Pros and cons of different expansion systems for manufacturing of an allogeneic cell therapy

7th Technical Meeting, Newcastle, 24 September 2013

Jef Pinxteren, Manager and Head R&D
MultiStem® cell therapy product: a multimodal biologic product

MultiStem® Cell Therapy

MultiStem® Product Profile

- Cell therapy product based on Multipotent Adult Progenitor Cell (MAPC) technology
- Developing for “off-the-shelf” administration – no tissue matching needed
- **Expanded product** with high production yield (e.g., millions of doses from each donor)
- **Long storage life** – can be kept in frozen form for years
- **Consistent safety profile**
- **Promotes healing and tissue repair** through **multiple mechanisms of action**
- Not a permanent transplant – cells cleared from the body over time

- Expanded, banked product
- Obtained from healthy, consenting adult donors
- Frozen storage
- Administered systemically (IV) or locally (catheter, injection, matrix/implant)
### Overview of the MultiStem® Production Process

<table>
<thead>
<tr>
<th>MultiStem®</th>
<th>Immunological</th>
<th>CARDIOVASCULAR</th>
<th>NEUROLOGICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD / Ulcerative Colitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematopoietic Stem Cell Transplant / GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid Organ Transplant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Artery Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neurological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traumatic Brain Injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal Cord Injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Allograft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5HT2c Agonists (obesity, other)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **IMMUNOLOGICAL**
- **CARDIOVASCULAR**
- **NEUROLOGICAL**

<table>
<thead>
<tr>
<th>Stage</th>
<th>2013 Data</th>
<th>2013-4 data</th>
<th>FDA discussions re: P2/3 design</th>
<th>FDA approved P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRECLINICAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IND/EQUIVALENT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHASE I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHASE II</td>
<td>2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHASE III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMMERCIAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Overview of the MultiStem® Production Process

Lot Release & Product Characterization Testing
- Sterility, Potency, Purity and Viability
- Stable Cytogenetics
- Absence of tumorigenic potential in vivo

Cell Isolation and Processing
- Isolate material from qualified donor
- Cell Expansion
- Closed system expansion

Cell Concentration
- Working Cell Bank and Clinical Dose Bank Creation
- Master Cell Bank Creation

Thaw and Administer
- Hundreds of thousands to millions of clinical doses
Cell Banking Approach

Isolate MultiStem®

Master Cell Bank
20 million/vial (200 vials)

4.10⁹ cells

Working Cell Bank
20 million/vial (200 vials)

800.10⁹ cells

Clinical Doses
>40 doses/vial
180.10⁶ cells/bag

288.10¹² cells
~1.6.10⁶ doses
• Requires working in a clean room
• Labor intensive and numerous open events
  ➢ Manual seeding, feeding and harvesting
• Limited process control
• Difficulties in achieving high reproducibility

• Includes xeno media components (e.g., serum)
Images from current MultiStem production process
• High capacity mfg which will allow for manufacturing runs in the 500 billion – 2 trillion cell range (decrease testing costs due to lot size)
• Removal of xeno/human source materials when possible (lowering of regulatory/safety risk)
• Determine optimal storage conditions for final product and generate stability data (no special requirements for storage at clinical site)
• Final container configuration that supports thaw – deliver use (bags, vials or other)
• Automate mfg process (reduce labor costs)
• Economy of scale for reagents (reduction in materials costs)
Technology Landscape for Scalable Manufacturing

<table>
<thead>
<tr>
<th>Scale (cells per lot)</th>
<th>Cell Culture</th>
<th>Volume Reduction &amp; Washing</th>
<th>Filling</th>
<th>Cryo-preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1B</strong></td>
<td>T-flask, Hyperflask, 10 Layer</td>
<td>Centrifugation</td>
<td>Manual</td>
<td>Bench Controlled Rate Freezer (CRF)</td>
</tr>
<tr>
<td><strong>10B</strong></td>
<td>10 &amp; 40 Layer, Hyperflask + TAP, Cell Cube</td>
<td>Centrifugation, Blood Processing Equipment</td>
<td>Manual Bags or Vials</td>
<td>Bench CRF</td>
</tr>
<tr>
<td><strong>50B</strong></td>
<td>40 Layer / Hyperstack + Robotics</td>
<td>Tangential Flow, Continuous Centrifugation</td>
<td>Automated Bag Fill, Vial Fill Line</td>
<td>Large-scale CRF</td>
</tr>
<tr>
<td><strong>100B</strong></td>
<td>Factory Automation of 40-layers &amp; HyperStack</td>
<td>Tangential Flow, Continuous Centrifugation</td>
<td>Vial Fill Line</td>
<td>Large-scale CRF</td>
</tr>
<tr>
<td><strong>500B</strong></td>
<td>Suspension Bioreactors</td>
<td>Continuous Centrifugation</td>
<td>Vial Fill Line</td>
<td>Scale-Out Large Scale CRF</td>
</tr>
</tbody>
</table>

