Strategies to Reduce ADA Response to Immunotoxin Therapy of Cancer

Ira Pastan
Recombinant Immunotoxin (RIT)

Pseudomonas Exotoxin A

Recombinant Immunotoxin (RIT)

Moxe

Targeting Fv

PE payload

Anti-CD22 Fv
Pseudomonas Exotoxin A

• Kills by a unique mechanism of action: inhibits protein synthesis
• Synergy with cytotoxic agents e.g. taxanes
• Kills drug resistant cells
• Kills resting or slow growing cells
• Very potent

• **Drawback** is it can be immunogenic.
  But was assured the **tolerance** problem would soon be solved and not to worry about immunogenicity.
How recombinant immunotoxins kill cells

Moxetumomab pasudotox: Anti-CD22(Fv)-PE38

Cell membrane

Clathrin-coated pit

Lysosome

Endoplasmic reticulum

Endosome

BCL-xL

Bak

Mcl-1

Inhibition of protein synthesis

NAD + EF\textsubscript{2} toxin

ADPr-EF\textsubscript{2}
Clinical Trials

Hairy Cell Leukemia targeting CD22
Moxetumomab pasudotox

Mesothelioma and Pancreatic Cancer targeting Mesothelin
SS1P
LMB-100
Goal

• Review clinical data
• Review strategies we have taken to reduce immunogenicity.
• B Cell epitopes: Masanori Onda, Satoshi Nagata
• T Cell Epitopes: Ronit Mazor
Moxetumomab pasudotox
Lumoxiti  Medimmune/AZ

Anti-CD22  PE38

Salvatore et al 2002 CCR
CD22

- Cell surface lineage-restricted differentiation antigen
- Only present on mature B-cells and B-cell malignancies
- Highly Expressed on **Hairy Cell Leukemia**
Example of a complete Response

HCL PATIENT, 50 µg/Kg QOD x 3

Days on Moxetumomab pasudotox protocol
Responses in Drug Resistant Hairy Cell Leukemia

No dose limiting toxicities at 50 ug/kg

<p>| | |</p>
<table>
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<tr>
<td>Number of Patients</td>
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<td>Complete Response</td>
<td>26 (54%)</td>
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<tr>
<td>Partial Response</td>
<td>16 (33%)</td>
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88% overall response rate

Phase 3 Trial conducted be Medimmune met endpoint. It is now approved by FDA as Lumoxiti
The Neutralizing Antibody Problem

% of Patients with Neutralizing Antibodies

Cycle Number

HCL (n=146)

Hassan et al
Kreitman et al
Wayne et al
Summary

• RITs can cause meaningful responses in Hairy Cell Leukemia where significant ADA formation is absent or low or delayed.

• What about solid tumors?
Mesothelin

• Cell surface glycoprotein

• Differentiation antigen expressed only on mesothelial cells of pleura, peritoneum & pericardium

• Mesothelin is highly expressed in many cancers

SS1P Phase I Study

- Patients with advanced mesothelin positive cancers
- SS1P given as IV infusion every other day x 3 doses
- Maximum tolerated dose - 45 µg/kg x 3
- Dose-limiting toxicity was pleuritis and CLS

Tumor responses in 33 patients

- Minor response 4
- Stable disease 18
- Progressive disease 11

The Neutralizing Antibody Problem

Mesothelioma, SS1P (n=22)

Hem. Malignancies

HCL (n=146)

Pediatric ALL (n=23)

Hassan et al
Kreitman et al
Wayne et al
Conclusion

• SS1P by itself has very little clinical activity.
• Formation of High Titer ADAs prevented retreatment
Solutions to ADA problem

• Treat patient with immune suppressive drugs
• Identify and remove B cell epitopes
• Identify and remove T cell epitopes
• Collaborate with Selecta to induce specific immunosuppression and induce tolerance
The Neutralizing Antibody problem

• Previous efforts to prevent formation of neutralizing antibodies and allow more doses to be given by treating patients with **high dose steroids, cyclosporine**, single agent **cytoxan** or **rituximab** have not worked.

• Fowler et al found that a combination of **pentostatin and cytoxan** allowed bone marrow engraftment in the transplantation setting without myelosuppression.
Pilot study of SS1P with pentostatin and cytoxan in advanced chemo-refractory mesothelioma

- Previously treated patients with treatment refractory and progressive disease

<table>
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<th>Day</th>
<th>Cytoxan (200 mg/day)</th>
<th>Pentostatin (4 mg/m²)</th>
<th>SS1P (35 or 45 µg/kg)</th>
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<td>(days 1-12)</td>
<td>1 5 9</td>
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Cycle 1 (day 1 to 30) | Cycle 2 & 3 (day 1 to 21)
Pentostatin/Cytoxan delays antibody response

One patient never developed antibodies and is still alive after 4 years.

