



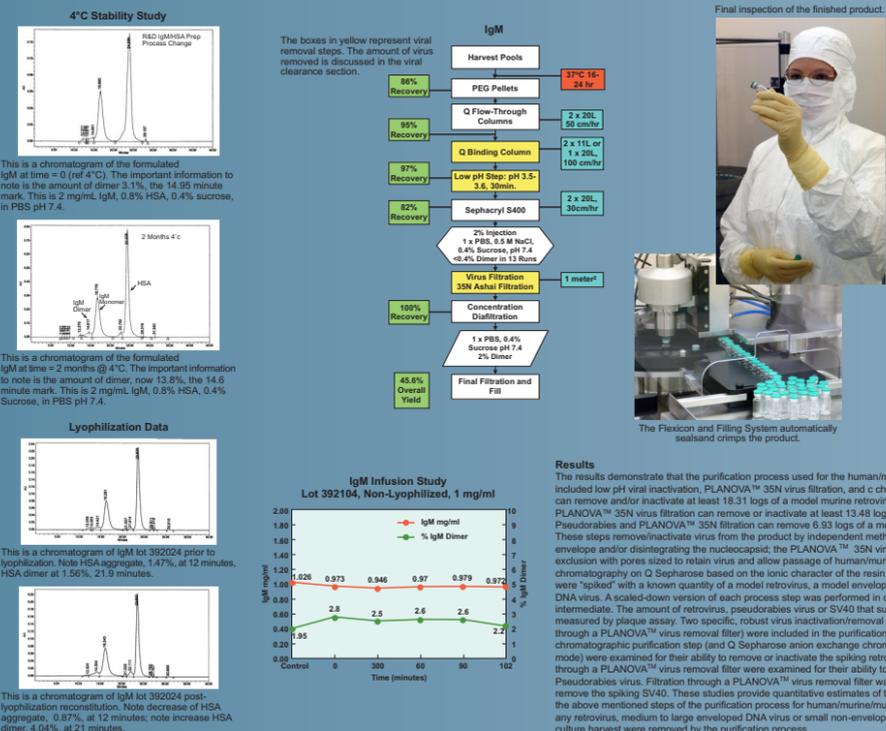
Biopharmaceutical Development Program (BDP) Projects

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Purification of IgM 216: A VH4-34-Encoded Monoclonal Antibody Directly Cytotoxic to Human B-Cell Lymphomas



Abstract
 Projects are assigned to the Biopharmaceutical Development Program (BDP), SAIC-Frederick, Inc., by the Biological Resource Branch (BRB) of the Developmental Therapeutics Program (DTP). Some projects are Intramural, Extramural, or a combination of both. These projects facilitate translation to the clinic of novel therapeutic interventions in order to evaluate clinical "proof of principle" of a new molecule or approach. There have been eleven cycles of RAID projects with more than 25 biologicals assigned to BDP through October 2002. Projects have included peptides, oligonucleotides, recombinant proteins, monoclonal antibodies and viral vectors for gene therapy. BDP capabilities include cell line development and characterization, cell culture/fermentation development, analytical development, purification and formulation development, cGMP manufacturing of clinical lots (including vialing), QC/QA release of clinical product, stability testing and preparation of regulatory documents suitable for Phase I & II human clinical trials. A selected set of projects is outsourced and managed by BDP scientific staff.

Viral Clearance Study
 Removal/Inactivation of Murine Retrovirus, Pseudorabies Virus and Simian Virus 40 During the Purification Procedure of 216 IgM Monoclonal Antibody (Project 392)

One robust virus removal step, one robust inactivation step and one chromatographic step used in the purification procedure of 216 IgM Monoclonal Antibody were evaluated for the removal/inactivation of a model medium to large enveloped RNA virus, murine xenotropic retrovirus. One robust viral removal step and one robust inactivation step were evaluated for the removal/inactivation of a model medium to large enveloped DNA virus, Pseudorabies, a herpesvirus. One robust viral removal step was evaluated for removal of a model small non-enveloped DNA virus, Simian virus 40 (SV40), a Polyomavirus.

Model Viruses/Assays
 The murine Xenotropic retrovirus (Xenotropic MuLV) used as the model in this study was quantitated by a plaque formation endpoint assay. Process buffer cytotoxicity assays were also performed using the same system. The Pseudorabies virus used as the model medium to large enveloped DNA virus in this study was quantitated by a plaque formation endpoint assay. Process buffer cytotoxicity assays were also performed using the same system. The Simian virus 40 used as the model small non-enveloped DNA virus in this study was quantitated by a plaque formation endpoint assay. Process buffer cytotoxicity assays were using the same system.

