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

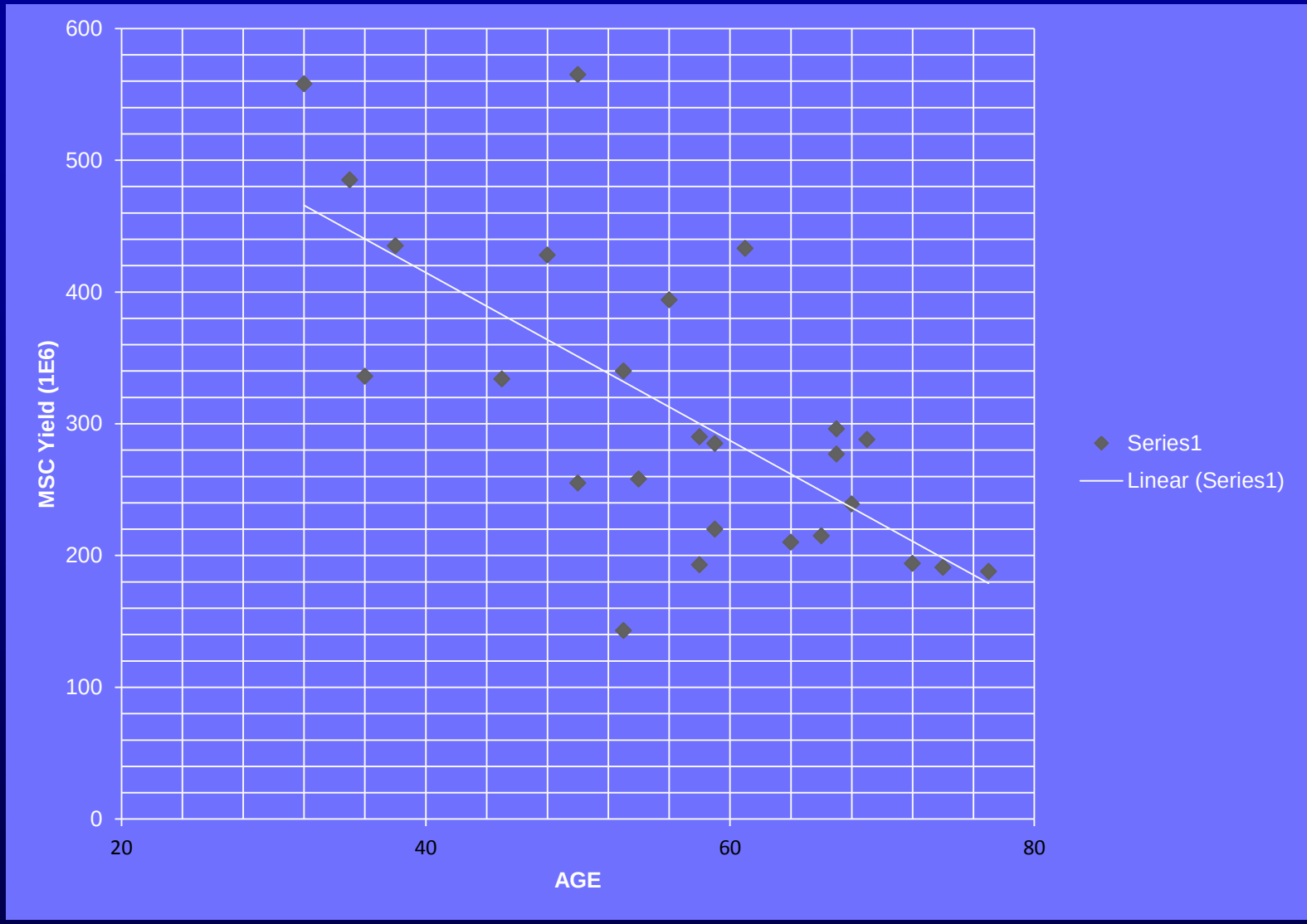
CMC Considerations

Source Control

- source of cells
- donor screening

Production of MSCs:

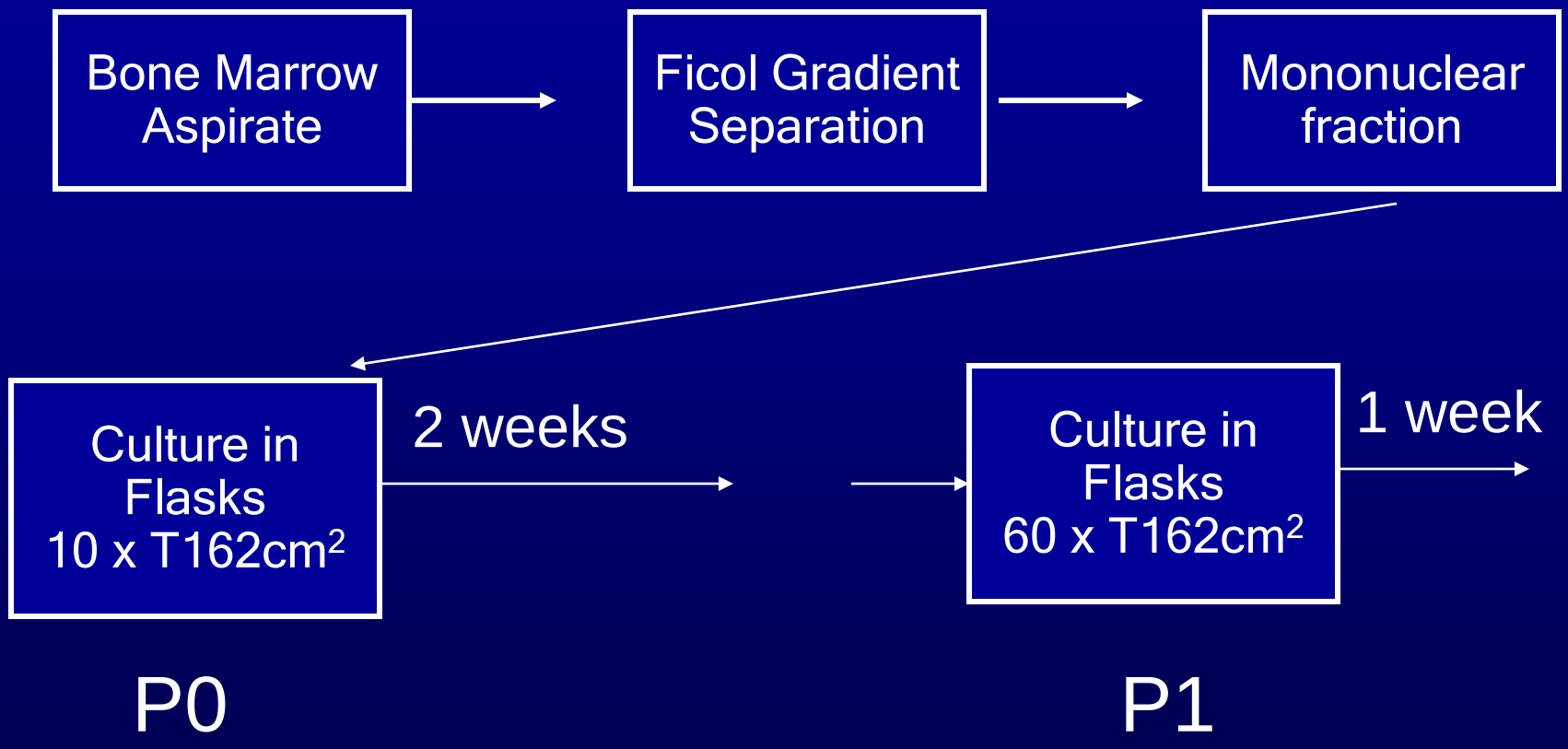
Heterogeneity of patient products


600
500
400
300
200
100
0
MSC Yield (1E6)
20
40
60
80
AGE
Series1
Linear (Series1)

CMC Considerations

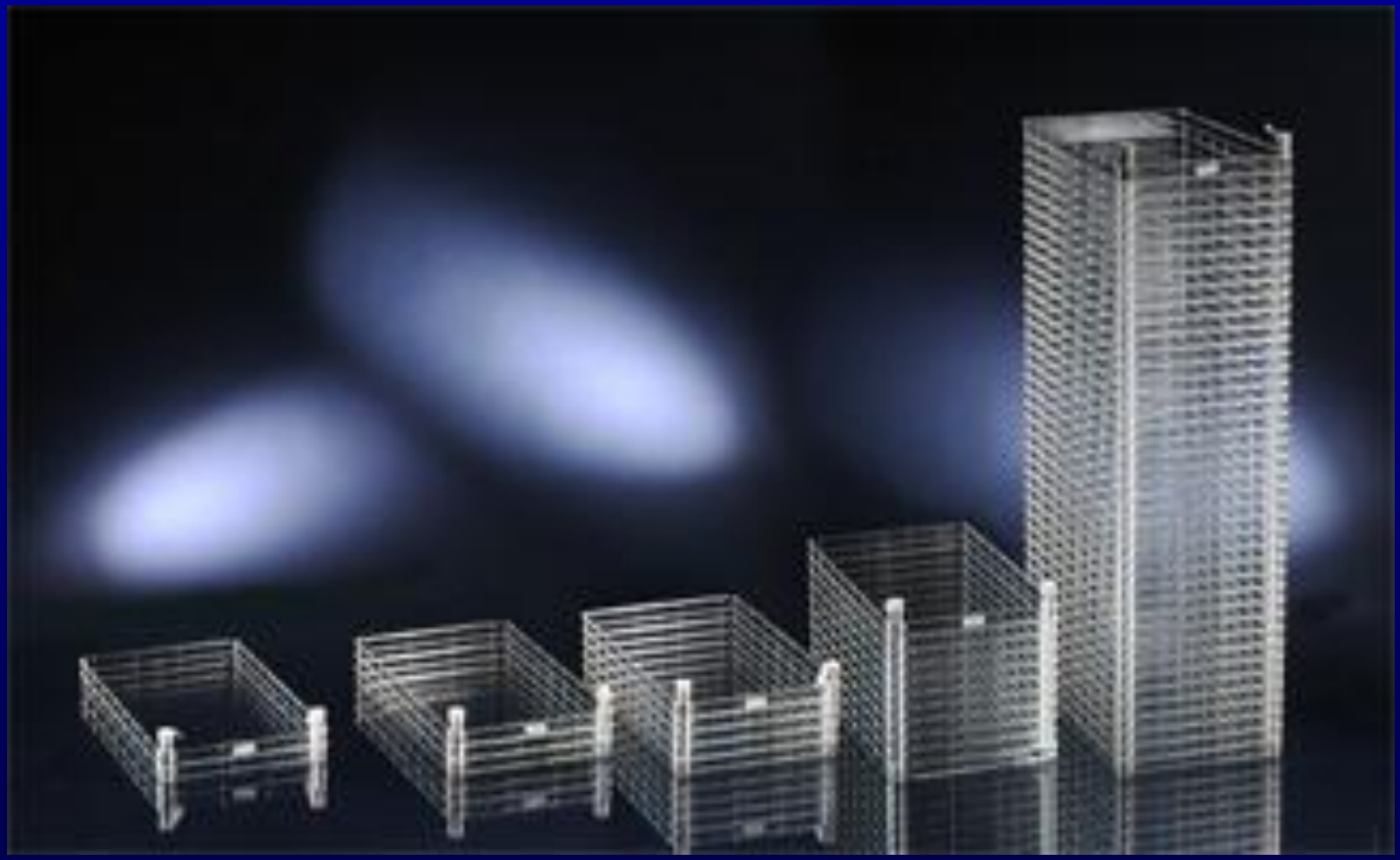
- Process controls
  - validation of production process
  - cGMPs