Delaying antibody responses is possible but it is complicated, inefficient and associated with toxicity.

Hassan, Pastan et al 2013
Serendipity
Patient 3 with extensive pleural mesothelioma:

Day 12  
3 months  
15 months
Patient 5 with widely metastatic peritoneal mesothelioma

Before treatment 1.6 months 8 months
Conclusions

• Cytoxan and pentostatin reduced ADA to SS1P allowing patients to receive more doses of SS1P

• Dramatic long lasting tumor responses in patients with extensive treatment refractory mesothelioma

• Immune modulation by pentostatin and cytoxan may play a role in anti-tumor responses
Approaches to reduce immunogenicity

1. PE38 binds to B cell
   - B cell epitope

2. PE38 is internalized and degraded
   - Decrease processing

3. PE38 peptides are presented to the T cell, which activates the B cell
   - T cell epitope

4. Activated B cell produces antibody against PE38
Strategy to decrease binding of toxin to receptor on B cells (Remove B cell Epitopes)

- Made large number of mabs in mice to PE38
- Mapped where they bound to the toxin
- Bulky amino acids like arg, gln glu
- Mutated to ala
- Showed mab binding was greatly decreased

Onda, Nagata et al
Identification of 7 major epitopes in PE38 by competition assay of 60 MAbs

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Each MAb = ⭐

60 MAbs x 60 MAbs = 3600 combinations

Competition %

![Heatmap representing competition assay results for 7 major epitopes in PE38 involving 60 MAbs.](image-url)
Location of 7 major B cell epitopes in PE38
Domain II is very protease sensitive and can be deleted.
First tried to de-immunize for Mice by identifying and removing mouse B Cell Epitopes
Decreased Immunogenicity of HA22-LR-8M in Mice
What about human B cell Epitopes?
Isolation of human antibodies to map the epitopes in RITs by Phage Display

*Patients*

Isolated B cells → PCR $V_H$ and $V_L$ → Display Fvs on phage → Display Fvs on phage → Panning → Immobilized RIT → Human Fvs to RIT
Location of Epitopes in Domain III

Front view

Green mouse

Red Human

Back View

Yellow both mouse and human
Collaboration with Roche to make a New RIT
Targeting Mesothelin: RG7787/LMB-100

LMB-100

• Humanized Fab
• Deletion of domain II which decrease CLS
• Point mutations in domain III to remove B and some T cell epitopes
LMB-100 in Lung Xenograft

Prolonged Treatment of Large Tumors

3 therapy cycles at 2mg/kg over a period of 5 weeks shrink 600 mm³ tumors.
Conclusion

• LMB-100 is somewhat less immunogenic than SS1P
• But all but 1 patient made antibodies after 2 cycles (6 doses)
• Need to do better
Where are the T cell epitopes in PE38?

Approach described by Sette and colleagues
Project led by Ronit Mazor
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Identification and elimination of T cell epitopes

- Improved sensitivity for low abundant T cells in naïve donors
- Eliminate irrelevant epitopes

Representative response

Strategy adopted from Osseroff et al. 2010

Mazor et al. PNAS 2012
Summary of ELISpot responses after *In Vitro* Expansion (n=50)

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<th>Peptide number</th>
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<th>Domain III</th>
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Donors

- Mesothelioma patients
  - 1
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35
  - 40
  - 45
  - 50
  - 55
  - 60
  - 65
  - 70
  - 75
  - 80
  - 85
  - 90
  - 95
  - 100
  - 105
  - 110

- HCL patients
  - 1
  - 5
  - 7

Peptide number

- 2a, 2b
- 5
- 6a, 6b
- 3 and 4

Responses:

- 0-3%
- 3-10%
- 10-20%
- >20%
## Epitopes summary

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* Activity for a single point mutation in HA22 RIT was evaluated in CA46 cell line

<sup>a</sup> donors and patients that responded to epitope 2A overlap with the patients and donors that responded to 2B.
4/8 epitopes were **successfully** predicted by the algorithm

4/8 False positive in this threshold

Mazor and Tai et al.
JIM 2015
4/8 epitopes were missed by the algorithm
T-cell epitopes in PE38 have variable presentation by both DR and DP