A. Compilation of log loss for Murine Xenotropic Retrovirus

Step	Log Reduction Value
Low pH Inactivation	6.75
PLANOVA™ 35N Filtration	>6.78
Q Sepharose	4.79
Total Log Reduction Value	>18.32

B. Compilation of log loss for Pseudorabies virus

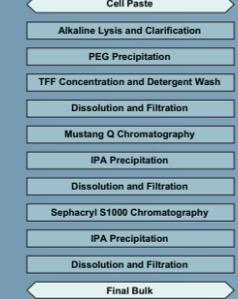
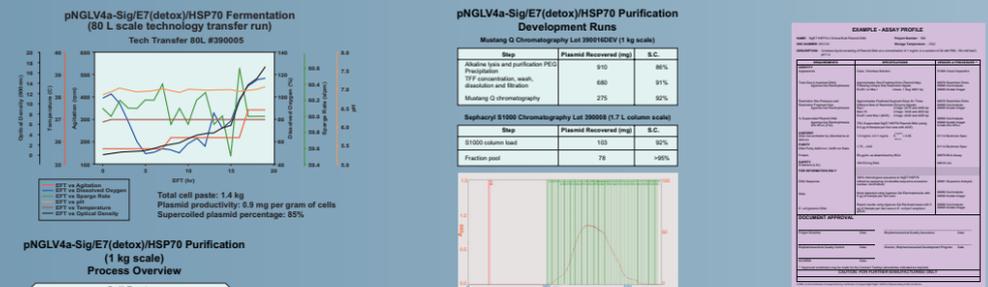
Step	Log Reduction Value
Low pH Inactivation	5.50
PLANOVA™ 35N Filtration	>7.61
Total Log Reduction Value	>13.51

C. Compilation of log loss for Simian virus 40

Step	Log Reduction Value
PLANOVA™ 35N Filtration	>5.53
Total Log Reduction Value	>6.93



Production of Plasmid DNA Expressing E7 for the Treatment of HPV-Associated Cervical Cancer and Pre-Cancer Lesions



Cell Lysis and Plasmid Extraction
 Alkaline lysis is carried out using a static mixer. The lysate is clarified with depth filtration using 10' 5 m filter unit. PEG 8000 is added to 8% to preferentially precipitate the plasmid DNA.

Tangential Flow Filtration
 TFF (0.1 μm) is used to concentrate and wash the DNA precipitate with detergent to remove endotoxin and other small molecular contaminants.

Mustang Q Chromatography
 Dissolved plasmid DNA is captured on the Mustang Q capsule (membrane with quaternary amine groups). The capsule is washed with buffer containing detergent and eluted with salt.

Sephacryl S1000 Chromatography
 S1000 column is used as a final polishing step to reduce the level of genomic DNA and non-supercoiled plasmid.

NIAD: Support to HIV Vaccine Development Program
 The plasmid DNA vaccine clinical manufacturing technology has been supporting the HIV vaccine development program via an international collaboration research and development program, Comprehensive International Program of Research on AIDS (CIPRA) at NIAD.

- BDP's project scientist was invited by the Vaccine and Prevention Research Program at NIAD to provide a presentation, entitled "Process Development For Plasmid DNA Vaccine and Gene Therapy Vectors" (February 18, 2004).
- BDP and BRB hosted a two-day visit for a Chinese delegation, as well as the Program Officer, Division of AIDS/NIAD. The discussions focused on GMP and technology training (May 3-4, 2004).
- BDP's project scientist was invited by NIAD to perform a pre-auditing assessment of two Chinese facilities (October 2004). NIAD is collaborating with the Chinese CDC for HIV vaccine clinical manufacturing.

NCI: Plasmid DNA Projects
 The BDP manages the technical operations of the NCI's Biological Resource Branch (BRB). The BDP manufactures biopharmaceuticals, including plasmid DNA vaccines and other biological products for the purpose of exploring "proof of principle" of novel therapeutic concepts in *in vitro* / *in vivo* models, as well as early clinical trials for cancer, AIDS and other diseases.

NGLV4a-Sig/E7(detox)/HSP70 DNA Vaccine for Treatment of HSV-Associated Cervical Cancer & Pre-Cancer Lesions

- Testing immunization with alpha-tetoprotein (APP) + GM-CSF Plasmid Prime and APP Adenoviral Vector Boost in Patients with Hepatocellular Carcinoma
- Vaccine encoding Calreticulin (CRT) Linked to E7 for the Treatment of Patients with Advanced Cervical Carcinoma
- DNA Vaccine encoding mouse tyrosinase related protein-2 for immunologic therapy for melanoma