MSC Manufacture



* **Target for manufacture 250 million MSC**

MSC Manufacture



MSC Manufacture

Cat. No.	165250	167695	140004	164327	170009	139446
Number of trays	1	2	4	10	10	40
Culture area, cm^2	632	1264	2528	6320	6320	25280
Suggested working volume, ml	200	400	800	2000	2000	8000

MSC Manufacture

Volume of BM	25ml
Starting cell count ($x10^6$)	588
Post ficol cell count ($x10^6$)	90
P0 – total cells ($x10^6$)	142
P1 - total cells ($x10^6$)	514

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

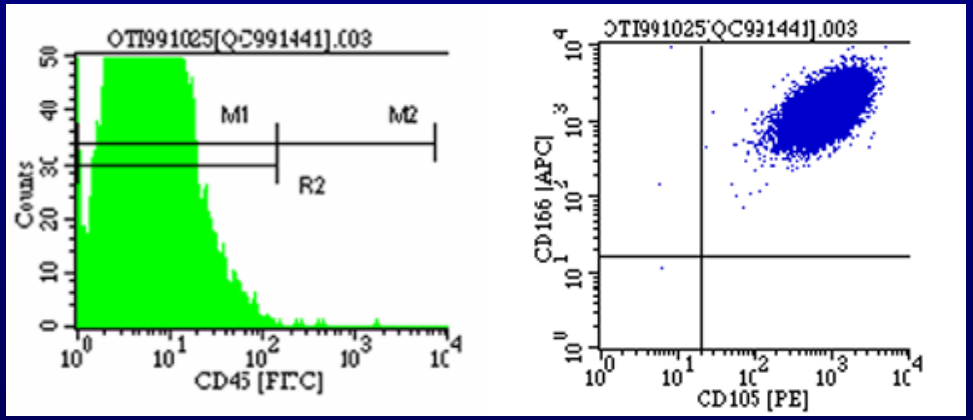
= CHARACTERIZATION

Final MSC Preparation Testing

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

Purity (FACS)