Courtesy of Jon Rowley, Lonza
CoGs Breakdown at Clinical and Commercial Scales (allogeneic product)

- Media: 24%
- RM: 3%
- Facility: 49%
- Labor: 3%
- Testing: 11%

~100,000 doses/yr

~1000 doses/yr

Courtesy of Jon Rowley, Lonza
The next stage

• **Stem Cell Research and Assay Development**
  - MultiStem identity / cell equivalency (distinguish between stem cells)
  - Molecular mechanisms of MultiStem function
  - Immunology
  - Pre-clinical animal studies

• **Evaluate novel expansion methods**
  - Quantum Cell System (Terumo)
  - Microcarrier/Stirred bioreactor

• **Development of xenobiotic free workflow**
Scale Up Manufacturing Options

40 Layer Cell Factory, Hyperstack – direct path to scale up with existing technologies
- Advantages
  - Scaled platform from current industry wide experience base
- Disadvantages
  - 2 dimensional platforms with large volume management requirements
  - Currently untested large volume reduction options
    - TFF – multiple manufacturers (Pall, Millipore)
    - kSep – promising but untested

Hollow Fiber Bioreactor; ATMI (closed system stacked pizza platform)
- Advantages
  - Provides scale out capacity with significant advantages
    - Eliminates requirement for large scale volume reduction
    - Closed system with increased safety for sterile breach
    - Significantly reduced labor costs
  - Allows in line testing for metabolites, control of gas/glucose in real time
- Disadvantages
  - Limited scalability within current configuration
  - Better suited for autologous or patient designated approach

Suspension bioreactor using microcarriers
- Advantages
  - 3D platform with minimal media exposure, likely most economical
  - Multiple microcarriers with controlled surface coating coming into market
  - Corning, BD, GE entering space
- Disadvantages
  - Macro-aggregates lead to complications in harvest; may be overcome with carrier size (Pluristem)
  - Difficulty in controlling oxygenation, shear parameters against particle size
• Phase III Registration trials must be run using commercial production process
• COGS a very important element of long term competitiveness
• 12 – 18 month period required to lock down process
  ➢ Pre-clinical equivalency and biosafety
  ➢ Process qualification and stability studies
  ➢ Clinical equivalency
• New manufacturing platforms developing with 3 to 6 month generation time!
Cell Factories, Hyperstack
kSep – counterflow centrifugation
• 12 microcarriers were tested for cell attachment in dishes
• The best 3 were tested in small 125 ml spinner flasks
• The best one was used for expansion in 3 liter bioreactor
  ➢ 12,000 cm² culture surface
  ➢ 2.4 liter of medium used

Primary Screen: 12  ➔  Secondary Screen: 3  ➔  Proof of concept: 1
Quantum System
• An automated integrated cell culture system
  ➢ Bioreactor, incubator, media and waste management
• Controlled cell expansion
  ➢ All steps can be programmed and monitored
• Decrease in labor
• Closed system
  ➢ Reduction in contamination potential
• CE marked and FDA listed device
Hollow fiber technology

• Self-contained bench top unit using a hollow fiber bioreactor
  ➢ Small device footprint (48.3 cm x 58.4 cm x 50 cm)
  ➢ Contains ≈ 10,000 fibers/bioreactor
  ➢ Total surface is 2.1 m² (3255 in²)
  ➢ Equivalent of 280 T75 flasks or one 40-stack
  ➢ Smaller surface to volume ratio requires media perfusion
  ➢ Metabolites and gas easily diffuse across fiber
Quantum cell expansion set

- Waste bag
- Bioreactor
- Tubing organizer
- Harvest bag
- Gas transfer module
Graphical user interface

