Translational Approaches for Clinical Development and Manufacturing of Cell-Based Immunotherapies

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VP-Business Development
Miltenyi Biotec
May 17, 2016
A Global Leader in Life Sciences & Cell Therapy

• Independent privately-owned company (>26 years in field)
• >1,600 employees with >400 people in R&D and engineering
• Vertically integrated company for instruments, consumables, & biologics
• Global operations (22 countries direct; >60 indirect)
• History of advancing basic & translational research
• Committed to enabling the commercialization of cell & gene therapy
World Class Expertise in Cell Processing

• Innovative reagents & instruments for R&D, GMP, and clinical use
• Integrated, automated workflows for *ex vivo* cell processing:
  - sample preparation
  - cell separation
  - cell activation
  - cell transduction
  - cell culture
  - cell analysis
• Enabling intellectual property portfolio
Comprehensive Portfolio

MACS Product Portfolio

- CliniMACS Portfolio
- MACS Cell Separation
- MACS Antibodies
- MACS Sample Preparation
- MACS Cell Culture
- MACS Cell Analysis
Large Scale GMP Manufacturing (Teterow, Germany)

- Antibodies & conjugates
- Proteins & peptides
- Media & buffers
- Aseptic vial filling
- Medical devices
- GMP consumables
- Plasmids, RNA
Lentigen Technology Inc
GMP Lentiviral Vector Production Facility (Gaithersburg, MD)

- **25,000 ft²**

- **GMP manufacturing suites**
  - One ISO 8 (Class 100,000) room
  - Two ISO 7 (Class 10,000) suites

- **Process development laboratories**

- **Flexible lentiviral vector production**
  - Custom vector design/synthesis
  - Pilot scale research lots
  - Large scale GMP-grade commercial lots

- **FDA master file supporting many prior and ongoing clinical studies**
In the Beginning – CliniMACS® Plus System

• Automated sterile cell separation system
• >900 instruments in the field
• >50,000 patient procedures performed
• CE marked Dec ’97 (CD34+ cell enrichment)
• IND/IDE status in US since ‘98
• US FDA HDE approval Jan ’14 (GVHD in AML)
• Wide variety of CRR-labeled & CE marked reagents for cell enrichment or depletion
## Validated Kits for GMP Cell Purification/Graft Engineering

### Stem Cells
- CD133
- CD34
- CD271

### T Cells
- CD3, CD4, CD8, CD25
- CD45RA
- CD62L
- CCS
  (antigen specific T Cells)
- CD137
- TCRab

### NK Cells
- CD56

### Monocytes
- CD14

### Dendritic Cells
- BDCA1
- BDCA4 (CD304)

### B Cells
- CD19

### Indirect Labelling (any cell type)
- Anti-Biotin Reagent

### Custom Reagents

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**Custom Reagents**
Next Generation Product Evolution

CliniMACS®Plus

CliniMACS®Prodigy
CliniMACS® Prodigy is Designed to Replace or Integrate ...
Integrated cell processing from starting cells to final cellular product
- Sample preparation
- Cell washing & gradient separation
- MACS cell separation
- Genetic modification
- Cell culture
- Final product formulation

Enabling complex processes
- Automated & controlled system
- Closed single-use tubing set
CliniMACS® Prodigy – Main Components

- Bag hangers
- Gas Mix Unit
- Pinch valves
- Peristaltic pump
- Liquid Sensor
- CentriCult Unit
- Bag Compartment
- Touch screen
- Magnet Unit
- Tube Sealer
- Bar code reader
Closed Centricult Chamber Delivers Sterility & Flexibility

- Total volume: 400 ml
- Centrifugation: up to 2500 rpm (400 g)
- Optimized cell pellet volumes (20 - 80 ml)

Cross section:

- Port at the bottom
- Port at the lid
- Connections
- Filter
- Double prism
Centicult Enables Automated Density Gradient Separation

- Separation of blood into different components
- Collection of different cell fractions: e.g. plasma, leucocytes, erythrocytes

Automatic layer detection system
Collection of erythrocytes after density gradient separation

- Plasma
- Leucocytes
- Erythrocytes
On-Board Centrifugation for Closed System Cell Processing

- CentriCult chamber
- Boundary-layer detection
- Microscopic cell examination
- Temperature control: 4 - 38°C
- Atmosphere control (CO₂, N₂, Air)
NK Cell Therapy: Clinical Approaches

Modulation of NK cell activity
- Antibodies (ADCC)
- Interleukins

NK cell adoptive therapy
- Freshly isolated donor NK cells infusions
- Expansion of donor NK cells

Gene modification of NK cells
- Manipulation of NK cells and expansion
Clinical Scale NK Cell Separation Strategies