$CD45^{-}$ $CD105^{+}$ $CD166^{+}$

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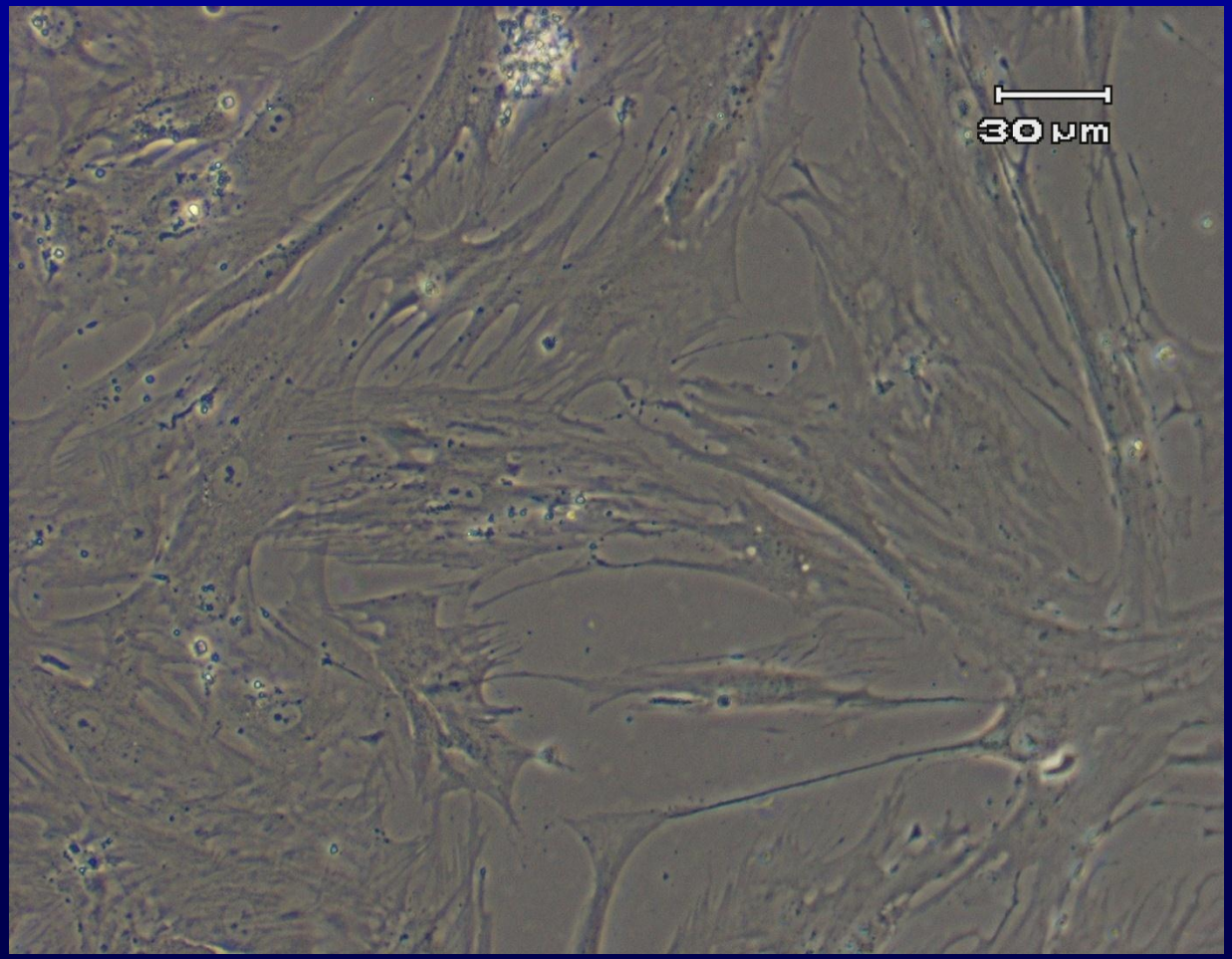
CMC Considerations

Identity

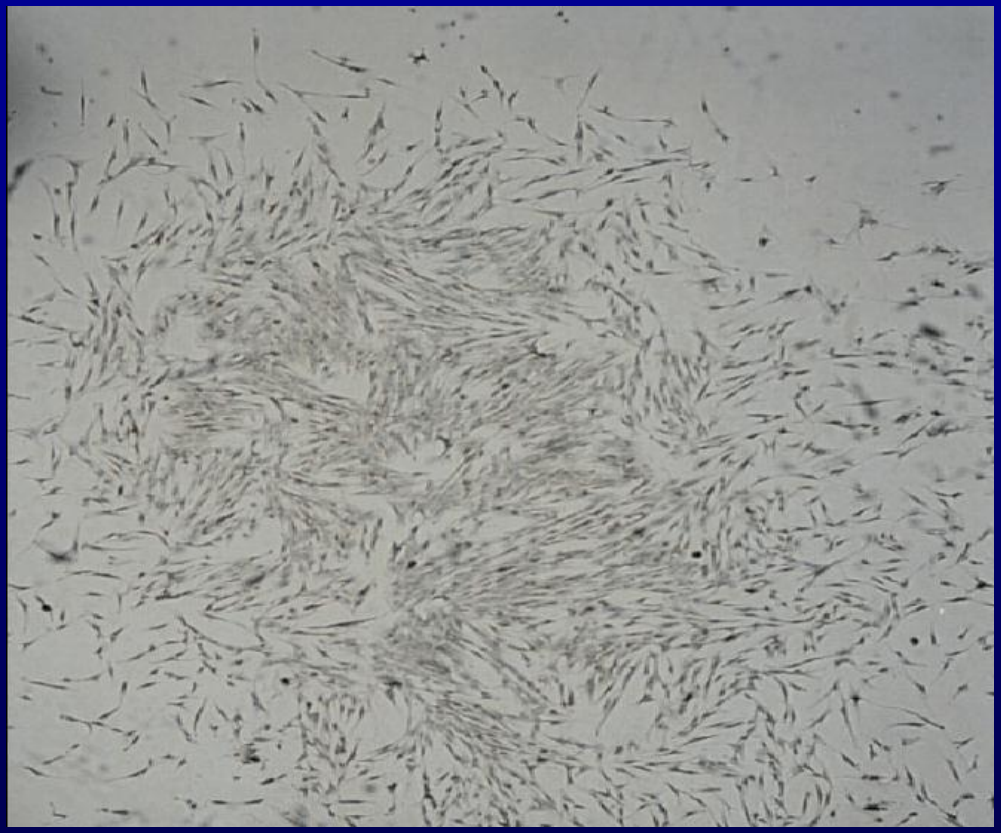
Is the product what you say it is?

