundant proliferation capacity in vitro without losing their main biological properties. Targeted therapy by using MSCs as the delivery vehicles of therapeutic genes is a new approach in the treatment of various types of cancers. Some of the distinct properties of MSCs, such as tumor-tropism, non-immunogenicity, stimulatory effect on the anti-inflammatory molecules, inhibitory effect on inflammatory responses, non-toxicity against the normal tissues, and easy processes for the clinical use, have formed the basis of attention to MSCs. They can be easily used for the treatment of damaged or injured tissues, regenerative medicine, and immune disorders. This review focused on the drugability of MSCs and their potential for the delivery of candidate anticancer genes. It also briefly reviewed the vectors and methods used for MSC-mediated gene therapy of malignancies. Also, the challenges, limitations, and considerations in using MSCs for gene therapy of cancer and the new methods developed for resolution of these problems are reviewed.

Maron, J. L. (2020). "Healing a Broken Heart: Can Stem Cell and Gene Therapy Regenerate and Repair the Myocardium?" Clin Ther **42**(10): 1847-1848.

Martinez-Serrano, A., et al. (2001). "Human neural stem and progenitor cells: in vitro and in vivo properties, and potential for gene therapy and cell replacement in the CNS." Curr Gene Ther **1**(3): 279-299.

The generation of unlimited quantities of neural stem and/or progenitor cells derived from the human brain holds great interest for basic and applied neuroscience. In this article we critically review the origins and recent developments of procedures developed for the expansion, perpetuation, identification, and isolation of human neural precursors, as well as their attributes. Factors influencing their in vitro properties, both under division and after differentiation conditions, are evaluated, with the aim of identifying properties common to the different culture systems reported. This analysis suggests that different culture procedures result in cells with different properties, or even in different cells being isolated. With respect to in vivo performance, present evidence obtained in rodents indicate that cultured human neural precursors, in general, are endowed with excellent integrative properties. Differentiation of the implanted cells, in particular in the case of adult recipients, seems not to be complete, and functionality still needs to be demonstrated. In relation to gene transfer and therapy, aspects currently underexplored, initial data support the view that human neural stem and progenitor cells may serve a role as a platform cell for the delivery of bioactive substances to the diseased CNS. Although a large deal of basic research remains to be done, available data illustrate the enormous potential that human neural precursors isolated, expanded, and characterized in vitro hold for therapeutic applications. In spite of this potential, maintaining a critical view on many unresolved questions will surely help to drive this research field to a good end, that is, the development of real therapies for diseases of the human nervous system.

Matsuda, K. M., et al. (1999). "Development of a modified selective amplifier gene for hematopoietic stem cell gene therapy." Gene Ther **6**(6): 1038-1044.

We have proposed a novel concept, ie selective expansion of transduced cells, to overcome the low efficiency of gene transfer into hematopoietic stem cells. Previously, a fusion gene encoding a

chimeric receptor (DeltaGCRER) between the mouse granulocyte colony-stimulating factor receptor (G-CSFR) and the hormone-binding domain of rat estrogen receptor was constructed as a 'selective amplifier gene'. Although the chimeric gene conferred estrogen-inducible proliferation on the transduced Ba/F3 cells, it also mediated differentiation of the retrovirally transduced 32D cells upon estrogen treatment. Since only a growth signal is required for our purpose, we further modified the DeltaGCRER gene to attenuate its differentiation signal. Based on the observation that tyrosine-703 in wild-type G-CSFR plays a pivotal role in transmitting the differentiation signal, phenylalanine was substituted for this residue in DeltaGCRER. When the resultant selective amplifier gene (DeltaY703F-GCRER gene) was expressed in 32D cells, sustained growth was supported by estrogen, while differentiation was suppressed. These cells ceased to grow upon estrogen withdrawal and differentiated with G-CSF treatment. The present findings suggested that DeltaY703F-GCRER may have desirable properties as a selective amplifier for hematopoietic stem cell expansion and gene therapy.

Matz, E. L. and R. P. Terlecki (2021). "Stem Cell and Gene-Based Therapy for Erectile Dysfunction: Current Status and Future Needs." Urol Clin North Am **48**(4): 611-619.

Erectile dysfunction affects an increasing number of men. The mainstays of management include oral medications, local erectogenic agents, and surgical placement of prosthetic devices. Newer technologies such as stem cell and gene therapy have been investigated as a means to restore spontaneous erectile capacity. Mesenchymal stem cells are thought to produce a local immunomodulatory and pro-repair milieu at the area of injury or needed repair. Gene therapy involves targeting the erectogenic pathway to augment factors involved in producing a natural erection. Such therapies are considered experimental and should be used in the setting of a clinical trial with appropriate oversight.

Matzner, U., et al. (2001). "Bone marrow stem cell gene therapy of arylsulfatase A-deficient mice, using an arylsulfatase A mutant that is hypersecreted from retrovirally transduced donor-type cells." Hum Gene Ther **12**(9): 1021-1033.

Arylsulfatase A (ASA)-deficient mice represent an animal model for the fatal lysosomal storage disease metachromatic leukodystrophy, which is characterized by widespread intralysosomal deposition of sulfatide. Bone marrow stem cell gene therapy in mice, using a retroviral vector mediating expression of wild-type human ASA, has the potential to ameliorate the visceral pathology, but improves the prevailing brain disease and neurologic symptoms only marginally. One factor that influences the efficacy of bone marrow transplantation therapy in lysosomal storage diseases is the secretion level of the therapeutic enzyme from donor-type cells. Here we test the potential of a hypersecreted glycosylation variant of ASA. Although this mutant lacks mannose 6-phosphate residues it is taken up by cells by a mannose 6-phosphate receptor-independent pathway and causes partial metabolic correction of ASA-deficient mouse cells. Retrovirally mediated transfer of the mutant cDNA into ASA-deficient mice results in the sustained expression of the transgene. Serum levels argue for an increased secretion of the glycosylation mutant also in vivo. Tissue levels were reduced to 2% in liver and up to 40% in kidney compared with animals treated with the wild-type enzyme, indicating reduced endocytosis. Thus, the limited uptake of the variant enzyme outweighs the putative advantageous effect of improved supply. Although the mutant enzyme is able to correct the metabolic defect partially, histological examinations did not reveal any reduction of sulfatide storage in treated animals. Surprisingly, analysis of neurologic symptoms indicated a significant improvement of the gait pattern.

McClain, L. E. and A. W. Flake (2016). "In utero stem cell transplantation and gene therapy: Recent progress and the potential for clinical application." Best Pract Res Clin Obstet Gynaecol **31**: 88-98.

Advances in prenatal diagnosis have led to the prenatal management and treatment of a variety of congenital diseases. Although surgical treatment has been successfully applied to specific anatomic defects that place the fetus at a risk of death or life-long disability, the indications for fetal surgical intervention have remained relatively limited. By contrast, prenatal stem cell and gene therapy await clinical application, but they have tremendous potential to treat a broad range of genetic disorders. If there are biological advantages unique to fetal development that favor fetal stem cell or gene therapy over postnatal treatment, prenatal therapy may become the preferred approach to the treatment of any disease that can be prenatally diagnosed and cured by stem cell or gene therapy. Here, we review the field including recent progress toward clinical application and imminent clinical trials for cellular and gene therapy.

Mei, S. H., et al. (2016). "Advances in Stem Cell and Cell-Based Gene Therapy Approaches for Experimental Acute Lung Injury: A Review of Preclinical Studies." Hum Gene Ther **27**(10): 802-812.

Given the failure of pharmacological interventions in acute respiratory distress syndrome (ARDS), researchers have been actively pursuing novel

strategies to treat this devastating, life-threatening condition commonly seen in the intensive care unit. There has been considerable research on harnessing the reparative properties of stem and progenitor cells to develop more effective therapeutic approaches for respiratory diseases with limited treatment options, such as ARDS. This review discusses the preclinical literature on the use of stem and progenitor cell therapy and cell-based gene therapy for the treatment of preclinical animal models of acute lung injury (ALI). A variety of cell types that have been used in preclinical models of ALI, such as mesenchymal stem cells, endothelial progenitor cells, and induced pluripotent stem cells, were evaluated. At present, two phase I trials have been completed and one phase I/II clinical trial is well underway in order to translate the therapeutic benefit gleaned from preclinical studies in complex animal models of ALI to patients with ARDS, paving the way for what could potentially develop into transformative therapy for critically ill patients. As we await the results of these early cell therapy trials, future success of stem cell therapy for ARDS will depend on selection of the most appropriate cell type, route and timing of cell delivery, enhancing effectiveness of cells (i.e., potency), and potentially combining beneficial cells and genes (cell-based gene therapy) to maximize therapeutic efficacy. The experimental models and scientific methods exploited to date have provided researchers with invaluable knowledge that will be leveraged to engineer cells with enhanced therapeutic capabilities for use in the next generation of clinical trials.

Meneghini, V., et al. (2017). "Generation of Human Induced Pluripotent Stem Cell-Derived Bona Fide Neural Stem Cells for Ex Vivo Gene Therapy of Metachromatic Leukodystrophy." Stem Cells Transl Med **6**(2): 352-368.

Allogeneic fetal-derived human neural stem cells (hfNSCs) that are under clinical evaluation for several neurodegenerative diseases display a favorable safety profile, but require immunosuppression upon transplantation in patients. Neural progenitors derived from patient-specific induced pluripotent stem cells (iPSCs) may be relevant for autologous ex vivo gene-therapy applications to treat genetic diseases with unmet medical need. In this scenario, obtaining iPSC-derived neural stem cells (NSCs) showing a reliable "NSC signature" is mandatory. Here, we generated human iPSC (hiPSC) clones via reprogramming of skin fibroblasts derived from normal donors and patients affected by metachromatic leukodystrophy (MLD), a fatal neurodegenerative lysosomal storage disease caused by genetic defects of the arylsulfatase A (ARSA) enzyme. We differentiated hiPSCs into NSCs (hiPS-NSCs) sharing molecular, phenotypic, and functional identity with hfNSCs, which we used as a "gold standard" in a side-by-side comparison when validating the phenotype of hiPS-NSCs and predicting their performance after intracerebral transplantation. Using lentiviral vectors, we efficiently transduced MLD hiPSCs, achieving supraphysiological ARSA activity that further increased upon neural differentiation. Intracerebral transplantation of hiPS-NSCs into neonatal and adult immunodeficient MLD mice stably restored ARSA activity in the whole central nervous system. Importantly, we observed a significant decrease of sulfatide storage when ARSA-overexpressing cells were used, with a clear advantage in those mice receiving neonatal as compared with adult intervention. Thus, we generated a renewable source of ARSA-overexpressing iPSC-derived bona fide hNSCs with improved features compared with clinically approved hfNSCs. Patient-specific ARSA-overexpressing hiPS-NSCs may be used in autologous ex vivo gene therapy protocols to provide long-lasting enzymatic supply in MLD-affected brains. Stem Cells Translational Medicine 2017;6:352-368.

Meng, X., et al. (2012). "Erythroid promoter confines FGF2 expression to the marrow after hematopoietic stem cell gene therapy and leads to enhanced endosteal bone formation." PLoS One **7**(5): e37569.

Fibroblast growth factor-2 (FGF2) has been demonstrated to be a promising osteogenic factor for treating osteoporosis. Our earlier study shows that transplantation of mouse Sca-1(+) hematopoietic stem/progenitor cells that are engineered to express a modified FGF2 leads to considerable endosteal/trabecular bone formation, but it also induces adverse effects like hypocalemia and osteomalacia. Here we report that the use of an erythroid specific promoter, beta-globin, leads to a 5-fold decrease in the ratio of serum FGF2 to the FGF2 expression in the marrow cavity when compared to the use of a ubiquitous promoter spleen focus-forming virus (SFFV). The confined FGF2 expression promotes considerable trabeculae bone formation in endosteum and does not yield anemia and osteomalacia. The avoidance of anemia in the mice that received Sca1(+) cells transduced with FGF2 driven by the beta-globin promoter is likely due to attenuation of high-level serum FGF2-mediated stem cell mobilization observed in the SFFV-FGF2 animals. The prevention of osteomalacia is associated with substantially reduced serum Fgf23/hypophosphatemia, and less pronounced secondary hyperparathyroidism. Our improved stem cell gene therapy strategy represents one step closer to FGF2-based clinical therapy for systemic skeletal augmentation.

Menon, A. and S. Vijayavenkataraman (2022). "Novel vision restoration techniques: 3D bioprinting, gene and stem cell therapy, optogenetics, and the bionic eye." Artif Organs **46**(8): 1463-1474.

BACKGROUND: Vision restoration has been one of the most sought-after goals of ophthalmology because of its inception. Despite these problems being tackled from numerous different perspectives, a concrete solution has not yet been achieved. An optimal solution will have significant implications on the patient's quality of life, socioeconomic status, and mental health. METHODS: This article will explore new and innovative approaches with one common aim-to restore functional vision for the visually impaired. These novel techniques include 3D bioprinting, stem cell therapy, gene therapy, implantable devices, and optogenetics. RESULTS: While the techniques mentioned above show significant promise, they are currently in various stages of development ranging from clinical trials to commercial availability. Restoration of minimal vision in specific cases has already been achieved by the different methods but optimization of different parameters like biocompatibility, spatiotemporal resolution, and minimizing the costs are essential for widespread use. CONCLUSION: The developments over the past decade have resulted in multiple milestones in each of the techniques with many solutions getting approved by the FDA. This article will compare these novel techniques and highlight the major advantages and drawbacks of each of them.

Meyer-Berg, H., et al. (2020). "Identification of AAV serotypes for lung gene therapy in human embryonic stem cell-derived lung organoids." Stem Cell Res Ther **11**(1): 448.

Gene therapy is being investigated for a range of serious lung diseases, such as cystic fibrosis and emphysema. Recombinant adeno-associated virus (rAAV) is a well-established, safe, viral vector for gene delivery with multiple naturally occurring and artificial serotypes available displaying alternate cell, tissue, and species-specific tropisms. Efficient AAV serotypes for the transduction of the conducting airways have been identified for several species; however, efficient serotypes for human lung parenchyma have not yet been identified. Here, we screened the ability of multiple AAV serotypes to transduce lung bud organoids (LBOs)-a model of human lung parenchyma generated from human embryonic stem cells. Microinjection of LBOs allowed us to model transduction from the luminal surface, similar to dosing via vector inhalation. We identified the naturally occurring rAAV2 and rAAV6 serotypes, along with synthetic rAAV6 variants, as having tropism for the human lung parenchyma. Positive staining of LBOs for surfactant proteins B and C confirmed distal lung identity and suggested the suitability of these vectors for the transduction of alveolar type II cells. Our findings establish LBOs as a new model for pulmonary gene therapy and stress the relevance of LBOs as a viral infection model of the lung parenchyma as relevant in SARS-CoV-2 research.

Miller, C. L., et al. (2002). "Feasibility of using autologous transplantation to evaluate hematopoietic stem cell-based gene therapy strategies in transgenic mouse models of human disease." Mol Ther **6**(3): 422-428.

Histoincompatibility between murine donors and recipients of bone marrow (BM) transplants reduces engraftment, and this compromises assessment of hematopoietic stem cells (HSCs) in certain transgenic mice. To study HSCs in the S+S-Antilles mouse model of human sickle cell disease (SCD), we developed an autotransplant protocol. Initial experiments showed no differences between S+S-Antilles mice and normal C57BL/6 (+/+) mice in their radiosensitivity or baseline hematopoietic progenitor numbers. The kinetics of red blood cell (RBC) replacement post-transplant in +/+ recipients of mixtures of transgenic and +/+ BM cells also showed no competitive advantage of the +/+ cells. BM cells were then aspirated from mice 4 days after 5-fluorouracil treatment, transduced with a green fluorescent protein (GFP)-encoding retrovirus, and transplanted into the same recipients that, just before transplant, were irradiated with 800 cGy. We subsequently detected high levels of GFP(+) RBCs (21-79%) and white blood cells (WBCs; 35-88%) in the blood for 11 months and showed that transduced HSCs regenerated in the primary mice also repopulated secondary mice. These findings provide a generally applicable protocol for performing autotransplants in mice and forecast the potential utility of this approach in assessing HSC-based gene therapy protocols in transgenic mouse models of many human diseases.

Miranda, S. R., et al. (2000). "Hematopoietic stem cell gene therapy leads to marked visceral organ improvements and a delayed onset of neurological abnormalities in the acid sphingomyelinase deficient mouse model of Niemann-Pick disease." Gene Ther **7**(20): 1768-1776.

