Immune Response in Stem Cell-based Therapy
27 September 2012
Questions & Answers

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1. Could you comment on the utility of ^{19}F labeling?	**Heike Daldrup-Link:** ^{19}F is not naturally present in biological tissues. Thus, cells labeled with ^{19}F provide a very specific signal on MRI scans, without confounding signal from "background tissue". Since the MRI signal and ^{19}F concentration are linearly related, ^{19}F-labeled cells can be quantified with this technique. A first ^{19}F agent for in vivo cell tracking has been recently FDA-approved (Celsense Inc). Current challenges include the generation of sufficient MR signal by ^{19}F labeled stem cells for in vivo detection. So far, ^{19}F labeling does not provide information about the viability of the labeled cells, i.e. both ^{19}F labeled viable and dead stem cells provide similar MR signal. A comprehensive review over ^{19}F cell labeling and cell-tracking studies is provided in Biomaterials 33 (2012) 8830-8840.
2. Please explain how you measured GAGS by MRI in cartilage?	**Heike Daldrup-Link:** Delayed Enhanced Magnetic Resonance Imaging of Cartilage (dGMERIC) is a technique for non-invasive monitoring of the GAG content of articular cartilage. It relies on the negative charge of both gadolinium-based MR contrast agents (e.g. Gd-DTPA) and GAG. Following intravenous Gd-DTPA injection and an appropriate delay, which allows the contrast agent to penetrate into cartilage, Gd-DTPA accumulates in cartilage areas with low GAG content. T1-relaxation times of cartilage, measured on dGMERIC scans, are indirectly proportional to gadolinium concentrations, and directly proportional to GAG concentration in cartilage, i.e. a low GAG level is associated with high gadolinium concentration and low T1-relaxation time and a high GAG level is associated with low gadolinium concentration and high T1-relaxation time.
3. To assess cytokine levels, do you recommend looking at protein or message levels?	**Amitabh Gaur:** We recommend looking at the level of proteins. Change of mRNA level may not always translate to the protein product. Thus measuring the protein level can give you a more accurate result. RNA can certainly be measured for specific cytokines and the data can be compared with the protein results.

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4. Genetic stability- what methods should be used, and what result would make the FDA nervous?	**Theresa Chen:** Genetic stability concerns may be immune related and might also be safety related. The potential concerns and methods to assess genetic stability for specific products should be discussed with FDA.
5. Will allogeneic MSCs trigger severe immune response?	**Amitabh Gaur:** It might trigger an immune response since it expresses allo-antigens. Interestingly, MSCs express immunosuppressive molecules to reduce this response. Experiments need to be done to prove it.
6. How different are the transplanted allogeneic vs. autologous MSCs regarding immunogenicity?	**Amitabh Gaur:** Autologous MSCs should be less immunogenic than Allogeneic MSCs. However, in mouse models, autologous MSCs have been shown to induce an immune response.
7. As of now with Allogeneic MSC's is there any Immune response reported? Also how to clinically predict this?	**Amitabh Gaur:** Schu et al., have shown that allo-MSCs induce formation of allo-antibodies with the capacity capable of causing complement-mediated lysis. (Schu S. etal. J of Cellular and Mol. Med. 2011 16(9):2094-2103) Nauta et al., have shown that allogeneic MSCs induce memory T-cell responses resulting in rejection of the stem cell graft. (Nauta A.J. et al. Blood 2006 108(6):2114-2120) One suggestion would be to measure the level of expression of MHC, immune stimulatory co-stimulatory molecules on the surface of donor MSCs by FACS; co-culturing the MSCs with recipient immune cells and their resulting activation could also help discover the immunogenic potential of the stem cells.
8. Please address how we model and assess the risks (to humans) of immunosuppression regimens in our pre-clinical (animal) studies	**Amitabh Gaur:** Assays that look for the risk of cancer (e.g., teratomas) and frequent and severe viral infections could be employed
9. Please address the acceptability of life-long immunosuppression for recipients of hESC-derived cells	**Amitabh Gaur:** Life-long immuno suppression is not recommended since it could induce cancer formation and result in debilitating viral infection in patients. **Theresa Chen:** The acceptability of life-long immunosuppression depends on the specific clinical situation and the risks and benefits associated with the specific immunosuppressant and the specific cellular product.

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10. Are there any specific in vitro assays the speakers could recommend to provide insights into immunogenicity of stem cell derived cells?	**Amitabh Gaur:** 1. Measuring Activation markers by flow cytometry: e.g. CD25, CD69, HLA-DR etc. Expression of HLA molecules, immuno co-stimulatory molecules and adhesion molecules on various stem cells. 2. Measuring specific T-cell immune responses: (using irradiated target cells, cell lysates, peptide or protein as stimulating antigens in an *in vitro* recall assay) Intracellular cytokine staining ELISPOT Proliferation assay (CFSE-based)
11. Does the immune profile of a typical MSC change under culture conditions?	**Amitabh Gaur:** There is evidence that suggests that culturing MSCs in the presence of certain growth factors leads to expression of higher levels of MHC molecules. (Please see the next question)
12. Can expansion of MSC occur without inducing MHC expression?	**Amitabh Gaur:** MSCs express MHC II molecules when expanded in the presence of growth factors e.g., FGF-2 and PDGF. (Bocelli-Tyndall C. et al. Arthritis & Rheumatism 2010 62(12):3815-3825) There is another report showing that growing MSC in a FCS-free medium is feasible without addition of growth factors. Those MSCs retained their immaturity. (Stute N. et al. Exp Hematol 2004 32(12):1212-25)
13. Once transplanted is there a difference between efficacy of cultured and non-cultured MSCs?	**Amitabh Gaur:** This is a good question, but it needs more experimentation to find the answer. We have not seen a report that has compared the results.