For MSCs can visually confirm identity by microscopy

ADHERENT MSC IN CULTURE



CFU-F Colony



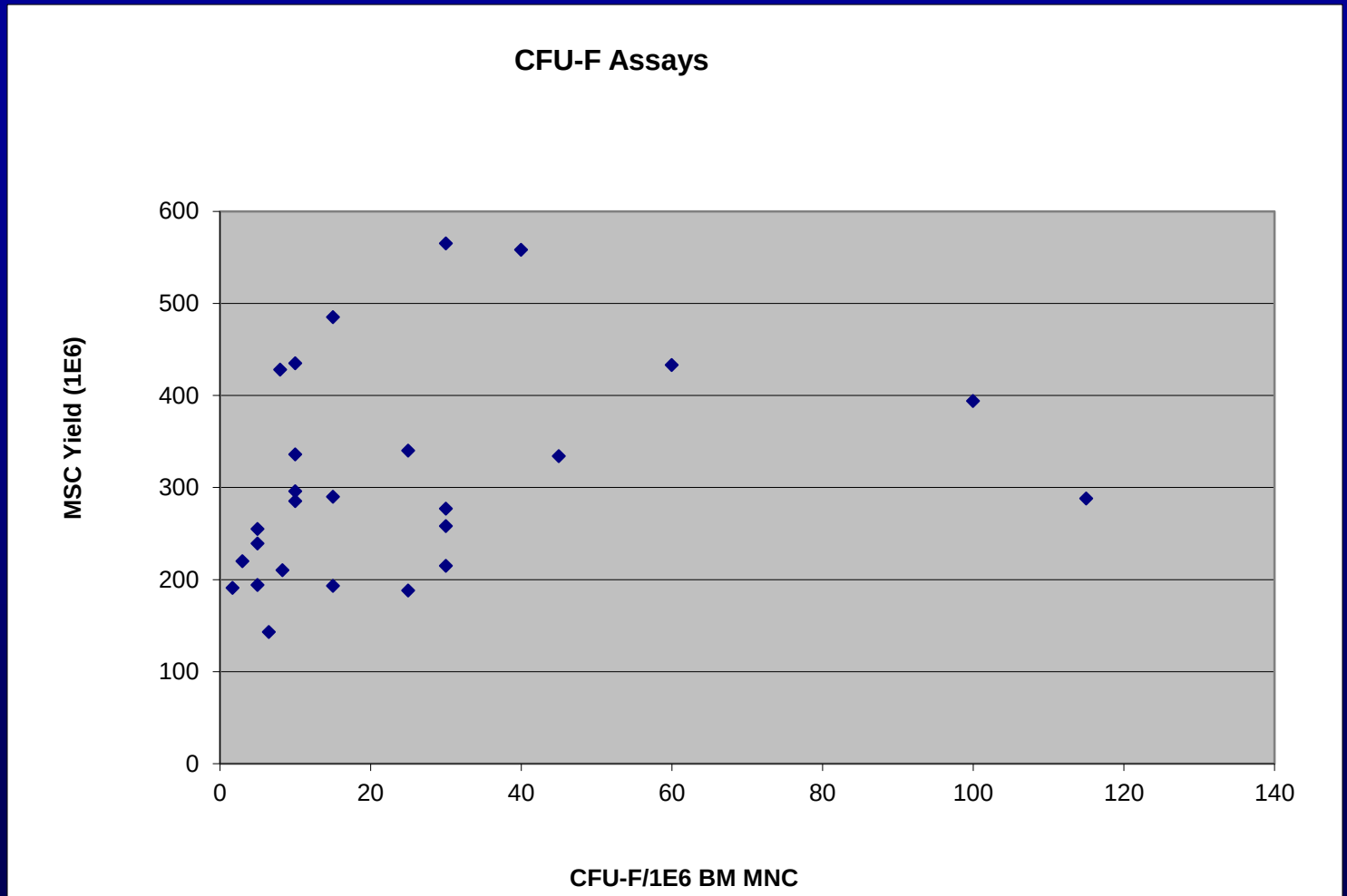
CMC Considerations

Quality

Potency

For MSCs – CFU-F

Flow analysis


CFU-F Assays
MSC Yield (1E6)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
CFU-F/1E6 BM MNC

