287. Improved Outcomes Following Drug-Resistant Immunotherapy in a Human Xenograft Model of Temozolomide-Resistant Glioblastoma Multiforme

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INTRODUCTION: Conventional treatment strategies for Temozolomide (TMZ)-resistant high-grade gliomas have been uniformly dismal. We have shown that TMZ-resistant tumors up-regulate stress-associated NKG2D ligands (NKG2DL) during the first hours after exposure to TMZ, thereby creating an opportunity for NKG2DL-directed cell therapies. We report improved survival using a combination of TMZ chemotherapy and gene modified TMZ-resistant γΔ T cells which we term Drug Resistant Immunotherapy (DRI). TMZ resistance is conferred by lentivector transfer of methylguanine methyltransferase (MGMT) enabling cytotoxic lymphocyte function during high-dose chemotherapy.

METHODS: Tumor NKG2DL expression and DRI cytotoxicity were assessed by flow cytometry. Intracranial (IC) glioma xenografts were established on Day 0 with either unmodified (P) or a TMZ resistant clone (T) of human primary GBM-X12 passaged exclusively in immunodeficient mice. Tumor-bearing mice were treated with intraperitoneal TMZ 60mg/kg on days 6, 8,13, and 15 followed by IC injection of 1.5 x 10^6 DRI 4h after each TMZ dose x 2 weeks. Control mice received non-modified cells, TMZ, or no therapy. Survival was assessed using Kaplan-Meier analysis.

RESULTS: Both GBM-X12P and X12T express NKG2DL MIC-A, MIC-B, and ULBP-4. DRI cells killed both tumors in vitro at approximately 80% at an effector: target ratio of 20:1 with no toxicity against cultured human astrocytes. Cell therapy alone did not improve survival beyond that of untreated mice. For the parent tumor X12P (Figure 1), TMZ therapy significantly improved median survival over untreated controls (29 vs. 59 days, $p < 0.0001$), and addition of DRI increased median survival to 120 days with 80% long term survivors. For the TMZ-resistant X12T (Figure 2), TMZ alone did not improve survival over untreated controls (22 vs. 27 days $p = 0.4827$). Addition of DRI significantly increased median survival from 22 days to 38 days ($p = 0.0004$) with 10% of animals surviving to day +119.

Figure 1
JX12P TMZ vs DRI

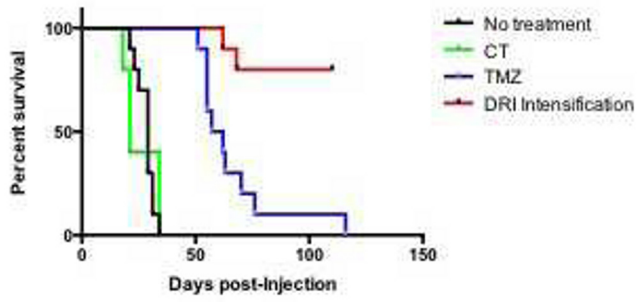
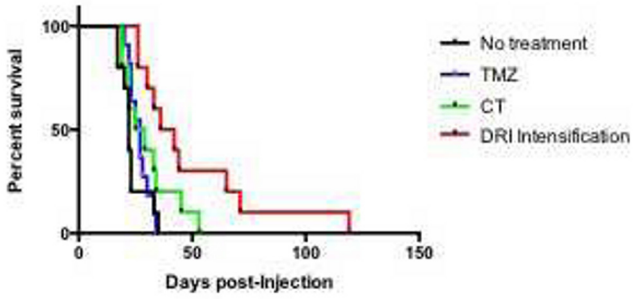


Figure 2
JX12T TMZ vs DRI



CONCLUSIONS: This is the first report of combined TMZ chemotherapy and drug-resistance modified γΔ T cell therapy resulting in a significant increase in time to progression and improvement in median and overall survival in immunodeficient mice bearing otherwise impervious human primary xenograft tumors using a strategy is readily adaptable to the clinical setting

Hematologic and Immunologic Diseases: Preclinical Development and Insights

288. Dual-Regulated Lentiviral Vector for Gene Therapy of X-Linked Chronic Granulomatous Disease

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Chronic Granulomatous Disease (CGD) is caused by defective NADPH oxidase function in phagocytes leading to increased susceptibility to fungal and bacterial infections. The majority of CGD is caused by mutations in the gp91phox gene. Gene therapy with hematopoietic stem cells (HSC) may represents a good alternative to allogenic haematopoietic stem cell transplantation. Past clinical trials for X-linked CGD with γ retroviral vectors resulted in transient clinical benefit but were limited by insertional oncogenesis and lack of persistent engraftment. To circumvent these issues we developed a strategy based on regulated, self-inactivating lentiviral vectors (LVs). We designed different gp91phox-expressing LVs: 1) PGK.gp91phox, in which gp91phox is driven by an ubiquitous cellular promoter; 2) MSP.gp91phox, to control the transgene expression at a transcriptional level using a myeloid specific promoter; 3) PGK.gp91phox_126T(2), in which we exploited the miRNA system, incorporating miR-126 target sequences, to prevent the transgene off-target expression in HSC; 4) MSP.gp91phox_126T(2), combining the posttranscriptional de-targeting with the MSP (dual regulated vector). These LVs were tested on human and murine HSC and in a mouse model of X-CGD. All vectors restored gp91phox expression and function in human X-CGD primary monocytes and differentiated myeloid cells. While unregulated LVs induced a transgene ectopic expression in CD34+ cells, transcriptionally and post-transcriptionally regulated LVs reduced this off-target expression while guaranteeing a good level

of expression in differentiated cells. With the dual-regulated vector, we achieved high levels of myeloid-specific transgene expression, entirely sparing the most primitive CD34+CD38-CD90+ HSC compartment. X-CGD mice transplanted with all vectors engrafted and restored gp91phox expression, with 20-70% of granulocytes and monocytes expressing human gp91phox, persisting up to 10 months after gene therapy. MSP-driven vectors were superior in maintaining regulation during BM development. Oxidase activity in corrected granulocytes was superior using MSP-driven vectors as compared to PGK. Gene therapy-treated mice were then infected with S.aureus in order to test their ability to clear one of the most recurrent bacteria causing infections in CGD patients. All gene therapy treated mice were able to clear pulmonary infection in contrast with untreated X-CGD untreated mice. The dual-regulated LV represents a promising approach for further clinical development of gp91phox therapeutic vectors.

