Evaluating the utility of adipose-derived mesenchymal stem cells for cell therapy of LGMD 2B

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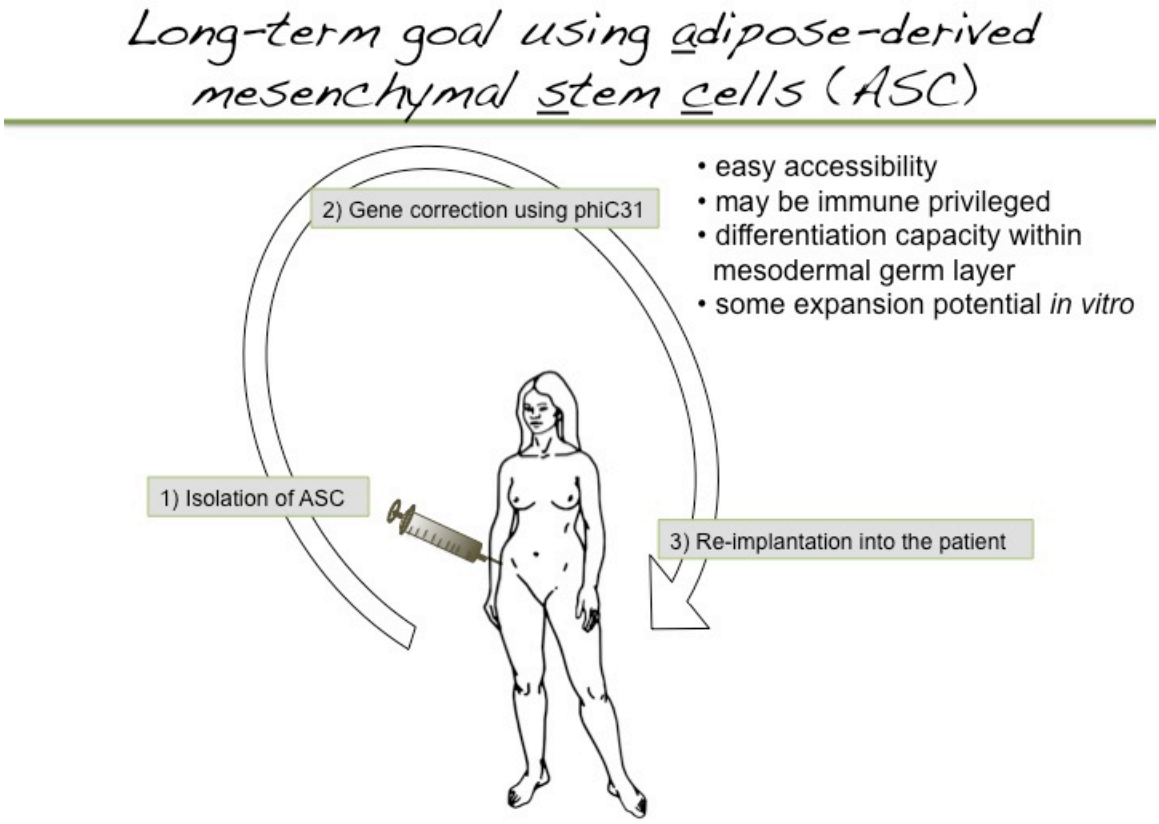
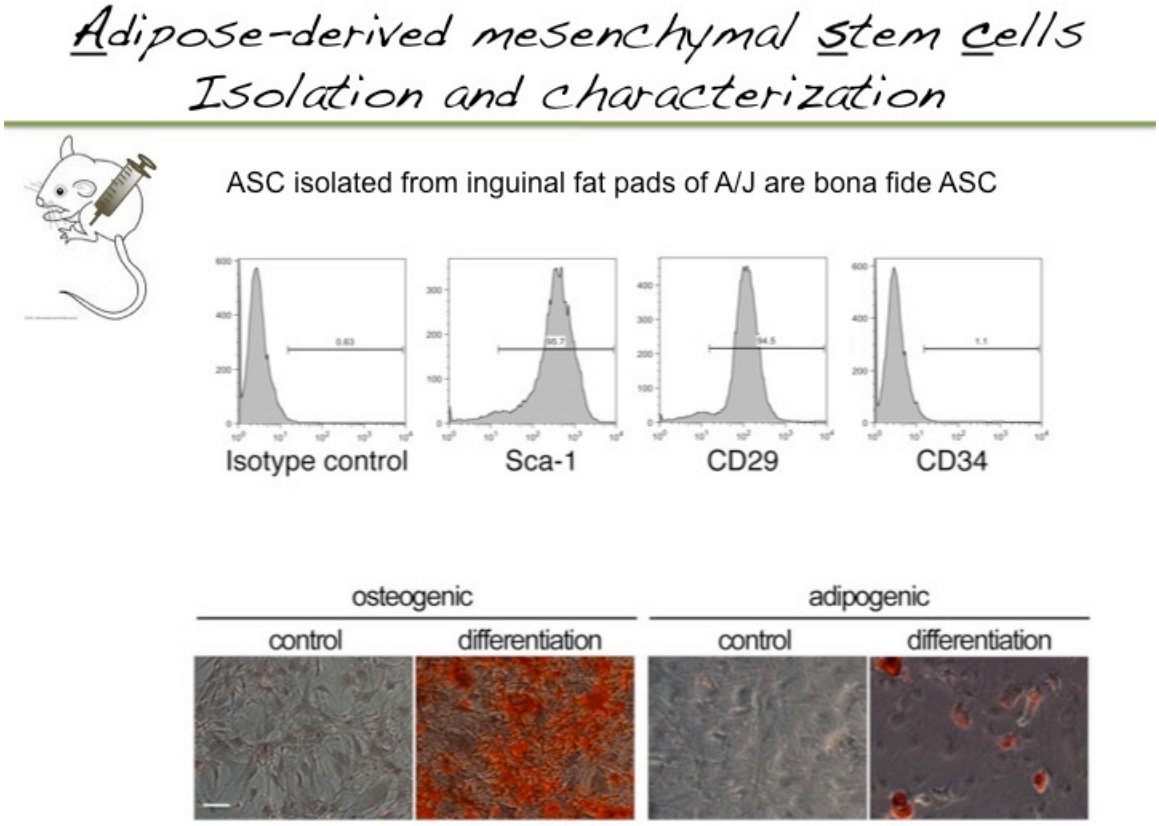
An appealing approach to address the muscle degeneration present in limb girdle muscular dystrophy 2B (LGMD 2B) is to repair the affected muscle with corrected stem cells derived from the patient. Our strategy involves using recombinase genome engineering methods to carry out dysferlin gene addition in candidate stem cells. In the process of determining which type of stem cell to use, we examined the potential of adipose-derived mesenchymal stem cells (ASC). These cells are abundant, expendable, relatively easily accessed, and appeared to have the ability to differentiate into muscle fibers.