Release Adherent Cells With Harvest

IC Inlet
Wash
IC Inlet Rate: 99 mL/min
IC Circulation Rate: 17 mL/min

Outlet
IC and EC Waste

EC Inlet
Wash
EC Inlet Rate: 147 mL/min
EC Circulation Rate: 1.7 mL/min

Pause
Stop

Next Step In
4 min 3 sec
Stop Condition
Exchange

Task
Configuration
About

Pressure (mmHg)
Inlet Outlet
0 IC 0
0 Differential 0
0 EC 0

37.0 °C
incubator OFF

7-01-2011 16:09
Release Adherent Cells With Harvest
Step 1/4

CaridianBCT®
• Graphical user interface makes operation very straightforward
• Reduced risk of errors
• Reduced labor
  ➢ Release and harvest of adherent cells takes 20 minutes
  ➢ Potential to run more cell expansion processes simultaneously
• Key steps in the process are automated
  ➢ e.g. Cell loading, feeding,...
  ➢ Possibility to adjust procedure and save as a custom task
Cell equivalency: Quantum vs plastic

- MultiStem cells were seeded in parallel on the Quantum and in standard culture conditions on cell culture plastic.
- Growth on the Quantum is monitored and feed rates may be adjusted based on glucose and lactate levels.

Harvest populations were tested on a set of QC assays.
**Cell equivalency: Quantum vs plastic**

**Inhibition of T cell proliferation**

**Cytogenetic analysis on CGH arrays**

**Differentiation**

**Flow cytometry**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Criterium</th>
<th>Standard culture</th>
<th>Quantum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD13</td>
<td>&gt; 90%</td>
<td>97.51%</td>
<td>99.45%</td>
</tr>
<tr>
<td>CD34</td>
<td>&lt; 2%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>CD45</td>
<td>&lt; 2%</td>
<td>0.05%</td>
<td>0.03%</td>
</tr>
<tr>
<td>CD49c</td>
<td>&gt; 90%</td>
<td>94.47%</td>
<td>98.71%</td>
</tr>
<tr>
<td>CD90</td>
<td>&gt; 90%</td>
<td>99.91%</td>
<td>99.92%</td>
</tr>
<tr>
<td>CD105</td>
<td>&gt; 90%</td>
<td>99.53%</td>
<td>95.76%</td>
</tr>
<tr>
<td>HLA I</td>
<td>&lt; 25%</td>
<td>5.39%</td>
<td>6.20%</td>
</tr>
<tr>
<td>HLA II</td>
<td>&lt; 2%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
</tbody>
</table>
# Reproducibility

<table>
<thead>
<tr>
<th># cells seeded ($10^6$)</th>
<th># cells harvested ($10^6$)</th>
<th>Run time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.45</td>
<td>752</td>
<td>146</td>
</tr>
<tr>
<td>7.28</td>
<td>782</td>
<td>148</td>
</tr>
<tr>
<td>8.57</td>
<td>753</td>
<td>144</td>
</tr>
</tbody>
</table>

3 independent runs from the same donor generate the same harvest yields.
MultiStem isolation from bone marrow

With prior density centrifugation

Whole bone marrow load

Whole BM load on Quantum as good as seeding BMMNC on cell culture plastic
Cell equivalency of MultiStem isolation: Quantum vs plastic

Inhibition of T cell proliferation

Differentiation

Flow cytometry

<table>
<thead>
<tr>
<th>Marker</th>
<th>Criterion</th>
<th>Plastic</th>
<th>Quantum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD13</td>
<td>&gt; 90%</td>
<td>99.65%</td>
<td>98.93%</td>
</tr>
<tr>
<td>CD34</td>
<td>&lt; 2%</td>
<td>0.19%</td>
<td>1.08%</td>
</tr>
<tr>
<td>CD45</td>
<td>&lt; 2%</td>
<td>0.42%</td>
<td>0.75%</td>
</tr>
<tr>
<td>CD49c</td>
<td>&gt; 90%</td>
<td>98.99%</td>
<td>99.07%</td>
</tr>
<tr>
<td>CD90</td>
<td>&gt; 90%</td>
<td>98.19%</td>
<td>99.17%</td>
</tr>
<tr>
<td>CD105</td>
<td>&gt; 90%</td>
<td>98.82%</td>
<td>99.11%</td>
</tr>
<tr>
<td>HLA I</td>
<td>&lt; 25%</td>
<td>17.95%</td>
<td>14.11%</td>
</tr>
<tr>
<td>HLA II</td>
<td>&lt; 2%</td>
<td>0.07%</td>
<td>0.10%</td>
</tr>
</tbody>
</table>

