Immunotoxin Therapy of Cancer
Successes and Challenges

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When naked antibodies fail, you can use them to target cytotoxic compounds to cancer cells.

Protein toxins are among the most active cytotoxic agents we know.

When protein toxins are attached to antibodies, they are called immunotoxins.

Early efforts in the immunotoxin field used whole antibodies conjugated to whole toxins.

Now we use antibody engineering and toxin engineering to produce recombinant immunotoxins in which the Fv portion of a mab is fused to a portion of the toxin.
WHAT IS A RECOMBINANT IMMUNOTOXIN?

It is a protein composed of the Fv portion of an antibody, chosen to react with a specific antigen on the surface of a cancer cell, fused to a toxin.

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

For the Fv, we use an antibody that reacts strongly with a cancer cell, but not essential normal cells (liver, kidney, nerves etc.)

To prevent unacceptable toxicities due to killing essential normal cells, we find it is best to use lineage restricted differentiation antigens that are expressed on cancers and the cells from which the cancer is derived.

CD22 is an excellent example.
PROPERTIES OF TOXINS

- High Potency (Long Evolution)
- Infrequent Resistance
- Not Mutagenic
- Not Toxic to Bone Marrow
- Disadvantage- Immunogenic
Pseudomonas Exotoxin A

- 66 kDa protein
- ADP ribosylates elongation factor 2
- Arrests protein synthesis
- Induces programmed cell death
- Very potent.
Mechanism of Immunotoxin Killing

Cell membrane

Clathrin Coated Pit

Endocytic Compartment

Endoplasmic Reticulum

Cell Death

Nucleus

ADP-r-EF2
CD22

- Differentiation Antigen
- 135 kDa B-cell restricted sialoglycoprotein
- Present on mature B-cells but not stem cells
Presence of CD22 on Tumors

- 100% of HCL - $4 \times 10^4$ sites/cell
- 99% of B-CLL - $1 \times 10^3$ sites/cell
- 70-85% of NHL - ?
- >90% of B-ALL - $5 \times 10^3$ per cell
HAIRY CELL LEUKEMIA

B-cell leukemia

2% of all Leukemias

Pancytopenia, Splenomegaly

Purine analogs (CdA, DCF)

High response rates
Not curative
Patients become resistant

HCLv (20%) primarily resistant

Very high CD22 expression
BL22 (CAT 3888) Structure

Anti-CD22

PE38
BL22 (CAT-3888) Phase I Protocol

Patients: Failed Standard Chemotherapies: CDA, Pentostatin, Interferon, Rituxan

Dosing: 30 min. infusion i.v. QOD x 3.

Start at 3 micrg/kg

MTD 40 micrg/kg,

Retreat: Every 21 days.
No progressive disease
No antibodies to BL22
Patients Treated in Phase 1 Trial

Non-Hodgkin’s Lymphoma 4
Chronic Lymphocytic Leukemia 11
Hairy Cell Leukemia 31

Total Phase I Patients Treated 46
Total Cycles = 249
CR OF HCL OR HCL-v TO BL22

HCL PATIENT #14
30 (C1-9)
40 (C10)
50 (C11)

HCL PATIENT #14

HCL PATIENT #23
40 (C1-2)

HCL PATIENT #25
40 (C1,2,4)
50 (C3)

HCL PATIENT #25

HCL PATIENT #26
50 (C1-2)
40 (C3)

HCLv PATIENT #14
30 (C1-9)
40 (C10)
50 (C11)

HCLv PATIENT #26

HCLv PATIENT #26
Blood Counts Before and After CAT-3888 Treatment
BL22 (CAT-3888) Phase I Study
Summary

Tumor responses in HCL (n= 31 patients)

1. Complete Response = 19
2. Partial response = 6
3. Objective responses 25/31 (81%)
4. Median Time to Progression of CR 36 months
Phase 2 Trial

- Confirmed High Response Rate
- Proof of Principle that Immunotoxins provide benefit in humans
- Life Saving and Durable Responses in patients with Advanced Drug Resistant Leukemia
Phase II CR Durability

Median CR duration = 18+ (5-41+) months
13/17 (76%) still in CR, median 20 (2-41+) months

Conclusion: Phase II CRs are at least as durable
Reality Check

- HCL is a rare disease, 1000 new cases yearly.
- Market is small.
- CD22 also expressed on CLL, NHL, ALL.