Types A and B Niemann-Pick disease (NPD) result from the deficient activity of acid sphingomyelinase (ASM). Currently, no treatment is available for either form of NPD. Using the ASM knockout (ASMKO) mouse model, we evaluated the effects of ex vivo hematopoietic stem cell gene therapy on the NPD phenotype. Thirty-two newborn ASMKO mice were preconditioned with low dose radiation (200

cGy) and transplanted with ASMKO bone marrow cells which had been transduced with an ecotropic retroviral vector encoding human ASM. Engraftment of donor-derived cells ranged from 15 to 60% based on Y-chromosome in situ hybridization analysis of peripheral white blood cells, and was achieved in 92% of the transplanted animals. High levels of ASM activity (up to five-fold above normal) were found in the engrafted animals for up to 10 months after transplantation, and their life-span was extended from a mean of 5 to 9 months by the gene therapy procedure. Biochemical and histological analysis of tissues obtained 4-5 months after transplantation indicated that the ASM activities were increased and the sphingomyelin storage was significantly reduced in the spleens, livers and lungs of the treated mice, major sites of pathology in type B NPD. The presence of Purkinje cell neurons was also markedly increased in the treatment group as compared with non-treated animals at 5 months after transplantation, and a reduction of storage in spinal cord neurons was observed. However, all of the transplanted mice eventually developed ataxia and died earlier than normal mice. Overall, these results indicated that hematopoietic stem cell gene therapy should be effective for the treatment of non-neurological type B NPD, but improved techniques for targeting the transplanted cells and/or expressed enzyme to specific sites of pathology in the central nervous system must be developed in order to achieve effective treatment for type A NPD.

Miwa, S., et al. (2020). "Efficient engraftment of genetically modified cells is necessary to ameliorate central nervous system involvement of murine model of mucopolysaccharidosis type II by hematopoietic stem cell targeted gene therapy." Mol Genet Metab **130**(4): 262-273.

Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disease (LSD) caused by a deficiency of the iduronate-2-sulfatase (IDS) that catabolizes glycosaminoglycans (GAGs). Abnormal accumulations of GAGs in somatic cells lead to various manifestations including central nervous system (CNS) disease. Enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) are the currently available therapy for MPS II, but both therapies fail to improve CNS manifestations. We previously showed that hematopoietic stem cell targeted gene therapy (HSC-GT) with lethal irradiation improved CNS involvement in a murine model of MPS II which lacks the gene coding for IDS. However, the strong preconditioning, with lethal irradiation, would cause a high rate of morbidity and mortality. Therefore, we tested milder preconditioning procedures with either low dose irradiation or low dose irradiation plus an anti c-kit monoclonal antibody (ACK2) to assess CNS effects in mice with MPS II after HSC-GT. Mice from all the HSC-GT groups displayed super-physiological levels of IDS enzyme activity and robust reduction of abnormally accumulated GAGs to the wild type mice levels in peripheral organs. However, only the mice treated with lethal irradiation showed significant cognitive function improvement as well as IDS elevation and GAG reduction in the brain. These results suggest that an efficient engraftment of genetically modified cells for HSC-GT requires strong preconditioning to ameliorate CNS involvement in cases with MPS II.

Moayeri, M., et al. (2005). "Correction of murine hemophilia A by hematopoietic stem cell gene therapy." Mol Ther **12**(6): 1034-1042.

A serious complication of current protein replacement therapy for hemophilia A patients with coagulation factor VIII (FVIII) deficiency is the frequent development of anti-FVIII inhibitor antibodies that preclude therapeutic benefit from further treatment. Induction of tolerance by persistent high-level FVIII synthesis following transplantation with hematopoietic stem cells expressing a retrovirally delivered FVIII transgene offers the possibility of permanently correcting the disease. Here, we transplanted bone marrow cells transduced with an optimized MSCV-based FVIII oncoretroviral vector into immunocompetent hemophilia A mice that had been conditioned with a potentially lethal dose of irradiation (800 cGy), a sublethal dose of irradiation (550 cGy), or a nonmyeloablative preparative regimen involving busulfan. Therapeutic levels of FVIII (42, 18, and 11% of normal, respectively) were detected in the plasma of the transplant recipients for the duration of the study (over 6 months). Moreover, subsequent challenge with recombinant FVIII elicited at most a minor anti-FVIII inhibitor antibody response in any of the experimental animals, in contrast to the vigorous neutralizing humoral reaction to FVIII that was stimulated in naive hemophilia A mice. These findings represent an encouraging advance toward potential clinical application and long-term amelioration or cure of this progressively debilitating, life-threatening bleeding disorder.

Mohajeri, M., et al. (2011). "FOXP3 gene expression in multiple sclerosis patients pre- and post mesenchymal stem cell therapy." Iran J Allergy Asthma Immunol **10**(3): 155-161.

Multiple Sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disorder of the central nervous system (CNS), which mainly affects young adults. Activated T lymphocytes promote the neuro-inflammatory cascade of MS by secreting pro-

inflammatory cytokines and play a significant role in its pathogenesis. T lymphocytes may trigger the inflammation, which in turn leads to axonal loss and neurodegeneration observed in the course of MS. Currently, there is no cure for MS, however, one of the most promising neuroprotective research tools consists of the use of bone marrow derived mesenchymal stem cells (MSC). This method promotes immune system regulation and possibly induces neurological repair and re-myelination of the damaged axons. Recent studies have shown that MSC exert an immune regulatory function and induce T regulatory-cell proliferation, therefore, it may serve as a potentially useful treatment for immune-mediated diseases such as MS. In this pilot study a group of MS patients underwent MSC therapy and we assayed the expression of an X-linked transcription factor, FoxP3, as a specific marker of T Regulatory cells in peripheral blood, prior to and after the treatment. Using q RT-PCR for measurement of expression of FoxP3 by peripheral blood mononuclear cells, we found that in all subjects, except for one, the expression of FoxP3 at 6 months after intrathecal injection of MSC was significantly higher than the levels prior to treatment. Such significant enhanced expression of FoxP3 associated with clinical stability. Findings from this pilot study further support the potential of bone marrow derived MSC for treatment of MS patients.

Mohammadi, M., et al. (2016). "Mesenchymal stem cell: a new horizon in cancer gene therapy." Cancer Gene Ther **23**(9): 285-286.

Cancer is one of the main problems in public health worldwide. Despite rapid advances in the diagnosis and treatment of cancer, the efficacy of current treatment strategies is still limited. There are promising new results in animal models whereby mesenchymal stem cells (MSCs) can be used as vehicles for targeted therapies. The use of MSCs as therapeutic biological vehicles in cell therapy has several advantages, including immune-silence, tumor tropism, easy and rapid isolation, ex vivo expansion, multilineage differentiation and the capacity to deliver a number of therapeutic agents. Some studies have shown that the microenvironment of the tumor provides a preferential niche for homing and survival of MSCs. Here, we have highlighted various applications of MSCs in cancer gene therapy.

Mohammadian, M., et al. (2016). "Mesenchymal stem cell-based gene therapy: A promising therapeutic strategy." Artif Cells Nanomed Biotechnol **44**(5): 1206-1211.

Mesenchymal stem cells (MSCs) are multipotent stromal cells that exist in bone marrow, fat, and so many other tissues, and can differentiate into a variety of cell types including osteoblasts, chondrocytes, and adipocytes, as well as myocytes and neurons. Moreover, they have great capacity for self-renewal while maintaining their multipotency. Their capacity for proliferation and differentiation, in addition to their immunomodulatory activity, makes them very promising candidates for cell-based regenerative medicine. Moreover, MSCs have the ability of mobilization to the site of damage; therefore, they can automatically migrate to the site of injury via their chemokine receptors following intravenous transplantation. In this respect, they can be applied for MSC-based gene therapy. In this new therapeutic method, genes of interest are introduced into MSCs via viral and non-viral-based methods that lead to transgene expression in them. Although stem cell-based gene therapy is a relatively new strategy, it lights a new hope for the treatment of a variety of genetic disorders. In the near future, MSCs can be of use in a vast number of clinical applications, because of their uncomplicated isolation, culture, and genetic manipulation. However, full consideration is still crucial before they are utilized for clinical trials, because the number of studies that signify the advantageous effects of MSC-based gene therapy are still limited.

Morgan, R. A., et al. (2017). "Hematopoietic Stem Cell Gene Therapy: Progress and Lessons Learned." Cell Stem Cell **21**(5): 574-590.

The use of allogeneic hematopoietic stem cells (HSCs) to treat genetic blood cell diseases has become a clinical standard but is limited by the availability of suitable matched donors and potential immunologic complications. Gene therapy using autologous HSCs should avoid these limitations and thus may be safer. Progressive improvements in techniques for genetic correction of HSCs, by either vector gene addition or gene editing, are facilitating successful treatments for an increasing number of diseases. We highlight the progress, successes, and remaining challenges toward the development of HSC gene therapies and discuss lessons they provide for the development of future clinical stem cell therapies.

Moriarty, N., et al. (2022). "A combined cell and gene therapy approach for homotopic reconstruction of midbrain dopamine pathways using human pluripotent stem cells." Cell Stem Cell **29**(3): 434-448 e435.

Midbrain dopamine (mDA) neurons can be replaced in patients with Parkinson's disease (PD) in order to provide long-term improvement in motor functions. The limited capacity for long-distance axonal growth in the adult brain means that cells are transplanted ectopically, into the striatal target. As a consequence, several mDA pathways are not re-

instated, which may underlie the incomplete restoration of motor function in patients. Here, we show that viral delivery of GDNF to the striatum, in conjunction with homotopic transplantation of human pluripotent stem-cell-derived mDA neurons, recapitulates brain-wide mDA target innervation. The grafts provided re-instatement of striatal dopamine levels and correction of motor function and also connectivity with additional mDA target nuclei not well innervated by ectopic grafts. These results demonstrate the remarkable capacity for achieving functional and anatomically precise reconstruction of long-distance circuitry in the adult brain by matching appropriate growth-factor signaling to grafting of specific cell types.

Moutsatsos, I. K., et al. (2001). "Exogenously regulated stem cell-mediated gene therapy for bone regeneration." Mol Ther **3**(4): 449-461.

Regulated expression of transgene production and function is of great importance for gene therapy. Such regulation can potentially be used to monitor and control complex biological processes. We report here a regulated stem cell-based system for controlling bone regeneration, utilizing genetically engineered mesenchymal stem cells (MSCs) harboring a tetracycline-regulated expression vector encoding the osteogenic growth factor human BMP-2. We show that doxycycline (a tetracycline analogue) is able to control hBMP-2 expression and thus control MSC osteogenic differentiation both in vitro and in vivo. Following in vivo transplantation of genetically engineered MSCs, doxycycline administration controlled both bone formation and bone regeneration. Moreover, our findings showed increased angiogenesis accompanied by bone formation whenever genetically engineered MSCs were induced to express hBMP-2 in vivo. Thus, our results demonstrate that regulated gene expression in mesenchymal stem cells can be used as a means to control bone healing.

Mu, X., et al. (2018). "siRNA Delivery with Stem Cell Membrane-Coated Magnetic Nanoparticles for Imaging-Guided Photothermal Therapy and Gene Therapy." ACS Biomater Sci Eng **4**(11): 3895-3905.

Biomimetic cell membrane coated nanoparticles (NPs) with desirable features have been extensively applied for various personalized biomedicine. However, there have not been relative explorations by employing the membrane nanocomplexes for small interfering RNA (siRNA) delivery. Herein, Fe(3)O(4)@PDA NPs with good photothermal capability were applied for efficient siRNA loading and delivery, which were then coated by mesenchymal stem cells (MSCs) to form a membrane. The data showed that MSCs membrane coated Fe(3)O(4)@PDA-siRNA NPs (Fe(3)O(4)@PDA-siRNA@MSCs) maintained the photothermal functionality and the capability of magnetic resonance imaging inherited from Fe(3)O(4)@PDA. The synthesized nanocomplexes exhibited excellent abilities in the delivery of siRNA into DU145 cells. Furthermore, Fe(3)O(4)@PDA-siRNA@MSCs NPs delivering siRNA against Plk1 gene could inhibit the expression of endogenous Plk1 gene and cause obvious apoptosis in DU145 cells. The synergistic combination of photothermal treatment and gene silencing showed obvious antitumor efficacy in a DU145 xenograft mice model. On the basis of preliminary in vitro and in vivo studies, Fe(3)O(4)@PDA-siRNA@MSCs NPs hold considerable promise as a carrier for gene and photothermal therapy.

Muller, A. M., et al. (2016). "Hypoxia-targeted 131I therapy of hepatocellular cancer after systemic mesenchymal stem cell-mediated sodium iodide symporter gene delivery." Oncotarget **7**(34): 54795-54810.

Adoptively transferred mesenchymal stem cells (MSCs) home to solid tumors. Biologic features within the tumor environment can be used to selectively activate transgenes in engineered MSCs after tumor invasion. One of the characteristic features of solid tumors is hypoxia. We evaluated a hypoxia-based imaging and therapy strategy to target expression of the sodium iodide symporter (NIS) gene to experimental hepatocellular carcinoma (HCC) delivered by MSCs.MSCs engineered to express transgenes driven by a hypoxia-responsive promoter showed robust transgene induction under hypoxia as demonstrated by mCherry expression in tumor cell spheroid models, or radioiodide uptake using NIS. Subcutaneous and orthotopic HCC xenograft mouse models revealed significant levels of perchlorate-sensitive NIS-mediated tumoral radioiodide accumulation by tumor-recruited MSCs using 123I-scintigraphy or 124I-positron emission tomography. Functional NIS expression was further confirmed by ex vivo 123I-biodistribution analysis. Administration of a therapeutic dose of 131I in mice treated with NIS-transfected MSCs resulted in delayed tumor growth and reduced tumor perfusion, as shown by contrast-enhanced sonography, and significantly prolonged survival of mice bearing orthotopic HCC tumors. Interestingly, radioiodide uptake into subcutaneous tumors was not sufficient to induce therapeutic effects. Our results demonstrate the potential of using tumor hypoxia-based approaches to drive radioiodide therapy in non-thyroidal tumors.

Murray, J. M., et al. (2009). "Mathematical modelling of the impact of haematopoietic stem cell-delivered gene therapy for HIV." J Gene Med **11**(12): 1077-1086.

BACKGROUND: Gene therapy represents a new treatment paradigm for HIV that is potentially delivered by a safe, once-only therapeutic intervention. METHODS: Using mathematical modelling, we assessed the possible impact of autologous haematopoietic stem cell (HSC) delivered, anti-HIV gene therapy. The therapy comprises a ribozyme construct (OZ1) directed to a conserved region of HIV-1 delivered by transduced HSC (OZ1+HSC). OZ1+HSC contributes to the CD4+ T lymphocyte and monocyte/macrophage cell pools that preferentially expand under the selective pressure of HIV infection. The model was used to predict the efficacy of OZ1 in a highly active antiretroviral therapy (HAART) naive individual and a HAART-experienced individual undergoing two structured treatment operations. In the standard scenario, OZ1+HSC was taken as 20% of total body HSC. RESULTS: For a HAART-naive individual, modelling predicts a reduction of HIV RNA at 1 and 2 years post-OZ1 therapy of 0.5 log(10) and 1 log(10), respectively. Eight years after OZ1 therapy, the CD4+ T-lymphocyte count was 271 cells/mm(3) compared to 96 cells/mm(3) for an untreated individual. In a HAART-experienced individual HIV RNA was reduced by 0.34 log(10) and 0.86 log(10) at 1 and 2 years. The OZ1 effect was maximal when both CD4+ T lymphocytes and monocytes/macrophages were protected from successful, productive infection by OZ1. CONCLUSIONS: The modelling indicates a single infusion of HSC cell-delivered gene therapy can impact on HIV viral load and CD4 T-lymphocyte count. Given that gene therapy avoids the complications associated with HAART, there is significant potential for this approach in the treatment of HIV.

Ourednik, V., et al. (2000). "Neural stem cells are uniquely suited for cell replacement and gene therapy in the CNS." Novartis Found Symp **231**: 242-262; discussion 262-249, 302-246.

In recent years, it has become evident that the developing and even the adult mammalian CNS contain a population of undifferentiated, multipotent cell precursors, neural stem cells, the plastic properties of which might be of advantage for the design of more effective therapies for many neurological diseases. This article reviews the recent progress in establishing rodent and human clonal neural stem cell lines, their biological properties, and how these cells can be utilized to correct a variety of defects, with prospects for the near future to harness their behaviour for neural stem cell-based treatment of diseases in humans.

