Review
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(e) Short Title: Cell and Gene Therapy

Introduction. Australia has been long recognized as punching above its weight in biomedical research. Australian scientists have achieved international recognition with the award of a Nobel Prize for Medicine particularly in the area of immunology and infectious disease: Sir Howard Florey (1945) for the discovery of penicillin; Sir Frank Macfarlane Burnet (1960) for the discovery of acquired immunological tolerance; Peter Doherty (1996) for cell mediated immune defence; Barry Marshall and Robin Warren (2005) for their discovery of the Helicobacter pylori bacterium causing stomach ulcers and gastritis. Australia boasts a vibrant research community with a diverse range of interests in the emerging field of cell therapies. International collaboration is actively supported by groups such as the International Society of Stem Cell Research, the International Society of Cellular Therapy and the Australasian Gene Therapy Society each hosting meetings in Australia this year. The fact that few cell therapies have been licenced by the FDA in the US indicates the youth of this field. This review showcases the preclinical, translational and clinical cell-based gene therapies, vaccines/immunotherapies and regenerative medicines that are currently under development in Australia.

(1) Cell-based Gene Therapy.

Cell-based gene therapy strategies used in Australia include:(a) corrective gene addition to restore dysfunctional gene activity; (b) immunotherapy and cell-based vaccines to augment the body's immune response against a disease; and (c) intracellular vaccination which introduces disease modifying or modulating genes such as antisense RNA, ribozymes or short interfering RNA. This section outlines gene therapy preclinical to clinical studies currently underway in Australia (see Table 1).

Johnson & Johnson Research P/L Sydney NSW has completed two Phase I gene transfer studies for human immunodeficiency virus (HIV) and also commenced a Phase II trial. A study in genetically identical twins, discordant for HIV-1 infection was initially performed [1]. $CD4^+$ enriched T cells from the HIV negative twin were divided into two equal portions, and transduced with either RRz2 (anti-HIV-1 ribozyme) or the LNL6 retroviral vector control, expanded and reinfused into the respective HIV-1-positive twin. Four patients were treated in the study. Macpherson et al [1] reported this gene therapy approach was safe and feasibile and the long-term survival of ribozyme (and control) modified T cells within the peripheral blood.

In another Phase I study, the ability to introduce a potential anti-HIV gene therapeutic into hematopoietic progenitor cells (HPC) was examined. Amado et al [2]showed the presence and expression of a retroviral vector encoding an anti-HIV-1 ribozyme in mature hematopoietic cells of different lineages, and de novo T cell development ensuing from the gene modified $CD34^+$ HPC. Sustained output of vector-containing mature myeloid and T cells was detected even in patients with multidrug-resistant infection. In addition, the study showed that the degree of persistence of gene-containing cells was dependent on transduced HPC dose. This report represented the first demonstration of long-term maintenance of a therapeutic transgene in AIDS.

Johnson & Johnson Pharmaceutical Research & Development with L.L.C. Tibotec Pharmaceutical Limited have commenced a "Randomized Phase II, double-blind, controlled trial to evaluate the safety and efficacy of autologous CD34+ hematopoietic progenitor cells transduced with placebo or an anti-HIV-1 ribozyme (OZ1) in patients with HIV-1 infection." This ongoing study which commenced in 2002 has accrued 74 patients (G Symonds; personal communication).

The **Gene Therapy Unit at The Children's Hospital Westmead in Sydney NSW** undertook a clinical study for the correction of the genetic disease X-linked severe combined immunodeficiency (SCID-X1). One SCID-X1 infant with intact natural

killer (NK) cells, a variant to the common SCID-X1 phenotype, was treated with autologous bone marrow $CD34^+$ HPC that were retrovirally transduced *ex vivo* with human gamma chain (gamma c), an integral component common to many interleukin receptors. A transient recovery in the peripheral blood T cells was accompanied by an early weight gain and rotavirus clearance from the gut. However, immune reconstitution remained incomplete and the infant receive a bone marrow transplant from a matched unrelated donor 26 months after gene therapy. It was proposed that the partial reconstitution was attributed to the relatively low dose of gene-corrected HPC re-infused; a viral infection during the early Phase of T cell reconstitution and the infant's NK^+ phenotype [3].

