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Safety management of iPSCs in hematopoietic regenerative medicine

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Several limitations prevent the use of iPSCs in hematology. A population of differentiated cells is always contaminated by residual undifferentiated iPSCs. Persistence of iPSCs in a ready-to graft cell population is a critical hurdle because they can form teratomas. Our goal was to develop potent tools to prevent iPSC-derived teratomas. iPSCs-specific expression of a suicide-gene under the control of an embryonic-promoter is supposed to specifically kill iPSCs when the appropriate pro-drug is added. We constructed lentivectors containing icaspase-9 gene and tdTomato under the control of an embryonic-pmiR302/367 promoter and transduced iPSCs. After cell-sorting of tdTomato + iPSCs, the promoter was specifically active in iPSCs. We established in vitro toxicity of the icaspase9 inducer AP20187 in iPSCs. We observed that td-Tomato + cells and TRA1-60 + cells decreases in a dose-dependent manner. Unfortunately, AP20187 treatment does not lead to complete eradication. Moreover, as suicide-gene approach will be used to eliminate iPSCs from hematopoietic cell population, we looked at AP20187 toxicity on cord-blood cells and observed an unexpected toxic effect. YM155, a surviving inhibitor, has been reported to be effective to eliminate iPSCs. We compared it to our gene-suicide approach and found to be more efficient without toxicity on cord-blood cells. We are currently testing its capability to prevent teratoma in vivo using iPSC-expressing luciferase. This study will be useful to improve the safety-management for iPSCs-based medicine.

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Interleukin-13 secretion by allogeneic mesenchymal stem cells reduces allograft-specific CD8 + T cell activation, induces M2a macrophage polarization, and promotes allogeneic cell graft survival in mice

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Transplantation of genetically engineered mesenchymal stem cells (MSCs) for in situ therapeutic protein delivery is an attractive alternative to (non-)viral gene delivery approaches for the modulation of the clinical course of several diseases and traumata. From an emergency point-of-view, the use of pre-engineered allogeneic MSCs as off-the-shelf cell preparations has numerous advantages over the use of patient-specific autologous MSCs. Although in vitro co-culture confirmed the immunomodulating properties of allogeneic MSCs on dendritic cell (DC) function, allogeneic MSC-primed DCs lost their immunomodulatory phenotype upon in vivo introduction. Similarly, although allogeneic MSCs administered intramuscularly or intracerebrally, but not intravenously, can survive 1 week following in vivo administration, they are irrevocably rejected by the host's immune system. In an attempt to modulate MSC allograft rejection, we transduced MSCs with an interleukin-13 (IL13)-expressing lentiviral vector. Upon intramuscular transplantation, IL13-expressing MSCs induced fewer alloantigen-reactive IFNγ- and/or IL2-producing CD8 + T cells than non-modified allografts. Histological analyses of intramuscular and intracerebral allografts further revealed that the robust infiltration of Iba1- and MHCII-expressing inflammatory cells was a common feature of all examined MSC allografts, whereas expression of the M2a-polarization markers Arg1, Ym1 and FIZZ1 was restricted to macrophages infiltrating IL13-producing implants. Finally, using in vivo bioluminescence imaging, we established that IL13-producing MSC allografts survive significantly longer than their wild-type counterparts upon both intracerebral and intramuscular implantation. In summary, this study demonstrates that both innate macrophage and adaptive CD8 + T cell immune responses are effectively modulated in vivo by locally secreted IL13, ultimately resulting in prolonged MSC allograft survival.

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Empty capsids and macrophage inhibition/depletion increase rAAV-transgene expression in joints of both healthy and arthritic mice

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Objective: rAAV5 is effective in delivering genes to the joint, however, the presence of macrophages in the inflamed joint might hamper gene delivery. We determined whether administration of agents that influence macrophage activity/number and/or addition of empty decoy capsids had an effect on rAAV5-transgene expression.

Material and Methods: Healthy or arthritic mice were injected with rAAV5.CMV.Fluc in both knee joints and monitored for luciferase expression (from 3 days - 6 months) by IVIS. Where indicated, empty capsids were co-administered with full particles. Macrophages were depleted or inhibited by systemic administration of clodronate liposomes or triamcinolone 48 hours prior to vector administration.

Results: Administration of rAAV5.CMV.Fluc in arthritic mice after the onset of inflammation resulted in lower expression of luciferase compared to vector administration before the onset of inflammation. Both macrophage depletion/inhibition or empty decoy capsid improved expression over a period of 4 weeks. The combination of macrophage inhibition and empty capsid resulted in a synergistic increase in gene expression (5.85 fold, $p = 0.001$), that was sustained for 6 months. We compared the efficacy of i.a versus i.m. triamciolone administration and found that enhancement of gene expression was independent of administration route. This effect was also observed in healthy animals.

Conclusions: We provide evidence that intra-articular macrophages are a barrier to efficient gene transfer, and that combination of triamcinolone and empty decoy capsids results in