Release Testing and Quality Control for Cellular Therapeutics

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Efforts to comply with Good Manufacturing Practices as applied to flow cytometric cell sorting of blood cell products

- SOPs and documentation to describe:
  - Quality Systems policies and procedures
  - Installation and operational qualification of devices
  - Equipment operation/maintenance
  - Manufacturing procedures/batch production records
  - Release testing procedures and specifications/batch release records
- Raw materials and supplies
  - Raw materials meet pre-established specifications based on intended use
  - Review of Vendor Quality Systems program to ensure product attributes
  - Certificates of analysis used to document material quality
  - In-house testing for performance and stability
- Downstream Procedures
  - Independent Quality Systems review of batch production and release records
  - Independent Medical Director review to ensure product is fit for intended use
  - Controlled product storage and archiving
Today’s lesson

• Use of antibody combinations to ensure cell product quality

• Critical quality attributes of antibodies used in-process and release
  - Specificity
  - Sensitivity
  - Stability
  - Performance requirements
  - Reproducibility
Antibodies for Selection, Sorting and Identity Assessment

- General parameters to consider for antibodies
  - GMP compliant preparative antibodies
  - Antibodies recognizing unique epitopes from preparative abs
  - Specificity in detecting target antigens
  - Compatibility with process and other reagents (antigen loss during fixation, spectral overlap, sensitivity)
  - ASR (analyte specific reagents) antibodies when available for release analysis. Validate RUO abs for analysis. Commercial FC controls where appropriate

Examples:
  - Use of an independent marker to establish purity (FoxP3 in Treg)
  - Use of antibodies recognizing non-cross inhibiting epitopes to CD34 and CD90 for sorting and analysis
Regulatory T-cell (Treg) Isolation Process

- Prophylaxis and treatment of Graft vs. Host Disease in BMT patients
- Tandem selection approach:
  - Clinical-scale CD25 immunomagnetic bead enrichment
  - High-speed FACS-based Treg enrichment by CD4+CD127\textsuperscript{low} phenotype (Vendor supplied reagents)
- Isolate therapeutic target doses of naturally occurring Treg (>1\times10^6/kg to 3\times10^6/kg)
- Reagents for independent verification of Treg content for product release
- Vendor initiated internal GMP Mab quality process
FoxP3+ Post-Sort Cell Analysis for Treg Purity

CD34 depleted PBSC
CD45+ gated

CD25+ Enriched
ungated

CD4+ CD127\textsuperscript{low} Sorted
CD45+ gated

*ASR CD45, CD3, CD4 performance on commercial FC controls*

CD25 Selection

CD4+CD127\textsuperscript{dim} Sorting

Independent measurement of CD4 and FoxP3

*CD4 clone L200 for sorting & SK3 for analysis*

*CD25 ab staining blocked by selection reagent*
Purification of Hematopoietic Stem Cells

- CD34+ cells are heterogeneous population of stem and progenitor cells (HSPC)
- CD34+ cell population also can contain tumor cells (breast cancer)
  - CD34+CD90+ phenotype define a more refined stem cell population depleted of tumor cells
- CD34+CD90+ population contains virtually all long term repopulating cells (ideal substrate for gene therapy)
- Selection, Sorting and Analysis reagents recognize same cell surface markers
- CMO produced sorting reagents for Stanford; Mabs used in previous trial
- Different release criteria may exist for different cell applications
High Purity HSC by Tandem Immunomagnetic CD34+ Enrichment and CD34+CD90+ Sorting

• High HSC purity enrichment based on co-expression of CD34 and CD90
  ▪ Reduced numbers of T cells (GVHD, allogeneic)
  ▪ Tumor cell reduction (relapse, autologous)
  ▪ Potential gene therapy improvements (autologous)

• Selection/sorting /analytical antibodies:

<table>
<thead>
<tr>
<th>Processing step</th>
<th>CD34 Clone</th>
<th>CD90 Clone</th>
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<tbody>
<tr>
<td>CD34 Enrichment</td>
<td>AC101</td>
<td>none</td>
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<tr>
<td>CD34/CD90 Sorting</td>
<td>PR3a</td>
<td>PR13</td>
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<tr>
<td>Post-sort quality assessment</td>
<td>8G12</td>
<td>5E10</td>
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Qualification of GMP Produced Monoclonal Antibody Reagents for Sorting