The **Gene and Stem Cell Therapy Program at the Centenary Institute of Cancer Medicine and Cell Biology, NSW and Royal Prince Alfred Hospital** have performed studies assess the efficacy of various adeno-associated virus (AAV) serotypes to transduce mesenchymal stromal cells (MSC) for use as cellular delivery vehicles in muscle repair. A preclinical animal study showed that AAV serotype 2 was the most efficient in transducing MSC isolated from humans and baboons. Baboon MSC transduced with AAV2, retained their potential to differentiate into adipocytes *in vitro*, and engraft injured muscle tissue of diabetic-severe combined immunodeficient (NOD-SCID) mice *in vivo*. The reporter gene was expressed in engrafted cells that displayed a muscle fibre morphology for 9 weeks following implantation [4].

(2) Vaccines.

Cell-based vaccines whether to prevent or treat disease take advantage of the immune system's network of cells and organs. Cell-based vaccines currently under development in Australia include the adoptive transfer of unmodified or gene modified immunological effector cells such as cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. Cell-based tumor vaccines may consist of or contain autologous or allogeneic tumor cells that may be genetically modified. Otherwise they are based on professional antigen-presenting cells such as dendritic cells (DC) loaded with tumor associated antigens (TAA). Alternatively, DCs may be fused with tumor cells or they may be genetically modified to express TAA and/or immunomodulatory genes. Cell-based vaccines are generally classified as cell therapies unless they have been gene modified and are classified as medicines. The following summarises the cell-based vaccine studies (preclinical to clinical) currently underway in Australia (see Table 1).

The **Cancer Immunotherapy Group at the Queensland Institute of Medical Research (QIMR)** undertook the first clinical application of retrovirally transduced melanoma cells [5]. With the Mater Adult Public Hospital they subsequently trialed autologous DC cultured *ex vivo* with irradiated autologous tumor cells, as a treatment for patients suffering advanced metastatic melanoma [6]. In this trial, 17 patients commenced immunotherapy, 12 of which received all six 'priming' vaccinations. Of these 12 patients, three had durable complete responses, three had partial responses, and the remaining six had progressive disease (WHO criteria). Importantly, the clinical results from this trial, and the lack of morbidity associated with treatment, have now been replicated in a follow-up study. A randomised, double blinded clinical trial using the same treatment for patients with an earlier stage metastatic melanoma has commenced. DC immunotherapy trials for other solid malignancies including Prostate cancer and Glioblastoma have also been initiated. This is in association with Q-Gen P/L at QIMR, a facility for the GMP production of Cell Therapies (N Martinez, Q-Gen; personal communication).

In a preclinical animal study the **Cancer Immunology Program at the Peter MacCallum Cancer Centre (Peter Mac)**, **Melbourne, Victoria** showed that gene modified T cells expressing a chimeric single-chain receptor co-delivering CD28 and T cell receptor zeta chain activation signals may be a promising treatment for cancer [7]. Primary human T cells were retrovirally transduced to express the chimeric receptor reactive with ErbB2. These modified T cells vigorously responded to ErbB2+ tumors in an antigen-specific manner *in vitro*. When infused into irradiated NOD-SCID tumor bearing mice, the modified T cells significantly delayed the growth of ErbB2+ human tumors. These studies showed that T cells may be genetically redirected to kill tumors.

In another preclinical mouse study, the **Cancer Immunology Program at the Peter Mac** with the **Department of Pathology, University of Melbourne**, **Parkville, Victoria** showed that chimeric Fc epsilon receptors combined with tumor specific IgE further enhanced the redirection of T cells for cancer gene therapy [8]. Human primary T cells were retrovirally transduced with the extracellular domain of Fc epsilon RI linked to the hinge and transmembrane domains of Fc gamma RII and the cytoplasmic domains of CD28 and T cell receptor zeta chain (chimeric Fc epsilon receptor). This study showed that modified T cells, in the presence of tumor-specific IgE antibody, can mediate effector function *in vitro* and *in vivo*.

The **PeterMac including the Centre for Blood Cell Therapies (CBCT)** with the **Ludwig Institute for Cancer Research**, **Melbourne** have commenced a translational/Phase 1 study investigating the infusion of autologous T cells retrovirally transduced with an anti-Lewis Y (LeY) chimeric receptor in patients with LeY^+ myeloid malignancies including multiple myeloma (MM). The anti-LeY chimeric surface receptor is composed of a humanised single chain antibody specific for LeY which is joined to human CD28 and the CD3-zeta molecules which relay a cancer killing T cell response upon binding to LeY on the surface of tumor cells [9, 10, 11]. The study will be further amended to allow inclusion of patients with myeloid malignancies.

