impairs long-term differentiation and proliferation of MGMT-P140K expressing stem cell clones. To address this question, serial transplantations of murine MGMT-P140K expressing hematopoiesis combined with repeated administrations of O6-BG and BCNU were done. After *ex vivo* gene transfer of an MGMT/IRES/eGFP encoding retroviral vector, bone marrow cells were transplanted into syngeneic C57 BL/6J mice and 1st, 2nd and 3rd generation recipient mice were subsequently treated every four weeks in order to exaggerate potential effects on long-term clonal behaviour. Lineage contribution of transduced hematopoiesis was monitored by FACS over a total of 17 rounds of selection and clonality by LAM-PCR over a total of 16 rounds of selection. In primary mice the percentage of transduced blood cells increased from 4.7 ± 0.8 % to 36.4 ± 9.8 % (n=12) and in secondary mice from 29.9 ± 7.2 % to 65.1 ± 8.7 % (n=18) after selection without persisting peripheral blood cytopenia. Lineage analysis showed an unchanged multilineage differentiation potential of transduced cells in 1st, 2nd and 3rd generation animals. LAM PCR analysis of peripheral blood revealed stable oligo- to polyclonal hematopoiesis in 1st, 2nd and 3rd generation mice. Evidence for predominant clones or clonal exhaustion was not observed. Interestingly, pairs of secondary transplanted mice that received bone marrow cells from identical donors showed very similar clonal composition, engraftment kinetics under selection and lineage contribution of the transduced hematopoiesis, indicating extensive self-renewal of transplantable stem cells in the primary mice resulting in a net symmetric refilling of the stem cell compartment. In summary, we demonstrate that even extended selection of MGMT-P140K expressing hematopoietic stem cells by repetitive chemotherapy does not affect their differentiation or proliferation potential and does not result in clonal exhaustion. Our results have important implications for the clinical use of MGMT selection strategies for amplification of a limited number of gene corrected clones in clinical gene therapy.

MS and CvK hold US-patent on LAM-PCR.

442. LV Expressing MR Reporter Genes Allows In Vivo Monitoring of Stem Cell Gene Therapy

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Somatic stem cells (SSC) have raised interest because of their therapeutic potential in both cell-based and gene therapy applications. Towards this goal, tracking the fate of either delivered cells or of genetically modified endogenous cells is of utmost importance. Diverse imaging approaches are available for cell tracking and among these MRI shows a greater resolution and allows direct anatomic correlation and long-term studies of dynamic cell migration on living animals. Superparamagnetic iron oxide (SPIO) has been used to label SSC in vitro and to make them detectable in vivo upon transplantation. However, major limitations of this approach are the progressive dilution of the contrast media among cell progeny and the need for ex vivo SPIO loading. We thus explored an alternative strategy based on the combination of lentiviral vectors (LV), which efficiently transduce SSC both ex vivo and in vivo and allow long-term expression in their progeny, and MR reporter genes, able to increase iron uptake and accumulation into different cell types.

We tested human tyrosinase and human ferritin chains in the context of bi-directional LVs carrying each MR reporter gene together with GFP.

These LVs were first used on cell lines to analyze protein expression, iron accumulation and to compare the different MR reporter genes. Transduced cells became detectable in T2 and T2* weighted images, concomitantly with the appearance of melanin or iron within cellular bodies, and fluorescence for GFP expression.

Similar experiments were performed on primary human SSC, like hematopoietic and neural stem cells. Viability, proliferation and differentiation capacity were preserved after gene transfer of the MR genes.

The new LVs were then injected both into the mouse brain striatum to mark resident neurons, and into the sub ventricular zone (SVZ) to label neural progenitor cells (NPC) and their progeny.

Injected mice underwent serial MR examinations by a 3 Telsa human scanner and were eventually analyzed for protein expression in the brain by immunofluorescence. MR reporter expression was detectable in vivo in the striatum from 1 week up to 3 months (latest time of analysis) from the time of vector injection. Pathology confirmed co expression of GFP and the reporter gene. Upon SVZ delivery, we are currently following the migration of genetically modified NPC progeny along the rostral migratory stream to the olfactory bulb. Overall these data suggest that expression of MR marker genes enables efficient cellular marking for MR localization studies and that LV transduction represents a promising strategy for in vivo long term monitoring of stem cell fate and their progeny. Furthermore, the use of MR reporter gene in a bi-directional vector together with gene of interest will allow studies coupling imaging to the administration of a bioactive molecule.

443. Successful Long-Term Doxycycline-Regulated Transgene Expression in the Retina of Nonhuman Primates Following Subretinal Injection of Recombinant AAV Vectors

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Adeno-associated viral gene therapy has shown promise for the treatment of inherited and acquired retinal disorders. In most applications, regulation of expression is a critical concern for both safety and efficacy. The purpose of our study was to evaluate the ability of the tetracycline-regulatable system to establish long-term transgene regulation in the retina of nonhuman primates. Three rAAV vectors expressing the tetracycline dependent transactivator (rtTA) under the control of either the ubiquitous CAG promoter or the specific RPE65 promoter (AAV2/5.CAG.TetOn.epo, AAV2/4.CAG.TetOn.epo, and AAV2/4.RPE65.TetOn.epo) were generated and administered subretinally to seven macaques. We demonstrated that repeated inductions of transgene expression in the nonhuman primate retina can be achieved using a Tet-inducible system via rAAV vector administration over a long period (2.5 years). Maximum erythropoietin (EPO) secretion in the anterior chamber depends upon the rAAV serotype and the nature of the promoter driving rtTA expression. We observed that the EPO isoforms produced in the retina differ from one another based on the transduced cell type of origin within the retina and also differ from both the physiological EPO isoforms and the isoforms produced by AAV transduced skeletal muscle.