- Establish utility of antibody using cell lines known to express relevant markers
  - KG-1a Cell line used for CD34 GMP Mab PR3a testing
  - Jurkat Cell line used for CD90 GMP Mab PR13 testing
- Test possible interactions between antibodies on cell lines and PBMC
  - CD34 selection and sorting: compare CD34 GMP sorting Mab with CD34 ab clones intended for immunomagnetic enrichment and for post-sort analysis
  - CD90 sorting: compare CD90 analysis clone 5E10 with CD90 GMP sorting Mab
- Markers expressed on other peripheral blood cell populations?
Qualification of Reagents for Selection and Analysis

Ensure minimal cross-inhibition of marker detection for either selection, sorting or analysis

- **CD34:**
  - Use of 8G12 (class III epitope binding) with AC101 (class II epitope binding) is well established
  - Anti-CD34 monoclonal for sorting (PR3a) has acceptable impact on post-sort identification of CD34+ cells with 8G12

- **CD90:**
  - Anti-CD90 monoclonal for sorting (PR13) has acceptable impact on post-sort identification of CD90+ cells with 5E10
  - 5E10 ab reagents available as RUO
    - Quality and validation plan needed to test vendors and lot #s
Qualification of GMP Produced Monoclonal Antibody Sorting Reagent Testing

<table>
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<tr>
<th>Reagent</th>
<th>Testing</th>
<th>Target Cells</th>
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<tr>
<td>PR13</td>
<td>Specificity and sensitivity on CD34+CD90+ cells</td>
<td>Mobilized PB, Marrow</td>
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<tr>
<td>(α-CD90)</td>
<td>Clinical Scale binding titration</td>
<td>Jurkats</td>
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</table>
Performance and Specificity: Cross-inhibition of Selection and Sorting Reagents

Saturate KG1a CD34 epitopes with AC101 ab

Sequential PR3a-APC staining

Confirm AC101 binding

Compare w/PR3a-APC alone staining:
AC101 pre-binding has Insignificant effect on PR3a MFI
Performance and Sensitivity:
Cross-inhibition of Sorting and Analysis Reagents

Kg1a

CD34 Clone 8G12

Sequential PR3a 8G12

Isotypes

PR3a-APC

Jurkat

CD90 Clone 5E10

Sequential PR13a 5E10

Isotypes

Small, acceptable impact on analysis ab staining

PR13-PE
Sorting Reagent Selectivity and Specificity

Jurkat  KG1a  Mobilized PBMC  Mobilized PBMC CD34+ Gated

*CD90 expressed on other peripheral blood cells: use CD34+ HSC gating
Reagent Performance

GMP sorting Mab needed to stain ~200X10^6 CD34-enriched cells at 50X10^6 cells/ml

Considerations

- **Balance between Sensitivity and Non-Specific Binding**
  - Some non-specific PR3a binding observed at higher concentrations
Reagent Stability Testing

- Test sensitivity, specificity, performance and reproducibility during long-term reagent storage
- Reagent testing consistent with expected use
- Establish binding curves on representative cell lines if possible
- Specificity and selectivity on target PB cells
- Compare curves achieved at specified time points to assess reagent stability under specified storage conditions
  - \(-65^\circ C\) vs. RT
- How much variation in curves allowed?
  - Impact of biologic variation in stained cells?
  - Need to define what variations and changes are acceptable
  - No hard & fast rules exist:
Conclusion: Set *Internal* Standards for Reagent Use

- Ensure reasonable patient safety
- For reagent testing, procedures must specify:
  - Test conditions with validated SOPs
  - Acceptable expected ranges
  - Timing of stability assessments
  - In-house standards for specific therapeutic use
- Cells used in assays: cell lines vs. peripheral blood samples
- Cell preparation conditions to assure uniformity (good luck)
  - Variations in peripheral blood samples
  - Subtle variations in cell line culture conditions
  - Process for determining impact of variations on reagent performance or assays
  (always a work in progress)
Acknowledgements

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