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GENE THERAPY AND HAEMATOPOIETIC STEM CELL TRANSFER AS A STRATEGY TO TREAT AUTOIMMUNITY

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Autoimmune diseases result from a failure in immune tolerance and charac-
terized by a chronic immune response to self. A major challenge for treating
autoimmune diseases is the provision of a targeted therapy to promote disease
specific tolerance. We know that exposure to self-antigens during lymphocyte
development is a normal process of inducing self-tolerance. Furthermore, it has
been shown that ectopic expression of antigens can promote immune tolerance.

With this in mind, we have pursued a gene therapy strategy that involves ex vivo
manipulation of BM cells followed by transplantation to preconditioned recipients
to promote antigen specific tolerance. Our model system involves the develop-
ment of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice following immunization with MOG35-55 peptide. As a model for human multiple sclerosis, we have generated data to support the clinical feasibility of utilizing gene therapy as a strategy to treat autoimmunity. We have shown that BM transduced with retrovirus encoding MOG not only protect mice from developing EAE but can also be used in a protocol that reverses and maintains long-term remission. Mice in remission are resistant to relapse following challenge with MOG35-55 peptide. We report that this process can be achieved with low-level chimerism following the use of lower toxicity, non-myeloablative conditioning of recipients, an important aspect for clinical translation. The mechanisms of tolerance include the deletion of MOG specific T cells in the thymus and B cells in the bone marrow. This abstract will present a compilation of published and unpublished data that illustrates the application of this strategy to promoting tolerance and treating autoimmunity.

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THERAPEUTIC EFFECT OF TRAIL-SECRETING MESENCHYMAL STEM CELLS COMBINED WITH TEMOZOLOMIDE AGAINST MALIGNANT GLIOMA

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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. In this study, we demonstrated that the chemo-therapeutic agent temozolomide (TMZ), which is currently used in clinical practice, potentiates TRAIL-induced apoptosis. 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 administra-
tion of each agent alone. TMZ effectively increased the sensitivity to TRAIL-induced apoptosis via the upregulation of the death receptor 5 and the downregulation of anti-apoptotic proteins. In addition, this combined treatment resulted in a substantial increase in caspase activation. Furthermore, in vivo survival experiments and bioluminescence imaging analyses performed in mice bearing intracranial gliomas showed that treatment using MSC-TRAIL combined with TMZ had greater therapeutic efficacy than did single-agent treatments. Overall, these results suggest that the combination of clinically relevant TMZ and MSC-delivered TRAIL is a novel therapeutic strategy for improving the treatment of malignant gliomas.

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THERAPEUTIC ANGIOGENESIS - DESIGN AND FIRST RESULTS OF PRE-CLINICAL STUDY

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Background: ‘Therapeutic angiogenesis’ is a technique to induce cell-based ther-
apneic angiogenesis in ischemic tissue. As an approach to ‘therapeutic angiogenesis’, we established a cell-based, non-viral gene-transfer medicinal product using primary fibroblasts to temporarily produce bFGF and VEGF165. This is used as a form of pharmacological local preconditioning before tissue ischemia occurs. Building on experience gained under non-GLP conditions, we designed a GLP pre-clinical study, taking into account the protocol of animal studies, and the safety and efficacy of new therapeutic techniques.

Results: The eukaryotic expression vectors harbouring VEGF and bFGF cDNAs were transfected into rat primary skin fibroblasts mediated by Amaxa Nucleofector. The evaluation of the transfection efficiency and transport conditions for genetically modified fibroblasts (GMF) to the GLP facility was carried out. Clinical therapeutic effects after the application of GMF were determined in an ischemic flap model in rats. Planimetric measurements detected a reduction of flap necrosis in the flap model, reaching nearly 40% on several occasions, after two weeks if GMF were applied.

In addition to this proof of concept, we designed the following working packages (within the preclinical study: toxicity study, tumorigenicity study, biodistribution/plasmid migration, target tissue selectivity, and environmental shedding. The design of the preclinical studies was approved by the Regulatory Authorities.

The expression of therapeutic factors in vitro and the detection of therapeu-
tic plasmids as active substances in rats in vivo were detected using a specific real time PCR. We could only trace a few plasmids in some organs over a period of 12 weeks.

Conclusions: Thus, in our present work we designed a preclinical study for ‘Therapeutic Angiogensers’ and universal use techniques that allow the recognition of the potential risks of gene therapies. The proof of concept study helps us to identify the relationship between the dosage and therapeutic effects.