The PeterMac established the CBCT to support a range of cell and tissue manipulation including gene modification under GMP conditions and was the first TGA licenced facility in Australia. In addition to the above study, the CBCT is undertaking two further Phase 1 trials: adjuvant monocyte-derived DC vaccination (pulsed with autologous myeloma lysate) post autologous stem cell transplant in patients with MM; autologous monocyte-derived DC (pulsed with viral peptide) immunotherapy for persistent Hepatitis C virus infection (D Wall, personal communication).

The **Institute of Hematology Royal Prince Alfred Hospital (RPAH)** and the **Cancer Immunology Group, University of Sydney** have commenced a translational study for the development of a tumor antigen-specific NK cell therapy for multiple myeloma and Waldenstrom's macroglobulinaemia. In this study the NK-92 cell line, a potent effector cytotoxic cell line, will be transfected with a gene expressing a receptor for human CD106 (VCAM-1) that will enable it to home to the bone marrow and to breach the tumor 'barrier' which is a major limiting step in immune rejection of tumors. At the same time, NK-92 will be transfected with a gene expressing a receptor for specific tumor surface marker such as human CD38 that will enable it to specifically target and kill the tumor cells [12]. In this ongoing study the NK-92 immunotherapy cytotoxicity will be assessed *in vitro* and clinical efficacy against MM will be assessed *in vivo* (D Sze, personal communication).

(3) Regenerative Medicine.
Regenerative medicine involves the use of healthy living cells to replace diseased, dying or missing cells or tissues. Cells may be minimally manipulated or expanded in cell culture (with or without modification) to obtain a sufficient cell "dose" to provide a therapeutic effect. Table 2 summarises some of the research in regenerative medicine from preclinical to clinical studies currently undertaken in Australia.

The **Victor Chang Cardiac Research Institute** and **St Vincent's Hospital**, **Sydney NSW** have recently completed a Phase Ib-IIa interventional study into the treatment of chronic refractory ischemic heart disease (IHD) with granulocyte-colony stimulating factor (G-CSF) mobilised autologous $CD133^+$ progenitor cells [13, 14, 15, 16]. G-CSF itself may promote angiogenesis via stem cell or cytokine-related pathways. This study assessed the safety and efficacy of: G-CSF to mobilise endothelial progenitors in chronic IHD patients [17]; intracoronary infusion of G-CSF mobilised $CD133^+$ endothelial progenitor cells to treat chronic IHD. The administration of G-CSF was an open-label, uncontrolled intervention. The intracoronary infusion of G-CSF mobilised $CD133^+$ cells was a randomised double-blind placebo controlled intervention. During the periods of G-CSF administration (2 cycles 3 months apart), patients underwent controlled myocardial 'ischemia-induction' by supervised exercise stress testing. It was hypothesised that ischemia could induce angiogenic and progenitor cell-homing cytokines and facilitate trafficking of the mobilised progenitors to the heart. Patients also underwent leucapheresis immediately after the second cycle of G-CSF. Patients were then randomised to receive an intracoronary infusion of $CD133^+$ or unselected cells. Preliminary data analyses have shown provisional safety, and an improvement in outcomes as a result of G-CSF administration + 'ischemia induction'. Specifically, patients showed significant improvements in angina, nitrate use and exercise stress test performance (all $p < 0.01$) as a result of G-CSF administration + 'ischemia-induction'. Analysis of the intracoronary cell infusion data is ongoing (H Tao, personal communication).

The Department of Medicine St Vincent's Hospital Fitzroy, University of Melbourne, and Royal Victorian Eye and Ear Hospital, Melbourne Victoria with Columbia University, New York, USA have shown improvement in patients with chronic IHD that have received intra-coronary high-dose $CD34^+$ stem cell therapy [18]. This study used G-CSF to mobilise $CD34^+$ cells for harvest by leucapheresis enabling the intracoronary reinfusion of large $CD34^+$ cell numbers. $CD34^+$ cells were separated (Isolex 300) in a GMP facility prior to reinfusion. All IHD patients showed a sustained reduction in anginal symptoms and improved quality of life scores following reinfusion. The 12-month follow-up angiography showed significant improvement, indicating sustained myocardial neovascularisation. However, complications arose in two patients, one developing an acute coronary syndrome and the other a lentigo maligna. These results demonstrate the feasibility of G-CSF mobilisation, leucapheresis and intracoronary transfer of $CD34^+$ stem cells in patients with chronic IHD, but longer-term studies are required to ensure the safety and efficacy of this protocol.