Ozawa, K., et al. (2008). "Cell and gene therapy using mesenchymal stem cells (MSCs)." J Autoimmun **30**(3): 121-127.

Mesenchymal stem cells (MSCs) are considered to be a promising platform for cell and gene therapy for a variety of diseases. First, in the field of hematopoietic stem cell transplantation, there are two applications of MSCs: 1) the improvement of stem cell engrafting and the acceleration of hematopoietic reconstitution based on the hematopoiesis-supporting ability; and 2) the treatment of severe graft-versus-host disease (GVHD) based on the immunomodulatory ability. Regarding the immunosuppressive ability, we found that nitric oxide (NO) is involved in the MSC-mediated suppression of T cell proliferation. Second, tumor-bearing nude mice were injected with luciferase-expressing MSCs. An in vivo imaging analysis showed the significant accumulation of the MSCs at the site of tumors. The findings suggest that MSCs can be utilized to target metastatic tumors and to deliver anti-cancer molecules locally. As the third application, MSCs may be utilized as a cellular vehicle for protein-supplement gene therapy. When long-term transgene expression is needed, a therapeutic gene should be introduced with a minimal risk of insertional mutagenesis. To this end, site-specific integration into the AAVS1 locus on the chromosome 19 (19q13.4) by using the integration machinery of adeno-associated virus (AAV) would be particularly valuable. There will be wide-ranging applications of MSCs to frontier medical treatments in the near future.

Portnow, J., et al. (2017). "Neural Stem Cell-Based Anticancer Gene Therapy: A First-in-Human Study in Recurrent High-Grade Glioma Patients." Clin Cancer Res **23**(12): 2951-2960.

Purpose: Human neural stem cells (NSC) are inherently tumor tropic, making them attractive drug delivery vehicles. Toward this goal, we retrovirally transduced an immortalized, clonal NSC line to stably express cytosine deaminase (HB1.F3.CD.C21; CD-NSCs), which converts the prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU).Experimental Design: Recurrent high-grade glioma patients underwent intracranial administration of CD-NSCs during tumor resection or biopsy. Four days later, patients began taking oral 5-FC every 6 hours for 7 days. Study treatment was given only once. A standard 3 + 3 dose escalation schema was used to increase doses of CD-NSCs from 1 x 10(7) to 5 x 10(7) and 5-FC from 75 to 150 mg/kg/day. Intracerebral microdialysis was performed to measure brain levels of 5-FC and 5-FU. Serial blood samples were obtained to assess systemic drug concentrations as well as to perform immunologic correlative studies.Results: Fifteen patients underwent study treatment. We saw no dose-limiting toxicity

(DLT) due to the CD-NSCs. There was 1 DLT (grade 3 transaminitis) possibly related to 5-FC. We did not see development of anti-CD-NSC antibodies and did not detect CD-NSCs or replication-competent retrovirus in the systemic circulation. Intracerebral microdialysis revealed that CD-NSCs produced 5-FU locally in the brain in a 5-FC dose-dependent manner. Autopsy data indicate that CD-NSCs migrated to distant tumor sites and were nontumorigenic.Conclusions: Collectively, our results from this first-in-human study demonstrate initial safety and proof of concept regarding the ability of NSCs to target brain tumors and locally produce chemotherapy. Clin Cancer Res; 23(12); 2951-60. (c)2016 AACR.

Powers, J. M. and G. D. Trobridge (2013). "Identification of Hematopoietic Stem Cell Engraftment Genes in Gene Therapy Studies." J Stem Cell Res Ther **2013**(Suppl 3): S3:004-.

Hematopoietic stem cell (HSC) therapy using replication-incompetent retroviral vectors is a promising approach to provide life-long correction for genetic defects. HSC gene therapy clinical studies have resulted in functional cures for several diseases, but in some studies clonal expansion or leukemia has occurred. This is due to the dyregulation of endogenous host gene expression from vector provirus insertional mutagenesis. Insertional mutagenesis screens using replicating retroviruses have been used extensively to identify genes that influence oncogenesis. However, retroviral mutagenesis screens can also be used to determine the role of genes in biological processes such as stem cell engraftment. The aim of this review is to describe the potential for vector insertion site data from gene therapy studies to provide novel insights into mechanisms of HSC engraftment. In HSC gene therapy studies dysregulation of host genes by replication-incompetent vector proviruses may lead to enrichment of repopulating clones with vector integrants near genes that influence engraftment. Thus, data from HSC gene therapy studies can be used to identify novel candidate engraftment genes. As HSC gene therapy use continues to expand, the vector insertion site data collected will be of great interest to help identify novel engraftment genes and may ultimately lead to new therapies to improve engraftment.

Prelle, K., et al. (2002). "Pluripotent stem cells--model of embryonic development, tool for gene targeting, and basis of cell therapy." Anat Histol Embryol **31**(3): 169-186.

Embryonic stem (ES) cells are pluripotent cell lines with the capacity of self-renewal and a broad differentiation plasticity. They are derived from pre-implantation embryos and can be propagated as a homogeneous, uncommitted cell population for an almost unlimited period of time without losing their pluripotency and their stable karyotype. Murine ES cells are able to reintegrate fully into embryogenesis when returned into an early embryo, even after extensive genetic manipulation. In the resulting chimeric offspring produced by blastocyst injection or morula aggregation, ES cell descendants are represented among all cell types, including functional gametes. Therefore, mouse ES cells represent an important tool for genetic engineering, in particular via homologous recombination, to introduce gene knock-outs and other precise genomic modifications into the mouse germ line. Because of these properties ES cell technology is of high interest for other model organisms and for livestock species like cattle and pigs. However, in spite of tremendous research activities, no proven ES cells colonizing the germ line have yet been established for vertebrate species other than the mouse (Evans and Kaufman, 1981; Martin, 1981) and chicken (Pain et al., 1996). The in vitro differentiation capacity of ES cells provides unique opportunities for experimental analysis of gene regulation and function during cell commitment and differentiation in early embryogenesis. Recently, pluripotent stem cells were established from human embryos (Thomson et al., 1998) and early fetuses (Shamblott et al., 1998), opening new scenarios both for research in human developmental biology and for medical applications, i.e. cell replacement strategies. At about the same time, research activities focused on characteristics and differentiation potential of somatic stem cells, unravelling an unexpected plasticity of these cell types. Somatic stem cells are found in differentiated tissues and can renew themselves in addition to generating the specialized cell types of the tissue from which they originate. Additional to discoveries of somatic stem cells in tissues that were previously not thought to contain these kinds of cells, they also appear to be capable of developing into cell types of other tissues, but have a reduced differentiation potential as compared to embryo-derived stem cells. Therefore, somatic stem cells are referred to as multipotent rather than pluripotent. This review summarizes characteristics of pluripotent stem cells in the mouse and in selected livestock species, explains their use for genetic engineering and basic research on embryonic development, and evaluates their potential for cell therapy as compared to somatic stem cells.

Prockop, D. J., et al. (2003). "One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues." Proc Natl Acad Sci U S A **100 Suppl 1**(Suppl 1): 11917-11923.

Most recent evidence suggests that the process of tissue repair is driven by stem-like cells that reside in multiple tissues but are replenished by precursor

cells from bone marrow. Among the candidates for the reparative cells are the adult stem cells from bone marrow referred to as either mesenchymal stem cells or marrow stromal cells (MSCs). We recently found that after MSCs were replated at very low densities to generate single-cell-derived colonies, they did not exit a prolonged lag period until they synthesized and secreted considerable quantities of Dickkopf-1, an inhibitor of the canonical Wnt signaling pathway. We also found that when the cells were cocultured with heat-shocked pulmonary epithelial cells, they differentiated into epithelial cells. Most of the MSCs differentiated without evidence of cell fusion but up to one-quarter underwent cell fusion with the epithelial cells. A few also underwent nuclear fusion. The results are consistent with the interesting possibility that MSCs and similar cells repair tissue injury by three different mechanisms: creation of a milieu that enhances regeneration of endogenous cells, transdifferentiation, and perhaps cell fusion.

Psatha, N., et al. (2016). "Optimizing autologous cell grafts to improve stem cell gene therapy." Exp Hematol **44**(7): 528-539.

Over the past decade, stem cell gene therapy has achieved unprecedented curative outcomes for several genetic disorders. Despite the unequivocal success, clinical gene therapy still faces challenges. Genetically engineered hematopoietic stem cells are particularly vulnerable to attenuation of their repopulating capacity once exposed to culture conditions, ultimately leading to low engraftment levels posttransplant. This becomes of particular importance when transduction rates are low or/and competitive transplant conditions are generated by reduced-intensity conditioning in the absence of a selective advantage of the transduced over the unmodified cells. These limitations could partially be overcome by introducing megadoses of genetically modified CD34(+) cells into conditioned patients or by transplanting hematopoietic stem cells hematopoietic stem cells with high engrafting and repopulating potential. On the basis of the lessons gained from cord blood transplantation, we summarize the most promising approaches to date of increasing either the numbers of hematopoietic stem cells for transplantation or/and their engraftability, as a platform toward the optimization of engineered stem cell grafts.

Psatha, N., et al. (2014). "Superior long-term repopulating capacity of G-CSF+plerixafor-mobilized blood: implications for stem cell gene therapy by studies in the Hbb(th-3) mouse model." Hum Gene Ther Methods **25**(6): 317-327.

High numbers of genetically modified hematopoietic stem cells (HSCs) equipped with enhanced engrafting potential are required for successful stem cell gene therapy. By using thalassemia as a model, we investigated the functional properties of hematopoietic stem and progenitor cells (HSPCs) from Hbb(th3)/45.2(+) mice after mobilization with G-CSF, plerixafor, or G-CSF+plerixafor and the engraftment kinetics of primed cells after competitive primary and noncompetitive secondary transplantation. G-CSF+plerixafor yielded the highest numbers of HSPCs, while G-CSF+plerixafor-mobilized Hbb(th3)/45.2(+) cells, either unmanipulated or transduced with a reporter vector, achieved faster hematologic reconstitution and higher levels of donor chimerism over all other types of mobilized cells, after competitive transplantation to B6.BoyJ/45.1(+) recipients. The engraftment benefit observed in the G-CSF+plerixafor group was attributed to the more primitive stem cell phenotype of G-CSF+plerixafor-LSK cells, characterized by higher CD150(+)/CD48 expression. Moreover, secondary G-CSF+plerixafor recipients displayed stable or even higher chimerism levels as compared with primary engrafted mice, thus maintaining or further improving engraftment levels over G-CSF- or plerixafor-secondary recipients. Plerixafor-primed cells displayed the lowest competiveness over all other mobilized cells after primary or secondary transplantation, probably because of the higher frequency of more actively proliferating LK cells. Overall, the higher HSC yields, the faster hematological recovery, and the superiority in long-term engraftment indicate G-CSF+plerixafor-mobilized blood as an optimal graft source, not only for thalassemia gene therapy, but also for stem cell gene therapy applications in general.

Qasim, W., et al. (2005). "T cell suicide gene therapy to aid haematopoietic stem cell transplantation." Curr Gene Ther **5**(1): 121-132.

Graft versus host disease (GVHD) is a T cell mediated phenomenon that arises following allogeneic haematopoietic stem cell transplantation, and may be particularly severe in the context of human leukocyte antigen (HLA) mismatched procedures. Although GVHD can be largely abrogated through T cell depletion, such measures result in loss of graft potency and reduced anti-viral and anti-leukaemic effects. The genetic modification of T cells to carry a suicide gene mechanism has been advocated as means of allowing T cells to be harnessed for their beneficial effects, and safely eliminated in the event of significant GVHD. The feasibility of the strategy has been demonstrated in clinical studies using T cells modified by retroviral transduction to encode the herpes simplex thymidine kinase (HSVTK) gene to treat patients with haematological malignancies. However, a number of limitations associated with current protocols have

become apparent. Most notably, the process of retroviral transduction, which requires pre-activation of T cells, appears to impair subsequent functional potential. Efforts are now directed towards circumventing the pre-activation requirements of retroviral vectors by using alternative lentiviral systems, in association with improved suicide gene/prodrug combinations.

Qiu, B., et al. (2010). "Dual transfer of GFP gene and MGd into stem-progenitor cells: toward in vivo MRI of stem cell-mediated gene therapy of atherosclerosis." Acad Radiol **17**(5): 547-552.

RATIONALE AND OBJECTIVES: The aim of this study was to develop a new technique, the use of magnetic resonance (MR) imaging (MRI) to monitor gene/MR-cotransferred stem-progenitor cells (SPCs) recruited to atherosclerosis. MATERIALS AND METHODS: First, a green fluorescent protein (GFP) gene and a T1 MR contrast agent (motexafin gadolinium [MGd]) were cotransferred into neural or bone marrow (BM)-derived SPCs. GFP expression and MGd signal were confirmed by fluorescent microscopy and quantified by flow cytometry. Cell viability and proliferation were then evaluated by trypan blue exclusion and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay, and GFP/MGd-transferred cells were imaged using 1.5-T and 9.4-T MR scanners. For in vivo validation, GFP/MGd-cotransferred beta-galactosidase-BM SPCs were transplanted to apolipoprotein E-knockout mice, and cell migration to atherosclerotic aortas was monitored using 9.4-T micro-MRI with subsequent histologic correlations. RESULTS: Fluorescent microscopy demonstrated simultaneous GFP expression and MGd signals in cotransferred-cells. Quantitative flow cytometry showed GFP-positive cells at 47 +/- 25% and 56 +/- 12% and MGd-positive cells at 96 +/- 6% and 57 +/- 11% for neural stem cells and BM cells, respectively. Cell viability and metabolic rates of cotransferred cells were 86 +/- 4% and 84 +/- 12%, respectively. In vivo MRI revealed high MR signals of the aortic walls in GFP/MGd-transferred mice, which were confirmed by histologic correlations. CONCLUSION: This study has initially proven the new concept of MRI for plaque-specific, cell-mediated gene expression of atherosclerosis.

Qiu, X., et al. (2012). "Combined strategy of mesenchymal stem cell injection with vascular endothelial growth factor gene therapy for the treatment of diabetes-associated erectile dysfunction." J Androl **33**(1): 37-44.

This study was designed to investigate the effect of vascular endothelial growth factor 164 adenovirus (Ad-VEGF(164))-transfected mesenchymal stem cells (MSC) on improving erectile function in diabetic rats. Forty-five male Sprague-Dawley rats were injected with streptozotocin to develop type 1 diabetes, whereas 10 served as normal controls. Diabetic rats were randomly divided into 3 groups: rats that underwent intracavernous injection with phosphate-buffered saline (DM+PBS), unmodified MSCs (DM+MSC), and Ad-VEGF(164)-transfected MSCs (DM+VMSC). Normal controls received intracavernous injection of PBS. Four weeks after injection, erectile function was measured by cavernous nerve electrostimulation. Penile tissue was harvested for histology and enzyme-linked immunoassay. Prior to injection, high expression of VEGF was confirmed in Ad-VEGF(164)-transfected MSCs by enzyme-linked immunoassay. Four weeks after injection, the erectile function, as well as the content of smooth muscle and endothelium in corpus cavernosum increased significantly in the MSC-injected groups compared with the DM+PBS group. There was a significant improvement of erectile function, the content of smooth muscle and endothelium, and the VEGF concentration in the corpus cavernosum in the DM+VMSC group compared with the DM+MSC group. Our study validates the effect of intracavernous injection of MSCs for diabetes-associated erectile dysfunction in an animal model. The combined strategy of MSC injection with VEGF gene therapy-enhanced therapy of MSCs for the treatment of diabetes-associated erectile dysfunction.

Rachakatla, R. S., et al. (2007). "Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors." Cancer Gene Ther **14**(10): 828-835.

Umbilical cord matrix stem (UCMS) cells are unique stem cells derived from Wharton's jelly, which have been shown to express genes characteristic of primitive stem cells. To test the safety of these cells, human UCMS cells were injected both intravenously and subcutaneously in large numbers into severe combined immunodeficiency (SCID) mice and multiple tissues were examined for evidence of tumor formation. UCMS cells did not form gross or histological teratomas up to 50 days posttransplantation. Next, to evaluate whether UCMS cells could selectively engraft in xenotransplanted tumors, MDA 231 cells were intravenously transplanted into SCID mice, followed by intravenous transplantation of UCMS cells 1 and 2 weeks later. UCMS cells were found near or within lung tumors but not in other tissues. Finally, UCMS cells were engineered to express human interferon beta--designated 'UCMS-IFN-beta'. UCMS-IFN-beta cells were intravenously transplanted at multiple intervals into SCID mice

bearing MDA 231 tumors and their effect on tumors was examined. UCMS-IFN-beta cells significantly reduced MDA 231 tumor burden in SCID mouse lungs indicated by wet weight. These results clearly indicate safety and usability of UCMS cells in cancer gene therapy. Thus, UCMS cells can potentially be used for targeted delivery of cancer therapeutics.

