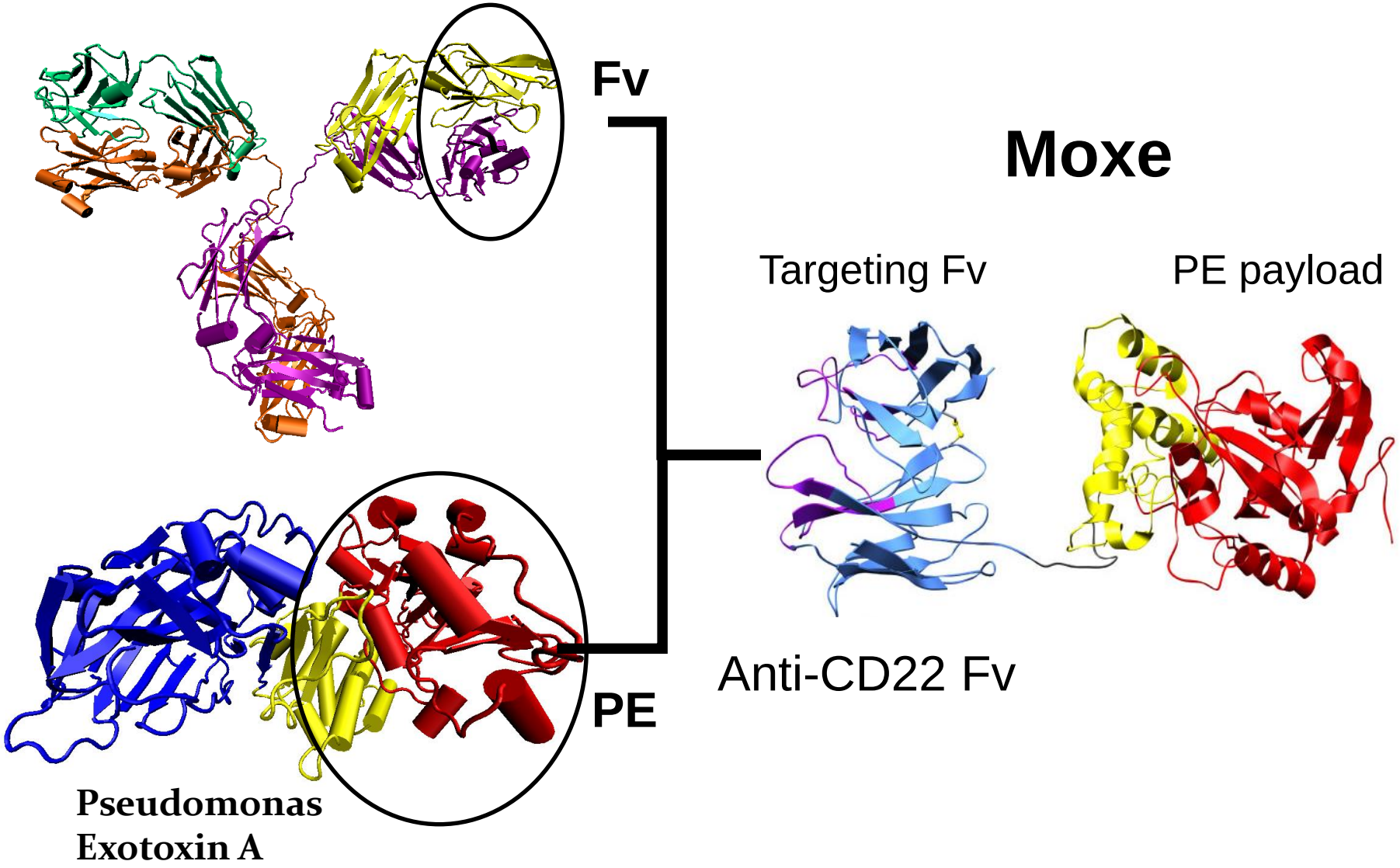
Strategies to Reduce ADA Response to Immunotoxin Therapy of Cancer

Ira Pastan

NATIONAL CANCER INSTITUTE

LMB!
Laboratory of Molecular Biology

Recombinant Immunotoxin (RIT)