The **Eskitis Institute for Cell and Molecular Therapies, National Adult Stem Cell Centre Griffith University** with **Princess Alexandra Hospital, Brisbane, QLD** are exploring the regeneration of human spinal cord injuries in paraplegic patients following transplantation with autologous olfactory ensheathing cells into the damaged spinal cord. This trial will test for: adverse reactions from the procedure; and whether olfactory ensheathing cells will allow axonal penetration and regeneration of the injured spinal cords. Half the patients received the cell implant, the control group did not. Human nasal mucosa was obtained by biopsy from the nasal septum. Biopsies were processed and the dissociated cells were cultured over

4 weeks to build up an adequate supply of ensheathing cells [19]. The study should be completed in August 2007 and the results will be published (A Mackay-Sim, personal communication).

The **Australian Stem Cell Centre in conjunction with the PeterMac in Melbourne, Victoria** have completed a Phase I study to assess the safety and efficacy of transplanting *ex vivo* expanded autologous $CD34^+$ cells to aid in the recovery of patients treated with multiple courses of high dose combination chemotherapy for breast cancer [20]**.** This study showed that *ex vivo* expanded $CD34^+$ cell product under GMP conditions could be administered without adverse events or post transplant complications. *Ex vivo* expanded cells contributed to faster rates of neutrophil recovery, significantly fewer episodes of febrile neutropenia and a significant reduction in platelet transfusion requirements (D Haylock, personal communication).

The **Division of Hematology and Hanson Institute, Institute of Medical** and **Veterinary Sciences, Adelaide, South Australia**, are undertaking a Phase I trial to assess the effect of infused mesenchymal stem cells (MSC) from HLA-identical, HLA- haploidentical or unrelated donors in patients with steroid resistant grades II-IV acute graft-versus-host disease (GVHD). Adherent MSC from volunteer bone marrow were expanded using a standardised protocol in a GMP facility for 4 – 6 weeks prior to infusion [21]. Data is currently under evaluation (I Lewis, personal communication).

The **Diabetes Transplant Unit at Prince of Wales Hospital and University of New South Wales in Sydney** is developing cell therapies to replace insulin-producing cells in insulin-dependent diabetics. The cells to be grafted are derived from: (a) the pancreas of humans who donate their pancreas after death; (b) human embryonic stem cells; (c) human stem cells derived from non-embryonic tissues, such as cord blood and the fetal pancreas; (d) genetically modified non-pancreatic cell lines, especially from the liver; (e) the pancreas of an animal, especially the pig. Clinical trials have commenced with insulin-producing cells isolated from donor human pancreas, which have been placed in microcapsules made of alginate to overcome the need for anti-rejection therapy [22]. Preclinical studies are being conducted with insulin-producing cells isolated from the fetal pig pancreas. Human embryonic stem cells have been derived from spare fertilised eggs, and it is anticipated that attempts will be made to make patient-specific embryonic stem cell lines (B Tuch, personal communication).

The **Ray & Bill Dobney Cell & Tissue Therapies (CTT) facility at Royal Perth Hospital, Western Australia** is a newly established GMP manufacturing unit is conducting two regenerative medicine studies: a Phase I study focused on GVHD associated with allogeneic stem cell transplantation; and translational studies investigating the utility of MSC in controlling autoimmune and hematological disorders. In the Phase I trial assessing the safety and efficacy of MSC in GVHD, MSC harvested from the bone marrow of donors were expanded and co-infused with the hemopoietic graft. The CTT is also conducting a translational study investigating the role of MSC as an immunosuppressive therapy for autoimmune and hematological disease including immune thrombocytopenic purpura and systemic lupus erythematosis and also for chronic lymphocytic leukemia. In conjunction with Princess Margaret Hospital, the CTT is assessing the ability of NK cells to treat leukemia and lymphoma. A specific HLA mismatch between donor and recipient may prevent both the recurrences of leukemia and GVHD. The presence of this mismatch enables donor NK cells to seek out and destroy any residual leukemic cells. This study will examine *in vitro* methods of preferentially expanding the minor

subset of NK cells that are alloreactive to be used as a therapy for treating hematological malignancy (M Sturm, personal communication)

The Spine Service, Dept of Orthopaedic Surgery, St. George Clinical School UNSW and the Dept of Hematology and Stem Cell Transplantation, St Vincent's Hospital, Sydney NSW have designed a randomised, placebo-controlled, double-blind, Phase I clinical trial to evaluate safety and feasibility of autologous bone marrow derived stem cell transplantation into the intervertebral disc to treat disc degeneration in patients with low back pain (H Tao, personal communication [23]).