Rachakatla, R. S., et al. (2008). "Combination treatment of human umbilical cord matrix stem cell-based interferon-beta gene therapy and 5-fluorouracil significantly reduces growth of metastatic human breast cancer in SCID mouse lungs." Cancer Invest **26**(7): 662-670.

Umbilical cord matrix stem (UCMS) cells that were engineered to express interferon-beta (IFN-beta) were transplanted weekly for three weeks into MDA 231 breast cancer xenografts bearing SCID mice in combination with 5-fluorouracil (5-FU). The UCMS cells were found within lung tumors but not in other tissues. Although both treatments significantly reduced MDA 231 tumor area in the SCID mouse lungs, the combined treatment resulted in a greater reduction in tumor area than by either treatment used alone. These results indicate that a combination treatment of UCMS-IFN-beta cells and 5-FU is a potentially effective therapeutic procedure for breast cancer.

Radtke, S., et al. (2020). "Mouse models in hematopoietic stem cell gene therapy and genome editing." Biochem Pharmacol **174**: 113692.

Gene therapy has become an important treatment option for a variety of hematological diseases. The biggest advances have been made with CAR T cells and many of those studies are now FDA approved as a routine treatment for some hematologic malignancies. Hematopoietic stem cell (HSC) gene therapy is not far behind with treatment approvals granted for beta-hemoglobinopathies and adenosine deaminase severe combined immune deficiency (ADA-SCID), and additional approbations currently being sought. With the current pace of research, the significant investment of biotech companies, and the continuously growing toolbox of viral as well as non-viral gene delivery methods, the development of new ex vivo and in vivo gene therapy approaches is at an all-time high. Research in the field of gene therapy has been ongoing for more than 4 decades with big success stories as well as devastating drawbacks along the way. In particular, the damaging effect of uncontrolled viral vector integration observed in the initial gene therapy applications in the 90s led to a more comprehensive upfront safety assessment of treatment strategies. Since the late 90s, an important read-out to comprehensively assess the quality and safety of cell products has come forward with the mouse xenograft model. Here, we review the use of mouse models across the different stages of basic, pre-clinical and translational research towards the clinical application of HSC-mediated gene therapy and editing approaches.

Rafi, M. A. (2011). "Gene and stem cell therapy: alone or in combination?" Bioimpacts **1**(4): 213-218.

INTRODUCTION: Both gene and stem cell therapies hold great promise in the treatment of many genetic diseases and are currently focus of interest for many investigators. While both approaches are offering great and valuable treatment options for devastating and life-threatening diseases, they hold much greater promise in combination. METHODS: As there are multiple options in selecting gene transfer vehicles among the non-viral and viral vectors, there are also many options among the different transplantable cell types ranging from lineage-restricted progenitor cells to multipotent and pluripotent stem cells. Here, combination of the gene therapy and stem cell therapy is discussed. RESULTS: Several suc-cessful gene and stem cell therapies have been reported both in animal and human trials. Combination of the gene therapy and stem cell therapy can be carried out sequentially where the cell transplantation and the in vivo gene therapy are accomplished one after the other; or, as it is more commonly practiced, they can be carried out as ex vivo gene therapy where the transplantable cells are genetically modified outside the body before being transplanted into the body. CONCLUSION: The combination of the stem-cell technology with gene therapy has the potential of providing both regenerative tissue and therapeutic material simultaneously; therefore, having the benefits of both technologies.

Richard, E., et al. (2004). "Hematopoietic stem cell gene therapy of murine protoporphyria by methylguanine-DNA-methyltransferase-mediated in vivo drug selection." Gene Ther **11**(22): 1638-1647.

Erythropoietic protoporphyria (EPP) is an inherited defect of the ferrochelatase (FECH) gene characterized by the accumulation of toxic protoporphyrin in the liver and bone marrow resulting in severe skin photosensitivity. We previously described successful gene therapy of an animal model of the disease with erythroid-specific lentiviral vectors in the absence of preselection of corrected cells. However, the high-level of gene transfer obtained in mice is not translatable to large animal models and humans if there is no selective advantage for genetically modified hematopoietic stem cells (HSCs) in vivo. We used bicistronic SIN-lentiviral vectors coexpressing EGFP or FECH and the G156A-mutated O6-methylguanine-DNA-methyltransferase (MGMT) gene, which allowed efficient in vivo selection of transduced HSCs after O6-benzylguanine and BCNU

treatment. We demonstrate for the first time that the correction and in vivo expansion of deficient transduced HSC population can be obtained by this dual gene therapy, resulting in a progressive increase of normal RBCs in EPP mice and a complete correction of skin photosensitivity. Finally, we developed a novel bipromoter SIN-lentiviral vector with a constitutive expression of MGMT gene to allow the selection of HSCs and with an erythroid-specific expression of the FECH therapeutic gene.

Rosengart, T. K., et al. (2012). "Cardiac biointerventions: whatever happened to stem cell and gene therapy?" Innovations (Phila) **7**(3): 173-179.

Angiogenic gene therapy and stem cell administration represent two "biologic" interventions for the treatment of cardiac disease that were first introduced more than 15 years ago but still have not achieved approval for clinical use for the treatment of myocardial ischemia and heart failure. Challenges that have been encountered in the clinical testing of these new treatment strategies have included a lack of placebo controls in phase I surgical trials and the incorporation of potentially ineffectual agent delivery via intracoronary routes. Although enthusiasm for these approaches may therefore have ebbed, new refinements in these technologies and insights into their appropriate clinical testing suggest that a resurgence of interest in these "biointerventions" may be expected in the near future.

Rosenzweig, M., et al. (1996). "In vitro T lymphopoiesis: a model system for stem cell gene therapy for AIDS." J Med Primatol **25**(3): 192-200.

Stable introduction of therapeutic genes into hematopoietic stem cells has the potential to reconstitute immunity in individuals with HIV infection. However, many important questions regarding the safety and efficacy of this approach remain unanswered and may be addressed in a non-human primate model. To facilitate evaluation of expression of foreign genes in T cells derived from transduced hematopoietic progenitor cells, we have established a culture system that supports the differentiation of rhesus macaque and human CD34+ bone marrow derived cells into mature T cells. Thymic stromal monolayers were prepared from the adherent cell fraction of collagenase digested fetal or neonatal thymus. After 10-14 days, purified rhesus CD34+ bone marrow-derived cells cultured on thymic stromal monolayers yielded CD3+CD4+CD8+, CD3+CD4+CD8-, and CD3+CD4-CD8+ cells. Following stimulation with mitogens, these T cells derived from CD34+ cells could be expanded over 1,000-fold and maintained in culture for up to 20 weeks. We next evaluated the ability of rhesus CD34+ cells transduced with a retroviral vector containing the marker gene neo to undergo in vitro T cell differentiation. CD34+ cells transduced in the presence of bone marrow stroma and then cultured on rhesus thymic stroma resulted in T cells containing the retroviral marker gene. These studies should facilitate both in vitro and in vivo studies of hematopoietic stem cell therapeutic strategies for AIDS.

Rossini, L., et al. (2022). "New Indications for Hematopoietic Stem Cell Gene Therapy in Lysosomal Storage Disorders." Front Oncol **12**: 885639.

Lysosomal storage disorders (LSDs) are a heterogenous group of disorders due to genetically determined deficits of lysosomal enzymes. The specific molecular mechanism and disease phenotype depends on the type of storage material. Several disorders affect the brain resulting in severe clinical manifestations that substantially impact the expectancy and quality of life. Current treatment modalities for LSDs include enzyme replacement therapy (ERT) and hematopoietic cell transplantation (HCT) from allogeneic healthy donors, but are available for a limited number of disorders and lack efficacy on several clinical manifestations. Hematopoietic stem cell gene therapy (HSC GT) based on integrating lentiviral vectors resulted in robust clinical benefit when administered to patients affected by Metachromatic Leukodystrophy, for whom it is now available as a registered medicinal product. More recently, HSC GT has also shown promising results in Hurler syndrome patients. Here, we discuss possible novel HSC GT indications that are currently under development. If these novel drugs will prove effective, they might represent a new standard of care for these disorders, but several challenges will need to be addresses, including defining and possibly expanding the patient population for whom HSC GT could be efficacious.

Rotin, L. E., et al. (2023). "A systematic review comparing allogeneic hematopoietic stem cell transplant to gene therapy in sickle cell disease." Hematology **28**(1): 2163357.

INTRODUCTION: Allogeneic hematopoietic stem cell transplant (HSCT) and gene therapy (GT) are two potentially curative approaches for sickle cell disease (SCD), but they have never been compared in clinical trials. OBJECTIVE: To compare the safety and efficacy of HSCT and GT to assist clinicians and patients in making informed treatment decisions. METHODS: Phase I-III clinical trials and case reports/series were included. Regimens included HSCT from all stem cell sources, lentiviral gene therapy, and gene editing, with any conditioning regimen. We searched Medline and EMBASE databases as of 1st June 2020 for studies reporting HSCT and GT

outcomes in SCD. The Newcastle-Ottawa scale was used to assess the risk of bias. Descriptive statistics and post-hoc imputation for standard deviations of mean change in FEV1 and FVC were performed. RESULTS: In total, 56 studies (HSCT, n = 53; GT, n = 3) representing 1,198 patients met inclusion criteria (HSCT, n = 1,158; GT, n = 40). Length of follow-up was 3,881.5 and 58.7 patient-years for HSCT and GT, respectively. Overall quality of evidence was low, with no randomized controlled trials identified. Two-year overall survival for HSCT was 91%; mortality was 2.5% for GT. Acute chest syndrome and vaso-occlusive episodes were reduced post-HSCT and GT. Meta-analysis was not possible due to lack of comparator and heterogeneity in outcome measures reporting. Very few studies reported post-transplant end-organ function. Six secondary malignancies (5 post-HSCT, 1 post-GT) were reported. DISCUSSION: Reporting of SCD-related complications and patient-important outcomes is lacking for both strategies. We advocate for standardized reporting to better compare outcomes within and between treatment groups.

Russell, A. L., et al. (2021). "Non-genotoxic conditioning facilitates hematopoietic stem cell gene therapy for hemophilia A using bioengineered factor VIII." Mol Ther Methods Clin Dev **21**: 710-727.

Hematopoietic stem and progenitor cell (HSPC) lentiviral gene therapy is a promising strategy toward a lifelong cure for hemophilia A (HA). The primary risks associated with this approach center on the requirement for pre-transplantation conditioning necessary to make space for, and provide immune suppression against, stem cells and blood coagulation factor VIII, respectively. Traditional conditioning agents utilize genotoxic mechanisms of action, such as DNA alkylation, that increase risk of sterility, infection, and developing secondary malignancies. In the current study, we describe a non-genotoxic conditioning protocol using an immunotoxin targeting CD117 (c-kit) to achieve endogenous hematopoietic stem cell depletion and a cocktail of monoclonal antibodies to provide transient immune suppression against the transgene product in a murine HA gene therapy model. This strategy provides high-level engraftment of hematopoietic stem cells genetically modified ex vivo using recombinant lentiviral vector (LV) encoding a bioengineered high-expression factor VIII variant, termed ET3. Factor VIII procoagulant activity levels were durably elevated into the normal range and phenotypic correction achieved. Furthermore, no immunological rejection or development of anti-ET3 immunity was observed. These preclinical data support clinical translation of non-genotoxic antibody-based conditioning in HSPC LV gene therapy for HA.

Ryu, C. H., et al. (2012). "Valproic acid enhances anti-tumor effect of mesenchymal stem cell mediated HSV-TK gene therapy in intracranial glioma." Biochem Biophys Res Commun **421**(3): 585-590.

Suicide gene therapy of glioma based on herpes simplex virus type I thymidine kinase (HSV-TK) and prodrug ganciclovir (GCV) suffers from the lack of efficacy in clinical trials, which is mostly due to low transduction efficacy and absence of bystander effect in tumor cells. Recently, stem cells as cellular delivery vehicles of prodrug converting gene has emerged as a new treatment strategy for malignant glioma. In this study, we evaluated the anti-glioma effect of suicide gene therapy using human bone marrow mesenchymal stem cells expressing HSV-TK (MSCs-TK) combined with valproic acid (VPA), which can upregulate the gap junction proteins and may enhance the bystander effect of suicide gene therapy. Expression of HSV-TK in MSCs was confirmed by RT-PCR analysis and the sensitivity of MSCs-TK to GCV was assessed. A bystander effect was observed in co-cultures of MSCs-TK and U87 glioma cells by GCV in a dose-dependent manner. VPA induced the expression of the gap junction proteins connexin (Cx) 43 and 26 in glioma cell and thereby enhanced the bystander effect in co-culture experiment. The enhanced bystander effect was inhibited by the gap junction inhibitor 18-beta-glycyrrhetinic acid (18-GA). Moreover, the combined treatment with VPA and MSCs-TK synergistically enhanced apoptosis in glioma cells by caspase activation. In vivo efficacy experiments showed that combination treatment of MSCs-TK and VPA significantly inhibited tumor growth and prolonged the survival of glioma-bearing mice compared with single-treatment groups. In addition, TUNEL staining also demonstrated a significant increase in the number of apoptotic cells in the combination treated group compared with single-treatment groups. Taken together, these results provide the rational for designing novel experimental protocols to increase bystander killing effect against intracranial gliomas using MSCs-TK and VPA.

Sadelain, M. (2002). "Globin gene transfer for the treatment of severe hemoglobinopathies: a paradigm for stem cell-based gene therapy." J Gene Med **4**(2): 113-121.

The prospect of treating blood disorders with genetically modified stem cells is highly promising. This therapeutic approach, however, raises a number of fundamental biological questions, spanning several research fields. Further investigation is required to better understand how to isolate and efficiently transduce hematopoietic stem cells (HSCs), while preserving optimal homing and self-renewing properties; how to design safe vectors permitting

controlled expression of the transgene products; and how to promote host repopulation by engrafted HSCs. This article addresses basic issues in stem cell-based gene therapy from the perspective of regulating transgene expression, taking globin gene transfer for the treatment of severe hemoglobinopathies as a paradigm.

Sagar, R., et al. (2019). "Fetal stem cell transplantation and gene therapy." Best Pract Res Clin Obstet Gynaecol **58**: 142-153.

The present chapter summarizes our current knowledge on fetal stem cell and gene therapy. It focuses on these therapeutic alternatives in regard to past experiences and ongoing and planned studies in humans. Several methodological challenges are discussed that may have wide implications on how these methods could be introduced in clinical practices. Although still promising, the methods are afflicted with very special requirements not least in regard to safety and ethical questions. Furthermore, careful monitoring and extended follow-up of the child and his/hers mother who receive prenatal stem cell or gene treatments are of outmost importance. Taken these prerequisites into consideration, it is natural that this type of experimental fetal therapies requires collaboration between different disciplinaries and institutions within medicine.

Saghizadeh, M., et al. (2014). "Normalization of wound healing and stem cell marker patterns in organ-cultured human diabetic corneas by gene therapy of limbal cells." Exp Eye Res **129**: 66-73.

Overexpression of c-met and suppression of matrix metalloproteinase-10 (MMP-10) and cathepsin F genes was previously shown to normalize wound healing, epithelial and stem cell marker patterns in organ-cultured human diabetic corneas. We now examined if gene therapy of limbal cells only would produce similar effects. Eight pairs of organ-cultured autopsy human diabetic corneas were used. One cornea of each pair was treated for 48 h with adenoviruses (Ad) harboring full-length c-met mRNA or a mixture (combo) of Ad with c-met and shRNA to MMP-10 and cathepsin F genes. Medium was kept at the limbal level to avoid transduction of central corneal epithelium. Fellow corneas received control Ad with EGFP gene. After additional 5 (c-met) or 10 days (combo) incubation, central corneal epithelial debridement with n-heptanol was performed, and wound healing times were determined microscopically. Corneal cryostat sections were immunostained for diabetic and putative limbal stem cell markers, alpha3beta1 integrin, nidogen-1, fibronectin, laminin gamma3 chain, DeltaNp63alpha, keratins 14, 15, and 17, as well as for activated signaling intermediates, phosphorylated EGFR, Akt, and p38. Limbal c-met overexpression significantly accelerated healing of 8.5-mm epithelial wounds over EGFP controls (6.3 days vs. 9.5 days, $p < 0.02$). Combo treatment produced a similar result (6.75 days vs. 13.5 days, $p < 0.03$). Increased immunostaining vs. EGFP controls for most markers and signaling intermediates accompanied c-met gene or combo transduction. Gene therapy of limbal epithelial stem cell compartment has a beneficial effect on the diabetic corneal wound healing and on diabetic and stem cell marker expression, and shows potential for alleviating symptoms of diabetic keratopathy.

