What Is a Recombinant Immunotoxin?

- It is a protein composed of the Fv portion of an antibody that reacts with an antigen on the surface of a cell fused to a toxin.

- For the toxin, one uses a 38 kDa portion of Pseudomonas exotoxin A that is missing its cell-binding domain.

- The Fv replaces the cell-binding domain of the toxin and directs the immunotoxin to the cancer cell.

BL22 Targets CD22 on B-Cell Lymphomas and Leukemias

- CD22 is a lineage-restricted glycoprotein that is expressed on B lymphocytes.

- CD22 is expressed in at least 70% of B-cell lymphomas and leukemias.

Process Development for Therapeutic Immunotoxin Production

The antibody fragments, heavy chain fused with toxin Toxin and light chain, were expressed in E. coli under regulation of T7 promoter. Fermentation has been performed using non-animal source nutrients and yields ~100 mg/L of recombinant protein expression. Inclusion bodies were recovered and refolded to form intermolecular disulfide bonds to link the heavy and light chains. Refolding has been scaled up to 200 L scale at 0.1 mg/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 consistency in terms of yield around 70%, and overall quality meets cGMP requirements, including final purity over 98% and low endotoxin level.

We have successfully developed a complete, simple, and scalable clinical manufacturer process for immunotoxin production. The upstream process has eliminated animal original raw material, and consistent process profile and productivity have been achieved. Compared with conventional purification protocol for immunotoxins, a novel hydrophobic chromatography has been incorporated into 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 peak. 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.

Toxicology Studies for BL22

- Monkeys were administered 0.1 or 2.0 mg/kg/dose i.v. every other day for 3 days.

- On days 4-6, animals that received 2.0 mg/kg/dose DOD x 3 had an elevated heart rate (150-170 at baseline to 220) and a small increase in mean arterial pressure (90-95 at baseline to 95-110).

- Monkeys that received either dose were lethargic beginning on day 5.

- Leukocytosis was noted at both doses; animals that received 2.0 mg/kg/dose also had increased numbers of immature neutrophils.

- Serum BUN was mildly elevated (2x) in animals given 2.0 mg/kg/dose on days 2-6. There were no significant changes in the percent of the mononuclear cell populations that were positive for CD20 or CD22 antigens.

- Adequate hepatic, renal, and pulmonary function.

- Evidence of CD22 positivity on the malignant cells.

- Absence of CNS disease.

- Duration: 30 minutes infusion i.v. QOD x 3.

- Retreat: patients without neutralizing antibodies or progressive disease.

- Immunotoxin Expression in HA-22 Purification Results (QFF)

- Comparison of Two Purification Methods

- Production Process for Immunotoxin

- Production Process Flow Chart for Immunotoxin

- Immunotoxins Are of Interest to Outside Parties

Commercialization efforts pertaining to HA-22 and BL22 are presently underway via a CRADA with Genencor, Inc.