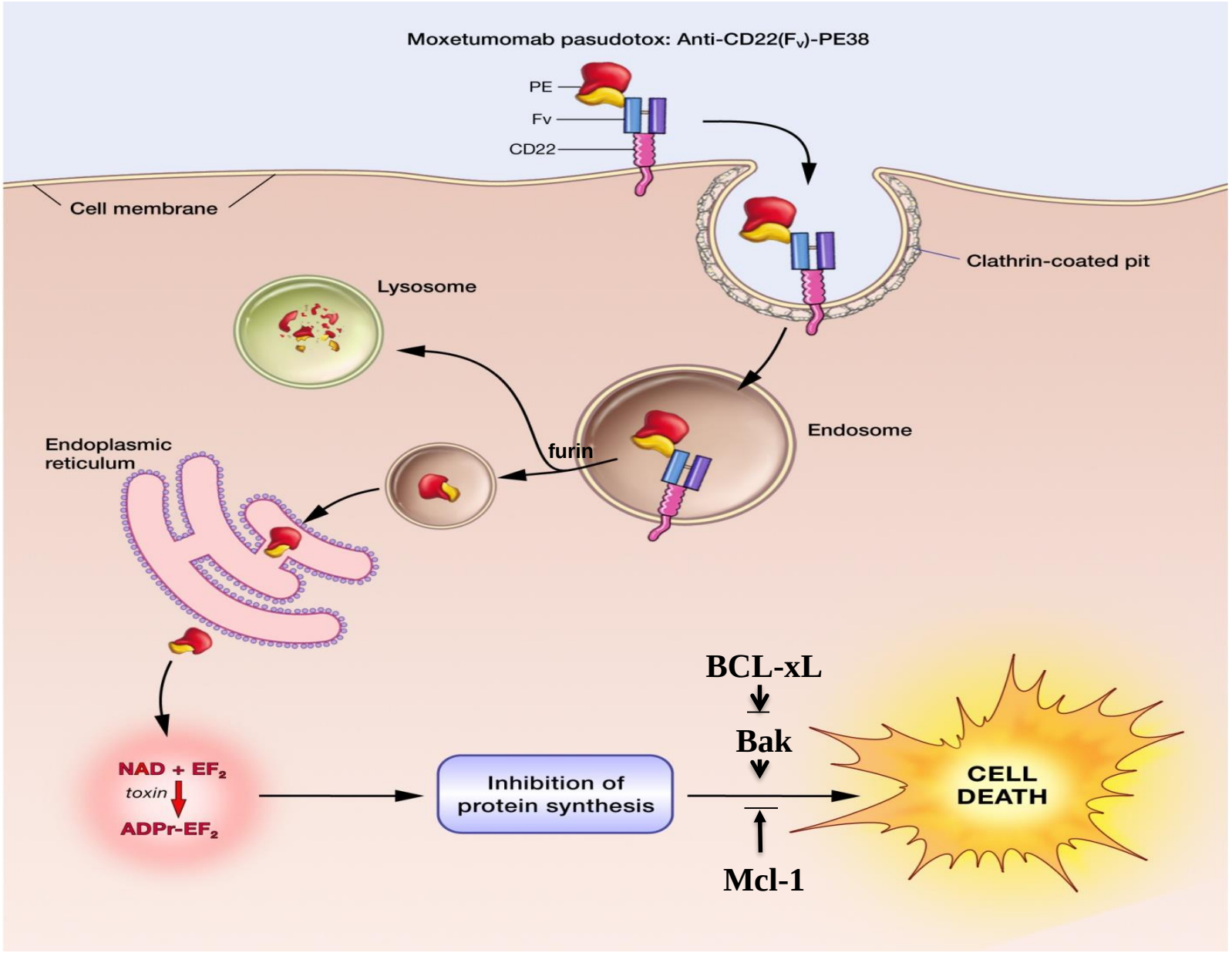
Pseudomonas Exotoxin A

- 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



Clinical Trials

Hairy Cell Leukemia targeting **CD22**

Moxetumomab pasudotox

Mesothelioma and **Pancreatic Cancer** targeting **Mesothelin**