289. Safe and Effective Gene Therapy for Wiskott - Aldrich Syndrome Using an Insulated Lentiviral Vector

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Wiskott–Aldrich Syndrome (WAS) is a severe X-linked immunodeficiency disorder characterized by recurrent infection, thrombocytopenia, and autoimmunity resulting from mutations within the WAS gene. The encoded gene product, WAS protein (WASp), is a scaffold protein important for activation of the actin cytoskeleton downstream of multiple hematopoietic receptors. Development of safe and effective hematopoietic stem cell (HSC) gene therapy for WAS would provide a valuable treatment alternative to allogeneic bone marrow transplantation. Of critical importance for such an approach is the capacity to achieve sufficient WASp expression in relevant lineages to correct the multiple deficits, while avoiding development of autoimmune disease. We previously tested a panel of self-inactivating (SIN) lentiviral (LV) vectors expressing human WAS via alternative internal promoters and observed optimal expression and rescue using the modified γ-retroviral derived LTR, MND. To improve the potential safety and stability of expression, we incorporated a 650bp chimeric insulator from the chicken β-globin locus into this LV. Using in vitro assays the insulated LV exhibited minimal LMO2 transactivation and no evidence for immortalization of cultured HSC. Next, we generated a stable high-titer producer clone expressing this candidate clinical LV, CL20-i650–MND-WAS. Here, we evaluate the efficacy and safety of the insulted vs uninsulated LV (CL20-MND-WAS) in a large cohort of primary and secondary transplanted Was-/- mice. Both vectors enabled stable engraftment of WASp expressing cells in all hematopoietic lineages, positive selection of WASp+ cells, and rescued functional and developmental T and B cell defects without evidence for toxicity. Inclusion of the insulator allowed for higher expression levels at lower numbers of viral integration per cell; a feature that was further exaggerated in secondary recipients. Finally, in parallel, we evaluated CL20-i650–MND LV expressing a fluorescent marker using CD34+ autologous HSC transplants in a non-human primate model (Macaca nemestrina) and observed long term stable polyclonal engraftment of LV expressing hematopoietic cells for up to 2 yr. Based on these extensive pre-clinical and toxicity studies we anticipate that CL20-i650–MND-WAS LV will provide sustained rescue of platelet and lymphocyte development and function in vivo (facilitated, in part, via the insulator) without evidence of toxicity. We plan to directly test this hypothesis in an upcoming WAS LV clinical trial.

290. Baboon Envelope Pseudo Typed Lentiviral Vectors Mediate High-Level Gene Transfer in Human B Cells Allowing Secretion of FIX at Therapeutic Levels in NSG Mouse

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B lymphocytes are attractive targets for gene therapy of genetic diseases associated with B-cell dysfunction. In addition, long-lasting transgene expression in B cells is of particular interest for immunotherapy by its potential to induce specific immune activation or tolerance. Moreover, B cells are potent specialized protein secreting cells. One obstacle though is that primary B cells are poorly transduced by VSVG pseudotyped lentiviral vectors (LVs) even when stimulated through the BCR to induce proliferation. We demonstrated that this is due to the poor expression of the low density lipid (LDL) receptor, identified as the VSV receptor (Amirache et al, 2014).

Since we recently showed that baboon retroviral envelope pseudotyped LVs (BaEV) outperform VSV-G-LVs for gene transfer into cytokine-stimulated and resting hematopoietic stem cells, we evaluated them here for gene transfer into primary human B cells. Upon B cell receptor stimulation, BaEV-LVs transduced up to 70% of the B cells. As expected, VSV-G-LVs were unable to transduce activated nor unstimulated resting human B cells efficiently, even at high vector doses (MOI= 100, VSV-G-LV < 5% transduction). Remarkably, BaEV-LVs permitted highly efficient transduction of 30% of resting B cells. Moreover, they transduced as well memory as naive B cells without inducing phenotypic changes. In addition, BaEV-LVs permitted up to 80% transduction of human plasmocytes. Adaptive transfer of mature BaEV-LV transduced human B cells into NSG mice allowed differentiation into plasmablasts and plasma B cells, both characterized by a high level gene marking in the lymphnodes, spleen and bone marrow. These results encouraged us to evaluate BaEV-LV mediated gene transfer of Factor IX into human B cells for treatment of heamophelia, a severe blood disorder. We produced high-titer BaEV-LVs carrying a codon optimized FIX with a hyperactivating FIX-R338L mutation. These BaEV-FIX-LVs efficiently transduced plasmocytic B cell lines leading to high-level FIX secretion (20-70% of normal levels in human serum). Transduction of primary human B cells followed by adaptive transfer into NSG mice resulted in therapeutic levels of FIX in the serum (7-25% of normal FIX levels) at 3 to 4 weeks of engraftment. Moreover, a faster coagulation of the blood proved that FIX was functional.

Concluding, the BaEV-LVs might represent in the future valuable tools for therapeutic protein secretion from autologous B cells for treatment of heamophelia and other disorders in vivo.