Saghizadeh, M., et al. (2011). "Alterations of epithelial stem cell marker patterns in human diabetic corneas and effects of c-met gene therapy." Mol Vis **17**: 2177-2190.

PURPOSE: We have previously identified specific epithelial proteins with altered expression in human diabetic central corneas. Decreased hepatocyte growth factor receptor (c-met) and increased proteinases were functionally implicated in the changes of these proteins in diabetes. The present study examined whether limbal stem cell marker patterns were altered in diabetic corneas and whether c-met gene overexpression could normalize these patterns. METHODS: Cryostat sections of 28 ex vivo and 26 organ-cultured autopsy human normal and diabetic corneas were examined by immunohistochemistry using antibodies to putative limbal stem cell markers including ATP-binding cassette sub-family G member 2 (ABCG2), N-cadherin, DeltaNp63alpha, tenascin-C, laminin gamma3 chain, keratins (K) K15, K17, K19, beta(1) integrin, vimentin, frizzled 7, and fibronectin. Organ-cultured diabetic corneas were studied upon transduction with adenovirus harboring c-met gene. RESULTS: Immunostaining for ABCG2, N-cadherin, DeltaNp63alpha, K15, K17, K19, and beta(1) integrin, was significantly decreased in the stem cell-harboring diabetic limbal basal epithelium either by intensity or the number of positive cells. Basement membrane components, laminin gamma3 chain, and fibronectin (but not tenascin-C) also showed a significant reduction in the ex vivo diabetic limbus. c-Met gene transduction, which normalizes diabetic marker expression and epithelial wound healing, was accompanied by increased limbal epithelial staining for K17, K19, DeltaNp63alpha, and a diabetic marker alpha(3)beta(1) integrin, compared to vector-transduced corneas. CONCLUSIONS: The data suggest that limbal stem cell compartment is altered in long-term diabetes. Gene therapy, such as with c-met overexpression, could be able to restore normal function to diabetic corneal epithelial stem cells.

Salgar, S. K., et al. (2004). "Viral interleukin-10-engineered autologous hematopoietic stem cell therapy: a novel gene therapy approach to prevent graft rejection." Hum Gene Ther **15**(2): 131-144.

The Epstein-Barr virus-encoded protein BCRF1 (viral interleukin [vIL]-10) is a biologically active homologue of cellular interleukin (IL)-10. In this study, a novel gene therapy approach to prolong allograft survival was designed. Autologous (syngeneic) hematopoietic progenitor/stem cell-enriched (HSC; lineage(-ve)) population derived from CBA/J mouse bone marrow were transduced with retrovirus encoding vIL-10 gene (vIL-10-HSC), ex vivo; vIL-10-HSC were injected (4-6 x 10(6) cells intravenously) into lethally (9.5 Gy) or sublethally (4 Gy) irradiated CBA/J mice. Six weeks after vIL-10-HSC administration, vascular heterotopic heart (C57BL/6) transplantation was performed. Ex vivo, the vIL-10-HSC produced 5.4 +/- 0.5 ng of vIL-10 protein/2 x 10(5) cells per 24 hr. In vivo, serum vIL-10 production was 187 +/- 205 pg/ml during 3-10 weeks after vIL-10-HSC administration. Cardiac allograft survival was prolonged ($p < 0.004$) in lethally (71 +/- 40 days) and sublethally (114 +/- 15 days) irradiated mice that received vIL-10-HSC compared to controls that received unengineered (UE) HSC or vector DNA-engineered HSC (12-16 days). However, secondary skin graft (C57BL/6) survival was not prolonged in cardiac allograft-tolerant animals. In the vIL-10-HSC-administered group, graft histopathology demonstrated mild arteritis/venulitis (grade 0.7) and rejection (grade 1.0). Intragraft expression of costimulatory molecules (B7.1, B7.2), cytokines (IL-2, IL-4, mIL-10, interferon [IFN]-gamma), and inducible nitric oxide synthase (iNOS) molecules was markedly lower in vIL-10-HSC-treated tolerant grafts that survived more than 100 days compared to vector DNA-HSC- or UE-HSC-treated controls. Furthermore, T lymphocytes derived from vIL-10-HSC-treated tolerant recipients demonstrated hyporeactivity to donor antigens in mixed lymphocyte cultures. Administration of autologous vIL-10-engineered HSC prior to organ transplantation prolonged cardiac allograft survival significantly.

Santore, M. T., et al. (2009). "Prenatal stem cell transplantation and gene therapy." Clin Perinatol **36**(2): 451-471, xi.

At the present time, the most likely and eminent application of stem cell therapy to the fetus is in utero hematopoietic stem cell transplantation (IUHCT), and this stem cell type will be discussed as a paradigm for all prenatal stem cell therapy. The authors feel that the most likely initial application of IUHCT will use adult HSC derived from bone marrow (BM) or peripheral blood (PB), and will focus this article on this specific approach. The article also reviews the experimental data that support the capacity of IUHCT to induce donor-specific tolerance.

Schug, C., et al. (2019). "Radiation-Induced Amplification of TGFB1-Induced Mesenchymal Stem Cell-Mediated Sodium Iodide Symporter (NIS) Gene (131)I Therapy." Clin Cancer Res **25**(19): 5997-6008.

PURPOSE: The innate tumor homing potential of mesenchymal stem cells (MSCs) has been used for a targeted delivery of the theranostic sodium iodide symporter (NIS) transgene into solid tumors. We have previously shown that external beam radiotherapy (EBRT) results in the enhanced recruitment of NIS-expressing MSCs into human hepatocellular carcinoma (HuH7). In parallel, the tumor-associated cytokine TGFB1 becomes strongly upregulated in HuH7 tumors in response to EBRT. EXPERIMENTAL DESIGN: We therefore evaluated the effects of combining focused EBRT (5 Gy) with MSC-mediated systemic delivery of the theranostic NIS transgene under control of a synthetic TGFB1-inducible SMAD-responsive promoter (SMAD-NIS-MSCs) using (123)I-scintigraphy followed by (131)I therapy in CD1 nu/nu mice harboring subcutaneous human hepatocellular carcinoma (HuH7). RESULTS: Following tumor irradiation and SMAD-NIS-MSC application, tumoral iodide uptake monitored in vivo by (123)I-scintigraphy was enhanced as compared with nonirradiated tumors. Combination of EBRT and SMAD-NIS-MSC-mediated (131)I therapy resulted in a significantly improved delay in tumor growth and prolonged survival in therapy mice as compared with the combined therapy using CMV-NIS-MSCs or to control groups receiving EBRT or saline only, or EBRT together with SMAD-NIS-MSCs and saline applications. CONCLUSIONS: MSC-based NIS-mediated (131)I therapy after EBRT treatment dramatically enhanced therapeutic efficacy when a TGFB1-inducible SMAD-responsive promoter was used to drive NIS expression in adoptively applied MSCs. The remarkable therapeutic effect seen is thought to be linked in large part to the enhanced TGFB1 produced in this context, which leads to a highly selective and focused amplification of MSC-based NIS expression within the tumor milieu.

Schug, C., et al. (2018). "External Beam Radiation Therapy Enhances Mesenchymal Stem Cell-Mediated Sodium-Iodide Symporter Gene Delivery." Hum Gene Ther **29**(11): 1287-1300.

The tumor-homing properties of mesenchymal stem cells (MSC) have led to their development as delivery vehicles for the targeted delivery of therapeutic genes such as the sodium-iodide symporter (NIS) to solid tumors. External beam radiation therapy may represent an ideal setting for the application of engineered MSC-based gene therapy, as tumor

irradiation may enhance MSC recruitment into irradiated tumors through the increased production of select factors linked to MSC migration. In the present study, the irradiation of human liver cancer cells (HuH7; 1-10 Gy) showed a strong dose-dependent increase in steady-state mRNA levels of CXCL8, CXCL12, FGF2, PDGFB, TGFB1, THBS1, and VEGF (0-48 h), which was verified for most factors at the protein level (after 48 h). Radiation effects on directed MSC migration were tested in vitro using a live cell tracking migration assay and supernatants from control and irradiated HuH7 cells. A robust increase in mean forward migration index, mean center of mass, and mean directionality of MSCs toward supernatants was seen from irradiated as compared to non-irradiated tumor cells. Transferability of this effect to other tumor sources was demonstrated using the human breast adenocarcinoma cell line (MDA-MB-231), which showed a similar behavior to radiation as seen with HuH7 cells in quantitative polymerase chain reaction and migration assay. To evaluate this in a more physiologic in vivo setting, subcutaneously growing HuH7 xenograft tumors were irradiated with 0, 2, or 5 Gy followed by CMV-NIS-MSC application 24 h later. Tumoral iodide uptake was monitored using (123)I-scintigraphy. The results showed increased tumor-specific dose-dependent accumulation of radioiodide in irradiated tumors. The results demonstrate that external beam radiation therapy enhances the migratory capacity of MSCs and may thus increase the therapeutic efficacy of MSC-mediated NIS radionuclide therapy.

Schwarzer, A., et al. (2021). "Predicting genotoxicity of viral vectors for stem cell gene therapy using gene expression-based machine learning." Mol Ther **29**(12): 3383-3397.

Hematopoietic stem cell gene therapy is emerging as a promising therapeutic strategy for many diseases of the blood and immune system. However, several individuals who underwent gene therapy in different trials developed hematological malignancies caused by insertional mutagenesis. Preclinical assessment of vector safety remains challenging because there are few reliable assays to screen for potential insertional mutagenesis effects in vitro. Here we demonstrate that genotoxic vectors induce a unique gene expression signature linked to stemness and oncogenesis in transduced murine hematopoietic stem and progenitor cells. Based on this finding, we developed the surrogate assay for genotoxicity assessment (SAGA). SAGA classifies integrating retroviral vectors using machine learning to detect this gene expression signature during the course of in vitro immortalization. On a set of benchmark vectors with known genotoxic potential, SAGA achieved an accuracy of 90.9%. SAGA is more robust and sensitive and faster than previous assays and reliably predicts a mutagenic risk for vectors that led to leukemic severe adverse events in clinical trials. Our work provides a fast and robust tool for preclinical risk assessment of gene therapy vectors, potentially paving the way for safer gene therapy trials.

Selleri, S., et al. (2011). "In vivo T-cell dynamics during immune reconstitution after hematopoietic stem cell gene therapy in adenosine deaminase severe combined immune deficiency." J Allergy Clin Immunol **127**(6): 1368-1375 e1368.

BACKGROUND: Gene therapy (GT) with hematopoietic stem cells is a promising treatment for inherited immunodeficiencies. OBJECTIVES: Limited information is available on the relative contribution of de novo thymopoiesis and peripheral expansion to T-cell reconstitution after GT as well as on the potential effects of gene transfer on hematopoietic stem cells and lymphocyte replicative lifespan. We studied these issues in patients affected by adenosine deaminase severe combined immune deficiency after low-intensity conditioning and reinfusion of retrovirally transduced autologous CD34(+) cells. METHODS: Immunophenotype, proliferative status, telomere length, and T-cell receptor excision circles were investigated at early and late time points (up to 9 years) after GT treatment. Control groups consisted of pediatric healthy donors and patients undergoing allogeneic bone marrow transplantation (BMT). RESULTS: We observed no telomere shortening in the bone marrow compartment and in granulocytes, whereas peripheral blood naive T cells from both GT and BMT patients showed a significant reduction in telomere length compared with healthy controls. This was in agreement with the presence of a high fraction of actively cycling naive and memory T cells and lower T-cell receptor excision circles. CONCLUSION: These data indicate that T-cell homeostatic expansion contributes substantially to immune reconstitution, like BMT, and is not associated with senescence in the stem cell compartment.

Seo, S. J., et al. (2013). "Gene delivery techniques for adult stem cell-based regenerative therapy." Nanomedicine (Lond) **8**(11): 1875-1891.

Over the past decade, stem cells have been considered to be a promising resource to cure and regenerate damaged or diseased tissues with research extending from basic studies to clinical application. Furthermore, genetically modified stem cells have the potential to reduce tumorigenic risks and achieve safe tissue formation. Recent advances in genetic modification of stem cells have rendered these cells more accessible and stable. The successful genetic

modification of stem cells relies heavily on designing vector systems, either viral or nonviral vectors, which can efficiently deliver therapeutic genes to the cells with minimum toxicity. Currently, viral vectors showing high transfection efficiencies still raise safety issues, whereas safer nonviral vectors exhibit extremely poor transfection in stem cells. Here, we attempt to review and discuss the main factors raising concern in previous reports, and devise strategies to solve the issues in gene delivery systems for successful stem cell-targeting regenerative therapy.

Sergijenko, A., et al. (2013). "Myeloid/Microglial driven autologous hematopoietic stem cell gene therapy corrects a neuronopathic lysosomal disease." Mol Ther **21**(10): 1938-1949.

Mucopolysaccharidosis type IIIA (MPSIIIA) is a lysosomal storage disorder caused by mutations in N-sulfoglucosamine sulfohydrolase (SGSH), resulting in heparan sulfate (HS) accumulation and progressive neurodegeneration. There are no treatments. We previously demonstrated improved neuropathology in MPSIIIA mice using lentiviral vectors (LVs) overexpressing SGSH in wild-type (WT) hematopoietic stem cell (HSC) transplants (HSCTs), achieved via donor monocyte/microglial engraftment in the brain. However, neurological disease was not corrected using LVs in autologous MPSIIIA HSCTs. To improve brain expression via monocyte/microglial specificity, LVs expressing enhanced green fluorescent protein (eGFP) under ubiquitous phosphoglycerate kinase (PGK) or myeloid-specific promoters were compared in transplanted HSCs. LV-CD11b-GFP gave significantly higher monocyte/B-cell eGFP expression than LV-PGK-GFP or LV-CD18-GFP after 6 months. Subsequently, autologous MPSIIIA HSCs were transduced with either LV-PGK-coSGSH or LV-CD11b-coSGSH vectors expressing codon-optimized SGSH and transplanted into MPSIIIA mice. Eight months after HSCT, LV-PGK-coSGSH vectors produced bone marrow SGSH (576% normal activity) similar to LV-CD11b-coSGSH (473%), but LV-CD11b-coSGSH had significantly higher brain expression (11 versus 7%), demonstrating improved brain specificity. LV-CD11b-coSGSH normalized MPSIIIA behavior, brain HS, GM2 ganglioside, and neuroinflammation to WT levels, whereas LV-PGK-coSGSH partly corrected neuropathology but not behavior. We demonstrate compelling evidence of neurological disease correction using autologous myeloid driven lentiviral-HSC gene therapy in MPSIIIA mice.

Sessa, M., et al. (2016). "Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial." Lancet **388**(10043): 476-487.