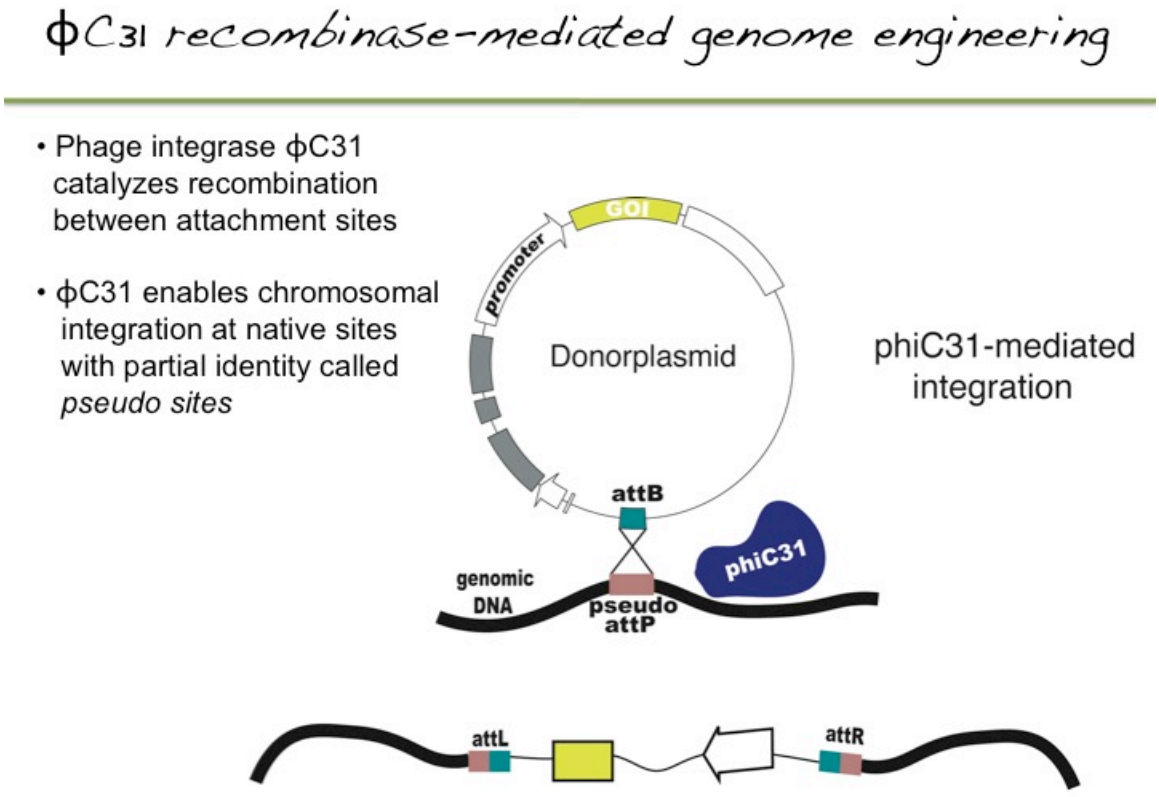


To test their applicability for a stem cell therapy, we isolated AD-MSC from the inguinal fat pads of mice and used FACS analysis to verify that the cells carried

the expected cell surface markers, such as Sca-1 and CD29, and lacked expression of CD34.



The cells were nucleofected with plasmid DNA, to introduce the dysferlin-expressing therapeutic plasmid, along with a plasmid encoding phiC31 integrase. This phage recombinase enables sequence-specific integration into the mammalian genome.

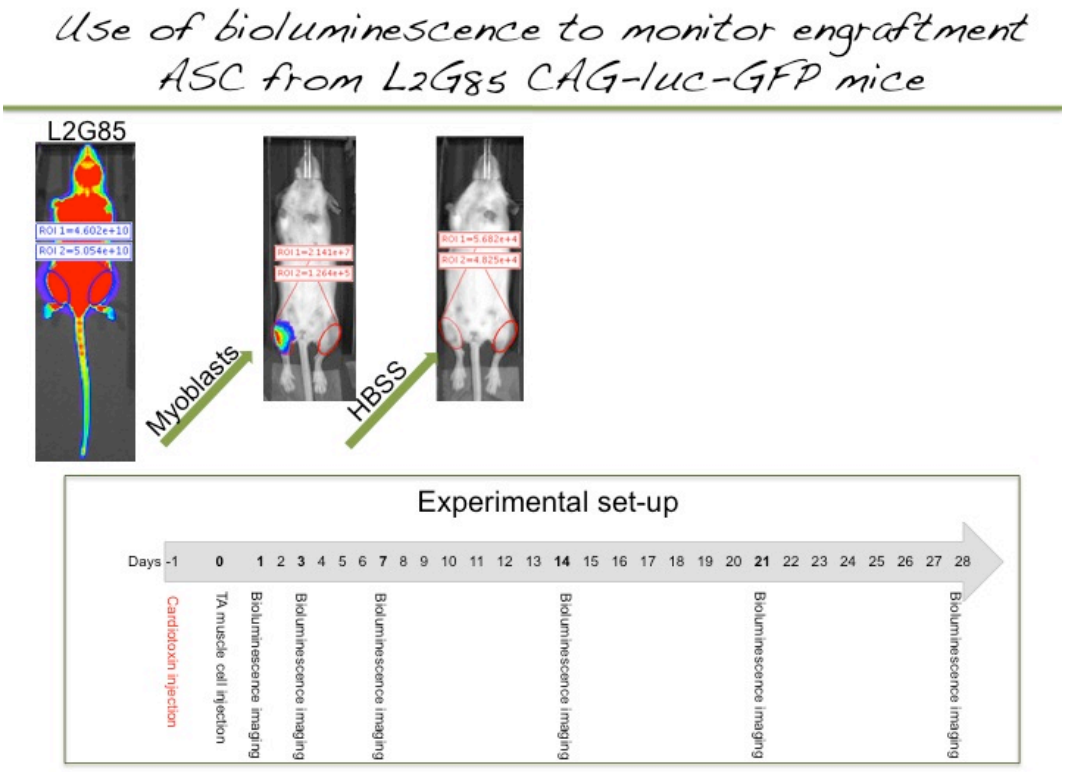


While we obtained good transfection efficiency (30 – 60%), the cells did not grow well after nucleofection, so it was not possible to obtain sufficient corrected cells for transplantation. The transient expression of reporter plasmids carrying a luciferase gene under the control of different promoters showed a 100-fold loss over the first few days. Moreover, since ASC are primary cells, they have a limited lifespan in culture. This feature makes it impossible to work with a clone of cells having one, characterized integration site. Therefore, a population of cells having a mixture of integration sites would need to be used, which could create an insertional mutagenesis risk.

