Development and Manufacturing of a Biobetter Therapeutic: A Case Study

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ZZ Biotech Company History

- Parent company, Socratech, LLC founded by Berislav Zlokovic and Selim Zilkha to advance *novel neurologic agents* toward clinical use

- After Socratech licenses 3K3A-APC from the John Griffin lab at The Scripps Research Institute, ZZ Biotech, LLC is spun out to focus on development of 3K3A-APC, a Biobetter version of wt-APC (Xigris) for *ischemic stroke*

- ZZ Biotech, LLC remains a partially owned subsidiary of Socratech, with 4 additional fundraising rounds from angel investors and first VC money committed; Phase 1 trial completed Dec. 2012
Pathogenic Triad of Ischemic Stroke Exposes Need For Multifunctional Stroke Agent

Vasculo-neuronal-inflammatory Pathogenic Triad Following Ischemic Stroke

- Vascular Damage
- Neuro-inflammation
- Neuronal Injury and/or Neuro-degeneration

Benefits of 3K3A-APC

- Protects multiple cell types
  - Neurons
  - Vascular cells (endothelium, pericytes, vascular smooth muscle cells)
  - Glia
- Anti-inflammatory activity

APC and its mutants address all components of neurovascular unit breakdown during stroke

Why Not Use Wild-Type APC?

“Bleeding is the most commonly reported adverse reaction in patients receiving Xigris therapy. Patients administered Xigris as treatment for severe sepsis experience many events which are potential sequelae of severe sepsis and may or may not be attributable to Xigris therapy.

In severe sepsis clinical trials, there were no types of non-bleeding adverse events suggesting a causal association with Xigris. In the PROWESS study, serious bleeding events were observed during the 28-day study period in 3.5% of Xigris-treated and 2.0% of placebo-treated patients. The difference in serious bleeding occurred primarily during infusion.”

—Xigris package insert
Wild-Type APC Has Benefits, But Poses Serious Bleeding Risk

**Anticoagulant and Cytoprotective Pathways of APC**

- **Anticoagulation**
  - Inactivation of $fV_i$ and $fVIII_i$
  - APC
  - $fV_i$ and $fVIII_i$
  - Protein cofactors
  - Lipid cofactors
  - Membrane

- **Cytoprotection**
  - Activation of PAR-1
  - APC
  - EPCR
  - PAR-1
  - Pleiotropic cytoprotective effects

**Commentary**

- **Cytoprotection**
  - Anti-apoptosis
  - Anti-inflammation
  - Alteration of gene expression
  - Protection of endothelium

- **Anticoagulation leads to bleeding risk**

- **3K3A-APC keeps benefits while decreasing risk**

“Reduced-Anticoagulant” APC Variant: 3K3A-APC—A Biobetter

Anticoagulant
(< 8% activity)

Cytoprotective
(full activity)

From: Moznier et al, Griffin. Blood 2004
3K3A-APC vs. Wild-Type APC in Cynomolgus Monkeys

**ΔaPTT**

- Change in coagulation parameter in Cynomolgus monkeys dosed with 3K3A-APC and Xigris (recombinant wt-APC, Lilly)

- ~10% residual anti-coagulation activity
  - 0.2 mg/kg: 9.4x
  - 1.0 mg/kg: 11.9x

*Dramatically reduced risk of bleeding at planned dose*

Combination of 3K3A-APC with tPA expands the tPA treatment window

Adhesive Removal Test

- 10 male Wistar rats/dose group
- tPA (10 mg/kg) and 3K3A-APC (7 mg/kg) dosed 4 hours after embolic stroke
  - When dosed earlier, tPA shows significant benefit on its own

Source: Stroke. 2012 Sep;43(9):2444-9
3K3A-APC + tPA combination therapy more potent than either alone

Foot-Fault Test

- * P<0.05 vs. saline
- † P<0.05 vs. tPA
- § P<0.05 vs. 3K3A

Neurological Severity Score

- * P<0.05 vs. saline
- † P<0.05 vs. tPA
- § P<0.05 vs. 3K3A

Source: Stroke. 2012 Sep;43(9):2444-9
Synergistic Effects of 3K3A-APC + tPA Lead to Reductions in Lesion and Hemorrhage Volume

**Lesion Volume**

<table>
<thead>
<tr>
<th></th>
<th>% of contralateral hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>tPA</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>3K3A-APC</td>
<td>30 ± 1.5</td>
</tr>
<tr>
<td>3K3A-APC + tPA</td>
<td>25 ± 1.2</td>
</tr>
</tbody>
</table>

* P<0.05 vs. saline
† P<0.05 vs. tPA
§ P<0.05 vs. 3K3A

**Hemorrhage Volume**

<table>
<thead>
<tr>
<th></th>
<th>μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>50</td>
</tr>
<tr>
<td>tPA</td>
<td>100</td>
</tr>
<tr>
<td>3K3A-APC</td>
<td>30</td>
</tr>
<tr>
<td>3K3A-APC + tPA</td>
<td>20</td>
</tr>
</tbody>
</table>

* P<0.05 vs. saline
† P<0.05 vs. tPA
§ P<0.05 vs. 3K3A

Source: Stroke. 2012 Sep;43(9):2444-9
Development & Manufacturing
Who is Laureate Biopharmaceutical Services?