- But these malignancies have lower CD22: HCL $4 \times 10^4$, CLL 1-2$\times 10^3$, ALL 5$\times 10^3$, NHL? Need to increase activity.
- This can be done by increasing affinity.
Fv of BL22 showing CDRs in yellow and a hot spot in blue.

The SSY was mutated to THW and an immunotoxin made.
Increased Binding of Mutant immunotoxin HA22 (CAT-8015) to CD22-Ig Coated BIAcore Chips

HA22 ($K_D = 4.5 \times 10^{-10} \text{ M}$)

BL22 ($K_D = 1.2 \times 10^{-8} \text{ M}$)
HA22 (CAT-8015): High affinity mutant of BL22

- Mutation: SSY → THW at 100, 100a, 100b
- Median cytotoxicity in CLL ↑ 5-fold
- Phase I Studies: CLL; HCL; NHL
RESPONSE TO LOW-DOSE HA22 IN HCL

- **HCL**
- **ANC**
- **PLT**

**CELLS / mm³ x 10⁻³**

- Pre   Post
- HH01   5 ug/Kg x3   PR
- HH02   5 ug/Kg x3   SD
- HH03   5 ug/Kg x3   PR
- HH04   10 ug/Kg x3   CR

**Hgb (g/dL)**

- Pre   Post
- CR 25%,  PR 50%

Graph legend:
- HH01: Red, 5 ug/Kg x3, PR
- HH02: Green, 5 ug/Kg x3, SD
- HH03: Yellow, 5 ug/Kg x3, PR
- HH04: Cyan, 10 ug/Kg x3, CR
CLL patient HL05 treated with HA22 10 ug/Kg x3
Summary

- Antibody and protein engineering were used to make and improve immunotoxins.

- BL22 (CAT 3888) produced a high rate of durable remissions in HCL.

- Phage display and Hot Spot mutagenesis were used to improve affinity and activity resulting in HA22 (CAT 8015), now in phase 1 trials and showing activity at low dose levels.
Solid Tumor Studies

Targeted therapy of mesothelin expressing tumors using the anti-mesothelin immunotoxin

SS1P
Mesothelin

- Is a 40 kDa PI linked cell surface glycoprotein.
- It is a differentiation antigen only present on normal mesothelial cells.
Mesothelin Expression

Normal tissues

• Mesothelial cells of pleura, pericardium and peritoneum
• Absent in important organs: heart, lungs, liver, kidneys and nervous tissue

Human tumors

• Non-mucinous Ovarian Cancer 66 – 74%
• Epithelial Mesotheliomas > 90%
• Pancreatic Adenocarcinoma > 90%
• Adenocarcinoma of lung 60-90%
• Other cancers stomach, cervical
Mesothelin immunostaining in mesothelioma
SS1P (CAT-8015) Structure

Anti-Mesothelin

PE38
Preclinical Studies

Pleuritis was DLT and was found at autopsy of monkeys
Phase I Studies of SS1P
Anti-Mesothelin Immunotoxin in Advanced Malignancies

Trial 1- Hassan I.V. Infusion QOD Dosing

Trial 2- Kreitman Continuous 10 day infusion
Toxicities in SS1P Trial

1. Dose Limiting pleuritis with chest pain and shallow breathing resulting in hypoxia due to targeting of mesothelial cells in the pleura as predicted by our monkey model.

2. No pericarditis
SS1P : Tumor Response

<table>
<thead>
<tr>
<th>Tumor response</th>
<th>Patients (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor response</td>
<td>4</td>
</tr>
<tr>
<td>Resolution of ascites</td>
<td>2</td>
</tr>
<tr>
<td>Stable disease</td>
<td>16 (in many patients lasting several months)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>11</td>
</tr>
</tbody>
</table>
Patient 432

Objective tumor response in a patient with peritoneal mesothelioma

Baseline  Post Cycle 1  Post Cycle 2
SS1P Phase I Study: Conclusions

• SS1P is well tolerated with pleuritis as the DLT at high doses (60 µg/kg/dose)
• The MTD of SS1P QOD x 3 schedule is 45 µg/kg/dose
• No pericardial toxicity
• Good SS1P blood levels (>500 ng/ml) and prolonged half-life (10 hours). Half life of BL22 is 2 -3 hours
• Anti-tumor activity noted in several heavily pretreated patients
Remarkable Synergy Observed when Chemotherapy and Immunotoxins combined
Anti-tumor Synergy when Taxol and Immunotoxin SS1P are Combined
## Summary of Synergy Results