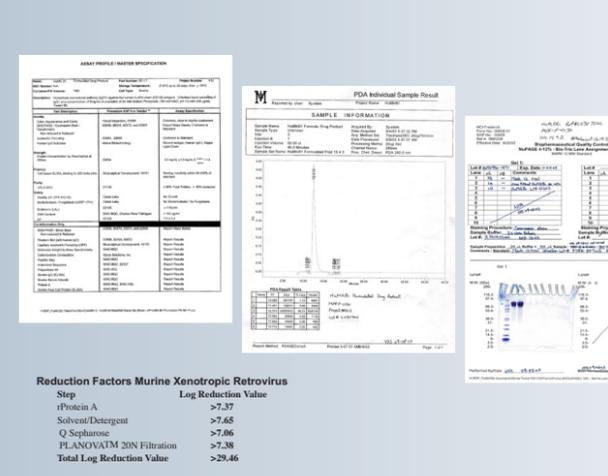
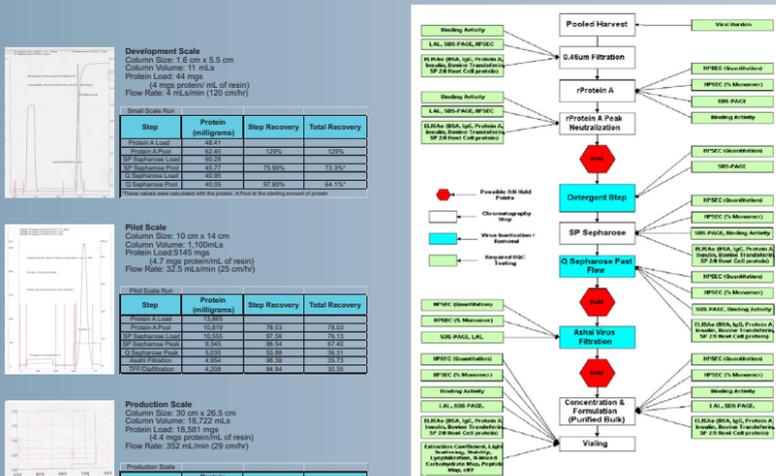
Process Improvements

Change Method	Project 8/2004	Method Described in Issue	Project 8/2004	
Process review	<ul style="list-style-type: none"> 4-18h Change column chromatography Accurate precipitation 	<ul style="list-style-type: none"> Change resin Change flow chromatography 	<ul style="list-style-type: none"> Change resin Change flow chromatography 	<ul style="list-style-type: none"> Change resin Change flow chromatography

Clinical Production of HuMik1

Abstract
 In this report we present our approach for the development of a cGMP-compliant process for the production of a monoclonal antibody destined for human clinical trials. Humanized Mik1 is a monoclonal antibody directed toward the IL-2/IL-15 receptor. It blocks all IL-15 activity, making it an attractive reagent for human clinical trials where abnormal expression of IL-15 is known to occur. The purity and quality of material produced at laboratory-scale, intermediate-scale and full manufacturing scale produced of comparable purity and potency. As required by the FDA, additional steps were included in the purification process to remove or inactivate viral contaminants. The results of a viral clearance study show that the final purification process was capable of removing more than 29 logs of a model retrovirus.

Introduction
 IL-15 appears to be involved in a number of human diseases as an inflammatory cytokine. Abnormalities in IL-15 expression have been demonstrated in multiple sclerosis and arthritis. The IL-2/IL-15 receptor represents an attractive target for pharmacologic intervention to achieve immunosuppression. Studies with murine antibodies have shown to be effective in prolonging allograft survival in monkeys and man. The use of murine antibodies in man is limited for a variety of reasons, including immunogenicity and failure of murine antibodies to recruit human effector cells. To ultimately examine the potential efficacy of blocking the IL-15 receptor, prolonged exposure to saturating amounts of antibody needs to be evaluated. HuMik1 is the only available humanized antibody directed against the IL-2/IL-15 receptor that blocks all IL-15 reactivity. In support of planned human clinical trials by the Metabolism Branch of the National Cancer Institute, we have developed a high-yield cGMP-compliant process for the production and purification of HuMik1. We demonstrate that the purification process worked out during laboratory development could be translated quickly into cGMP manufacturing and the production of more than 40 grams of clinical-grade antibody. We also show that in addition to producing a product of exceptional biochemical purity, the process was sufficiently robust to remove/inactivate more than 29 logs of a model murine retrovirus, thereby enhancing the safety of the final product.



