



Development and Manufacturing of a Biobetter Therapeutic: A Case Study

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on Biosimilars and Biowaivers
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ZZ Biotech Company History

2000

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

2006

- 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*

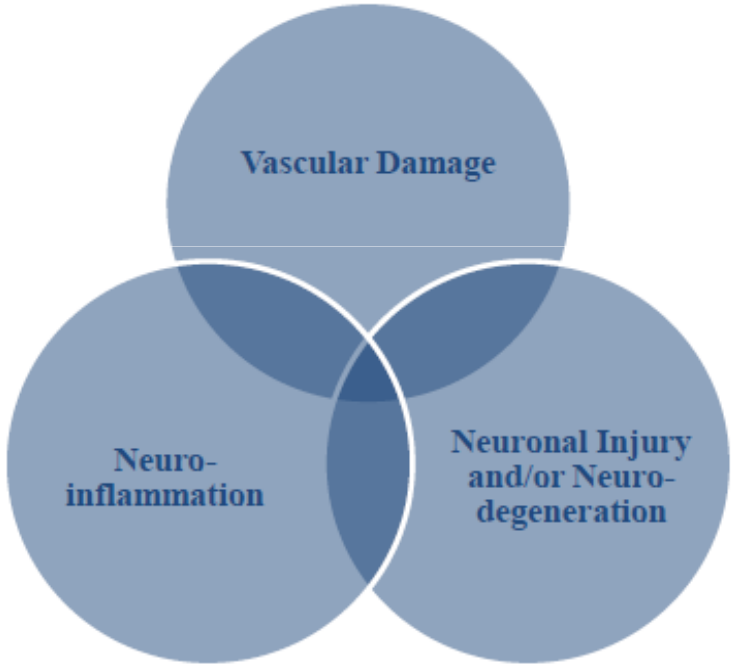
2013

- 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

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Pathogenic Triad of Ischemic Stroke Exposes Need For Multifunctional Stroke Agent

Vasculo-neuronal-inflammatory Pathogenic Triad Following Ischemic Stroke



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

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Source: Trends Neurosci. 2011 Apr;34(4):198-209 PMID: 21353711

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.

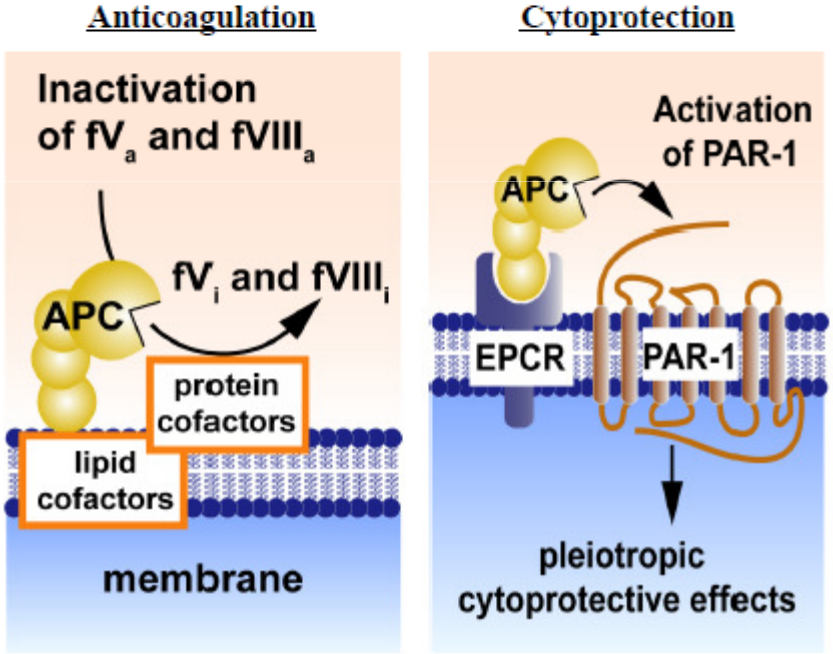
In severe sepsis clinical trials, there were *no types of non-bleeding adverse events* suggesting a causal association with Xigris. In the PROWESS study, *serious bleeding events* were observed during the 28-day study period in 3.5% of Xigris-treated and 2.0% of placebo-treated patients. The difference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

—Xigris package insert

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Wild-Type APC Has Benefits, But Poses Serious Bleeding Risk

Anticoagulant and Cytoprotective Pathways of APC



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

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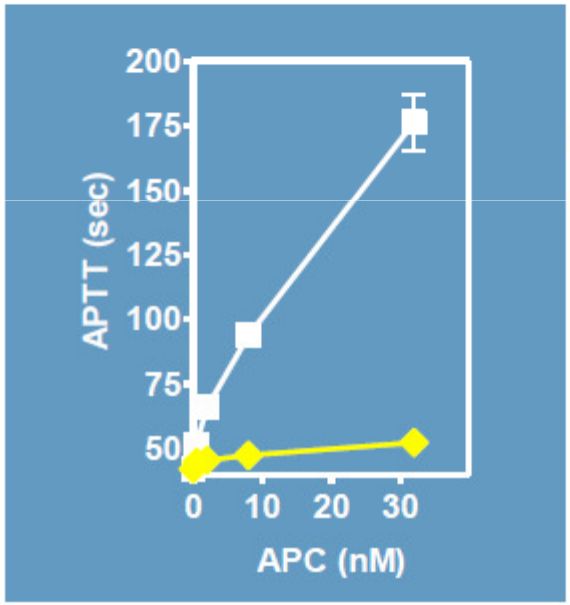
Source: Blood. 2007 Apr 15;109(8):3161-72. Epub 2006 Nov 16 PMID: 17110453