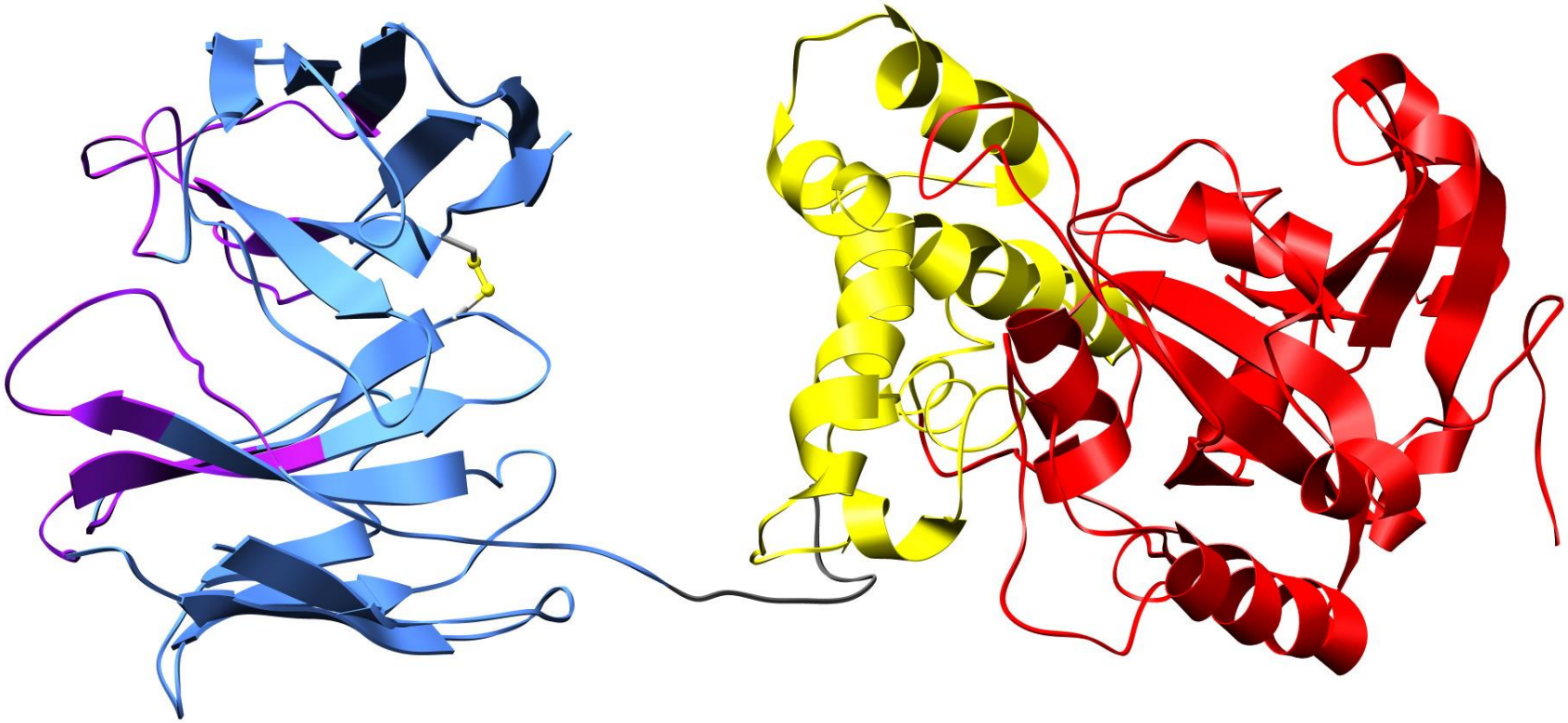
SS1P

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

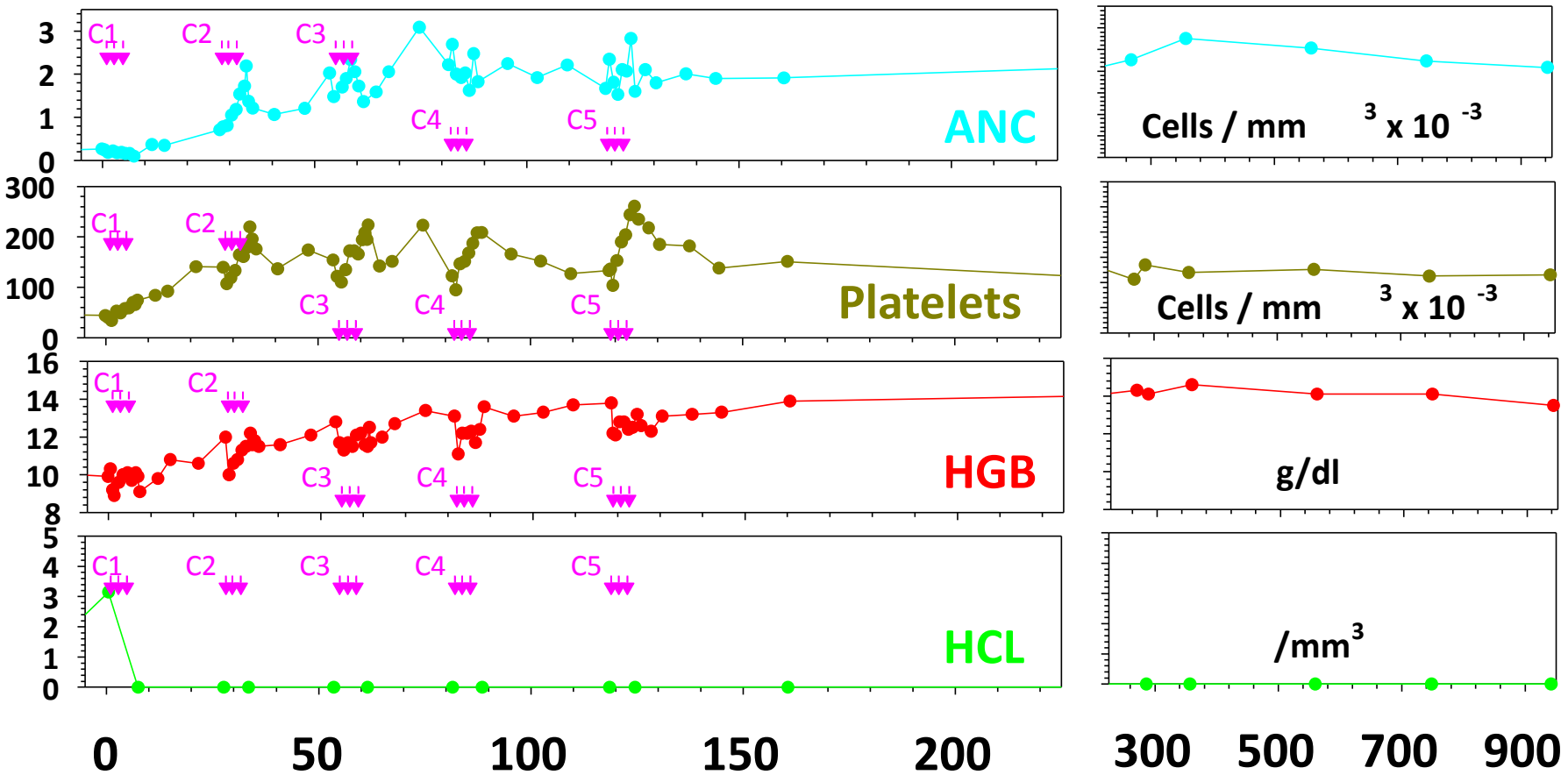
Salvatore et al 2002 CCR

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 ug/Kg QOD x 3