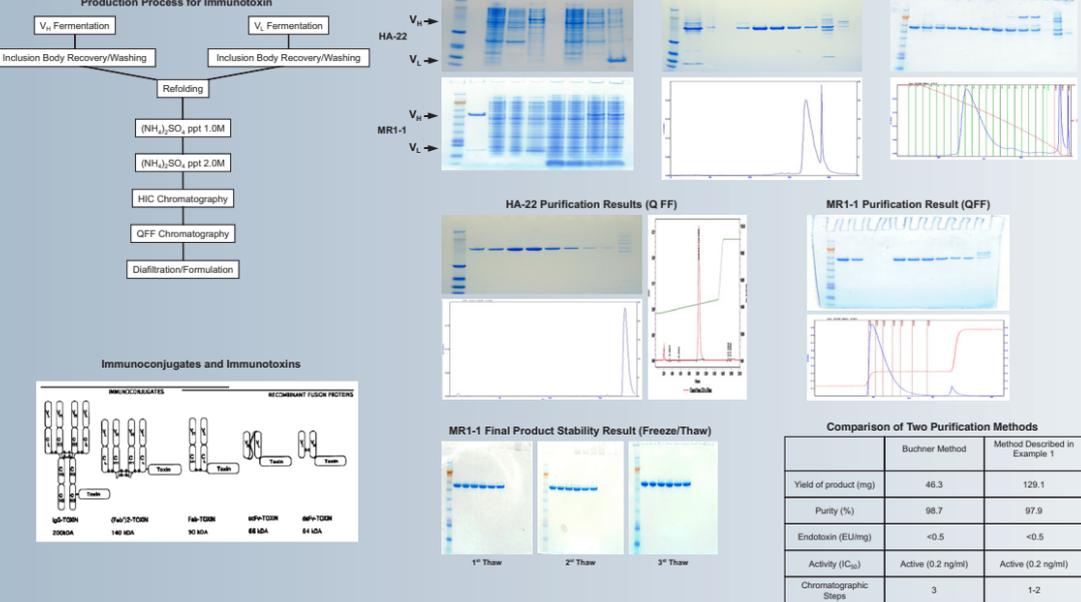
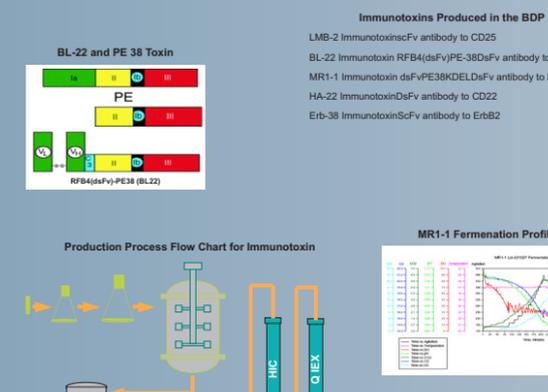
Production of an Immunotoxin

Abstract
 Antibody fragments including single chain antibody (scFv), have demonstrated greater tumor penetration, more specific tumor cell targeting, and lower immunogenicity compared with intact antibodies. Utilizing the antibody fragments conjugated with toxin (termed immunotoxin) is a new approach to attack cancer cells. Several SCFvs are currently under clinical trials. It has become crucial to provide clinical material and solve technical issues in the production yield, quality of the product, and process scale-up. Here we report a generic process of producing therapeutic immunotoxin for phase I and II clinical trials.

The antibody fragments, heavy chain fused with Pseudomonas exotoxin and light chain, were expressed in E. coli under regulation of T7 promoter. Fermentation has been performed using non-animal source nutrients and yields ~100mg/L of recombinant protein expression. Inclusion bodies were recovered and refolded to form inter-molecular disulfide bonds to link the heavy and light chains. Refolding has been scaled-up to 200L scale at 0.1mg/ml protein concentration.

The refolded material was precipitated with ammonium sulfate followed by hydrophobic interaction and ion-exchange chromatography. Purification process has shown robustness and consistent in terms of yield about 70%, as well as overall quality meets cGMP requirements including final purity over 98% and low endotoxin level.

Introduction
 Utilizing the antibody fragments conjugated with toxin (termed immunotoxin) is a new approach to attack cancer cell. Several such scFvs are currently under clinical trials. It has become crucial to provide clinical material and solve technical issues in the production yield, quality of the product, and process scale up. Here we report a generic process of producing therapeutic immunotoxin for phase I and II clinical trials, including fermentation, recovery, and purification at production scale. In addition, the purification process for the immunotoxin has been improved resulting in 3 fold increased purification yield compared with current conventional method.



Conclusion
 We have successfully developed a complete, simple and scalable clinical manufacturing process for immunotoxin production. The upstream process has eliminated animal origin raw material, and consistent process profile and productivity have been achieved. Compared with conventional purification procedures for immunotoxin, a novel hydrophobic chromatography has been incorporated in the process to replace Mono-Q or Source 150 ion exchange chromatography. The product can be clearly separated from other impurities as eluted in different peaks. By using this protocol an almost tripled yield of final product is achieved with simplified procedure and lowered cost. This novel purification method can also be applied to other similar antibody conjugated toxins as product, and hence should facilitate manufacturing immunotoxin anti-cancer drugs in large scale.

NOTE: Commercialization efforts pertaining to HA-22 and BL-22 are presently underway via a CRADA with Genencor, Inc.

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