First, we demonstrated that microglia cells can be gene modified *in vitro* to efficiently express and secrete a model single chain variable fragment (scFv) antibody, which then correctly binds to its target antigen when immobilised on a synthetic surface or expressed on the plasma membrane of HEK cells. Subsequently, we transduced HSCs with lentiviral particles carrying the scFv construct and optimised protocols to differentiate them into microglia cells *in vitro* to investigate the ability of HSC-derived microglia to efficiently secrete a functional antibody. The same HSCs were also transplanted into conditioned mice to analyse antibody production in the brain upon HSC engraftment and differentiation into microglia-like cells *in vivo*.

Microglia cells play a central role in neuroinflammation and they are particularly enriched in certain pathologies. The ability to harness their localisation for the target delivery of therapeutic antibodies could dramatically improve the prognosis of serious neurological conditions.

OR07

Acquisition of somatic mutations after hematopoietic stem cell gene therapy varies among cell lineages and is modulated by vector genotoxicity and the activity of key cellular senescence gene

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The hematopoietic system of patients undergoing Hematopoietic Stem and Progenitor Cell (HSPC) Gene Therapy (GT) is fully restored when autologous engineered HSPCs are reinfused into the patient. During this process, HSPCs go through a high level of proliferation until the hematopoietic reconstitution is complete. The impact of proliferation in HSPCs on cellular fitness and safety remains an open question. Moreover, the accumulation of somatic mutations in vivo could show differences in different hematopoietic lineages depending on their susceptibility to the negative effects elicited by the DNA damage response. Furthermore, oncogene activation in human HSPCs has been shown to trigger a chronic inflammatory response leading to hematopoietic decay.

Here we studied the clonality and the accumulation of somatic mutations in different hematopoietic lineages and during hematopoietic reconstitution in mice subjected to HSPC-GT. Indeed, wild type C57 mice were transplanted with bone marrow-derived lineage negative (Lin-) cells from WT mice or tumor-prone *Cdkn2a*$^{-/-}$ mice which lack p16^{INK4A} and p19^{ARF} proteins and thus have no barriers against proto-oncogene activation. Moreover, to evaluate if genotoxic integrations may increase the probability of acquiring somatic mutation upon oncogene activation, Lin- cells were transduced with a genotoxic LV harboring the strong retroviral enhancer/promoter Spleen Focus Forming Virus in the LTR (LV.SF.LTR) or the safer GT-like non-genotoxic LV (SIN.LV.PGK).

Mice receiving WT Lin- cells treated with LV.SF.LTR (N = 25) or SIN.LV.PGK (N = 24) did not develop tumors, while mice transplanted with *Cdkn2a*/LV.SF.LTR-marked cells (N = 24) developed tumors significantly earlier compared to mock (N = 20, $p < 0.0001$) and mice receiving *Cdkn2a*/SIN.LV.PGK-treated cells (N = 23, $p < 0.0001$). To evaluate the clonal dynamics of hematopoietic reconstitution, vector integration sites (IS) were identified by by Sonication Mediated Integration Site (SLiM) PCR from peripheral blood, lymphoid (B and T) and myeloid cells collected every 4 weeks post-transplantation. Somatic mutations were identified by analyzing the mouse genomic portion flanking each IS using VarScan2. Overall, we detected >200,000 IS, corresponding to more than 135 Mb of genomic sequence information. We introduced a new Mutation Index (MI), which normalizes the number of mutations by clones and coverage to assess mutation accumulation rates. By this approach, we found that the MI increased over time in LV.SF.LTR-treated mice and was significantly higher when compared to SIN.LV.PGK-treated mice ($p < 0.001$). Notably, myeloid clones exhibited a higher frequency of mutation accumulation compared to T and B cell lineages. This phenomenon was further exacerbated in Cdkn2a/LV.SF.LTR-marked cells, indicating that the absence of barriers to proto-oncogene activation and the presence of genotoxic insertions result in progressive somatic mutation accumulation and insertional mutagenesis.

These results demonstrate for the first time that by combining the assessment of acquired mutations with IS analysis at the single clone level we can identify differential accumulations of somatic mutations in different hematopoietic lineages in vivo which depend on the genotoxic potential of the vector used and the ability of the genetically modified cells to sense and react to genotoxic lesions.

OR08

In vivo hematopoietic stem cell gene therapy using BaEVRLess-pseudotyped retroviral vectors

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In vivo hematopoietic stem cell (HSC) gene therapy has several potential advantages over *ex vivo* gene therapy, such as eliminating the need for stem cell harvest, *ex vivo* cell manipulation and conditioning of the patient. The VSVg envelope glycoprotein is commonly used for the pseudotyping of retroviral vectors but is not well suited for *in vivo* application due to its serum sensitivity and poor ability to mediate gene transfer into quiescent HSCs. In contrast, the baboon endogenous retrovirus (BaEV) glycoprotein, and its derivative BaEVRLess, mediate efficient gene transfer into resting HSCs and are serum resistant. Here, we explore the potential of BaEVRLess-pseudotyped retroviral particles for *in vivo* HSC gene therapy. Initially, to overcome problems during virus production related to high fusogenic activity of the BaEVRLess envelope, we generated a stable BaEVRLess-packaging cell line carrying knockout