NK cells
1st step: CD3 depletion
2nd Step: CD56 enrichment

NK cells and NKT cells
One-step: CD56 enrichment

NK cells and accessory cells
One-step: CD3/CD19 Depletion

NK cells γδ T cells and accessory cells
One-step: TCRαβ/CD19 Depletion
Clinical Scale NK Cell Workflow

- **NK cell isolation**
  - CD3 depletion and CD56 enrichment
  - CD56+ enrichment
  - CD3/CD19 depletion

- **NK cell expansion**
  - *NK MACS Medium GMP*
  - Cytokines GMP (IL-2, IL-12, IL-15, IL-21)
  - Prodigy expansion FPS

- **Freezing and thawing**
  - Multiple infusions of NK cells
  - Freezing bags

- **Quality control**
  - Flow cytometry: NK cell analysis (REA antibodies)

- **Follow-up**
  - Flow cytometry: NK cell analysis (REA antibodies)

* Available 2017
# CD3 Depletion with CD56 Enrichment

Köhl et al. Frontiers in Oncology, 2013

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Köhl (A)</th>
<th>Köhl (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x CD3 depl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x CD56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 13</td>
<td></td>
<td>n = 18</td>
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### Leukapheresis

<table>
<thead>
<tr>
<th></th>
<th>WBC (x10e9) median</th>
<th>WBC (x10e6) median</th>
<th>CD3- CD56+ (%) median</th>
<th>CD3- CD56+ median</th>
<th>range</th>
<th>CD3- CD56+ (%) median</th>
<th>CD3- CD56+ median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Köhl (A)</strong></td>
<td>18,9</td>
<td>18907</td>
<td>8,5 (5,4 – 13,7)</td>
<td>15,26x10e8</td>
<td>6,73-29,75</td>
<td>94,9</td>
<td>7,12x10e8</td>
<td>2,34x10e8 – 1,8x10e9</td>
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<tr>
<td><strong>Köhl (B)</strong></td>
<td>19,3</td>
<td>19338</td>
<td>7,5 (2,8 - 14,9)</td>
<td>17,01x10e8</td>
<td>6,12-47,90</td>
<td>92,2</td>
<td>7,45x10e8</td>
<td>2,84x10e8 – 2,7x10e9</td>
</tr>
</tbody>
</table>

### Final product

<table>
<thead>
<tr>
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<th>CD3- CD56+ (%) median</th>
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<tr>
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<td>94,9</td>
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<tr>
<th></th>
<th>CD3+ T cells median</th>
<th>CD3+ T cells range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Köhl (A)</strong></td>
<td>0,12x10e6</td>
<td>&lt; dl – 1,50</td>
</tr>
<tr>
<td><strong>Köhl (B)</strong></td>
<td>0,31x10e6</td>
<td>&lt; dl – 6,99</td>
</tr>
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</table>
NK MACS Medium (Research Grade)

Optimized culture medium for cultivation, activation, and expansion of human NK cells

- Superior proliferation compared to home-brew and competitor media
NK MACS Medium (Research Grade)

Starting from PBMCs, expansion of unwanted cells (T cells, NKT cells) is minimal after 14 days culture.
Clinical Grade Products for NK Cell Culture

Isolated NK cells + TexMACS GMP Medium + Cytokines GMP:

- IL-2
- IL-12
- IL-15
- IL-21

Example:

Fully automated expansion and activation of clinical-grade natural killer cells for adoptive immunotherapy

MARKUS GRANZIN1, STEPHANIE SOLTENBORN1, SABINE MÜLLER1, JUTTA KOLLET1, MARIA BERG2, ADELHEID CERVENKA2, RICHARD W. CHILDS3 & VOLKER HUPPERT4

1Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, 2Innate Immunity Group, German Cancer Research Center, Heidelberg, Germany, and 3National Heart, Lung and Blood Institute, Hematology Branch, Bethesda, Maryland, USA
Clinical Grade Products for NK Cell Culture (Coming Soon)

Isolated NK cells

+

NK Medium GMP*

+ 

PBMCs

Cytokines GMP:
✓ IL-2
✓ IL-12
✓ IL-15
✓ IL-21

* Available 2017
MACS Cytokines: Three Quality Grades for Easy Translation

Research grade

Premium grade

GMP grade

Basic Research  Pre-clinical Research  Clinical Trials  Clinic

Highly regulated
Clinical Grade NK Cells for Immunotherapy

Fully automated expansion and activation of clinical-grade natural killer cells for adoptive immunotherapy

MARKUS GRANZIN\(^1,2\), STEPHANIE SOLTENBORN\(^1\), SABINE MÜLLER\(^1\), JUTTA KOLLET\(^1\), MARIA BERG\(^3\), ADELHEID CERWENKA\(^2\), RICHARD W. CHILDS\(^3\) & VOLKER HUPPERT\(^1\)