The **Institute of Hematology and the Cell and Molecular Therapy Laboratories, Royal Prince Alfred Hospital (RPAH) Sydney NSW** are designing a Phase I trial to standardise the treatment of allogeneic hematopoietic stem cell transplantation (HSCT) induced GVHD using mesenchymal stromal cells (MSC). Whilst there is now a moderate experience with the use of MSCs to treat patients with refractory GVHD with some success [24], there is variability in the methods of culture, number of cells infused and passage number at which the cells are given. One of the greatest concerns currently relates to MSC safety owing to an increasing number of studies demonstrating that even medium-term culture *ex vivo* may lead to phenotypic and genotypic changes including neoplasia. This study will investigate the issues of cell dose and passage number with the view to establishing the optimal combination for the treatment of these patients (S Larsen, personal communication).

In a translational study the **Department of Pathology University of Melbourne and Paediatrics Royal Children's Hospital, Melbourne Victoria** are investigating the ability of cord blood-derived stem cells to form lung epithelial cells for the treatment of lung disease, in particular cystic fibrosis (CF). Unrestricted somatic stem cells (USSC) were successfully generated from 3 out of 9 cord blood samples. USSC showed the ability to undergo tri-lineage differentiation (neuronal, bone and epithelial). The differentiated epithelial cells expressed SPC, and at certain time points the cystic fibrosis transmembrane conductance regulator (CFTR). If the differentiated epithelial cells prove to be functional, and a safe method to engraft these cells into the lung can be determined, in the first instance HLA identical sib cords will be used to treat the affected sibling with CF. The goal is to eventually move to using units of cord blood from unrelated donors from the public cord blood bank, which is particularly relevant to older patients with CF (F Zaibak, personal communication).

In a preclinical study the **Department of Anatomy and Human Biology and the Centre for Opthalmology and Visual Science, University of Western Australia** have enhanced the revascularization (and hence speed of regeneration) of transplanted whole muscles by transducing muscles with the vascular endothelial growth factor (VEGF) gene and a reporter GFP gene before transplantation, using an AAV into the tibialis anterior muscles of adult mice. One month following injection whole muscle auto-transplantation was performed. After grafting, GFP expression persisted only in a few surviving myofibers in the periphery of the pretreated muscles, although abundant VEGF expression was seen in myogenic cells in all grafted muscle. Although only small numbers of rAAV-transduced myofibers were present, whole muscle grafts preinjected with rAAV were significantly more vascular than saline injected and uninjected control muscle grafts. Furthermore, rAAV-injected whole muscle transplants showed greater regeneration (myotube formation) compared with the uninjected control muscle transplants. This study showed that rAAV-mediated VEGF expression persists only in myofibers that survive the necrosis induced by muscle transplantation; however, this amount of VEGF significantly increased revascularisation and regeneration of a whole muscle transplants [25].

Discussion: Australian researchers are developing promising cell therapies particularly for HIV, myocardial regeneration in chronic ischemic heart disease and to promote hematological recovery in patients receiving cancer chemotherapy. The preclinical and translational studies described herein demonstrate the broad interest in cell-based gene therapies for the correction of genetic disease, cancer treatment and regenerative medicine. This has been achieved despite several hurdles of the industry that are common world-wide including: complex manufacturing processes requiring aseptic production with safeguards against adventitious agent contamination; the requirement for production to be undertaken in expensive clean room facilities of which there are only three publically owned licenced facilities currently in operation (another seven are in development) in Australia; the need to ensure the products remain genetically stable and do not acquire mutation; a complex regulatory environment focused around the Therapeutic Goods Administration (TGA; the FDA equivalent in Australia). However, the future of cell and gene therapies Down Under looks promising.

