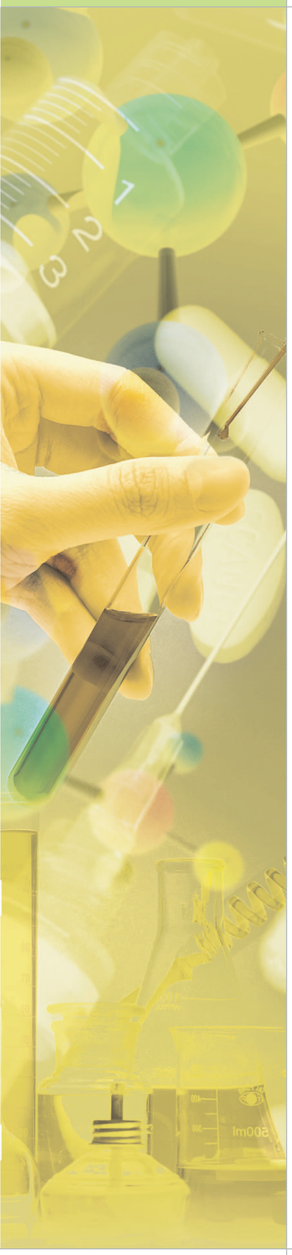
SUCCESS STORY

CD22-Directed Immunotoxins

A COLLABORATIVE EFFORT BETWEEN DTP AND DR. IRA PASTAN, CENTER FOR CANCER RESEARCH, NCI

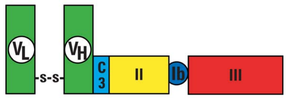


What Is a Recombinant Immunotoxin?

- 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.
- For the Fv, one uses an antibody that reacts strongly with a cancer cell but not essential normal cells (e.g., liver, kidney, nerves).
- The Fv replaces the cell-binding domain of the toxin and directs the immunotoxin to the cancer cell.

BL22

BL22 Targets CD22 on B-Cell Leukemias and Lymphomas

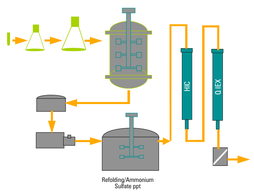


- CD22 is a lineage-restricted glycoprotein that is expressed on B lymphocytes.
- CD22 is expressed in at least 70% of B-cell lymphomas and leukemias.

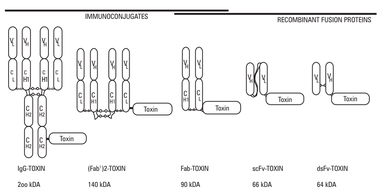
Process Development for Therapeutic Immunotoxin Production

The antibody fragments, heavy chain fused with *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 ~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.

Production Process Flow Chart for Immunotoxin



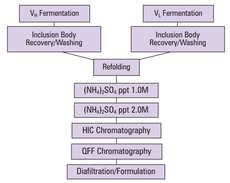
Immunoconjugates and Immunotoxins



The refolded material was precipitated with ammonia 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 manufacturing 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 procedure for immunotoxin, a novel hydrophobic chromatography has been incorporated into the process to replace Mono-Q or Source 15Q 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.

Production Process for Immunotoxin



Comparison of Two Purification Methods

	Buchner Method	New Method
Yield of product (mg)	46.3	129.1
Purity (%)	98.7	97.9
Endotoxin (EU/mg)	< 0.5	< 0.5
Activity (IC_{50})	Active (0.2 ng/ml)	Active (0.2 ng/ml)
Chromatographic steps	3	1-2
Microbial content	0	0

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 4–9, animals that received 2.0 mg/kg/dose QOD x 3 had an elevated heart rate (150–170 at baseline to 220) and a small increase in mean arterial pressure (80–90 at baseline to 90–110).
- 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.
- Reversible, moderate bone marrow hyperplasia, minimal myocardial degeneration and necrosis, and mild focal renal tubule necrosis were noted at 2.0 mg/kg/dose.
- Plasma levels averaged 2.5 and 53 μg/mL in monkeys treated with 0.1 and 2.0 mg/kg/dose, respectively.
- The maximum tolerated dose of BL22 in monkeys was > 2.0 mg/kg/dose (> 24 mg/m^2/dose) QOD x 3. This dose is approximately 30-fold higher on a mg/kg equivalent basis than the dose required for a therapeutic response in mice bearing human CD22$^+$ xenografts. However, non-life-threatening, reversible renal and cardiac lesions were noted in monkeys given 1.0 mg/kg/dose.
- The recommended starting dose for the phase I clinical trial is 2.0 μg/kg/dose QOD x 3, based on the fact that man is typically much more sensitive than cynomolgus monkeys to the effects of *Pseudomonas* immunotoxins, and the sensitivity of man in comparison to the mouse and monkey has been unpredictable.

