Mesenchymal Stem Cells (MSC)

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MESENCHYMAL STEM CELLS

- Multipotent stem cells originally defined in the bone marrow
- Equivalent to stromal cells identified back in the 1960’s by Dexter and colleagues
- Grown from BM mononuclear cells by their adherence to plastic in tissue culture flasks
MESENCHYMAL STEM CELLS

- The International Society for Cellular Therapy position paper:
- Defined the minimal criteria for defining multipotent mesenchymal stromal cells.
- Plastic-adherent cells expressing CD105, CD73 and CD90, but not CD45, CD34, CD14, CD11b, CD79alpha, CD19 or HLA-DR.
- MSC must differentiate to osteoblasts, adipocytes and chondrocytes in vitro.
MSC Manufacture

Autologous versus Allogeneic

- Autologous cells considered safer because there are no issues with immune rejection of graft versus host
- Autologous MSCs require a minimum of 5 weeks for isolation, expansion and release. This limits their application
CMC Considerations

Source Control

source of cells
donor screening

Production of MSCs:
Heterogeneity of patient products
CMC Considerations

Process controls
validation of production process
cGMPs
MSC Manufacture

Bone Marrow Aspirate → Ficol Gradient Separation → Mononuclear fraction

Culture in Flasks 10 x T162cm² → 2 weeks → Culture in Flasks 60 x T162cm² → 1 week

P0 → P1

* Target for manufacture 250 million MSC
MSC Manufacture
<table>
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<th>Cat. No.</th>
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<th>164327</th>
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<td>Number of trays</td>
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<td>4</td>
<td>10</td>
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<tr>
<td>Culture area, cm²</td>
<td>632</td>
<td>1264</td>
<td>2528</td>
<td>6320</td>
<td>6320</td>
<td>25280</td>
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<tr>
<td>Suggested working volume, ml</td>
<td>200</td>
<td>400</td>
<td>800</td>
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<td>8000</td>
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## MSC Manufacture

<p>| | |</p>
<table>
<thead>
<tr>
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<tr>
<td><strong>Volume of BM</strong></td>
<td>25ml</td>
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<tr>
<td><strong>Starting cell count (x10^6)</strong></td>
<td>588</td>
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<tr>
<td><strong>Post ficol cell count (x10^6)</strong></td>
<td>90</td>
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<tr>
<td><strong>P0 – total cells (x10^6)</strong></td>
<td>142</td>
</tr>
<tr>
<td><strong>P1 - total cells (x10^6)</strong></td>
<td>514</td>
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</table>
CMC Considerations

Product testing
- should ensure product safety
- should ensure consistency of process and final product
- should predict in vivo activity
- is guided by detailed understanding of the manufacturing process and product

= CHARACTERIZATION
Final MSC Preparation Testing

- Release testing
  - Sterility
  - Endotoxin
  - Mycoplasma
  - Viability
  - Cell Concentration
  - Purity (FACS)

Purity (FACS)
CD45− CD105+ CD166+

![Flow cytometry chart showing CD45 and CD105 expression]
CMC Considerations

Identity

Is the product what you say it is?

For MSCs can visually confirm identity by microscopy
ADHERENT MSC IN CULTURE
CFU-F Colony
CMC Considerations

Quality

Potency

For MSCs – CFU-F
Flow analysis
CFU-F Assays

MSC Yield (1E6)

CFU-F/1E6 BM MNC
CMC Considerations

Purity

Ideal product has high levels of desired cells with a low level of unwanted cells

Typically MSC products > 95% CD105+

> 95% CD45 –ve

< 1% CD3+ cells
ADHERENT MSC IN CULTURE
CMC Considerations

Strength

How much?
How will you dose?

Dose finding studies needed to identify effective dose.
Studies to date have given up to 200M MSCs without safety issues
Delivery of Cell Products

• Intravenous injection (IV) – BMT products
• Sub cutaneous (subQ) – drugs
• Direct injection to tissue
  – Heart – catheter delivery
    » post by-pass surgery
Surgical Injection of MSCs
DELIVERY OF CELL PRODUCTS TO HEART TISSUE

• Ideally we want the volume to be delivered to be minimal

• To deliver a large number of cells in a small volume means the cell must be prepared at a very high cell concentration. Eg 40M MSC/ml

• This can result in a viscous cell product which can result in clumping and other complications
DELIVERY OF CELL PRODUCTS TO HEART TISSUE

• Preparing cell products results in cell loss
  - transfer to a sterile cup to fill syringes
  - filling syringes, removing air
  - priming catheters (200 ul deadspace = 4% of the product)

• With a minimal volume of cells, will you inject the same number of sites with a smaller volume OR inject the same number of cells into fewer sites??
CMC Considerations

Lot release
Final MSC Preparation Testing

- Release testing
  - Sterility
  - Endotoxin
  - Mycoplasma
  - Viability
  - Cell Concentration
  - Purity (FACS)

Purity (FACS)
CD45⁻ CD105⁺ CD166⁺
MANUFACTURING ISSUES

• Different cell yields with different patients

• Some patients fail to grow

• Excess product – should this be stored for future use of the patient, or discarded?

• BM products for placebo patients – should these be stored for the patients future use?
CFU-F

Normal donors

Patients

AGE
Initial Observations

• Many patients requiring CABG surgery are unable to wait for production of MSC. One option could be to use allogeneic MSC for this patient group.

• Delivery of concentrated cell products (40 million cells per ml) can result in clumping of products.

• Delivering cell doses offers challenges.
  – Losses with thawing and washing
  – Losses with transfer to syringes and elimination of air bubbles
  – Loss of cells at the site of injection
Sources of MSC

- Bone Marrow
- Adipose Tissue
- Cord Blood Products
- Placenta
- Warten’s Jelly
- Amniotic Fluid
- Other tissues