Angiogenesis assay
Cells from 3 donors tested

- Donor 1
  - 3 consecutive runs on the Quantum starting from bone marrow in run 1
  - Cells cultured on regular plastic
- Donor 2
  - 2 runs starting from the same seeding stock (run 1 & 2)
  - Run with different type of coating which was not successful (run 3)
  - Cells cultured on regular plastic
- Donor 3
  - Isolation on the Quantum
  - Isolation on plastic
  - MSC isolated on plastic

- HUVEC as control
Sample relations based on 25602 genes with sd/mean > 0.1
Conclusions

- Hollow fiber’s large surface area maximizes cell expansion in a minimal amount of space
- MultiStem cells can be isolated and expanded on the Quantum
- MultiStem cells show the same growth kinetics and retain all cell characteristics
- Automation: ease of use and better control
- Decrease in labor: 2 hours of lab work to generate an average of 800 million cells
- Expensive clean rooms not required
• MultiStem and MSC derived from same donor bone marrow
• Differences are of epigenetic origin
• Further describing MultiStem identity
  ➢ Transcriptome
  ➢ miRnome
  ➢ Methylome
  ➢ Proteome
- PCA to monitor cell equivalency of different cell culture methods

Transcriptome: comparability after culture manipulation

- Plastic expanded
- Quantum expanded
- HUVEC

First Principal Component

- MSC

Second Principal Component

- Xeno-free condition 1
- Xeno-free condition 2
- Bioreactor expanded
- MultiStem cells
- MSC cells

First Principal Component

Second Principal Component
**MultiStem versus MSC: Gene Set Enrichment analysis**

<table>
<thead>
<tr>
<th>Term</th>
<th>%</th>
<th>Fold Enrichment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell cycle</td>
<td>14.59343</td>
<td>3.412511219</td>
<td>1.29E-55</td>
</tr>
<tr>
<td>M phase</td>
<td>9.128083</td>
<td>5.035222668</td>
<td>5.46E-51</td>
</tr>
<tr>
<td>cell cycle phase</td>
<td>10.12408</td>
<td>4.438083274</td>
<td>2.10E-50</td>
</tr>
<tr>
<td>cell cycle process</td>
<td>11.69385</td>
<td>3.756301398</td>
<td>4.75E-47</td>
</tr>
<tr>
<td>M phase of mitotic cell cycle</td>
<td>7.168189</td>
<td>5.80759355</td>
<td>2.21E-46</td>
</tr>
<tr>
<td>mitosis</td>
<td>7.102997</td>
<td>5.859141967</td>
<td>1.87E-46</td>
</tr>
<tr>
<td>nuclear division</td>
<td>7.102997</td>
<td>5.859141967</td>
<td>1.87E-46</td>
</tr>
<tr>
<td>mitotic cell cycle</td>
<td>9.030811</td>
<td>4.429953427</td>
<td>5.80E-43</td>
</tr>
<tr>
<td>organelle fission</td>
<td>7.102997</td>
<td>5.628870012</td>
<td>1.36E-44</td>
</tr>
<tr>
<td>DNA metabolic process</td>
<td>10.0268</td>
<td>3.596563213</td>
<td>2.24E-37</td>
</tr>
<tr>
<td>cell division</td>
<td>7.319742</td>
<td>4.50323366</td>
<td>1.99E-36</td>
</tr>
<tr>
<td>DNA replication</td>
<td>5.631192</td>
<td>5.379231201</td>
<td>2.67E-32</td>
</tr>
<tr>
<td>chromosome segregation</td>
<td>3.151121</td>
<td>7.060421948</td>
<td>4.11E-24</td>
</tr>
<tr>
<td>DNA repair</td>
<td>5.294996</td>
<td>3.383993216</td>
<td>1.74E-18</td>
</tr>
<tr>
<td>response to DNA damage stimulus</td>
<td>6.150697</td>
<td>2.992927786</td>
<td>4.87E-19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Term</th>
<th>%</th>
<th>Fold Enrichment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>extracellular structure protein</td>
<td>2.706394</td>
<td>3.18027313</td>
<td>5.07E-11</td>
</tr>
<tr>
<td>biological adhesion</td>
<td>7.423045</td>
<td>2.027167884</td>
<td>5.57E-11</td>
</tr>
<tr>
<td>cell adhesion</td>
<td>7.3616</td>
<td>2.013269968</td>
<td>1.05E-10</td>
</tr>
<tr>
<td>extracellular matrix organization</td>
<td>2.089575</td>
<td>3.846289543</td>
<td>2.56E-10</td>
</tr>
<tr>
<td>skeletal system development</td>
<td>3.972857</td>
<td>2.384532868</td>
<td>2.58E-09</td>
</tr>
<tr>
<td>negative regulation of cell proliferation</td>
<td>4.107177</td>
<td>2.178794949</td>
<td>5.39E-08</td>
</tr>
<tr>
<td>regulation of cell proliferation</td>
<td>7.201364</td>
<td>1.752369798</td>
<td>3.41E-07</td>
</tr>
<tr>
<td>regulation of cell motion</td>
<td>2.409467</td>
<td>2.031073371</td>
<td>9.67E-07</td>
</tr>
<tr>
<td>response to wounding</td>
<td>5.128091</td>
<td>1.852111061</td>
<td>3.50E-06</td>
</tr>
<tr>
<td>response to mechanical stimulus</td>
<td>1.125131</td>
<td>3.843822113</td>
<td>6.83E-06</td>
</tr>
</tbody>
</table>