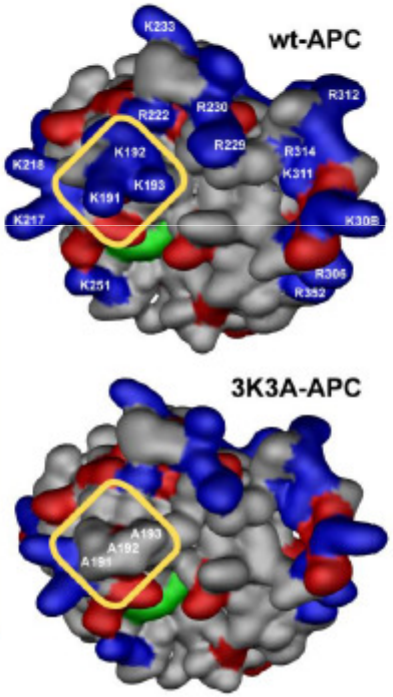
"Reduced-Anticoagulant" APC Variant: 3K3A-APC—A Biobetter

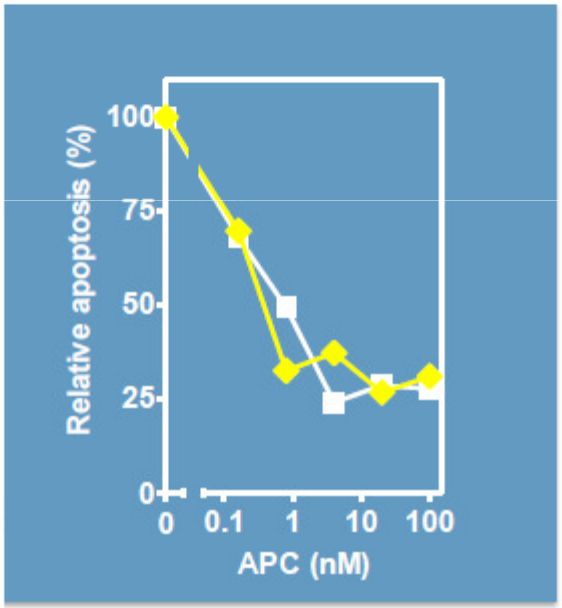
Anticoagulant
(< 8% activity)

3K3A-APC: 189-DSKKKLA-195

Cytoprotective
(full activity)