Responses in Drug Resistant Hairy Cell Leukemia

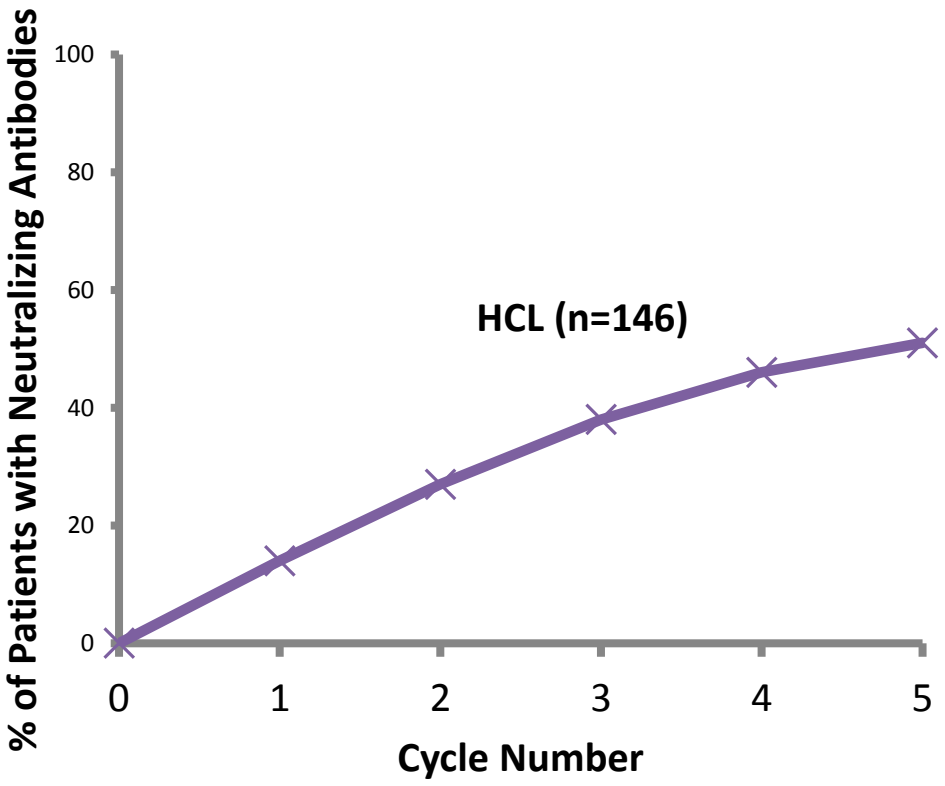
No dose limiting toxicities at 50 ug/kg

Number of Patients	**48**
Complete Response	**26 (54%)**
Partial Response	**16 (33%)**