Phase I Trial of BL22

BL22 Protocol

Eligibility

- Patients with B-cell leukemia or lymphoma.
- Evidence of CD22 positivity on the malignant cells.
- Adequate hepatic, renal, and pulmonary function.
- Absence of CNS disease.
- Dosing: 30-minute infusion i.v. QOD x 3.
- Retreat: patients without neutralizing antibodies or progressive disease.

High Complete Remission Rate to BL22 in Drug-Resistant Hairy Cell Leukemia

PREVIOUS TREATMENT	TOTAL CYCLES	DOSE LEVEL	RESP	DURATION
CdAx3	4	10–20	*CR*	20+ mo
IFN,CdAx2,FLUDARA,RITUX,DCFx3	11	30–50	*CR*	8 mo
SPLEN,CdAx1,IFN	2	30	*MR**	4 mo
CdAx2,IFN	3	30	*CR*	18+ mo
CdAx3,IFN	5	30	*CR*	12 mo
CdA	2	30	*CR*	14+ mo
CdAx2,IFN,DCFx1	2	40	*CR*	12+ mo
CHL,PDN,SPLEN,IFN,CdAx2,DCFx1	5	40–50	*CR*	10+ mo
CdA,SPLEN	3	40–50	*CR*	11+ mo
CdAx2,PDN,IFN	2	50	*CR*	7 mo
CdA,IFN,SPLEN	2	50	*CR*	9+ mo
SPLEN,CdAx3	2	40	*CR*	9+ mo

*PRE-EXISTING ANTIBODIES

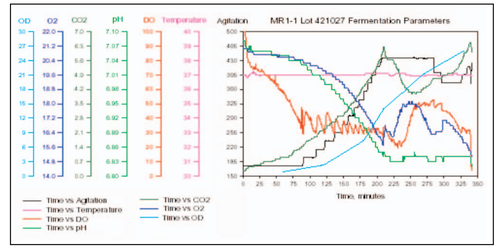
In Patients Completing 10–50 μg/Kg x3: *CR 11/12 (92%)*

Production Methods Used for BL22 Extended to Other Immunotoxins

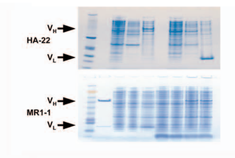
Immunotoxins Produced in the BDP

LMB-2	Immunotoxin	ScFv antibody to CD25
BL-22	Immunotoxin RFB4(dsFv)PE-38	DsFv antibody to CD22
MR1-1	Immunotoxin dsFvPE38KDEL	DsFv antibody to EGFR VIII
HA-22	Immunotoxin	DsFv antibody to CD22
Erb-38	Immunotoxin	ScFv antibody to ErbB2

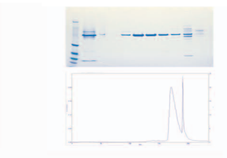
MR1-1 Fermentation Profile



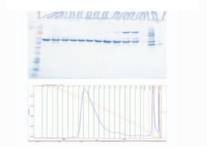
Immunotoxin Expression in *E. coli*



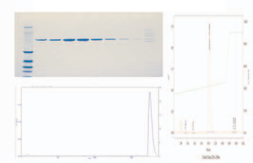
HA-22 Purification Results (HIC)



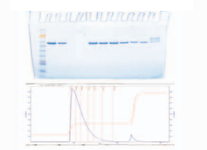
MR1-1 Purification Results (HIC)



HA-22 Purification Results (QFF)



MR1-1 Purification Result (QFF)



MR1-1 Final Product Stability Result (Freeze/Thaw)

Immunotoxins Are of Interest to Outside Parties

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