**MultiStem**:
- **Proliferation**
- Telomerase activity maintenance

**MSC**:
- MSC more mature?
Telomerase activity is preserved in XF-MS.
Application of miRNA expression data

- Identify markers by comparing differential expression between Multistem and MSC
  - Extract ‘key’ miRNAs
  - Consistent effect in 3 donors
  - Develop multiplex qPCR as xeno-free equivalency assays

- Gene Set Enrichment Analysis
  - Identify mRNA targets of miRNA
  - Gene Set enrichment / Pathway analysis
“omics screening”

miRNA screening:

Using two different platforms, array and PCR-based, we are currently exploring the possibility of using miRNA profiles to confirm the uniqueness of our product.
1: miRNome + data analysis

<table>
<thead>
<tr>
<th></th>
<th>qPCR</th>
<th>Array</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total targets</td>
<td>755</td>
<td>1400</td>
</tr>
<tr>
<td>Detected targets</td>
<td>207</td>
<td>847</td>
</tr>
<tr>
<td>Differential expression</td>
<td>160</td>
<td>140</td>
</tr>
<tr>
<td>Donor independent, FC &gt; 1.5</td>
<td>67</td>
<td>32</td>
</tr>
</tbody>
</table>

2: MultiStem marker identification

**Positive markers**

- MS > MSC
  - qPCR: 18
  - Array: 10
  - overlap: 5

**Negative markers**

- MSC > MS
  - qPCR: 33
  - Array: 6
  - overlap: 11

3: Validate 16 miRs by qPCR in independent donors → top markers

4: Use panel of top markers in cell comparability testing
• miRNA marker expression is maintained during:
  - Regular expansion
  - Xeno-free expansion
  - Bioreactor expansion

![Graph showing miRNA expression qPCR results for different conditions and donors.](image)
Genome-wide DNA methylation analysis

Find epigenetic markers for distinguishing MultiStem from competing products and proof epigenetic stability during expansions
Methylome: Multi-layered epigenetic regulation of MultiStem expansion capacity
Application of ‘omics’ data in product development

- MS miRNAs
- Gene methylation
- mRNA
- Protein

Molecular characterization
- Comparability XF media
- Pathway analysis
- Single cell expression

Therapeutic activity
- Exosome analysis
- Cell-cell interaction

Optimize stem cell population
Acknowledgments

ReGenesys: Bart Vaes, David Craeye, Annelies Bogaert, Aline Visser, Saartje Walbers, Peter Sterkendries, Marian Crabbé, Liesbeth Vandenpoel, Kristel Gijbels, Ellen Van Houtven, Lien Timmerman

Atherson: Bob Deans, Tony Ting, Jon Rowley, Willie Mays

www.regenesys.eu