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NOVEL APPROACH TO GENERATE CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS USING GENETICALLY MODIFIED T CELL PRECURSORS

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Generation of genetically modified T cells targeting cancer cells is a novel approach with proven success in animal models and early phase human trials. A patient’s own or matched donor T cells can be genetically modified in the laboratory to target antigens expressed on tumour cells through the introduc-
tion of chimeric antigen receptor (CAR) genes. Tumour-targeted T cells must persist for a sufficient period of time to result in successful tumour elimination. Mature CAR-T cells, however, rapidly differentiate into short-lived effector cells that limit anti-tumour activity in vivo. We hypothesised that continuous generation of mature T cells from genetically modified T cell precursors may circumvent this shortcoming. Human T cell precursors have been generated using CD19+ haematopoietic progenitor cells cultured with immobilised D4 Notch ligand promoting T cell differentiation. To generate CAR-T cell precursors, CD34+ cells are being retrovirally transduced with CAR-gene. CAR-T cell precursors targeting CD19+ will be tested in ‘humanised’ mouse model of B-cell leukaemia. We expect that adoptive transfer of CAR-T cell precursors will provide an improved persistence and enhanced anti-leukemic effect compared to mature CAR-T cells.

Unlike non-MHC-matched mature CAR-T cells possessing intact allogeneic responses, T cells produced by allo-T cell precursors undergoing natural selection in the host’s thymus, develop tolerance to the host antigens. Therefore, non-MHC-matched T cell precursors can be used for any patient regardless HLA-match. Our approach enables the generation and storage of relatively small quantities of T cell precursors for universal use techniques to large numbers of mature CAR-T cells. The ability to generate off-the-shelf third party T cell precursors that target tumours provides a new application to the use of cord blood and provides the rationale for the establishment of a bio-bank of different CAR-T cell precursors to treat cancer in transplant patients.

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BONE MARROW-DERIVED MESENCHYMAL STEM CELLS EXPRESSING INTERFERON-GAMMA INHIBIT PROLIFERATION OF CHRONIC MYELOID LEUKEMIA CELLS IN VITRO

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Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder caused by the BCR/ABL gene rearrangement. To date, the only curative therapy for CML is allogeneic stem cell transplantation. However, significant morbidity and mortality are associated with the procedure and the need for a matched donor makes this option not available to the majority of the patients. Currently, various studies have been carried out to develop an alternative approach for CML treatment, for example targeted gene delivery of therapeutic cytokines. In this study, the feasibility of using bone marrow-derived mesenchymal stromal cells (BM-MSC) in delivering interferon-gamma (IFN-\(\gamma\)) gene for targeted CML therapy was explored. BM-MSC were transfected with IFN-\(\gamma\) gene via nucleofection. The transfection efficiency was determined from intracellular IFN-\(\gamma\) expression by flow cytometry and was found to be at 54% (\(P<0.05\)). The in vitro expression of IFN-\(\gamma\) mRNA and protein in BM-MSC were also analysed at an interval of 24 h, up to 5 days post nucleofection, via real-time PCR and ELISA, respectively. Real-Time PCR data analysis showed a significant up-regulation of IFN-\(\gamma\) mRNA in nucleofected BM-MSC when compared to non-transfected BM-MSC (control). These results corresponded with the expression of IFN-\(\gamma\) protein in the supernatant of nucleofected BM-MSC, which was sustained at high level for 5 days as compared to the control group. We further investigated the effects of IFN-\(\gamma\) produced in nucleofected BM-MSC on the proliferation of CML cell line (K562) in vitro. 61% of K562 growth inhibition were observed after seven days of co-culture with nucleofected BM-MSC (\(P<0.05\)). In conclusion, our results indicate that the production of IFN-\(\gamma\) by nucleofected BM-MSC successfully inhibited the proliferation of K562 cells in vitro. MSC as vehicle in IFN-\(\gamma\) gene delivery could be further explored as a promising treatment option for CML patients.

The self-renewal, tumor-tropic migration ability and low immunogenicity of mesenchymal stem cells (MSCs) make it a potential delivery candidate for suicide genes for anti-tumor therapy, but scarcely put into translational study mainly because of unreliable supply of well-characterized cellular products. Regarding this technical difficulty, we generated immortalized human fetal bone marrow-derived MSCs (hfBMSCs) expressing herpes simplex virus thymidine kinase (TK) by conventional lentiviral transduction method. Functional expression of TK was evaluated by cytotoxicity in the presence of its prodrug, ganciclovir (GCV). SV40-TK-hfBMSCs exhibited comparable proliferation, surface phenotype expression, multi-differentiation potential and tumor-tropic migration ability as hfBMSCs. Mixed lymphocyte reaction demonstrated that SV40-TK-hfBMSCs did not induce significant proliferation of lymphocytes isolated from healthy adults. By in vivo live imaging and measurement of tumor volume, repeated injection of the SV40-TK-hfBMSCs (1 \(\times\) 10e6/kg, i.v.) and subsequent consecutive GCV administration (30 mg/kg, i.p.) could effectively suppresses tumor growth in DU145 or PC3 human prostate tumor xenograft nude mice model, without causing weight loss as in the group receiving treatment of doxorubicin, a conventional chemotherapeutic. Our findings provide a proof-of principle that immortalization of human fetal MSCs expressing suicide gene represents a safe and novel way to pursue the clinical development of MSCs as tumor specific vehicle for gene therapy. Optimization in cryopreservation and package could allow its clinical application in an off-the-shelf manner.