Human Platelet Lysate requirements for cell therapy expansion in support of clinical trials

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The Issue

• cGMP manufacture of cell therapies needs to avoid the use non-human animal supplements.

  – Current options:
    • Defined media
      – Limited utility to date
    • AB serum
      – Not optimal for culture of some cells
      – Supply concerns
    • Human platelet lysate (HpL)
      – Extensive literature demonstrating utility of HpL for cell culture
      – Multiple companies/organizations marketing or developing HpL product formulations
        » There is variability in manufacture of HpL
        » Establishing a minimum set of criteria for HpL production/characterization is needed
Why platelets?

- The growth factors contained within platelets are key factors for tissue repair and regeneration, vital to inducing cells to grow rapidly, but not abnormally in the body.
HpL VS FBS Effect On CB MSC Proliferation

![Bar graph showing cell number (x10^3) over time (48hr to 144hr) for 2% FBS and 2% Clearlsate treatments.](imageurl)
General Manufacturing Scheme for HpL

Platelets in peripheral blood → Platelets harvested → Platelets Washed → Platelets flash frozen

Finished product: HpL

Multiple filtrations → Centrifugation → Platelets thawed, membranes rupture

San Diego Blood Bank
HpL Starting Material

– Typically expired platelets collected for transfusion are used as starting material
  • Collected at accredited (AABB, FACT) facilities with that follow State and CFR guidelines for clinical blood products
    – The appropriate consent must be obtained from donors prior to collection and use
    – IDM and sterility tested prior to release for manufacture of HpL
    – Assures a consistent, safe, traceable starting material with QA/QC oversight
    – Need to finalize how “old” can expired platelets can be and still be acceptable for use
HpL Production Process

• Standardization of policies and procedures for HpL manufacturing processes assure production of a safe, consistent HpL product

• Manufacturing processes should be cGMP compliant, with Quality oversight
  – Environment, equipment, personnel, manufacturing supplies
    • Monitoring, validation, training, qualification

• Implementing these practices for HpL production is a key support for cGMP manufacture of the final cell therapy product
Lot to Lot Consistency Assessment

hMSC proliferation at Day 6

Amplification Yield

- FBS+bFGF
- FBS-bFGF
- Lot 1 Clearsate
- Lot 2 Clearsate
- Lot 3 Clearsate

2.5%  5%  8%
Product Characterization

- A Certificate of Analysis (CoA) should be included with each HpL manufacturing lot
  - Additives*
  - Lot number
  - Protein concentration
  - Endotoxin
  - IDM
  - Mycoplasma
  - Sterility
  - Expiration Date
  *if used, for heparin, porcine source may be an issue

Certificate of Analysis and Release

Product: Clearest™ (Human Platelet Lysate) from saline-washed Human Platelets, GMP process, no non-Human biologicals or anti-coagulants added.

- Clearest™ produced by: San Diego Blood Bank
- Lot Number: DN____________________
- Clearest™ Storage Conditions: ≤ -20°C
- Not for Transfusion Into Human
- Not for Human or Animal Consumption

All human platelet units, prior to selection for Clearest™ production, were tested and found to be negative or non-reactive for the following:

<table>
<thead>
<tr>
<th>Platelets Used To Manufacture Clearest Tested For:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Detection (Unrelated antibodies to red cells)</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus (HIV) 1 &amp; 2 plus O</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
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<tr>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>Human T-Lymphotropic Viruses I and II</td>
</tr>
<tr>
<td>Trypanosoma pallidum (Syphilis)</td>
</tr>
<tr>
<td>Trypanosoma cruzi (Chagas)</td>
</tr>
<tr>
<td>West Nile Virus (WNV)</td>
</tr>
<tr>
<td>Zika Virus (ZIKV)</td>
</tr>
<tr>
<td>Bacterial Contamination</td>
</tr>
<tr>
<td>Mycoplasma</td>
</tr>
<tr>
<td>Endotoxin</td>
</tr>
</tbody>
</table>

Final Lysate Product:

<table>
<thead>
<tr>
<th>Sterility Tested: Bacterial/Fungal/anaerobic &amp; aerobic</th>
<th>METHOD</th>
<th>SPECIFICATION*</th>
<th>RESULT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Fungal Anamorphic</td>
<td>Direct PX</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*All batches of Human Platelet Lysate have been sterile filtered and sterility tested and found to be N = Negative/Non-reactive prior to release. Sterility testing performed at San Diego Blood Bank.

Quality Assurance/Compliance Department Review and Lot Release
This above lot number tested using the methods listed has been found negative or non-reactive and is approved for release.

Signature: ____________________________  Printed Name and Title: ____________________________  Release Date: ____________________________
HpL Product Characterization

• Stability
  – A stability plan may be needed to determine shelf life of HpL products for a specific storage condition
    • -20 or -80 C storage conditions
  – Stability may be assessed with an in vitro cell proliferation potency assay
    • May need to use FBS standard or develop an HpL reference lot as comparator
Potency Assessment

• Used to support stability studies
• Used for HpL lot release criteria
  – Potential cell types to be screened:
    • MSCs, HSCs, other
  – Cell counts taken at multiple time points
  – Data compared to a reference standard
HpL Quality Control and Assurance

- QA/QC oversight is a key aspect of HpL production to support cGMP cell therapy manufacture
  - Release of expired platelets as starting material for HpL manufacturing
  - Manufacturing standardization to assure safety and lot-to-lot consistency of the product
Suggestions for FDA to Consider

• A draft guidance for sourcing platelets that are the starting material for HpL production
  – Transfusion-grade Platelets sourced from cGMP facilities with appropriate accreditations, Consent and Quality oversight
    • Assures safety and consistency of starting material, typically expired platelet product and still acceptable for HpL manufacture

• A cGMP compliant HpL manufacturing process preferred with Quality program oversight supporting environmental, equipment, personnel and materials management

• A CoA with each manufacturing lot of HpL having the minimum criteria listed below:
  – Sterility, protein concentration, IDM, endotoxin, mycoplasma, lot number, expiration date, additives (if used)
  – Optional: Growth factor(s) content, potency assay results