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Immunotoxin</th>
<th>Target</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431/K5</td>
<td>SS1P</td>
<td>Mesothelin</td>
<td>Taxol</td>
</tr>
<tr>
<td>A431/K5</td>
<td>SS1P</td>
<td>Mesothelin</td>
<td>CDDP</td>
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<tr>
<td>A431/K5</td>
<td>SS1P</td>
<td>Mesothelin</td>
<td>Cytoxan</td>
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<tr>
<td>A431/K5</td>
<td>SS1P</td>
<td>Mesothelin</td>
<td>Gemcitabine</td>
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<tr>
<td>CA46</td>
<td>HA22</td>
<td>CD22</td>
<td>Taxol</td>
</tr>
<tr>
<td>CA46</td>
<td>HA22</td>
<td>CD22</td>
<td>Adriamycin</td>
</tr>
<tr>
<td>KB (Hela)</td>
<td>HB21(Fv)PE40</td>
<td>TFR</td>
<td>Taxol</td>
</tr>
<tr>
<td>KB (Hela)</td>
<td>SS1P</td>
<td>Mesothelin</td>
<td>Taxol</td>
</tr>
</tbody>
</table>
No Synergy with Taxol Resistant Tumor

Taxol sensitive

Taxol resistant

Zhang
Conclusions

• Tumor must be drug sensitive to observe synergy.

• Type of chemotherapy does not matter.

• Chemotherapy is working on tumor cells and not on blood vessels or other cells in the tumor matrix.
2003 model of solid tumor expressing mesothelin
Model of Solid Tumor Expressing Mesothelin after SS1P Treatment – Weinstein Model

- Afferent capillary
- Efferent capillary
- SS1P
- membrane-bound mesothelin
- tumor cell

The diagram illustrates the distribution of SS1P and mesothelin within the tumor cells and their interaction with the capillary network.
Levels of immunotoxin SS1P in Blood and Tumor

SS1P

membrane-bound mesothelin

tumor cell

100nM

17nM

Afferent capillary

Efferent capillary
2007 Model of Solid Tumor in Which Mesothelin is Shed into the Blood

Diagram showing the movement of shed mesothelin from tumor cells through the blood vessels. The diagram includes labels for afferent capillary, efferent capillary, shed mesothelin, membrane-bound mesothelin, and tumor cell.
Concentrations of Shed Mesothelin in Blood and Tumor

- Afferent capillary
- Efferent capillary
- Shed mesothelin
- Membrane-bound mesothelin
- Tumor cell

Concentrations:
- 4nM
- 60nM
Levels of Mesothelin and SS1P in Tumor Extracellular Fluid (ECF) and in Serum

![Bar chart showing concentrations of mesothelin and SS1P in Tumor ECF and Serum.](chart.png)
Immunotoxin molecules bind to outer cells of the tumor and also to shed mesothelin.

- SS1P
- shed mesothelin
- membrane-bound mesothelin
- tumor cell
Effect of Taxol on KB Tumor Morphology

Untreated  Taxol Day 2  Taxol Day 3
Taxol Arrests Growth and Lowers Mesothelin Levels in KB Tumors and in Blood

tumor size

mesothelin in ECF

mesothelin in serum
Taxol fails to arrest growth or decrease mesothelin levels in Taxol resistant KB-8-5 tumors

Yujian Zhang
After Taxol there are fewer tumor cells and much less shed mesothelin in the tumor and in the blood.
As a consequence the immunotoxin can now reach all the tumor cells.
Conclusion 1

- Effective chemotherapy kills tumor cells, disrupts their organization within the tumor mass and lowers mesothelin levels, probably by slowing synthesis and allowing shed mesothelin to escape more efficiently.

- This allows immunotoxins (and probably other immunoconjugates) to bind to and kill more tumor cells.
Conclusion 2

• Synergy only occurs if tumor cells are sensitive to chemotherapy and to the immunotoxin.

• Important implications for clinical trials

• Should combine immunotoxin and chemotherapy before tumor becomes drug resistant
Future

• A phase 2 trials in which SS1P will be combined with Alimta (pemetrexed) and cisplatin will open soon.
Support

NCI Center for Cancer Research
Cambridge Antibody Technology
Enzon