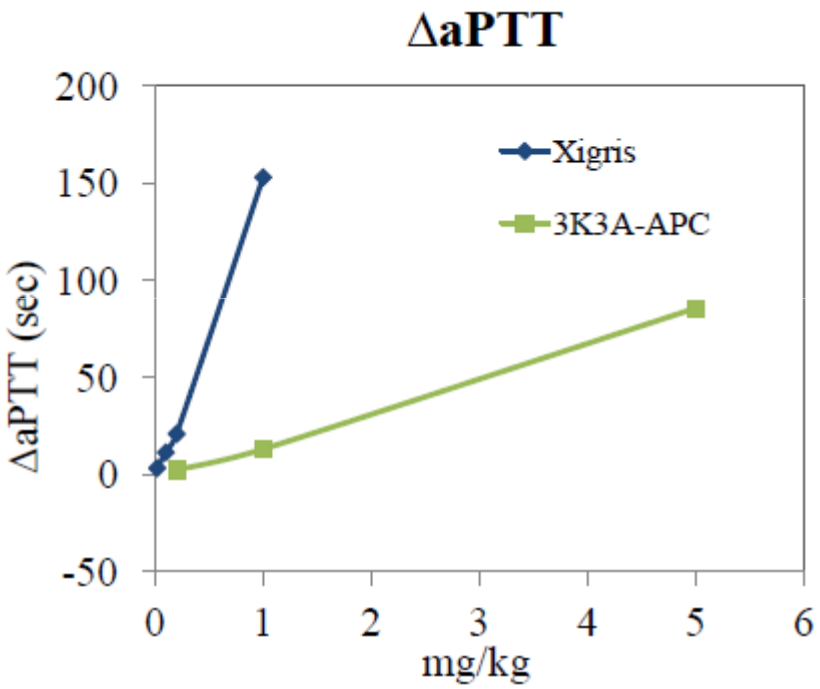






From: Mosnier et al, Griffin. *Blood* 2004

3K3A-APC vs. Wild-Type APC in Cynomolgus Monkeys



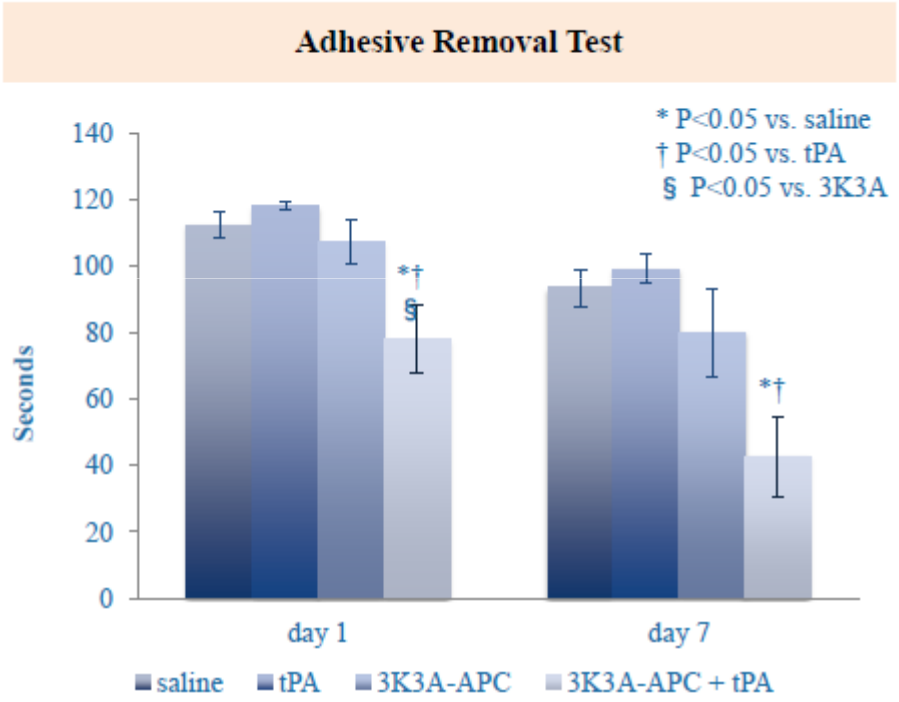
- Change in coagulation parameter in Cynomolgus monkeys dosed with 3K3A-APC and Xigris (recombinant wt-APC, Lilly)
- ~10% residual anti-coagulation activity
  - 0.2 mg/kg: 9.4x
  - 1.0 mg/kg: 11.9x

Dramatically reduced risk of bleeding at planned dose

Williams, P.D., Zlokovic, B.V., Griffin, J.H., Pryor, K.E., Davis, T.P. Preclinical Safety and Pharmacokinetic Profile of 3K3A-APC, a Novel, Modified Activated Protein C for Stroke, *Current Pharmaceutical Design* (2012) 18 (27), 4215-4222.

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



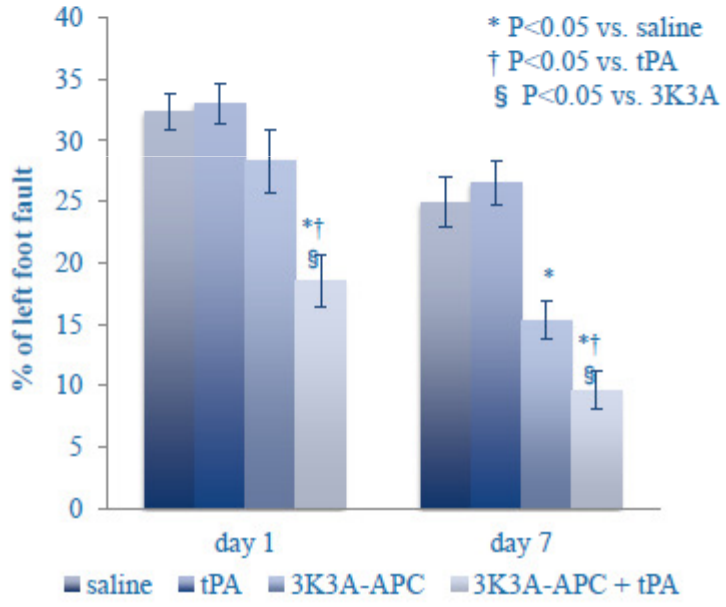
- 10 male Wistar rats/dose group
- tPA (10 mg/kg) and 3K3A-APC (2 mg/kg) dosed **4 hours** after embolic stroke
  - When dosed earlier, tPA shows significant benefit on its own

Source: Stroke. 2012 Sep;43(9):2444-9

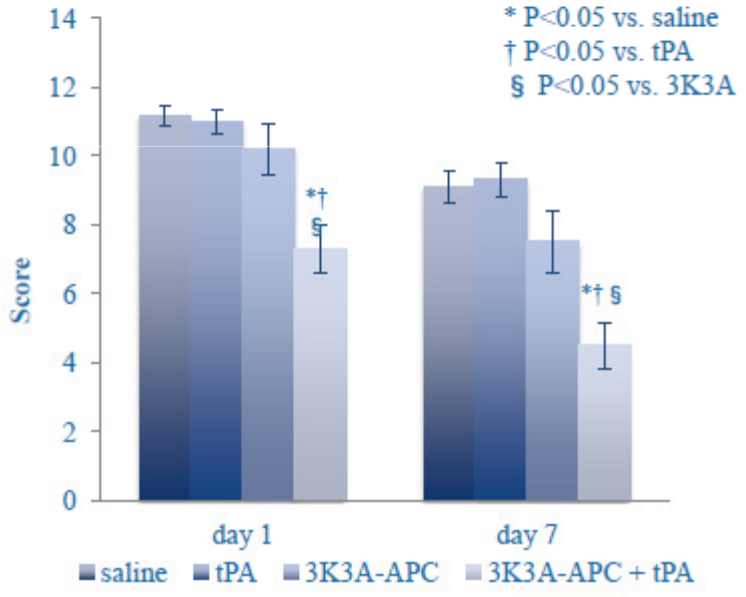
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3K3A-APC + tPA combination therapy more potent than either alone