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Table 1. Cell-based Gene Therapy

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Human immuno-deficiency virus (**HIV)**	Phase 1	Completed	Long-Term Survival and Concomitant Gene Expression of Ribozyme-Transduced CD4+ T-Lymphocytes in HIV infected Patients.	Determine the safety and ribozyme persistence in HIV infected patient delivered by gene modified T cells.	CD4+ enriched T cells from the HIV negative twin.	T cells from the HIV negative twin were transduced with either RRz2 (anti-HIV-1 ribozyme) or the LNL6 retroviral vector control prior to infusion.	Retroviral gene therapy for HIV/AIDS was shown to be safe and feasibile. Long-term survival of the ribozyme gene-containing T cells within the peripheral blood with concomitant long-term expression of the transgenes was also observed.	[1]
HIV	Phase 1	Completed	Anti-HIV hematopoietic progenitor cell (HPC)-delivered ribozyme in a Phase I study: myeloid and lymphoid reconstitution in HIV-1 infected patients	Determine the safety and ribozyme persistence in HIV infected patient delivered by gene modified HPC.	Autologous $CD34^+$ enriched HPC harvested from the peripheral blood following G-CSF mobilisation	Retroviral transduction of HPC with anti-HIV protective gene Rz2 or the control retroviral vector LNL6 prior to infusion.	The anti-HIV-1 ribozyme was detected in mature hematopoietic cells of different lineages. The study showed that the degree of persistence of gene-containing cells was dependent on transduced HPC dose. These findings support the concept of gene therapy as a modality to effect immune reconstitution with cells engineered to inhibit HIV replication.	[2]
HIV	Phase II Randomised Double-Blind, Controlled Trial	Ongoing	Evaluation of the Safety and Efficacy of Autologous $CD34^+$HPC Transduced With Placebo or an Anti-HIV-1 Ribozyme (OZ1) in Patients With HIV-1 Infection	Determine the safety and ribozyme persistence in HIV infected patient delivered by gene modified HPC.	Autologous $CD34^+$ enriched HPC	Retroviral transduction of HPC with anti-HIV protective gene OZ1 or a control prior to infusion.	Pending	
X-linked severe combined immuno-deficiency (SCID-X1)	Phase 1	Completed	Treatment of an infant with SCID-X1 by gene therapy in Australia	Determine the safety and efficacy of *ex vivo* corrective gene therapy for SCID-X1.	Autologous CD34+ enriched HPC	Retroviral transduction of HPC *ex vivo* with human gamma chain (gamma c) to correct SCID-X1	A transient reconstitution was achieved but remained incomplete. The infant receive a bone marrow transplant from a matched unrelated donor 26 months after gene therapy.	[3]

Table 1. Cell-based Gene Therapy (continued)

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Muscle repair	Pre-clinical animal study	Completed	Specific AAV serotypes facilitate efficient gene transfer into human and non-human primate mesenchymal stromal cells (MSC)	Determine the efficacy of various adeno-associated viral vector (AAV) serotypes to transduce MSCs isolated from humans and baboons.	Bone marrow MSC isolated from baboon or human	AAV serotypes 1, 2, 3, 4, 5, 6, and 8 carrying the beta-galactosidase reporter gene.	Optimum transduction of baboon MSC was achieved with AAV serotype 2 vector, Cells retained their potential to differentiate and engraft injured muscle tissue.	[4]
Late stage metastatic melanoma	Phase 1	Completed	Immune responses and clinical course of GM-CSF immunotherapy	Determine the safety and efficacy of GM-CSF-transduced autologous melanoma cells.	Irradiated, autologous melanoma	Retroviral transduction of autologous melanoma with GM-CSF	Vaccination resulted in the transient regression of most metastatic lesions. The anti-tumor effects and immune response were not detectable 2 months following the last vaccination.	[5]
Late stage metastatic melanoma	Phase 1/II	Completed		Determine the safety and efficacy of an autologous melanoma cell/dendritic cell vaccine for melanoma immunotherapy.	Autologous DC cultured *ex vivo* with irradiated autologous tumor cells	N/A	Durable complete clinical responses in a Phase I/II trial using an autologous melanoma cell/dendritic cell vaccine in a subset of patients.	[6]
ErbB2+ human tumors grown in irradiated NOD-SCID mice	Animal study	Completed	Immuno-therapy of Cancer Using Systemically Delivered Gene-Modified Human T cells	Evaluate methods of redirecting T cells to kill tumors using chimeric tumor-associated antigen (TAA) receptor gene modification.	Primary human T cells	Retroviral transduction of T cells with a chimeric receptor reactive with the ErbB2 TAA	Chimeric receptor modified T cells produced high levels of cytokines, proliferated vigorously and mediated the specific lysis of ErbB2+ tumors. In NOD-SCID tumor bearing mice, the modified T cells significantly delayed tumor growth.	[7]
Mouse CD8+ tumors grown in irradiated nonobese diabetic-severe combined immuno-deficient (NOD-SCID) mice	Animal study	Completed	Adoptive Transfer of Chimeric Fc epsilon RI Receptor Gene-Modified Human T Cells for Cancer Immuno-therapy	Evaluate methods of redirecting T cells to kill tumors using in an IgE-dependent mechanism.	Primary human T cells	Retroviral transduction of T cells with a chimeric Fc epsilon receptor recognizing mouse CD8.	Adoptive transfer of chimeric receptor-T cells incubated with anti-CD8 IgE mAb significantly enhanced the survival of irradiated NOD-SCID mice bearing CD8+ tumor compared with control mice.	[8]

