Injured Muscle

- Muscle stem / satellite cells are activated and proliferate.
- Cells, including inflammatory cells, transiently infiltrate the muscle bed.
- Post-mitotic satellite cells align and fuse to repair/form new muscle fibres.
Chemotherapeutic-based Enhancement of Stem Cell Engraftment

- **Alkylating Chemotherapy + Drug Resistant Donor Cells** - based on mechanisms established for Bone Marrow Transplantation
  - Efficient Elimination of Endogenous Cells
  - Creating Receptive & Favourable Niche for Donor Cells
  - Selective *in vivo* Expansion of the Protected Donor Cells
  - Feasibility in the Skeletal Muscle as a Solid Organ?
Muscle Stem Cell Transplantation - Improved Strategy
Selective Enrichment: The Mechanism

Wild-Type cell

BCNU

\[ \text{Alkylates DNA} \]

\[ \text{CH}_3 \]

\[ \text{MGMT} \]

\[ \text{CH}_3 \]

Cell survives

Wild-Type Cell

BCNU

\[ \text{Alkylates DNA} \]

\[ \text{CH}_3 \]

\[ \text{MGMT} \]

\[ 0^\circ \text{BG} \]

Cell death

MGMT-P140K * Expressing Cell

BCNU

\[ \text{Alkylates DNA} \]

\[ \text{CH}_3 \]

\[ \text{MGMT-} \]

\[ \text{P140K} \]

\[ \times \]

Cell survives

* Pegg (1990) Cancer Research
Selective Enrichment: The Mechanism

**SELECTIVE ENRICHMENT**

- Wild type
- MGMT-P140K

BCNU + O°BG
Resistance of MGMT(P140K) Expressing Cells \textit{in vitro}

Mouse myoblasts (C2C12) \hspace{2cm} Human myoblasts

Lee et al. (2009) \textit{Stem Cells}
Selective Enrichment of MGMT(P140K) Expressing Cells in vitro

Mouse C2C12 Myoblasts Transduced with Retroviral Vector Encoding MGMT(P140K)

Lee et al. (2009) Stem Cells
Normal Myogenic Differentiation of Myoblasts Transduced with MGMT(P140K)

Lee et al. (2009) Stem Cells
In Vivo Muscle Degeneration Model using Myotoxic agent Notexin

- Australian tiger snake venom.

- Phospholipase A$_2$ (PLA$_2$) enzyme causing:
  - Depolarisation / degradation of sarcolemma
  - Blockage of acetylcholine release at motor nerve terminals
  - Leaves blood vessels, nerves and basal lamina intact
  - Induces inflammation

Harris (2003) Toxicon
MGMT(P140K)$^{+ve}$ Regenerating Skeletal Muscles can be Protected from BCNU+O$^6$BG Treatment in vivo

Lee et al. (2009) Stem Cells
Myogenic Lineage

Genetic Hierarchy

Stem Cell

Pax3$^+$ and/or Pax7$^+$

Proliferation

Myogenic Specification

Myf5$^+$, MyoD$^+$

Differentiation

MyoD$^+$, Myogenin$^+$

Terminal Differentiation

Myogenin$^+$, MHC$^+$

Modified from Tajbakhsh (2003)
Current Opinion in Genetics & Development
Muscle Stem Cell Transplantation: CD34$^+$ Cells as Donors

- **CD34$^+$ Cells from Skeletal Muscle**
  - Shown to have stem cell like properties - *Pax3-GFP transgenic* (Montarras et al, Science, 2005)

*High regenerative capacity*

*freshly isolated $>>$ cultured*