CMC Considerations

Purity

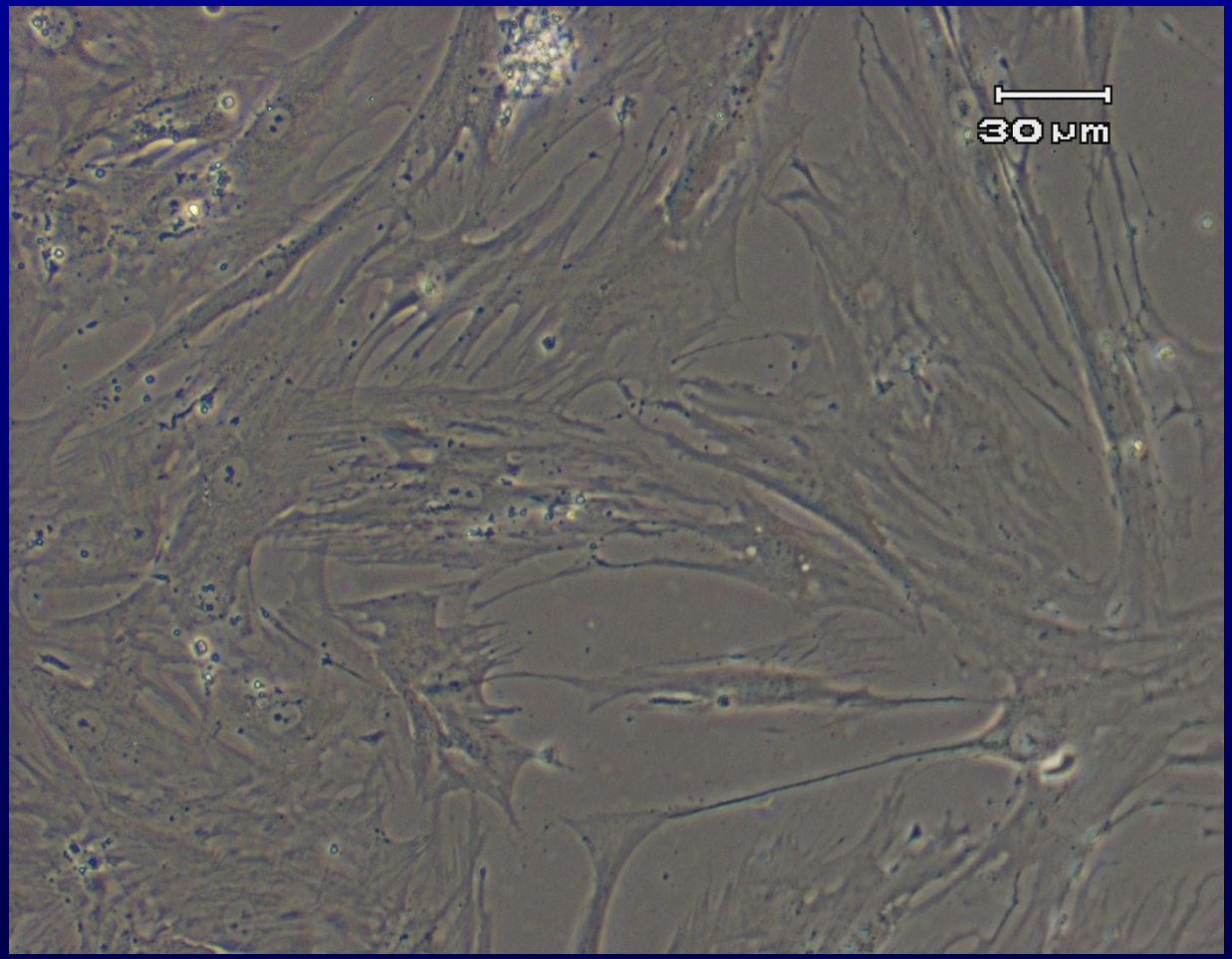
Ideal product has high levels of desired cells with a low level of unwanted cells

Typically MSC products > 95% CD105+

> 95% CD45 –ve

< 1% CD3+ cells

ADHERENT MSC IN CULTURE



CMC Considerations

Strength

- How much?
- How will you dose?

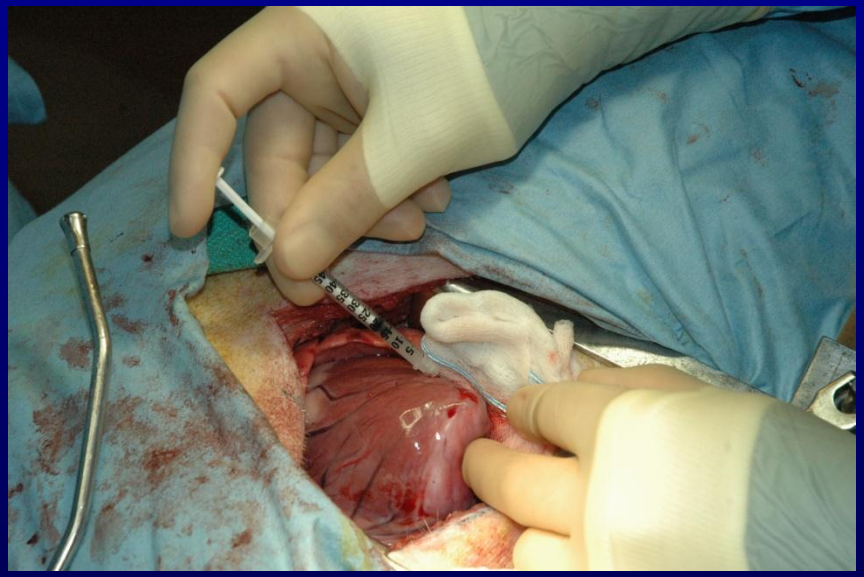
Dose finding studies needed to identify effective dose.