Foot-Fault Test



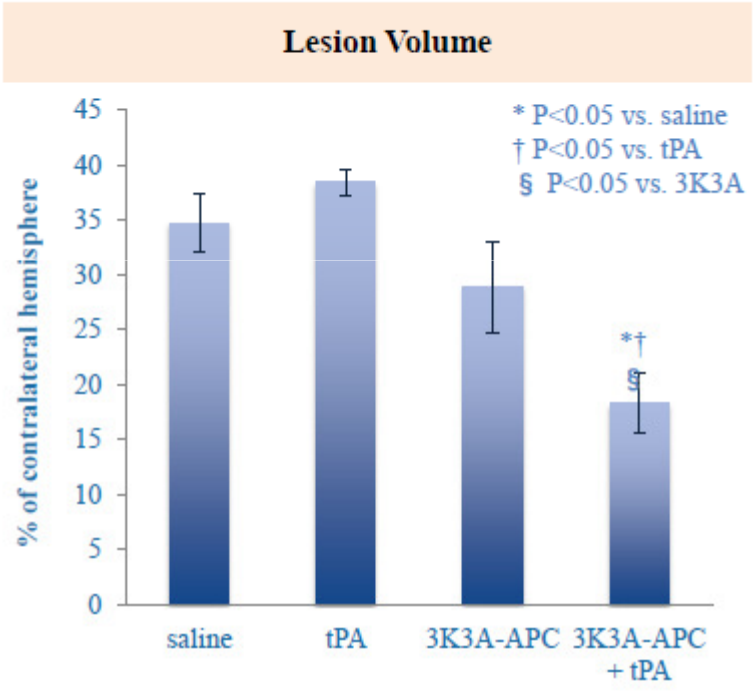
Neurological Severity Score

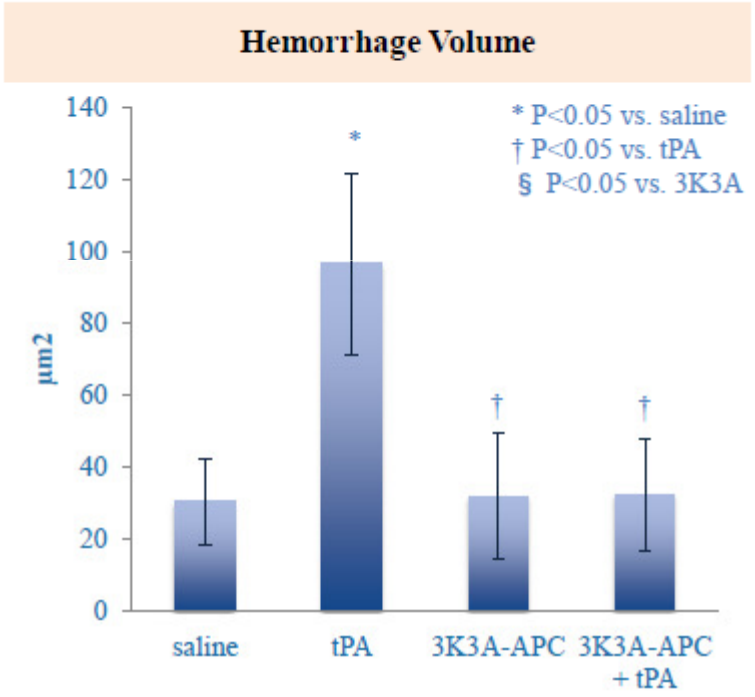


Source: Stroke. 2012 Sep;43(9):2444-9

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Synergistic Effects of 3K3A-APC + tPA Lead to Reductions in Lesion and Hemorrhage Volume





Source: Stroke. 2012 Sep;43(9):2444-9

Development & Manufacturing

LAUREATE BIOPHARMA

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

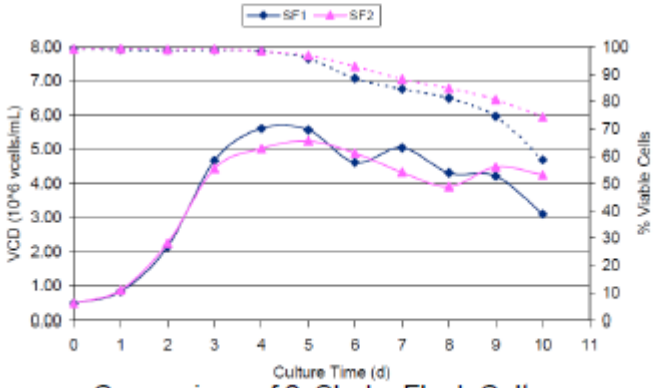


1000L Single-Use Bioreactor (SUB)

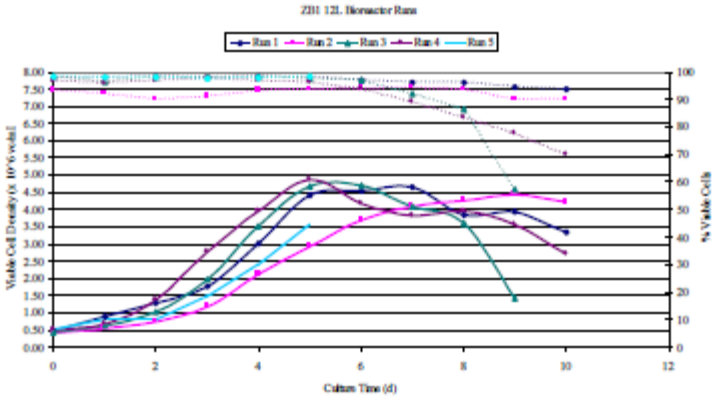
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Tech Transfer of Early Process

- 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.