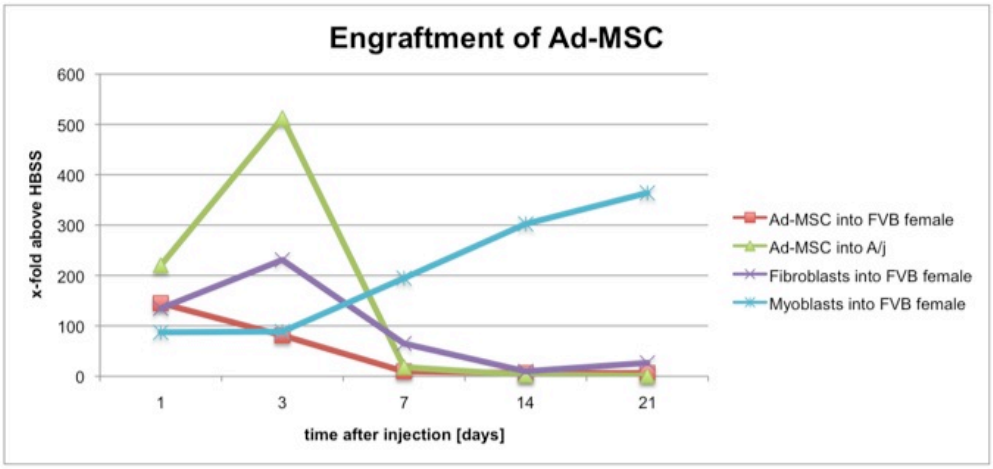
An additional roadblock with ASC was their poor ability to differentiate into muscle fibers, *in vitro* or *in vivo*. Although the ASC underwent osteogenic and adipogenic differentiation in vitro (see illustration on previous page), we were unable to demonstrate robust, reproducible differentiation of ASC into muscle

fibers using conventional differentiation conditions, such as co-culture with C2C12 cells.

Similarly, after injection into the TA muscle of cardiotoxin-treated mice, ASC showed a poor ability to engraft, unlike the myoblast positive controls.



Engraftment of ASC into FVB and A/J – Part I
Results



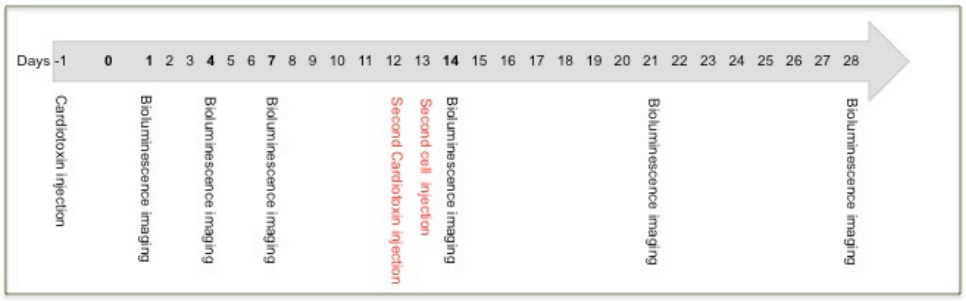
- Myoblasts as positive controls appeared to engraft and grow
- ASC numbers dropped between day 3 and 7
  ➔ensure against possible immune response

In order to exclude possible immune rejection, we also set up an engraftment experiment performing a daily injection of the immunosuppressant Tacrolimus. Moreover, we evaluated whether a second dose of cardiotoxin or a re-injection of cells could be beneficial for the engraftment. None of these procedures led to a significant improvement of the engraftment.

Engraftment of ASC into FVB and A/J - PartII
Experimental set-up

Goals:

- Second cardiotoxin (CTX) injection at d12; does re-injury enhance engraftment?
- Is a double dose (re-injection of cells) beneficial for engraftment?
- Use of Tacrolimus immunosuppressant to avoid rejection of FVB cells in A/J.



Engraftment of ASC into FVB and A/J - PartII
Results

- AD-MSC engrafted poorly
- 2nd CTX and cell dose was not beneficial
- Myoblasts were good positive control, required immunosuppression as expected

Due to their poor ability to grow after transfection, the inability to utilize a clone, and poor ability to differentiate into muscle *in vitro* and *in vivo*, we believe that AD-MSC are not an appropriate stem cell choice for this gene therapy/cell therapy approach in muscle. We believe that induced pluripotent stem cells may be a better choice.