\(^1\)Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, \(^2\)Innate Immunity Group, German Cancer Research Center, Heidelberg, Germany, and \(^3\)National Heart, Lung and Blood Institute, Hematology Branch, Bethesda, Maryland, USA

Granzin M, Cytoteraphy 2015
Automated NK Cell Expansion Using a Clinical Grade Protocol

Clinical grade protocol from Richard Childs

NK + IL-2 → EBV-LCL
Irradiated clinical grade feeder cell

Manual cultivation

Automation / Translation

Granzin M, Cytotherapy 2015
Comparison of Automatically- and Manually-Expanded NK Cells

Automated process includes
- Maintenance of culture conditions (CO₂, Temp)
- “feeding“ = medium exchange
- “shaking“ = gentle mixing of cells for high cell density cultivation
- Sampling for analysis
Automated NK Cell Expansion – Cell Numbers

Cell pellet after Expansion

Granzin M, Cytotherapy 2015
Automated NK Cell Expansion – Function

Killing of different leukemic cell lines

**K562**

**Raji**

**Daudi**

*Automated* | *Manual* | *Manual w/o EBV-LCL*
Antibody Dependent Cellular Cytotoxicity (ADCC)

Target Cell (Cancer Cell)

Effector Cell (NK cell & Monocyte)

Tumor Lysis by ADCC

Cytotoxicity

NK

NK+ Rituximab

Raji Automated

Raji Manual

Daudi Automated

Daudi Manual

% of Killed Target Cells

E:T Ratio

0 2 10

0 2 10

0 2 10

0 2 10

* 75 100

* 75 100

* 75 100

* 75 100

** 75 100

** 75 100

** 75 100

** 75 100

* 75 100

* 75 100

* 75 100

* 75 100
Coming soon….

Application sheet:

**Automated NK cell expansion program**

**Application**

Automated procedure for the expansion of natural killer (NK) cells using a set of programs for culture setup, culture, loading, sampling, and harvesting. This application sheet gives an overview of the required materials needed to perform the shown example of NK cell expansion. Further it provides an overview of the setup of the tubing set, the expansion workflow and the performance data.

**Specifications**

- **Program name:** Automated NK cell expansion
- **Program capacity:**
  - Starting number: 1.75 x 10⁶ CD56⁺ CD3⁻ NK cells (presented protocol)
  - Expanded NK cells: 1.1-3.2 x 10⁶ CD56⁻ CD3⁻ NK cells (presented protocol)
- **Cell density:** 0.5-1.5 x 10⁶ CD56⁻ CD3⁻ NK cells per ml
- **Sample volume:** 70-250 ml
- **Elution volume:** Approx. 100 ml
- **Process time:** 1-21 days (14-day protocol presented)

After the isolation of the cells, NK cell expansion can be adapted to individual expansion protocols and cultivation periods, for example, an expansion protocol starting with higher NK cell numbers. There are different options to purify NK cells prior to expansion. The shown expansion process starts with a low NK cell number generated via consecutive CD3⁺ cell depletion and CD56⁺ cell enrichment.

CD3⁺ cell depletion and CD56⁺ cell enrichment can be performed with both the ClinMACS® Plus instrument and the ClinMACS Prodigy. Both systems will generate higher numbers of purified NK cells than needed for this expansion protocol. These surplus cells can either be frozen or used for other downstream applications.
Comprehensive Regulatory Support

Proven Clinical Expert in Cell Therapy
- > 200 INDs/IDEs at US FDA
- > 150 clinical studies in EU & ROW
- > 20 Type II Master files at US FDA
- > 40 Product dossiers in EU
- FDA approved CliniMACS CD34 system

Certified to Meet Clinical Commercial Needs
- Certified as a pharmaceutical manufacturer
- ISO 13485
- ISO 9001
- GMP manufacturing license for monoclonal antibodies
A Comprehensive Cell & Gene Therapy Partner

• Novel cell processing automation for isolation/washing/expansion
  o CliniMACS Plus and Prodigy

• Existing GMP ancillary products portfolio
  o Antibodies, cytokines, and cell activation reagents
  o Media and media supplements

• GMP custom production capabilities
  o Custom antibodies, proteins, and nucleic acids
  o Lentiviral vectors (pilot and production scale)

• Comprehensive alliance R&D team for rapid partner development
  o Optimizing assays, protocols, expansion procedures, automation for partner-specific goals

• Enabling IP across the workflow of cell therapy manufacturing
  o Cell separation, activation, transduction, processing
  o Lentiviral vector manufacturing, novel pseudotypes, enhanced cell targeting/transduction