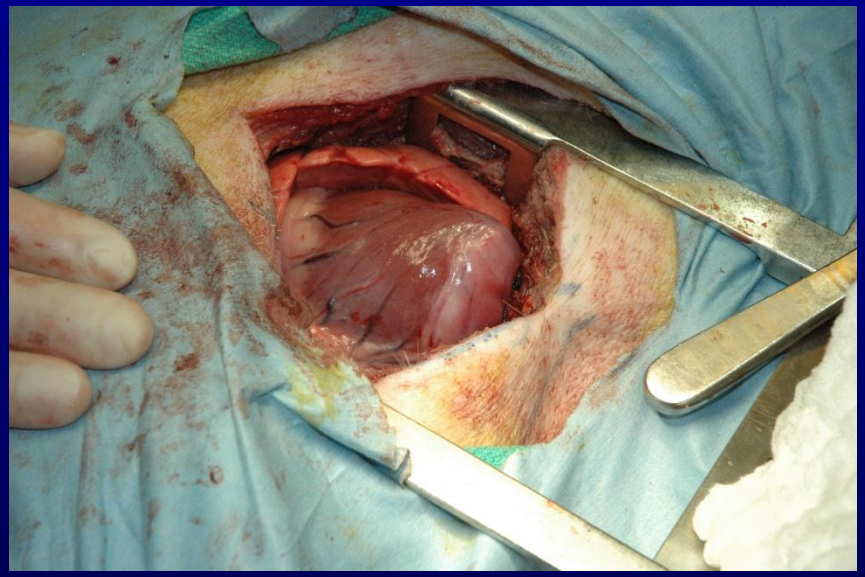
Studies to date have given up to 200M MSCs without safety issues