Mazor et al. AAPS 2017
## Alanine mutagenesis for core region (epitope 1)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
<th>Donor 5</th>
<th>Donor 6</th>
<th>Donor 7</th>
<th>Donor 8</th>
<th>Donor 9</th>
<th>Donor 10</th>
<th>Averages (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no peptide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6%</td>
</tr>
<tr>
<td>wt 15</td>
<td>LVALYLAARLSWNQV</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>L294A</td>
<td>AVALYLAARLSWNQV</td>
<td>6%</td>
<td>4%</td>
<td>8%</td>
<td>4%</td>
<td>25%</td>
<td>3%</td>
<td>7%</td>
<td>19%</td>
<td>27%</td>
</tr>
<tr>
<td>V295A</td>
<td>AVALYLAARLSWNQV</td>
<td>5%</td>
<td>103%</td>
<td>2%</td>
<td>27%</td>
<td>19%</td>
<td>13%</td>
<td>63%</td>
<td>14%</td>
<td>1%</td>
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<tr>
<td>A496G</td>
<td>LGYLAARLSWNQV</td>
<td>11%</td>
<td>118%</td>
<td>10%</td>
<td>24%</td>
<td>16%</td>
<td>19%</td>
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<td>31%</td>
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<tr>
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<td>LVAAYLAARLSWNQV</td>
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<td>2%</td>
<td>8%</td>
<td>8%</td>
<td>4%</td>
<td>10%</td>
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<td>2%</td>
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<td>6%</td>
<td>16%</td>
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<tr>
<td>L299A</td>
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<td>11%</td>
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<tr>
<td>A300G</td>
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<td>82%</td>
<td>91%</td>
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<tr>
<td>A301G</td>
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<td>R302A</td>
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<td>0%</td>
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<td>22%</td>
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<td>31%</td>
<td>86%</td>
<td>5%</td>
</tr>
<tr>
<td>L303A</td>
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<td>96%</td>
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<td>49%</td>
<td>108%</td>
<td>108%</td>
<td>79%</td>
<td>66%</td>
<td>78%</td>
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<tr>
<td>S304A</td>
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<td>109%</td>
<td>83%</td>
<td>106%</td>
<td>92%</td>
<td>121%</td>
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<tr>
<td>W305A</td>
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<td>70%</td>
<td>114%</td>
<td>72%</td>
<td>102%</td>
<td>124%</td>
<td>54%</td>
<td>116%</td>
<td>59%</td>
<td>305%</td>
</tr>
</tbody>
</table>

Gray represents <10%

A few different single mutations diminish the response to peptide15

Mazor et al. 2012
Structures of SS1P and LMB-T20 targeting Mesothelin
T20 has greatly diminished T-cell activation and no new T-cell epitopes.

T20 has a decrease in T cell resp. of 90% in normal donors and of 83% in patients.
Deimmunized immunotoxin targeting Mesothelin more active than SS1P

<table>
<thead>
<tr>
<th>Immunotoxin</th>
<th>Cancer type</th>
<th>IC$_{50}$ (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMB-T20 (anti- Mesothelin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell line</td>
<td></td>
<td>SS1P</td>
</tr>
<tr>
<td>HAY</td>
<td>Mesothelioma</td>
<td>13</td>
</tr>
<tr>
<td>Patient RH29</td>
<td>Mesothelioma</td>
<td>635</td>
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<tr>
<td>KLM1</td>
<td>Pancreas</td>
<td>62</td>
</tr>
<tr>
<td>MKN 28</td>
<td>Stomach</td>
<td>21</td>
</tr>
</tbody>
</table>

Mazor et al. 2015 MCT
Proof of Concept

Is the elimination of T cell epitopes sufficient to prevent ADA in a mouse?
T cell epitope mapping of PE38 peptides in BALB/c identifies two epitopes.

Mazor et al. 2015 Cell Mol Immunol
Immunization with LMB-T20 does not induce formation of Ag-specific IgG
Not Yet In the Clinic
Collaboration with Selecta Bioscience

**Synthetic vaccine particles (SVP-Rapamycin) for specific Immuno-suppression and Tolerance Induction**

- poly(lactidecoglycolide) (PLGA)
- Taken up by APC
- Contain Rapamycin
- Successfully prevented immunogenicity of: Factor VIII, pegsiticase, and adalimumab in mice, rats and monkeys
- SVP-Rapamycin + pegsiticase currently in Phase 2 clinical trials
Clinical Trial

• Collaboration with Selecta in which we will combine LMB-100 with SVP-nanoparticles in patients with mesothelioma to see how many cycles can be safely given
The combination of LMB-100 + SVP prevents formation of neutralizing ADA induces tolerance and in mice.
SVP-Rapamycin

- Block primary responses
- Induce tolerance
- Block recall (secondary) responses
- Allow repeated dosing of immunotoxins in mice with normal immune systems
- Now in clinical trials in mesothelioma
Take Away

• Low titers of antibodies do not prevent repeated dosing and efficacy.
• High titers block efficacy.
• Removing B cell epitopes is useful in blocking immunogenicity in mice but not humans.
• Removing T cell epitopes is useful in mice.
• Have not tested T cell epitope modified immunotoxin in humans.
Take Away

• Focusing on SEL-212
THE END