Comparison of 2xShake Flask Cultures

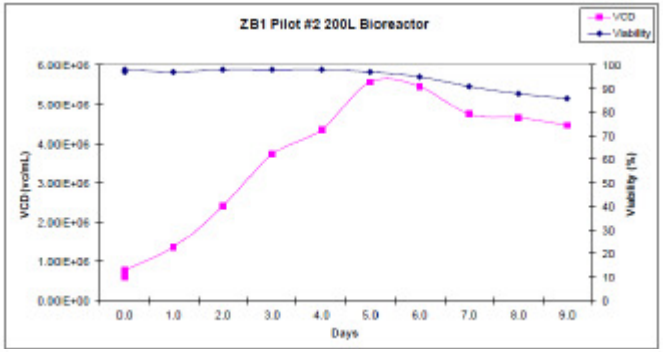


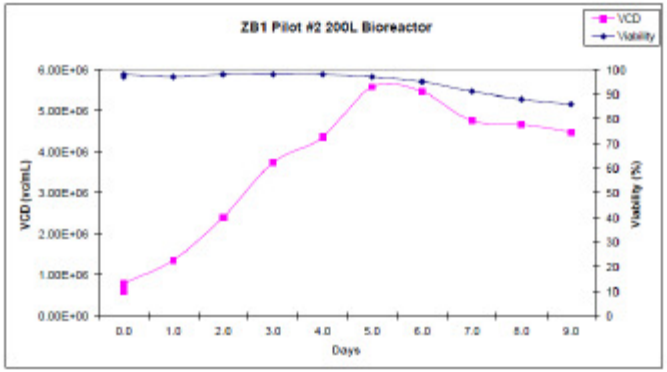
Comparison of 5x12L Bioreactor Runs

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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

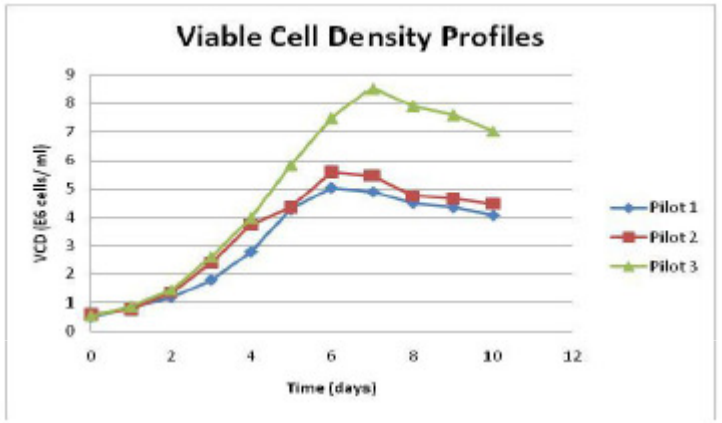


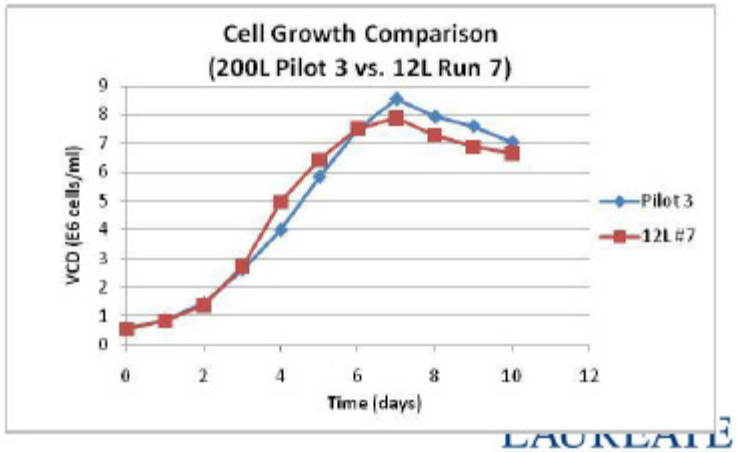


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Variation of Cell Growth Traced to Lot of Culture Media

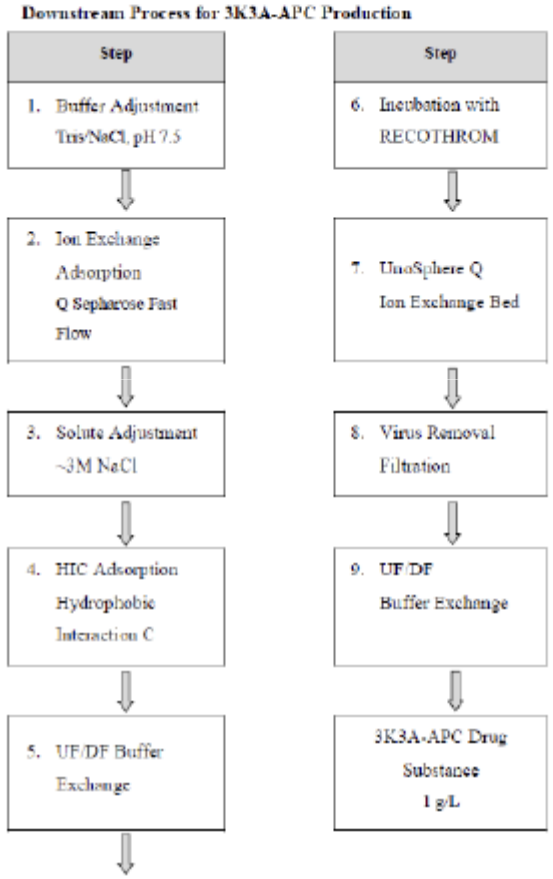
- Performed additional 12L run with media from pilot run #3 that showed similar higher growth
- Additional growth had minimal effect on titer
- Purification yields comparable to previous runs