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

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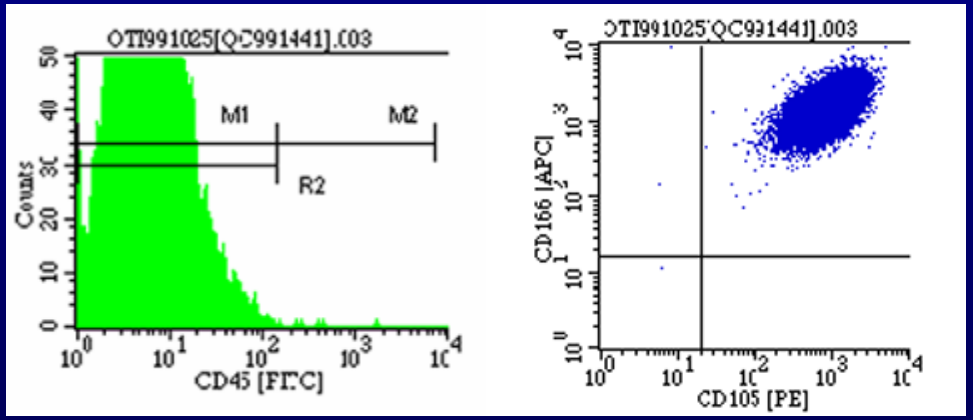
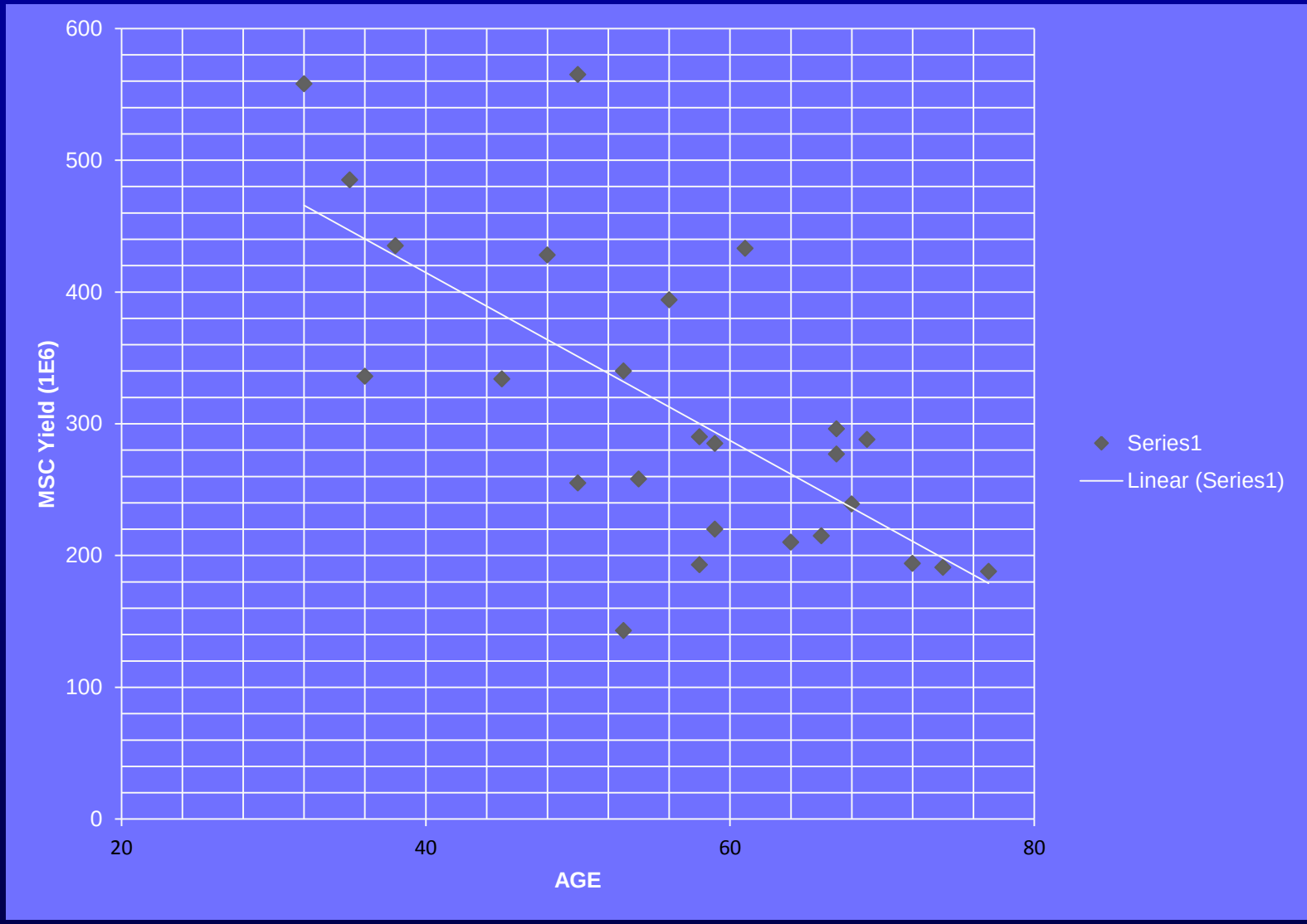


MANUFACTURING ISSUES

- 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?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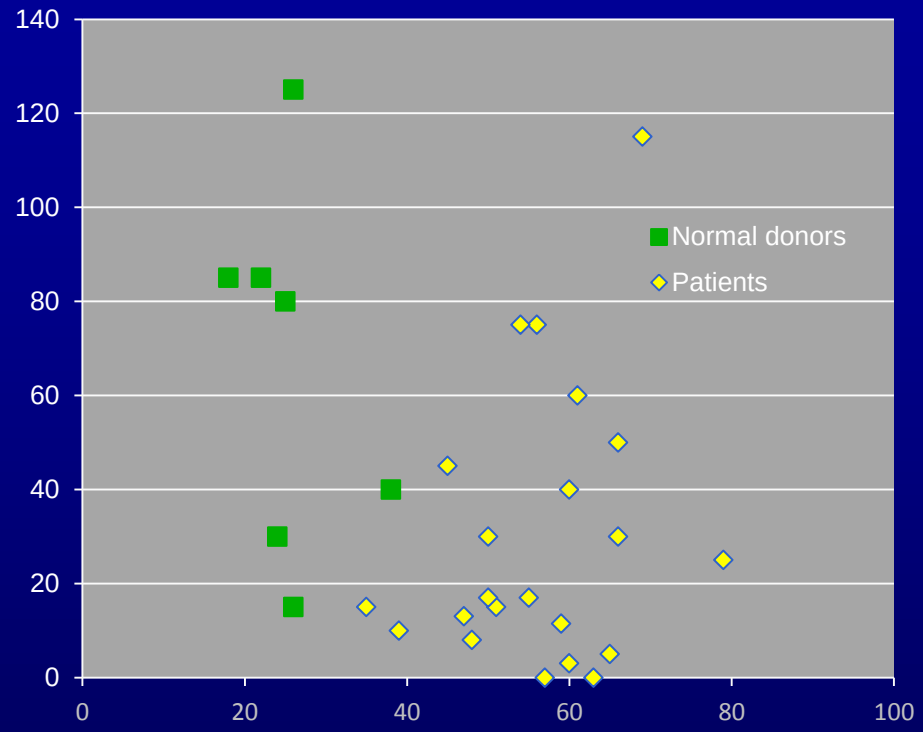
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Normal donors
Patients

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