BACKGROUND: Metachromatic leukodystrophy (a deficiency of arylsulfatase A [ARSA]) is a fatal demyelinating lysosomal disease with no approved treatment. We aimed to assess the long-term outcomes in a cohort of patients with early-onset metachromatic leukodystrophy who underwent haemopoietic stem-cell gene therapy (HSC-GT). METHODS: This is an ad-hoc analysis of data from an ongoing, non-randomised, open-label, single-arm phase 1/2 trial, in which we enrolled patients with a molecular and biochemical diagnosis of metachromatic leukodystrophy (presymptomatic late-infantile or early-juvenile disease or early-symptomatic early-juvenile disease) at the Paediatric Clinical Research Unit, Ospedale San Raffaele, in Milan. Trial participants received HSC-GT, which consisted of the infusion of autologous HSCs transduced with a lentiviral vector encoding ARSA cDNA, after exposure-targeted busulfan conditioning. The primary endpoints of the trial are safety (toxicity, absence of engraftment failure or delayed haematological reconstitution, and safety of lentiviral vector-tranduced cell infusion) and efficacy (improvement in Gross Motor Function Measure [GMFM] score relative to untreated historical controls, and ARSA activity, 24 months post-treatment) of HSC-GT. For this ad-hoc analysis, we assessed safety and efficacy outcomes in all patients who had received treatment and been followed up for at least 18 months post-treatment on June 1, 2015. This trial is registered with ClinicalTrials.gov, number NCT01560182. FINDINGS: Between April, 2010, and February, 2013, we had enrolled nine children with a diagnosis of early-onset disease (six had late-infantile disease, two had early-juvenile disease, and one had early-onset disease that could not be definitively classified). At the time of analysis all children had survived, with a median follow-up of 36 months (range 18-54). The most commonly reported adverse events were cytopenia (reported in all patients) and mucositis of different grades of severity (in five of nine patients [grade 3 in four of five patients]). No serious adverse events related to the medicinal product were reported. Stable, sustained engraftment of gene-corrected HSCs was observed (a median of 60.4% [range 14.0-95.6] lentiviral vector-positive colony-forming cells across follow-up) and the engraftment level was stable during follow-up; engraftment determinants included the duration of absolute neutropenia and the vector copy number of the medicinal product. A progressive reconstitution of ARSA activity in circulating haemopoietic cells and in the cerebrospinal fluid was documented in all patients in association with a reduction of the storage material in peripheral nerve samples in six of seven patients. Eight patients, seven

of whom received treatment when presymptomatic, had prevention of disease onset or halted disease progression as per clinical and instrumental assessment, compared with historical untreated control patients with early-onset disease. GMFM scores for six patients up to the last follow-up showed that gross motor performance was similar to that of normally developing children. The extent of benefit appeared to be influenced by the interval between HSC-GT and the expected time of disease onset. Treatment resulted in protection from CNS demyelination in eight patients and, in at least three patients, amelioration of peripheral nervous system abnormalities, with signs of remyelination at both sites. INTERPRETATION: Our ad-hoc findings provide preliminary evidence of safety and therapeutic benefit of HSC-GT in patients with early-onset metachromatic leukodystrophy who received treatment in the presymptomatic or very early-symptomatic stage. The results of this trial will be reported when all 20 patients have achieved 3 years of follow-up. FUNDING: Italian Telethon Foundation and GlaxoSmithKline.

Shi, S., et al. (2019). "Bone Marrow-Derived Mesenchymal Stem Cell-Mediated Dual-Gene Therapy for Glioblastoma." Hum Gene Ther **30**(1): 106-117.

Bone-marrow mesenchymal stem cells (BMSCs) have been used for systemic delivery of therapeutic genes to solid tumors. However, the optimal treatment time post-BMSC implantation and the assessment of the long-term fate of therapeutic BMSCs post-tumor treatment are critical if such promising therapies are to be translated into clinical practice. An efficient BMSC-based therapeutic strategy has been developed that simultaneously allows killing of tumor cells, inhibiting of tumor angiogenesis, and assessment and eradication of implanted BMSCs after treatment of glioblastoma. BMSCs were engineered to co-express the angiogenesis inhibitor kringle 5 (K5) of human plasminogen, under the control of the cytomegalovirus promoter (CMV) and the human sodium-iodide symporter (NIS), involved in uptake of radioisotopes, under the control of early growth response factor 1 (Egr1), a radiation-activated promoter. A significant decrease in tumor growth and tumor angiogenesis and a subsequent increase in survival were observed when mice bearing glioblastoma were treated with (188)Re post-therapeutic intravenous BMSC implantation. Furthermore, the systemic administration of (188)Re post-tumor treatment selectively eliminated therapeutic BMSCs expressing NIS, which was monitored in real time by (125)I micro single photon emission computed tomography/computed tomography imaging. Meanwhile, the Egr1 promoter induced a (188)Re radiation positive feedback effect absorbed by NIS. After intravenous BMSC implantation, BMSCs levels in the tumor and lung both peaked on day 10 and decreased to the lowest levels on days 24 and 17, respectively. These findings suggest that day 17 post-BMSC implantation could be an optimal time for (188)Re treatment. These results provide a new adjuvant therapy mediated by BMSCs for glioblastoma treatment.

Shimada, Y., et al. (2022). "A novel preclinical model of mucopolysaccharidosis type II for developing human hematopoietic stem cell gene therapy." Gene Ther.

A hematopoietic stem cell (HSC) gene therapy (GT) using lentiviral vectors has attracted interest as a promising treatment approach for neuropathic lysosomal storage diseases. To proceed with the clinical development of HSC-GT, evaluation of the therapeutic potential of gene-transduced human CD34+ (hCD34+) cells in vivo is one of the key issues before human trials. Here, we established an immunodeficient murine model of mucopolysaccharidosis type II (MPS II), which are transplantable human cells, and demonstrated the application of those mice in evaluating the therapeutic efficacy of gene-modified hCD34+ cells. NOG/MPS II mice, which were generated using CRISPR/Cas9, exhibited a reduction of disease-causing enzyme iduronate-2-sulfatatase (IDS) activity and the accumulation of glycosaminoglycans in their tissues. When we transplanted hCD34+ cells transduced with a lentiviral vector carrying the IDS gene into NOG/MPS II mice, a significant amelioration of biochemical pathophenotypes was observed in the visceral and neuronal tissues of those mice. In addition, grafted cells in the NOG/MPS II mice showed the oligoclonal integration pattern of the vector, but no obvious clonal dominance was detected in the mice. Our findings indicate the promising application of NOG/MPS II mice to preclinical study of HSC-GT for MPS II using human cells.

Sinenko, S. A., et al. (2021). "Pluripotent stem cell-based gene therapy approach: human de novo synthesized chromosomes." Cell Mol Life Sci **78**(4): 1207-1220.

A novel approach in gene therapy was introduced 20 years ago since artificial non-integrative chromosome-based vectors containing gene loci size inserts were engineered. To date, different human artificial chromosomes (HAC) were generated with the use of de novo construction or "top-down" engineering approaches. The HAC-based therapeutic approach includes ex vivo gene transferring and correction of pluripotent stem cells (PSCs) or highly proliferative modified stem cells. The current progress in the technology of induced PSCs, integrating with the HAC

technology, resulted in a novel platform of stem cell-based tissue replacement therapy for the treatment of genetic disease. Nowadays, the sophisticated and laborious HAC technology has significantly improved and is now closer to clinical studies. In here, we reviewed the achievements in the technology of de novo synthesized HACs for a chromosome transfer for developing gene therapy tissue replacement models of monogenic human diseases.

Sobrino, S., et al. (2023). "Severe hematopoietic stem cell inflammation compromises chronic granulomatous disease gene therapy." Cell Rep Med **4**(2): 100919.

X-linked chronic granulomatous disease (CGD) is associated with defective phagocytosis, life-threatening infections, and inflammatory complications. We performed a clinical trial of lentivirus-based gene therapy in four patients (NCT02757911). Two patients show stable engraftment and clinical benefits, whereas the other two have progressively lost gene-corrected cells. Single-cell transcriptomic analysis reveals a significantly lower frequency of hematopoietic stem cells (HSCs) in CGD patients, especially in the two patients with defective engraftment. These two present a profound change in HSC status, a high interferon score, and elevated myeloid progenitor frequency. We use elastic-net logistic regression to identify a set of 51 interferon genes and transcription factors that predict the failure of HSC engraftment. In one patient, an aberrant HSC state with elevated CEBPbeta expression drives HSC exhaustion, as demonstrated by low repopulation in a xenotransplantation model. Targeted treatments to protect HSCs, coupled to targeted gene expression screening, might improve clinical outcomes in CGD.

Song, L. (2009). "A possible approach for stem cell gene therapy of Fanconi anemia." Curr Gene Ther **9**(1): 26-32.

Fanconi anemia (FA) is an inherited chromosomal recessive syndrome characterized by cellular hypersensitivity to DNA crosslinking agents and bone marrow failure, which cause aplastic anemia, and an increased incidence of malignancy. 13 complementation groups are currently discovered, and 13 distinct genes have been cloned (FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FNACI, FANCJ, FANCL, FANCM, FANCN). Stem cells can theoretically divide to other cells without limit as long as a person is still alive. The stem cells that form blood and immune cells are known as hematopoietic stem cells. Hematopoietic stem cells can be acquired from a Fanconi anemia patient, whereas genomic DNA can be obtained easily from blood cells of a normal person. Normal genes also can be synthesised by PCR method. Normal genomic DNA will be delivered into a patient's stem cells via microinjection or transfection after enzyme digestion; the defective genes might be repaired by homologous genetic recombination. The gene-corrected stem cells can be transplanted into the same patient finally. It is possible that human genomic DNA to be considered as materials for homologous genetic recombination to repair defective genes in vivo. This might be an efficient method for gene therapy, which has no or less immunological rejection for Fanconi anemia and some genetic diseases. Several related observations and experiments are discussed to support this possible means of stem cell gene therapy of Fanconi anemia.

Sorrentino, B. P. (2012). "Chemoprotection in brain tumor patients: another success for stem cell gene therapy." Mol Ther **20**(8): 1485-1487.

Spees, J. L., et al. (2004). "Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy." Mol Ther **9**(5): 747-756.

Adult stem cells from human bone marrow stroma, referred to as mesenchymal stem cells or marrow stromal cells (hMSCs), are attractive candidates for clinical use. The optimal conditions for hMSC expansion require medium supplemented with fetal calf serum (FCS). Some forms of cell therapy will involve multiple doses, raising a concern over immunological reactions caused by medium-derived FCS proteins. By a sensitive fluorescence-based assay we determined that 7 to 30 mg of FCS proteins are associated with a standard preparation of 100 million hMSCs, a dosage that probably will be needed for clinical therapies. Here we present ex vivo growth conditions for hMSCs that reduce the FCS proteins to less than 100 ng per 100 million hMSCs, approximately a 100,000-fold reduction. The cells maintain their proliferative capacity and sustain their ability for multilineage differentiation. Experiments in rats demonstrate that rat MSCs grown in 20% FCS induce a substantial humoral response after repeated administrations, whereas cells grown under the conditions described in this study reduce the immunogenicity in terms of IgG response over 1000-fold to barely detectable levels. Our results have the potential to dramatically improve cellular and genetic therapies using hMSCs and perhaps other cells.

Srivastava, A. and R. V. Shaji (2017). "Cure for thalassemia major - from allogeneic hematopoietic stem cell transplantation to gene therapy." Haematologica **102**(2): 214-223.

Allogeneic hematopoietic stem cell transplantation has been well established for several decades as gene replacement therapy for patients with

thalassemia major, and now offers very high rates of cure for patients who have access to this therapy. Outcomes have improved tremendously over the last decade, even in high-risk patients. The limited data available suggests that the long-term outcome is also excellent, with a >90% survival rate, but for the best results, hematopoietic stem cell transplantation should be offered early, before any end organ damage occurs. However, access to this therapy is limited in more than half the patients by the lack of suitable donors. Inadequate hematopoietic stem cell transplantation services and the high cost of therapy are other reasons for this limited access, particularly in those parts of the world which have a high prevalence of this condition. As a result, fewer than 10% of eligible patients are actually able to avail of this therapy. Other options for curative therapies are therefore needed. Recently, gene correction of autologous hematopoietic stem cells has been successfully established using lentiviral vectors, and several clinical trials have been initiated. A gene editing approach to correct the beta-globin mutation or disrupt the BCL11A gene to increase fetal hemoglobin production has also been reported, and is expected to be introduced in clinical trials soon. Curative possibilities for the major hemoglobin disorders are expanding. Providing access to these therapies around the world will remain a challenge.

Staal, F. J. T., et al. (2019). "Autologous Stem-Cell-Based Gene Therapy for Inherited Disorders: State of the Art and Perspectives." Front Pediatr **7**: 443.

Gene therapy using patient's own stem cells is rapidly becoming an alternative to allogeneic stem cell transplantation, especially when suitably compatible donors cannot be found. The advent of efficient virus-based methods for delivering therapeutic genes has enabled the development of genetic medicines for inherited disorders of the immune system, hemoglobinopathies, and a number of devastating metabolic diseases. Here, we briefly review the state of the art in the field, including gene editing approaches. A growing number of pediatric diseases can be successfully cured by hematopoietic stem-cell-based gene therapy.

Stanojevic, M., et al. (2022). "Viral infection in hematopoietic stem cell transplantation: an International Society for Cell & Gene Therapy Stem Cell Engineering Committee review on the role of cellular therapy in prevention and treatment." Cytotherapy **24**(9): 884-891.

Despite recent advances in the field of HSCT, viral infections remain a frequent causeof morbidity and mortality among HSCT recipients. Adoptive transfer of viral specific T cells has been successfully used both as prophylaxis and treatment of viral infections in immunocompromised HSCT recipients. Increasingly, precise risk stratification of HSCT recipients with infectious complications should incorporate not only pretransplant clinical criteria, but milestones of immune reconstitution as well. These factors can better identify those at highest risk of morbidity and mortality and identify a population of HSCT recipients in whom adoptive therapy with viral specific T cells should be considered for either prophylaxis or second line treatment early after inadequate response to first line antiviral therapy. Broadening these approaches to improve outcomes for transplant recipients in countries with limited resources is a major challenge. While the principles of risk stratification can be applied, early detection of viral reactivation as well as treatment is challenging in regions where commercial PCR assays and antiviral agents are not readily available.

Tolar, J., et al. (2011). "Stem cell gene therapy for fanconi anemia: report from the 1st international Fanconi anemia gene therapy working group meeting." Mol Ther **19**(7): 1193-1198.

Survival rates after allogeneic hematopoietic cell transplantation (HCT) for Fanconi anemia (FA) have increased dramatically since 2000. However, the use of autologous stem cell gene therapy, whereby the patient's own blood stem cells are modified to express the wild-type gene product, could potentially avoid the early and late complications of allogeneic HCT. Over the last decades, gene therapy has experienced a high degree of optimism interrupted by periods of diminished expectation. Optimism stems from recent examples of successful gene correction in several congenital immunodeficiencies, whereas diminished expectations come from the realization that gene therapy will not be free of side effects. The goal of the 1st International Fanconi Anemia Gene Therapy Working Group Meeting was to determine the optimal strategy for moving stem cell gene therapy into clinical trials for individuals with FA. To this end, key investigators examined vector design, transduction method, criteria for large-scale clinical-grade vector manufacture, hematopoietic cell preparation, and eligibility criteria for FA patients most likely to benefit. The report summarizes the roadmap for the development of gene therapy for FA.

Trobridge, G. D. (2011). "Genotoxicity of retroviral hematopoietic stem cell gene therapy." Expert Opin Biol Ther **11**(5): 581-593.

INTRODUCTION: Retroviral vectors have been developed for hematopoietic stem cell (HSC) gene therapy and have successfully cured X-linked severe combined immunodeficiency (SCID-X1), adenosine deaminase deficiency (ADA-SCID),

adrenoleukodystrophy, and Wiskott-Aldrich syndrome. However, in HSC gene therapy clinical trials, genotoxicity mediated by integrated vector proviruses has led to clonal expansion, and in some cases frank leukemia. Numerous studies have been performed to understand the molecular basis of vector-mediated genotoxicity with the aim of developing safer vectors and safer gene therapy protocols. These genotoxicity studies are critical to advancing HSC gene therapy. AREAS COVERED: This review provides an introduction to the mechanisms of retroviral vector genotoxicity. It also covers advances over the last 20 years in designing safer gene therapy vectors, and in integration site analysis in clinical trials and large animal models. Mechanisms of retroviral-mediated genotoxicity, and the risk factors that contribute to clonal expansion and leukemia in HSC gene therapy are introduced. EXPERT OPINION: Continued research on virus-host interactions and next-generation vectors should further improve the safety of future HSC gene therapy vectors and protocols.

Trobridge, G. D. and H. P. Kiem (2010). "Large animal models of hematopoietic stem cell gene therapy." Gene Ther **17**(8): 939-948.

Large animal models have been instrumental in advancing hematopoietic stem cell (HSC) gene therapy. Here we review the advantages of large animal models, their contributions to the field of HSC gene therapy and recent progress in this field. Several properties of human HSCs including their purification, their cell-cycle characteristics, their response to cytokines and the proliferative demands placed on them after transplantation are more similar in large animal models than in mice. Progress in the development and use of retroviral vectors and ex vivo transduction protocols over the last decade has led to efficient gene transfer in both dogs and nonhuman primates. Importantly, the approaches developed in these models have translated well to the clinic. Large animals continue to be useful to evaluate the efficacy and safety of gene therapy, and dogs with hematopoietic diseases have now been cured by HSC gene therapy. Nonhuman primates allow evaluation of aspects of transplantation as well as disease-specific approaches such as AIDS (acquired immunodeficiency syndrome) gene therapy that can not be modeled well in the dog. Finally, large animal models have been used to evaluate the genotoxicity of viral vectors by comparing integration sites in hematopoietic repopulating cells and monitoring clonality after transplantation.