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®)



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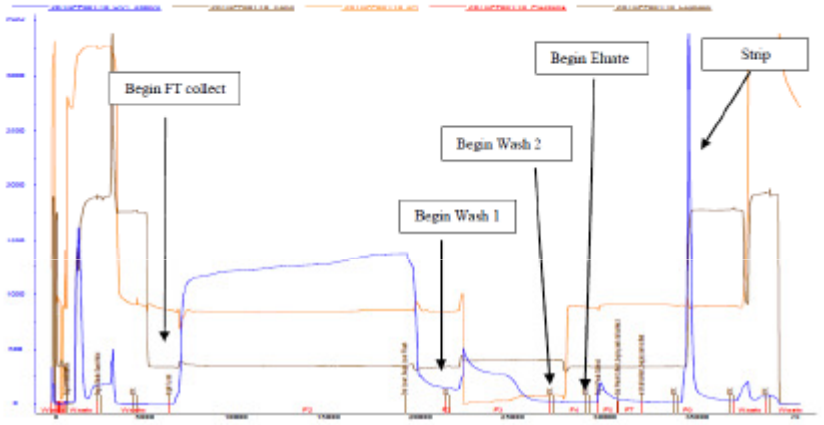
Results of Initial Runs of Transferred Process

- 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.

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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

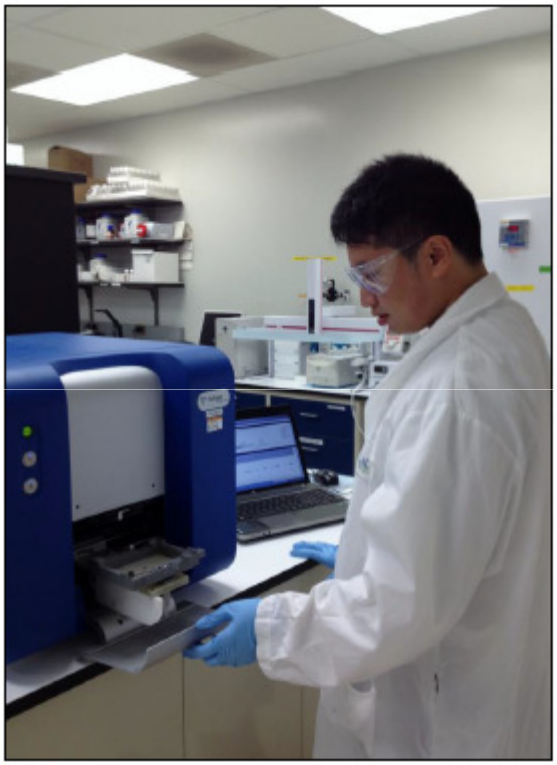


Q-Sepharose Chromatographic Profile showing additional wash step

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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



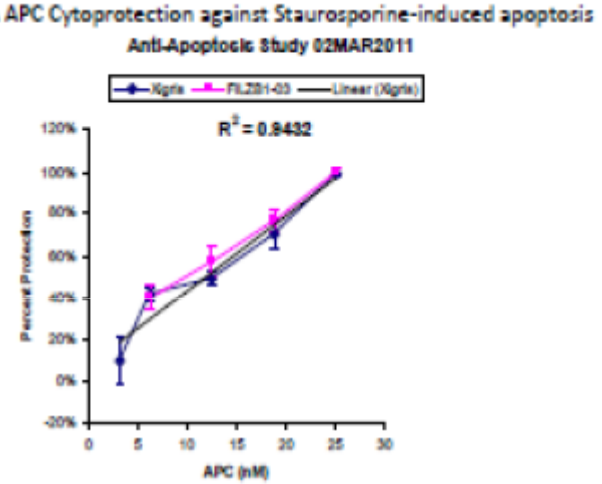
Analyst with Capillary Electrophoresis Instrument

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Cytoprotective Assay: 3K3A-APC vs. APC

- No statistical difference in anti-apoptotic activity between Xigris and 3K3A-APC

1. CYTOPROTECTION POTENCY



Nominal Conc (nM)	Cytoprotection (%)	Calculated Conc (nM)	% Activity	AVE	STD	CV
25	100.7%	29.6	118.5%	127.3%	11.8%	9.2%
18.75	76.7%	23.0	122.7%			
12.5	57.1%	17.6	140.6%			
6.25	40.2%	12.9	206.6%	Data not averaged in		