- Well-established CMO with decades of experience
  - Mab’s
  - Fusion proteins
  - Other recombinant proteins
  - All mammalian cell culture
  - Commercial and Clinical Production
- Full service
  - Vial of DNA/cells → vials of drug product
  - Process development & manufacturing
  - Release & stability testing
  - Regulatory filing
Tech Transfer of Early Process

- Developed by a CRO & ZZ Biotech research
- Recombinant production in CHO-dhfr cells
- Chemically defined media and feeds
  - Need for Vitamin K addition for post-translational modification of gamma-carboxy-glutamic acid (Gla)
- Ran shake flasks, 2L & 12L glass & Wave bioreactors.
Scale-Up to Pilot Scale

- Process scaled using standard parameters from 12L to 200L pilot runs
- Series of 200L runs performed to hone process
- Material used for formulation and toxicological studies
Variation of Cell Growth Traced to Lot of Culture Media

- Performed additional 12L run with media from pilot run #3 that showed similar higher growth
- Additional growth had minimal effect on titer
- Purification yields comparable to previous runs
Transferred in Purification Process

- Purification based on literature for Protein C, a series of ion-exchange and a hydrophobic interaction chromatography
- Load conditioning: Solvent-detergent step added upfront to inactivate potential enveloped viruses. No effect on subsequent Q step
- 1st Q-anion exchange step works in pseudo-affinity mode to selectively capture Gla form
- Note Protein C is activated to APC at Step 6 by addition of recombinant thrombin (Recothrom®)
Results of Initial Runs of Transferred Process

• Positives
  – Q-Sepharose capture step: Worked as expected, with 50-70% recovery due to removal of PC with reduced levels of Gla.
  – HIC & Thrombin activation: No issues

• Negatives
  – Viral Filtration: Losses due to clogging – product interaction with filter
  – Overall Yield: Low at < 10%
  – Product Quality: High levels (2000-4000 ppm) of Host-Cell Protein
  – Product Integrity: Post activation, 3K3A-APC is a protease that under certain conditions autodigests resulting in clips in peptide chain.
Improvements for Yield & Purity

• Yield:
  – Changed viral filter from dead-end to tangential-flow type to reduce binding
  – Autodigestion controlled by rapid processing and buffer modifications

• Purity
  – Added additional wash steps to Q-Sepharose and HIC

• Results
  – Product yield doubled
  – HCP reduced to ~100 ppm
Analytics

- Standard protein assays for protein integrity & purity
  - SDS-PAGE critical in assessing proteolytic clipping of peptide chain
- Specialty activity assays to show unique qualities of 3K3A-APC vs. APC:
  - Cytoprotective activity
  - Anticoagulation
- Assay for Gla content
Cytoprotective Assay: 3K3A-APC vs. APC

- No statistical difference in anti-apoptotic activity between Xigris and 3K3A-APC
Anticoagulation Assay: 3K3A-APC vs APC

- The anti-coagulant activity of 3K3A-APC is < 10% that of Xigris
Gla Assay

- Performed as a specialty amino-acid analysis
- Separation by CEX-HPLC
- Product met theoretical specification and was comparable to Xigris®

Table 1. Gla Content of Xigris and 3K3A-APC

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Mole Ratio</th>
<th>Xigris</th>
<th>3K3A-APC Lot: FLIZB1-01</th>
<th>3K3A-APC Lot: FLIZB1-02</th>
<th>3K3A-APC Lot: FLIZB1-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gla</td>
<td>Theoretical</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

* There is only one replicate of 3K3A-APC Lot FLIZB1-02.
Scale-Up to Manufacturing

- 2000L production scale in Bioreactors
- Larger columns and buffer volumes
- Need for continuous processing (3-shift) processing once 3K3A-APC activated
2000L vs 200L Bioreactor Run Profile

Production VCD Comparison

Production Viability Comparison

Titer Comparison
Protein Purification

- Reproduced pilot-run processes in terms of yield and quality
- Scaled columns by residence-time and capacity
- Bulk Drug Substance (BDS) met all specifications

Larger-scale chromatographic skid
Aseptic Filling into Vials

- Filled >4000 vials with 5 mL into 10 mL glass vials
- Product immediately inspected and frozen at -70°C
Quality Control Testing

- Both Drug Product (DP) and Bulk Drug Substance (BDS) met all specifications, including HCP and Gla levels, and assays for cytoprotective and anticoagulation activity.

Analyst with UPLC
Clinical Status
Current 3K3A-APC Program Status

- Robust preclinical efficacy data leads to high Stroke Therapy Academic Industry Roundtable (STAIR) quality scores
  - Recent work in aged female mice and spontaneously hypertensive rats gives 3K3A-APC a score of 10/10; combo therapy with tPA 9/10
- 14-Day mouse and monkey toxicology/PK studies complete
- GMP manufacturing process developed; investigational medicinal product manufactured for 2 clinical trials
- Phase 1 safety/PK study in healthy human volunteers complete
- Working with NeuroNEXT network to obtain NIH support of a Phase 2 safety/PK study in ischemic stroke patients receiving tPA, to commence in 2014; seeking additional funding support
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