Table 1. Cell-based Gene Therapy (continued)

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Myeloid malignancies including multiple myeloma	Translat-ional leading to a open-label, uncontroll ed Phase I study	Ongoing	Immunotherapy of myeloid malignancies.	Evaluate the tolerability, safety and biological parameters of an infusion of autologous peripheral blood T cells transduced with an anti-Lewis Y chimeric receptor gene in patients with Lewis Y positive myeloid malignancies.	Autologous peripheral blood T cells	Retroviral transduction of T cells with an anti-Lewis Y chimeric receptor gene	Translational Phase complete-progressing to CTX prior to clinical enrolment.	[11]
Multiple myeloma (MM) and Waldenstroms Macroglobulinaemia	Translat-ional	Ongoing	Developing a tumor antigen-specific natural killer cellular therapy.	Sufficient numbers of a retargeted NK-92 cells will be infused to target and kill MM.	NK-92 cells	Stable transfection of NK-92 cells with a receptor for CD106 (VCAM-1) to promote homing to the bone marrow and CD38 allowing tumor targeting and killing.	Cytotoxicity *in vitro* and efficacy *in vivo* will be assessed.	[12]

Table 2. Regenerative Medicine

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Chronic ischemic heart disease (IHD)	Phase Ib-IIa inter-ventional clinical study	Completed with full analysis ongoing	G-CSF mobilised autologous endothelial progenitor cells for the treatment of chronic IHD.	This study assessed the safety and efficacy of: (a) G-CSF to mobilise endothelial progenitors in chronic IHD patients; (b) intracoronary infusion of G-CSF mobilised CD133+ endothelial progenitor cells to treat chronic IHD.	G-CSF mobilised autologous $CD133^+$ endothelial progenitor cells	N/A	Preliminary analyses have shown provisional safety, and significant improvements in angina, nitrate use and exercise stress test performance (all $p < 0.01$) as a result of G-CSF administration + 'ischemia-induction' Analysis of the intracoronary cell infusion data is ongoing.	[13], [14], [15], [16], [17]
Chronic IHD	Phase I	Completed with full analysis ongoing	Intra-coronary high-dose CD34+ stem cells in patients with chronic IHD: A 12-month follow-up	This study assessed the safety and efficacy of G-CSF to mobilise $CD34^+$ cells from the bone marrow for harvest by leucapheresis enabling the intracoronary reinfusion of large $CD34^+$ cell numbers in patients with chronic IHD.	G-CSF mobilised autologous $CD34^+$ stem cells	N/A	All IHD patients showed improvement following reinfusion. The 12-month follow-up angiography showed significant improvement, indicating sustained myocardial neovascularisation. However, complications arose in two patients.	[18]
Spinal cord injury	Phase I with a 3 yr follow-up. Non-randomised, single-blinded, controlled study	Ongoing	The regeneration of human spinal cord injuries with olfactory glial cells.	This study will assess: the safety of the procedure; and whether olfactory ensheathing cells, harvested from the nose of the paraplegic patient, will allow injured spinal cord regeneration.	Human nasal mucosa ensheathing cells cultured *ex vivo.*	N/A	Pending completion in August 2007.	[19]
Metastatic breast cancer chemotherapy recovery	Phase I	Completed		This study assessed the safety and efficacy of transplanting *ex vivo* expanded autologous $CD34^+$ cells to aid in the recovery of patients treated with multiple courses of high dose combination chemotherapy for breast cancer.	Harvest, cryopreservation of autologous $CD34^+$ cells, *ex vivo* culture for 12 days under GMP.	N/A	*Ex vivo* expanded cells were administered without adverse events or post transplant complications and contributed to faster rates of neutrophil recovery, significantly fewer episodes of febrile neutropenia and significant reduction in platelet transfusion requirements.	[20]

Table 2. Regenerative Medicine (continued)