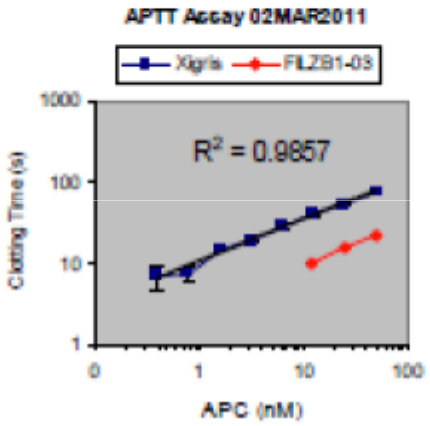
BIOPHARMA

Anticoagulation Assay: 3K3A-APC vs APC

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

ANTICOAGULANT ACTIVITY ASSAY

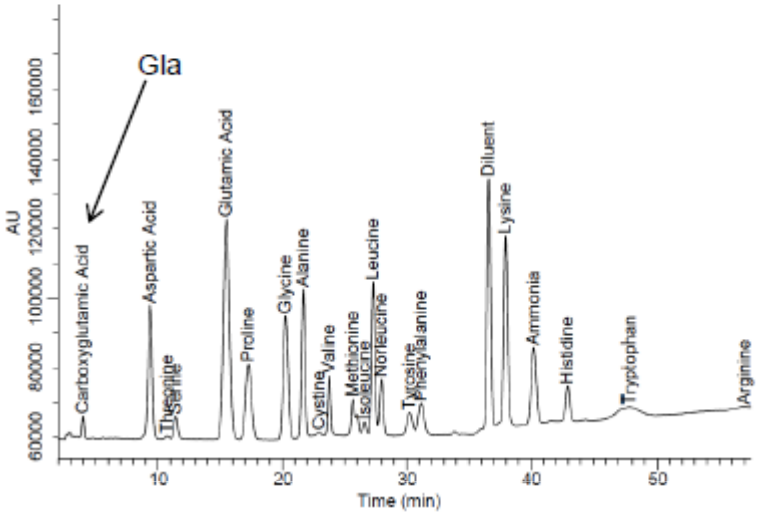
APTT Clotting assay with Laureate Pharma 200L Run #3 3K3A-APC FILZB1-03
0.39nM to 50nM Xigris Standards



Nominal Conc. (nM)	APTT Prolongation (sec)	Calculated Conc. (nM)	% Activity of Control	AVE	STD
50	22.0	4.27	8.5 %	8.1 %	0.6 %
25	15.2	2.08	8.3 %		
12.5	10.0	0.92	7.3 %		

Gla Assay

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



CEX-HPLC Profile

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

Amino Acid	Mole Ratio				
	Theoretical	Observed			
		Xigris	3K3A-APC Lot: FLIZB1-01	3K3A-APC Lot: FLIZB1-02	3K3A-APC Lot: FLIZB1-03
Gla	9	10	9	9	9

* 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



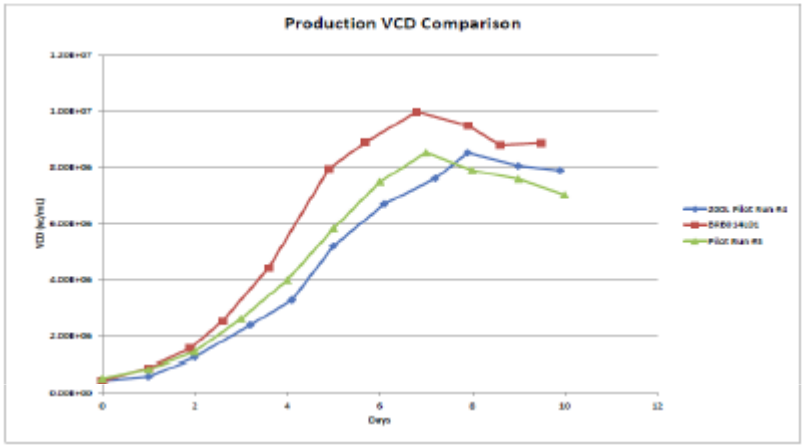
2000L ABEC Bioreactor

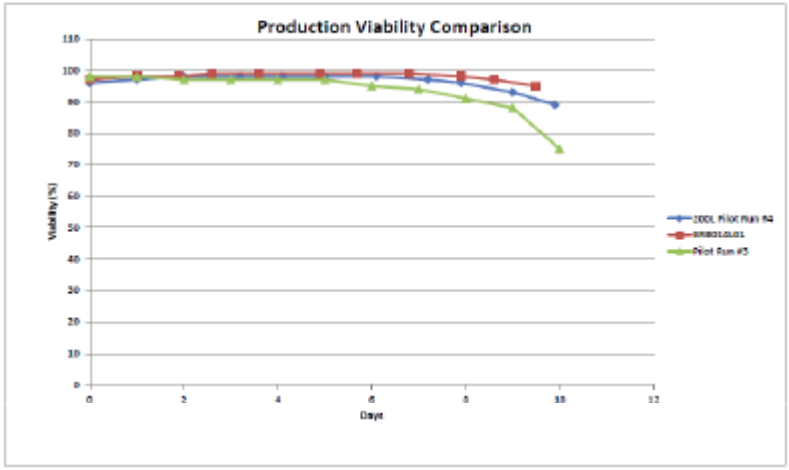


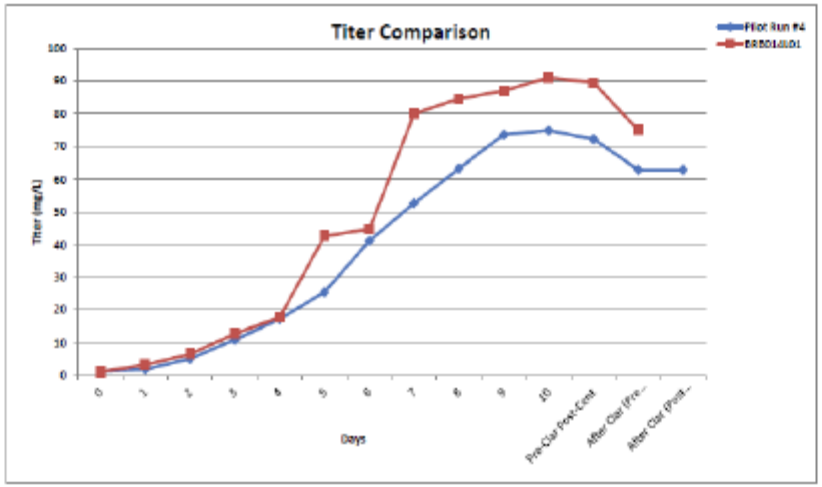
60 cm Chromoflow Column

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2000L vs 200L Bioreactor Run Profile



