



Prof
Jacques
Galipeau

We propose to employ a transgenic cell therapy strategy as treatment for hemophilia A. This study will examine a novel technological platform which addresses the weaknesses of a Hemophilia A cell therapy strategy published by DA Roth et al (NEJM, 2001) and avoids the pitfalls of a 'gene therapy' dependent on administration of potentially toxic viral vectors. We have published data that reveals that autologous marrow stromal cells (MSCs): 1. can be readily harvested from donors without the need of mobilization regimens, 2. are easily expanded in tissue culture in large number and, 3. are amenable to genetic engineering. We also have 'proof-of-concept' that supports the use of genetically-engineered MSCs to deliver therapeutic plasma proteins in vivo (N Eliopoulos et al. Gene Therapy 2003; 10: 4788). We propose that pharmacological delivery of factor VIII can be achieved by this method.

To pursue this objective we will investigate and characterize the ability of genetically-engineered MSCs to produce canine factor VIII (cFVIII) and validate their therapeutic ability in

Marrow stromal cells for transgenic cell therapy of hemophilia A

NOD/SCID mice and a canine model of hemophilia A. MSCs will be engineered with an existing FVIII-encoding lentiviral vector.

Our first objective will involve engineering human and canine MSCs to produce cFVIII and characterizing the functional biochemistry of the secreted protein in vitro. Human MSCs will be collected from normal volunteers and canine MSCs will be harvested from normal outbred dogs. MSCs from both species will be genetically engineered with a cFVIII-encoding lentiviral vector from Dr Lillicrap's laboratory. cFVIII-secreting clonal human and canine MSC subsets will be isolated and utilized to generate synthetic 'organoids' following a previously developed protocol. These synthetic endocrine devices will be implanted in NOD/SCID mice. Temporal analysis of plasma cFVIII levels in mice will be performed using a species-specific cFVIII immunoassay. The correlation between MSC implant size and cFVIII production will be assessed. The utility of engineered human MSCs will be validated in NOD/SCID mice only, whereas engineered canine MSCs will be tested in NOD/SCID mice as well as hemophilic dogs as described below. The experiments testing and comparing the utility of cFVIII-producing human and canine MSCs will determine whether human MSCs behave similarly to canine MSCs in vivo in NOD/SCID mice.

Lastly, based on the information gathered in the initial experiments in NOD/SCID mice (cell dose/kg, size of implant), we will harvest MSCs from at least three hemophilic dogs. These MSCs will serve to generate 'autologous' FVIII-producing implants for each hemophilic dog from which

the MSCs were harvested. Each test dog will be implanted with his own cFVIII engineered MSCs and clinical and biochemical end points will be monitored by Dr Lillicrap's team at Queen's University.

These data will serve as a rationale for future testing in humans. Since the required factor vectors have already been developed and extensive data describing the efficacy of synthetic 'organoid' platform documented, we believe it to be realistic to perform all the experimental work in mice within the first 18 months of funding and to complete testing in hemophilic dogs in year two.

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We will investigate the ability of genetically-engineered marrow stromal cells to produce canine FVIII and validate their therapeutic ability in NOD/SCID mice and a canine model of hemophilia A

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Lady Davis Institute for Medical Research
Canada