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Graft versus Host Disease (GVHD)	Phase I Interventional, single arm, safety and efficacy	Ongoing	Infusion of mesenchymal stem cells (MSC) from allogeneic donors in patients with steroid resistant grades II-IV acute GVHD associated with allogeneic hematopoietic stem cell transplantation (HSCT).	To establish the safety and efficacy of infusions of HLA-identical, HLA- haploidentical or unrelated MSC on steroid-resistant acute GVHD.	Isolation of MSC from volunteer BM, expansion for 4 – 6wks under GMP followed by infusion.	N/A	Currently being evaluated.	[21]
Type 1 Diabetes	Phase I Interventional, non-randomised	Ongoing	Encapsulated human islets as a therapy for type 1 diabetes	To overcome the need for insulin injections in people with type 1 diabetes by transplanting islets placed in microcapsules to prevent the need for anti-rejection drugs.	Isolation of donated islets, and encapsulate-ion of cells	N/A	Pending.	[22]
GVHD associated with allogeneic HSCT	Phase I Interventional	Ongoing	MSC for Allogeneic Hemopoietic Stem Cell Transplantation	Test for the safety and efficacy of MSC harvested from the bone marrow of donors to improve hemopoietic engraftment and reduce GVHD when co-infused with the hemopoietic graft.	Isolation, culture expansion, characterisation, cryopreservation, infusion of donor MSC	N/A	Pending.	
Autoimmune Disease and Chronic Lymphocytic Leukemia	Translational	Ongoing	MSC as an Immunosuppressive Therapy for Autoimmune and Hematological Disease	Examine the interaction of allogeneic cultured MSC and B cells from patients with these disorders.	Isolation, culture expansion, characterisation, cryopreservation and infusion of donor MSC	N/A	Pending.	

Table 2. Regenerative Medicine (continued)

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Leukemia and Lymphoma	Transl-ational	Ongoing	Natural Killer Cells to Treat Leukemia and Lymphoma	Investigate *in vitro* methods of expanding a minor subset of NK cells that are alloreactive for use as a therapy for treating hematological malignancy.	As above	N/A	Pending.	
Inter-vertebral disc degeneration	Phase I randomised, placebo-controlled,double-blind study	Awaiting clinical trial registration	Bone marrow derived stem cell transplantation in patients with low back pain and disc degeneration.	This study will assess the safety and feasibility of autologous bone marrow derived stem cells injected into the intervertebral disc to repair lower back disc degeneration.	Autologous bone marrow derived stem cell	N/A	Pending.	[23]
GVHD associated with allogeneic hematopoietic stem cell transplantation (HSCT)	Phase 1 study, randomised control study	Under design	Treatment of allogeneic HSCT induced GVHD using MSC.	This study will investigate cell dose and passage number with the view to establishing the optimal combination for the treatment of patients with refractory GVHD.	Isolation of MSC, expansion under GMP followed by infusion into patients.	N/A	Pending.	[24]
Lung disease, in particular cystic fibrosis (CF)	Pre-clinical	Ongoing	Cell therapy for cystic fibrosis	To determine if unrestricted somatic stem cells (USSC) generated from cord blood can be used to treat lung disease.	Cord blood was collected from mothers undergoing elective Caesarean section.	N/A	USSC were successfully generated from 3 out of 9 cord blood samples. These cells showed tri-lineage differentiation including epithelial cells expressing SPC, and at certain time points CFTR. The ability of these cells to correct the CF defect is currently under investigation.	

Table 2. Regenerative Medicine (continued)

Target Disease	Study Type	Study Status	Study Title	Study Purpose	Cell Therapy used	Gene Modification used	Outcome	Ref
Muscle regeneration	Pre-clinical animal study	Completed	Vascular Endothelial Growth Factor (VEGF) Gene Therapy in Skeletal Muscle before Transplantation Promotes Muscle Regeneration.	To determine whether rAAV delivered VEGF to skeletal muscle prior to transplantation promotes muscle regeneration via improved revascularisation.	The tibialis anterior muscles of mice were injected with rAAV encoding VEGF and GFP followed by whole muscle auto-transplantation.	Adeno-associated virus (rAAV)-mediated vascular endothelial growth factor (VEGF) gene therapy.	Muscles were sampled 7 days after autografting. Although only small numbers of rAAV-transduced myofibers were present, whole muscle grafts preinjected with rAAV were significantly more vascular and showed greater regeneration (myotube formation) than saline injected and uninjected control muscle grafts.	[25]