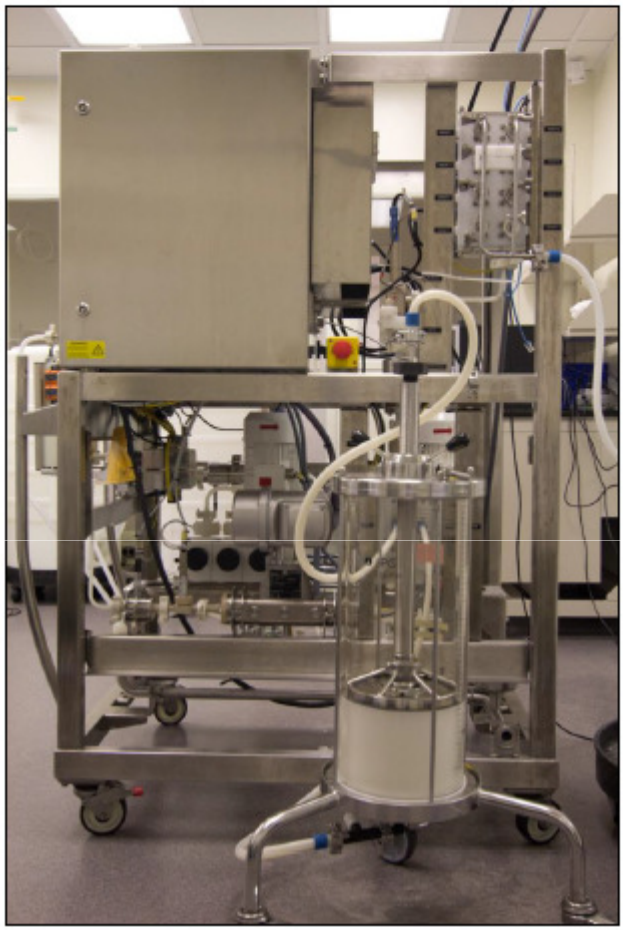




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Protein Purification

- 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

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Aseptic Filling into Vials

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

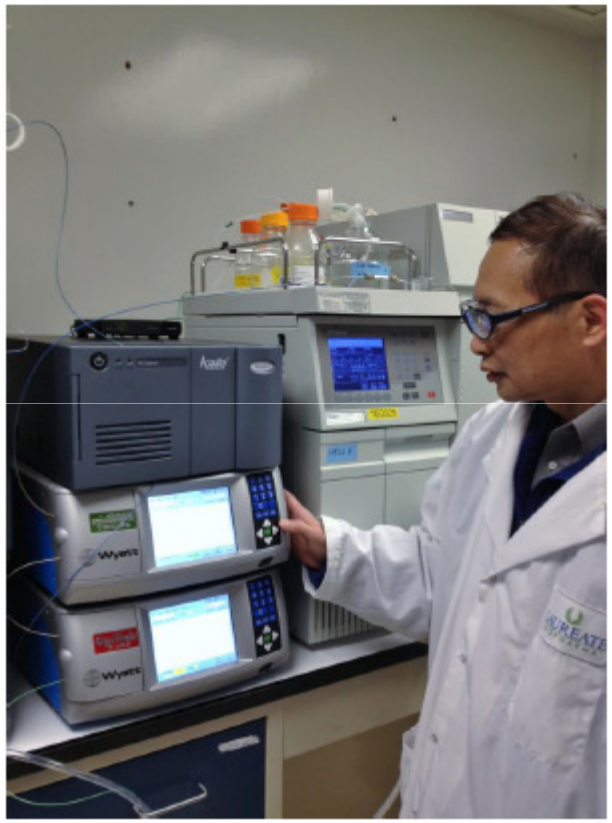


Aseptic Filled Vials of Media in Validation Run

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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

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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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Acknowledgments

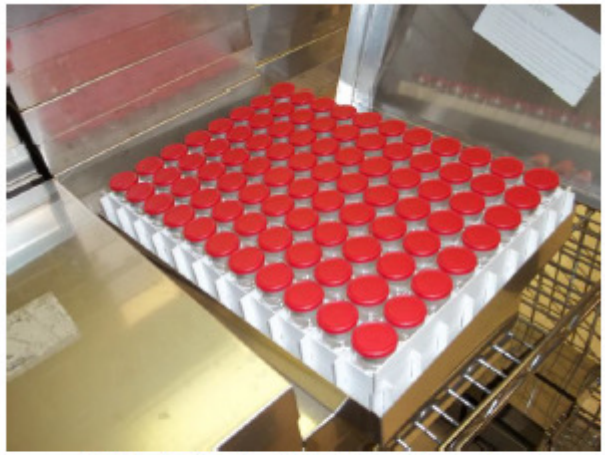
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For More Information



Filled 3K3A-APC Drug Product

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.50 in