Trobridge, G. D., et al. (2009). "Protection of stem cell-derived lymphocytes in a primate AIDS gene therapy model after in vivo selection." PLoS One **4**(11): e7693.

BACKGROUND: There is currently no effective AIDS vaccine, emphasizing the importance of developing alternative therapies. Recently, a patient was successfully transplanted with allogeneic, naturally resistant CCR5-negative (CCR5Delta32) cells, setting the stage for transplantation of naturally resistant, or genetically modified stem cells as a viable therapy for AIDS. Hematopoietic stem cell (HSC) gene therapy using vectors that express various anti-HIV transgenes has also been attempted in clinical trials, but inefficient gene transfer in these studies has severely limited the potential of this approach. Here we evaluated HSC gene transfer of an anti-HIV vector in the pigtailed macaque (Macaca nemestrina) model, which closely models human transplantation. METHODS AND FINDINGS: We used lentiviral vectors that inhibited both HIV-1 and simian immunodeficiency virus (SIV)/HIV-1 (SHIV) chimera virus infection, and also expressed a P140K mutant methylguanine methyltransferase (MGMT) transgene to select gene-modified cells by adding chemotherapy drugs. Following transplantation and MGMT-mediated selection we demonstrated transgene expression in over 7% of stem-cell derived lymphocytes. The high marking levels allowed us to demonstrate protection from SHIV in lymphocytes derived from gene-modified macaque long-term repopulating cells that expressed an HIV-1 fusion inhibitor. We observed a statistically significant 4-fold increase of gene-modified cells after challenge of lymphocytes from one macaque that received stem cells transduced with an anti-HIV vector ($p<0.02$, Student's t-test), but not in lymphocytes from a macaque that received a control vector. We also established a competitive repopulation assay in a second macaque for preclinical testing of promising anti-HIV vectors. The vectors we used were HIV-based and thus efficiently transduce human cells, and the transgenes we used target HIV-1 genes that are also in SHIV, so our findings can be rapidly translated to the clinic. CONCLUSIONS: Here we demonstrate the ability to select protected HSC-derived lymphocytes in vivo in a clinically relevant nonhuman primate model of HIV/SHIV infection. This approach can now be evaluated in human clinical trials in AIDS lymphoma patients. In this patient setting, chemotherapy would not only kill malignant cells, but would also increase the number of MGMTP140K-expressing HIV-resistant cells. This approach should allow for high levels of HIV-protected cells in AIDS patients to evaluate AIDS gene therapy.

Tsujimura, M., et al. (2019). "Cell-based interferon gene therapy using proliferation-controllable, interferon-releasing mesenchymal stem cells." Sci Rep **9**(1): 18869.

An important safety concern on cell-based gene therapy is that few methods have been available to control the proliferation and functioning of therapeutic protein-expressing cells after transplantation. We previously reported that the proliferation and functioning of the cells transfected with herpes simplex virus thymidine kinase (HSVtk) gene, a suicide gene, can be controlled by administration of ganciclovir. In this study, we tried to control the amount of murine interferon-gamma (IFN-gamma) secreted from transplanted murine mesenchymal stem cell line C3H10T1/2 cells to achieve safe cell-based IFN-gamma gene therapy for cancer. C3H10T1/2 cells were transfected with HSVtk- and murine IFN-gamma-expressing plasmid vectors to obtain C3H10T1/2/HSVtk/IFN-gamma cells. C3H10T1/2/HSVtk/IFN-gamma cells released IFN-gamma and were sensitive to ganciclovir. C3H10T1/2/HSVtk/IFN-gamma cells significantly suppressed the proliferation of murine adenocarcinoma cell line colon26 cells both in vitro and in vivo. Moreover, subcutaneous administration of ganciclovir to mice transplanted with NanoLuc luciferase-expressing C3H10T1/2/HSVtk cells for three consecutive days reduced the luminescence signals from the transplanted cells. These results indicate that the cell regulation system using HSVtk gene and ganciclovir can be useful for safe and efficient cell-based IFN-gamma gene therapy for cancer.

Tsujimura, M., et al. (2019). "Rapid Regulation of Human Mesenchymal Stem Cell Proliferation Using Inducible Caspase-9 Suicide Gene for Safe Cell-Based Therapy." Int J Mol Sci **20**(22).

The regulation of transplanted cell proliferation and function is important to achieve safe cell-based therapies. We previously reported that the proliferation and function of transplanted cells, which expressed the herpes simplex virus thymidine kinase (HSVtk) suicide gene, could be controlled by ganciclovir (GCV) administration. However, there are some concerns regarding the use of GCV. It is reported that the inducible caspase-9 (iC9) gene, a human caspase-9-derived genetically engineered suicide gene, rapidly induces cell apoptosis in the presence of apoptosis inducers, such as AP20187. In this study, we used a combination of the iC9 gene and AP20187 to achieve rapid regulation of transplanted cell proliferation. Cells from the human mesenchymal stem cell line UE7T-13 were transfected with the iC9 gene to obtain UE7T-13/iC9 cells. AP20187 significantly reduced the number of UE7T-13/iC9 cells within 24 h in a concentration-dependent manner. This reduction was much faster than the reduction of HSVtk-expressing UE7T-13 cells induced by GCV addition. Subcutaneous AP20187 administration rapidly reduced the luminescence signal from NanoLuc luciferase (Nluc)-expressing UE7T-13/iC9 cells transplanted into mice. These results indicate that the combined use of the iC9 gene and AP20187 is effective in rapidly regulating transplanted cell proliferation.

Witt, R., et al. (2017). "Fetal stem cell and gene therapy." Semin Fetal Neonatal Med **22**(6): 410-414.

Advances in our understanding of stem cells, gene editing, prenatal imaging and fetal interventions have opened up new opportunities for the treatment of congenital diseases either through in-utero stem cell transplantation or in-utero gene therapy. Improvements in ultrasound-guided access to the fetal vasculature have also enhanced the safety and efficacy of cell delivery. The fetal environment offers accessible stem cell niches, localized cell populations with large proliferative potential, and an immune system that is able to acquire donor-specific tolerance. In-utero therapy seeks to take advantage of these factors and has the potential to cure diseases prior to the onset of symptoms, a strategy that offers substantial social and economic benefits. In this article, we examine previous studies in animal models as well as clinical attempts at in-utero therapy. We also discuss the barriers to successful in-utero therapy and future strategies for overcoming these obstacles.

Wrobel, C., et al. (2021). "Access to the Apical Cochlear Modiolus for Possible Stem Cell-based and Gene Therapy of the Auditory Nerve." Otol Neurotol **42**(3): e371-e377.

OBJECTIVE: Loss of spiral ganglion neurons (SGN) is permanent and responsible for a substantial number of patients suffering from hearing impairment. It can derive from the degeneration of SGNs due to the death of sensory hair cells as well as from auditory neuropathy. Utilizing stem cells to recover lost SGNs increasingly emerges as a possible therapeutic option, but access to human SGNs is difficult due to their protected location within the bony impacted cochlea. Aim of this study was to establish a reliable and practicable approach to access SGNs in the human temporal bone for possible stem cell and gene therapies. METHODS: In seven human temporal bone specimen a transcanal approach was used to carefully drill a cochleostomy in the lateral second turn followed by insertion of a tungsten needle into the apical modiolus to indicate the spot for intramodiolar injections. Subsequent cone beam computed tomography (CBCT) served as evaluation for positioning of the marker and cochleostomy size. RESULTS: The apical modiolus could be exposed in all cases by a cochleostomy (1.6 mm2, standard deviation +/-0.23 mm2) in the lateral second turn. 3D reconstructions and analysis of CBCT revealed reliable positioning of the marker in the apical

modiolus, deviating on average 0.9 mm (standard deviation +/-0.49 mm) from the targeted center of the second cochlear turn. CONCLUSION: We established a reliable, minimally invasive, transcanal surgical approach to the apical cochlear modiolus in the human temporal bone in foresight to stem cell-based and gene therapy of the auditory nerve.

Wu, C. and C. E. Dunbar (2011). "Stem cell gene therapy: the risks of insertional mutagenesis and approaches to minimize genotoxicity." Front Med **5**(4): 356-371.

Virus-based vectors are widely used in hematopoietic stem cell (HSC) gene therapy, and have the ability to integrate permanently into genomic DNA, thus driving long-term expression of corrective genes in all hematopoietic lineages. To date, HSC gene therapy has been successfully employed in the clinic for improving clinical outcomes in small numbers of patients with X-linked severe combined immunodeficiency (SCID-X1), adenosine deaminase deficiency (ADA-SCID), adrenoleukodystrophy (ALD), thalassemia, chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome (WAS). However, adverse events were observed during some of these HSC gene therapy clinical trials, linked to insertional activation of proto-oncogenes by integrated proviral vectors leading to clonal expansion and eventual development of leukemia. Numerous studies have been performed to understand the molecular basis of vector-mediated genotoxicity, with the aim of developing safer vectors and lower-risk gene therapy protocols. This review will summarize current information on the mechanisms of insertional mutagenesis in hematopoietic stem and progenitor cells due to integrating gene transfer vectors, discuss the available assays for predicting genotoxicity and mapping vector integration sites, and introduce newly-developed approaches for minimizing genotoxicity as a way to further move HSC gene therapy forward into broader clinical application.

Wu, P., et al. (2015). "A gene expression based predictor for high risk myeloma treated with intensive therapy and autologous stem cell rescue." Leuk Lymphoma **56**(3): 594-601.

Myeloma is characterized by a highly variable clinical outcome. Despite the effectiveness of high-dose therapy, 15% of patients relapse within 1 year. We show that these cases also have a significantly shorter post-relapse survival compared to the others (median 14.9 months vs. 40 months, p = 8.03 x 10(-14)). There are no effective approaches to define this potentially distinct biological group such that treatment could be altered. In this work a series of uniformly treated patients with myeloma were used to develop a gene expression profiling (GEP)-based signature to identify this high risk clinical behavior. Gene enrichment analyses applied to the top differentially expressed genes showed a significant enrichment of epigenetic regulators as well as "stem cell" myeloma genes. A derived 17-gene signature effectively identifies patients at high risk of early relapse as well as impaired overall survival. Integrative genomic analyses showed that epigenetic mechanisms may play an important role on transcription of these genes.

Wu, X., et al. (2010). "Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy." Cell Tissue Res **340**(3): 549-567.

Muscle tissue represents an abundant, accessible, and replenishable source of adult stem cells for cell-based tissue and genetic engineering. A population of cells isolated from muscle exhibits both multipotentiality and self-renewal capabilities. Satellite cells, referred to by many investigators as muscle stem cells, are myogenic precursors that are capable of regenerating muscle and that demonstrate self-renewal properties; however, they are considered to be committed to the myogenic lineage. Muscle-derived stem cells (MDSCs), which may represent a predecessor of the satellite cell, are considered to possess a higher regeneration capacity and to exhibit better cell survival and a broader range of multilineage capabilities. Remarkably, MDSCs are not only able to differentiate into mesodermal cell types including the myogenic, adipogenic, osteogenic, chondrogenic, endothelial, and hematopoietic lineages, but also possess the potential to break germ layer commitment and differentiate into ectodermal lineages including neuron-like cells under certain conditions. This article reviews the current preclinical studies and potential clinical applications of MDSC-mediated gene therapy and tissue-engineering and methods for MDSC isolation, differentiation, and molecular characterization.

Xie, J., et al. (2010). "Repetitive busulfan administration after hematopoietic stem cell gene therapy associated with a dominant HDAC7 clone in a nonhuman primate." Hum Gene Ther **21**(6): 695-703.

The risk of genotoxicity of retroviral vector-delivered gene therapy targeting hematopoietic stem cells (HSCs) has been highlighted by the development of clonal dominance and malignancies in human and animal gene therapy trials. Large-animal models have proven invaluable to test the safety of retroviral vectors, but the detection of clonal dominance may require years of follow-up. We hypothesized that hematopoietic stress may accelerate the proliferation and therefore the detection of abnormal clones in these

models. We administered four monthly busulfan (Bu) infusions to induce hematopoietic stress in a healthy rhesus macaque previously transplanted with CD34+ cells transduced with retroviral vectors carrying a simple marker gene. Busulfan administration resulted in significant cytopenias with each cycle, and prolonged pancytopenia after the final cycle with eventual recovery. Before busulfan treatment there was highly polyclonal marking in all lineages. After Bu administration clonal diversity was markedly decreased in all lineages. Unexpectedly, we found no evidence of selection of the MDS1/EVI1 clones present before Bu administration, but a clone with a vector integration in intron 1 of the histone deacetylase-7 (HDAC7) gene became dominant in granulocytes over time after Bu administration. The overall marking level in the animal was increased significantly after Bu treatment and coincident with expansion of the HDAC7 clone, suggesting an in vivo advantage for this clone under stress. HDAC7 expression was upregulated in marrow progenitors containing the vector. Almost 5 years after Bu administration, the animal developed progressive cytopenias, and at autopsy the marrow showed complete lack of neutrophil or platelet maturation, with a new population of approximately 20% undifferentiated blasts. These data suggest that chemotherapeutic stress may accelerate vector-related clonal dominance, even in the absence of drug resistance genes expressed by the vector. This model may both accelerate the detection of abnormal clones to facilitate analysis of genotoxicity for human gene therapy, and help assess the safety of administering myelotoxic chemotherapeutic agents in patients previously engrafted with vector-containing cells.

Xu, H., et al. (2017). "Gene-modified Mesenchymal Stem Cell-based Therapy in Renal Ischemia-Reperfusion Injury." Curr Gene Ther **17**(6): 453-460.

Acute Kidney Injury (AKI) is a common syndrome in the clinic and has become a worldwide public health problem. Renal Ischemia-Reperfusion Injury (IRI) is the most common cause of AKI. So far, effective treatment is still lacking for renal IRI, resulting in a high mortality rate of AKI. Mesenchymal Stem Cells (MSCs), considered as a promising candidate for tissue repair and regenerative medicine have aroused an increasing concern in recent years for the capacity of self-renewal and multi-lineage differentiation. MSC-based therapy has drawn wide attention for its therapeutic potential in renal IRI. The administrated MSCs can alleviate the renal IRI and improve the renal function for its anti-inflammatory, immunomodulation properties. MSCs preferentially migrate into injured sites to play the role of tissue repair. Furthermore, MSCs can modify the microenvironment to promote the recovery of damaged renal tubular cells via paracrine factors. However, the poor kidney-directional homing and poor survival under ischemia environment have limited their beneficial effects. Genetic modification is an effective approach to increase the therapeutic action of MSCs. MSCs are modified with exogenous genes to enhance their innate properties. Here we review the current knowledge of gene-modified MSCs, their biological characteristics and applications in renal IRI.

Xu, J., et al. (2008). "Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice." J Pathol **214**(4): 472-481.

Bone marrow-derived mesenchymal stem cells (MSCs) can serve as a vehicle for gene therapy. Angiopoietin-1 (Ang1) is a critical factor for endothelial survival and vascular stabilization via the inhibition of endothelial permeability and leukocyte-endothelium interactions. We hypothesized that MSC-based Ang1 gene therapy might be a potential therapeutic approach for lipopolysaccharide (LPS)-induced lung injury. MSCs were isolated from 6 week-old inbred male mice and transduced with the Ang1 gene, using a lentivirus vector. The MSCs showed no significant phenotypic changes after transduction. In the in vivo mouse model, the LPS-induced lung injury was markedly alleviated in the group treated with MSCs carrying Ang1 (MSCs-Ang1), compared with groups treated with MSCs or Ang1 alone. The expression of Ang1 protein in the recipient lungs was increased after MSCs-Ang1 administration. The histopathological and biochemical indices of LPS-induced lung injury were improved after MSCs-based Ang1 gene treatment. MSCs-Ang1 administration also reduced pulmonary vascular endothelial permeability and the recruitment of inflammatory cells into the lung. Cells of MSC origin could be detected in the recipient lungs for 2 weeks after injection with MSCs. These results suggest that MSCs and Ang1 have a synergistic role in the treatment of LPS-induced lung injury. MSC-based Ang1 gene therapy may be developed as a potential novel strategy for the treatment of acute lung injury.

Xu, S., et al. (2022). "Engineered mesenchymal stem cell-derived exosomes with high CXCR4 levels for targeted siRNA gene therapy against cancer." Nanoscale **14**(11): 4098-4113.

Gene therapy has been used in a variety of diseases and shows brilliant anticancer or cancer suppression effects. Gene therapy is gradually evolving as the most compelling frontier hotspot in the field of cancer therapy. The current vehicles used in gene therapy have poor safety and low delivery efficiency, and thus, it is urgent to develop novel delivery vehicles

for gene therapy. Due to the excellent stability and biosafety of exosomes, their use as drug carriers for novel nucleic acid therapy is in full swing, revealing huge prospects for clinical application. Mesenchymal stem cells (MSCs) have a natural homing property and can spontaneously accumulate at injury sites, inflammation sites, and even tumour sites. This feature is attributed to a variety of tropism factors expressed on their surface; for example, CXC chemokine receptor type 4 (CXCR4) can specifically bind to the highly expressed stromal cell derived factor-1 (SDF-1) on the tumour surface, which is essential for accumulation of MSCs at the tumour site. The mesenchymal stem cells used in this study were genetically engineered to obtain exosomes with high CXCR4 expression as carriers for targeted gene-drug delivery, and then, the Survivin gene was loaded via electrotransformation to construct a brand-new gene-drug delivery system (CXCR4(high) Exo/si-Survivin). Finally, related in vivo and in vitro experiments were conducted. We observed that the new delivery system can efficiently aggregate at the tumour site and release siRNA into tumour cells, knocking down the Survivin gene in tumour cells in vivo and thereby inhibiting tumour growth. This new gene-drug delivery system has tremendous clinical transformation value and provides a new strategy for clinical treatment of tumours.

Yadak, R., et al. (2018). "Preclinical Efficacy and Safety Evaluation of Hematopoietic Stem Cell Gene Therapy in a Mouse Model of MNGIE." Mol Ther Methods Clin Dev **8**: 152-165.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder caused by thymidine phosphorylase (TP) deficiency resulting in systemic accumulation of thymidine (d-Thd) and deoxyuridine (d-Urd) and characterized by early-onset neurological and gastrointestinal symptoms. Long-term effective and safe treatment is not available. Allogeneic bone marrow transplantation may improve clinical manifestations but carries disease and transplant-related risks. In this study, lentiviral vector-based hematopoietic stem cell gene therapy (HSCGT) was performed in Tymp(-/-)Upp1(-/-) mice with the human phosphoglycerate kinase (PGK) promoter driving TYMP. Supranormal blood TP activity reduced intestinal nucleoside levels significantly at low vector copy number (median, 1.3; range, 0.2-3.6). Furthermore, we covered two major issues not addressed before. First, we demonstrate aberrant morphology of brain astrocytes in areas of spongy degeneration, which was reversed by HSCGT. Second, long-term follow-up and vector integration site analysis were performed to assess safety of the therapeutic LV vectors in depth. This report confirms and supplements previous work on the efficacy of HSCGT in reducing the toxic metabolites in Tymp(-/-)Upp1(-/-) mice, using a clinically applicable gene transfer vector and a highly efficient gene transfer method, and importantly demonstrates phenotypic correction with a favorable risk profile, warranting further development toward clinical implementation.

Yamada, Y., et al. (2006). "Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy." Biomaterials **27**(20): 3766-3781.

We investigated gene expression patterns and functional classifications regarding the clusters of human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs)--which possess a multipotent ability--because little is known about the precise moleculobiological clues by which these cells activate their differentiating ability or functionality to eventually form dentin and bone, respectively. We first verified the expressions of the alkaline phosphatase (ALP) gene, dentin matrix protein 1 (DMP-1), and dentinsialophosphoprotein (DSPP) by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and consequently discovered the high expressions of these genes. Total RNA was also followed by hybridization with a human microarray system consisting of 12,814 genes. Analyses of gene expression patterns indicated several genes which encode extracellular matrix components, cell adhesion molecules, growth factors, and transcription regulators. Functional and clustering analyses of differences in gene expression levels revealed cell signaling, cell communication, or cell metabolism. In the future, information on the gene expression patterns of hDPSCs and hMSCs might be useful in determining the detailed functional roles of the relevant genes and applicable to stem cell therapies, and these cells could also be used as multipotent cell sources for gene technology and tissue engineering technology.

Yamashita, T., et al. (2009). "Gene and stem cell therapy in ischemic stroke." Cell Transplant **18**(9): 999-1002.

Possible strategies for treating ischemic stroke include neuroprotection (preventing injured neurons from undergoing apoptosis in the acute phase of cerebral ischemia) and stem cell therapy (the repair of disrupted neuronal networks with newly born neurons in the chronic phase of cerebral ischemia). First, we estimated the neuroprotective effect of glial cell line-derived neurotrophic factor (GDNF) by administration of GFNF protein. GDNF protein showed a direct protective effect against ischemic brain damage.

Pretreatment of animals with adenoviral vector containing GDNF gene (Ad-GDNF) 24 h before the subsequent transient middle cerebral artery occlusion (MCAO) effectively reduced infarcted volume. Secondly, we studied the neuroprotective effect of a calcium channel blocker, azelnidipine, or a by-product of heme degradation, biliverdin. Both azelnidipine and biliverdin had a neuroprotective effect in the ischemic brain through their antioxidative property. Lastly, we developed a restorative stroke therapy with a bioaffinitive scaffold, which is able to provide an appropriate platform for newly born neurons. In the future, we will combine these strategies to develop more effective therapies for treatment of strokes.

Yan, Y., et al. (2009). "Establishment of medakafish as a model for stem cell-based gene therapy: efficient gene delivery and potential chromosomal integration by baculoviral vectors." Exp Cell Res **315**(13): 2322-2331.

Viral vectors hold promise and challenges in gene therapy. Specifically, we have previously shown that baculoviral (BV) vectors have a high efficiency of gene delivery in human embryonic stem (ES) cells. Here we report the development of a complementary system to further our evaluation by utilizing the laboratory fish medaka that has ES cell lines and tools for experimental analyses in vitro and in vivo. We show that BV vectors can give rise to almost 100% of transient gene delivery in the medaka ES cell line MES1. BV-transduced MES1 cells reproducibly (at approximately 10(-5)) produce GFP-expressing colonies that, upon manual isolation, develop into stable clones during 300 days of culture. Surprisingly, BV transduction can also mediate efficient gene integration in the medaka genome, as fluorescent in situ hybridization revealed the presence of the BV-delivered gfp transgene in multiple locations in nuclei and on various chromosomes of metaphase spreads. We show that BV transduction does not compromise the genome stability and pluripotency of MES1 cells. We conclude that BV can efficiently mediate gene delivery and chromosomal integration in medaka ES cells. Therefore, medaka provides a powerful system for analyzing the potential of BV-mediated gene delivery in stem cells and gene therapy.

Yang, J., et al. (2012). "Tumor tropism of intravenously injected human-induced pluripotent stem cell-derived neural stem cells and their gene therapy application in a metastatic breast cancer model." Stem Cells **30**(5): 1021-1029.

Human pluripotent stem cells can serve as an accessible and reliable source for the generation of functional human cells for medical therapies. In this study, we used a conventional lentiviral transduction method to derive human-induced pluripotent stem (iPS) cells from primary human fibroblasts and then generated neural stem cells (NSCs) from the iPS cells. Using a dual-color whole-body imaging technology, we demonstrated that after tail vein injection, these human NSCs displayed a robust migratory capacity outside the central nervous system in both immunodeficient and immunocompetent mice and homed in on established orthotopic 4T1 mouse mammary tumors. To investigate whether the iPS cell-derived NSCs can be used as a cellular delivery vehicle for cancer gene therapy, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected through tail vein into 4T1 tumor-bearing mice. The transduced NSCs were effective in inhibiting the growth of the orthotopic 4T1 breast tumor and the metastatic spread of the cancer cells in the presence of ganciclovir, leading to prolonged survival of the tumor-bearing mice. The use of iPS cell-derived NSCs for cancer gene therapy bypasses the sensitive ethical issue surrounding the use of cells derived from human fetal tissues or human embryonic stem cells. This approach may also help to overcome problems associated with allogeneic transplantation of other types of human NSCs.

Yannaki, E., et al. (2013). "Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus plerixafor in patients with beta-thalassemia major." Hum Gene Ther **24**(10): 852-860.

Successful stem cell gene therapy requires high numbers of genetically engineered hematopoietic stem cells collected using optimal mobilization strategies. Here we focus on stem cell mobilization strategies for thalassemia and present the results of a plerixafor-based mobilization trial with emphasis on the remobilization with granulocyte-colony stimulating factor (G-CSF)+plerixafor in those patients who had previously failed mobilization. Plerixafor rapidly mobilized CD34(+) cells without inducing hyperleukocytosis; however, 35% of patients failed to reach the target cell dose of >/=6x10(6) CD34(+) cells/kg. Four subjects who failed on either plerixafor or G-CSF were remobilized with G-CSF+plerixafor. The combination proved highly synergistic; the target cell dose was readily reached and the per-apheresis yield was significantly increased over initial mobilization, ultimately resulting in single-apheresis collections, despite a more than 50% reduction of the dose of G-CSF in splenectomized patients to avoid hyperleukocytosis. The total stem and progenitor cells mobilized in G-CSF+plerixafor patients were higher than in patients treated by plerixafor alone. Importantly, the G-CSF+plerixafor-mobilized cells displayed a

primitive stem cell phenotype and higher clonogenic capacity over plerixafor-mobilized cells. G-CSF+plerixafor represents the optimal strategy when very high yields of stem cells or a single apheresis is required. The high yields and the favorable transplantation features render the G-CSF+plerixafor-mobilized cells the optimal CD34(+) cell source for stem cell gene therapy applications.

Yannaki, E., et al. (2012). "Hematopoietic stem cell mobilization for gene therapy of adult patients with severe beta-thalassemia: results of clinical trials using G-CSF or plerixafor in splenectomized and nonsplenectomized subjects." Mol Ther **20**(1): 230-238.

The safety and efficacy of hematopoietic stem cell (HSC) mobilization was investigated in adult splenectomized (SPL) and non-SPL patients with thalassemia major, in two clinical trials, using different mobilization modes: granulocyte-colony-stimulating factor (G-CSF)-alone, G-CSF following pretreatment with hydroxyurea (HU), plerixafor-alone. G-CSF-mobilization was both safe and effective in non-SPL patients. However, in SPL patients the procedure resulted in excessive response to G-CSF, expressed as early hyperleukocytosis necessitating significant dose reduction, and suboptimal CD34(+) cells yields. One-month HU-pretreatment prevented hyperleukocytosis and allowed successful CD34(+) cell collections when an optimal washout period was maintained, but it significantly prolonged the mobilization procedure. Plerixafor resulted in rapid and effective mobilization in both SPL and non-SPL patients and was well-tolerated. For gene therapy of thalassemia, G-CSF or Plerixafor could be used as mobilization agents in non-SPL patients whereas Plerixafor appears to be the mobilization agent of choice in SPL adult thalassemics in terms of safety and efficacy.

Yannaki, E., et al. (2021). "Success Stories and Challenges Ahead in Hematopoietic Stem Cell Gene Therapy: Hemoglobinopathies as Disease Models." Hum Gene Ther **32**(19-20): 1120-1137.

Gene therapy is a relatively novel field that amounts to around four decades of continuous growth with its good and bad moments. Currently, the field has entered the clinical arena with the ambition to fulfil its promises for a permanent fix of incurable genetic disorders. Hemoglobinopathies as target diseases and hematopoietic stem cells (HSCs) as target cells of genetic interventions had a major share in the research effort toward efficiently implementing gene therapy. Dissection of HSC biology and improvements in gene transfer and gene expression technologies evolved in an almost synchronous manner to a point where the two fields seem to be functionally intercalated. In this review, we focus specifically on the development of gene therapy for hemoglobin disorders and look at both gene addition and gene correction strategies that may dominate the field of HSC-directed gene therapy in the near future and transform the therapeutic landscape for genetic diseases.

Yannaki, E. and G. Stamatoyannopoulos (2010). "Hematopoietic stem cell mobilization strategies for gene therapy of beta thalassemia and sickle cell disease." Ann N Y Acad Sci **1202**: 59-63.

Effective gene therapy for hemoglobinopathies will require high numbers of autologous gene-engineered hematopoetic stem cells to be reintroduced into the patients. Stem cell mobilization using G-CSF is the most convenient and effective approach to achieve this goal, but it can have severe side effects in sickle cell anemia and be potentially harmful in the case of severe thalassemia. Hence, the optimal way of collection of hematopoetic stem cells from patients with thalassemia and sickle cell disease needs to be determined. In this paper, we review the possible risks of G-CSF mobilization in hemoglobinopathies and we outline the approaches used in an on-going clinical trial in which pretreatment with hydroxyurea is used to reduce potential risks of G-CSF administration to patients with severe beta thalassemia.

Yin, P. T., et al. (2016). "Stem cell-based gene therapy activated using magnetic hyperthermia to enhance the treatment of cancer." Biomaterials **81**: 46-57.

Stem cell-based gene therapies, wherein stem cells are genetically engineered to express therapeutic molecules, have shown tremendous potential for cancer applications owing to their innate ability to home to tumors. However, traditional stem cell-based gene therapies are hampered by our current inability to control when the therapeutic genes are actually turned on, thereby resulting in detrimental side effects. Here, we report the novel application of magnetic core-shell nanoparticles for the dual purpose of delivering and activating a heat-inducible gene vector that encodes TNF-related apoptosis-inducing ligand (TRAIL) in adipose-derived mesenchymal stem cells (AD-MSCs). By combining the tumor tropism of the AD-MSCs with the spatiotemporal MCNP-based delivery and activation of TRAIL expression, this platform provides an attractive means with which to enhance our control over the activation of stem cell-based gene therapies. In particular, we found that these engineered AD-MSCs retained their innate ability to proliferate, differentiate, and, most importantly, home to tumors, making them ideal cellular carriers. Moreover, exposure of the engineered AD-MSCS to mild magnetic hyperthermia resulted in the selective expression of TRAIL from the engineered AD-MSCs and, as a result, induced

significant ovarian cancer cell death in vitro and in vivo.

Yokoo, T., et al. (2003). "Stem cell gene therapy for chronic renal failure." Curr Gene Ther **3**(5): 387-394.

Recently, stem cell research has attracted considerable attention because it could be used for the regeneration of damaged organs that are untreatable by conventional techniques, and several stem cells (or progenitor cells), such as endothelial stem cells and neural stem cells have been discovered. Following the progression of this field of research, the potential for stem cell gene therapy has increased and several therapeutic benefits have already been reported. Although this approach was originally investigated for fatal or hereditary diseases, chronic renal failure is also a candidate for stem cell gene therapy. We have proposed two different therapeutic strategies for chronic renal failure depending on whether the bone marrow stem cells differentiate and commit into mesenchymal or hematopoietic stem cells. In the case of diseases, which need reconstitution of residential renal cells, such as congenital enzyme deficiency diseases, mesenchymal stem cells should be transplanted, and in contrast, hematopoietic stem cells may be used for gene delivery for diseases, which need foreign cytokines and growth factors, such as glomerulonephritis. This article reviews the recent investigation on this tailor-made stem cell gene therapy for chronic renal failure and discusses the potential of this novel strategy and the major practical challenges of its clinical application.

Zonari, E., et al. (2017). "Efficient Ex Vivo Engineering and Expansion of Highly Purified Human Hematopoietic Stem and Progenitor Cell Populations for Gene Therapy." Stem Cell Reports **8**(4): 977-990.

Ex vivo gene therapy based on CD34(+) hematopoietic stem cells (HSCs) has shown promising results in clinical trials, but genetic engineering to high levels and in large scale remains challenging. We devised a sorting strategy that captures more than 90% of HSC activity in less than 10% of mobilized peripheral blood (mPB) CD34(+) cells, and modeled a transplantation protocol based on highly purified, genetically engineered HSCs co-infused with uncultured progenitor cells. Prostaglandin E(2) stimulation allowed near-complete transduction of HSCs with lentiviral vectors during a culture time of less than 38 hr, mitigating the negative impact of standard culture on progenitor cell function. Exploiting the pyrimidoindole derivative UM171, we show that transduced mPB CD34(+)CD38(-) cells with repopulating potential could be expanded ex vivo. Implementing these findings in clinical gene therapy protocols will improve the efficacy, safety, and sustainability of gene therapy and generate new opportunities in the field of gene editing.

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