88% overall response rate

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

The Neutralizing Antibody Problem



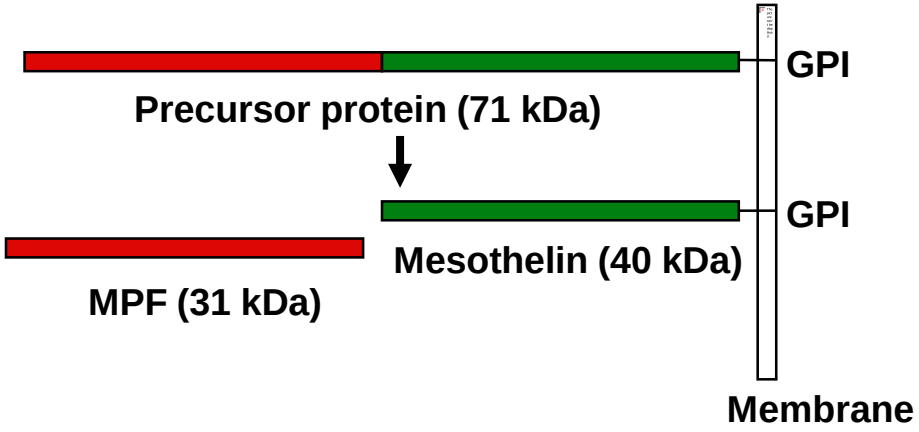
Hassan et al
Kreitman et al
Wayne et al

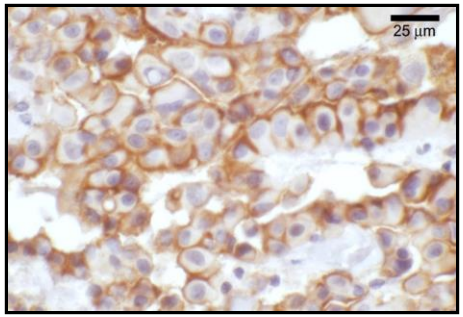
Summary

- RITs can cause meaningful responses in Hairy Cell Leukemia where significant ADA formation is **absent** or **low** or **delayed**.
- What about solid tumors?

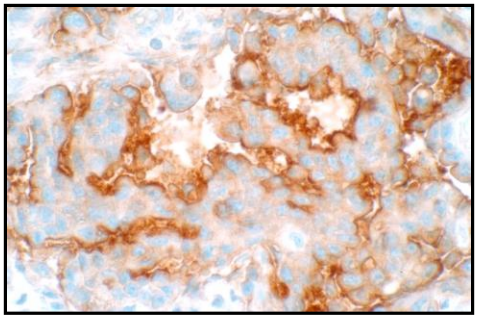
Mesothelin

- **Cell surface glycoprotein**
- **Differentiation antigen expressed only on mesothelial cells of pleura, peritoneum & pericardium**
- **Mesothelin is highly expressed in many cancers**

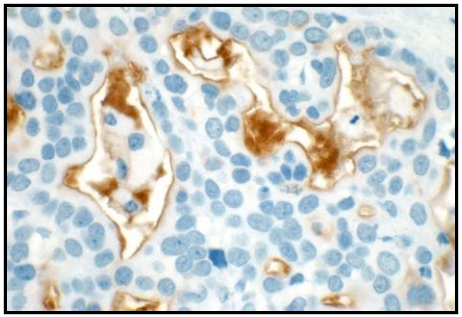




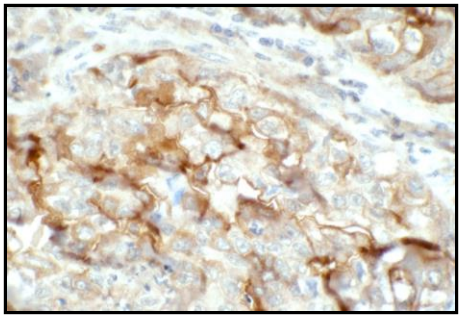
Mesothelioma



Ovarian Cancer



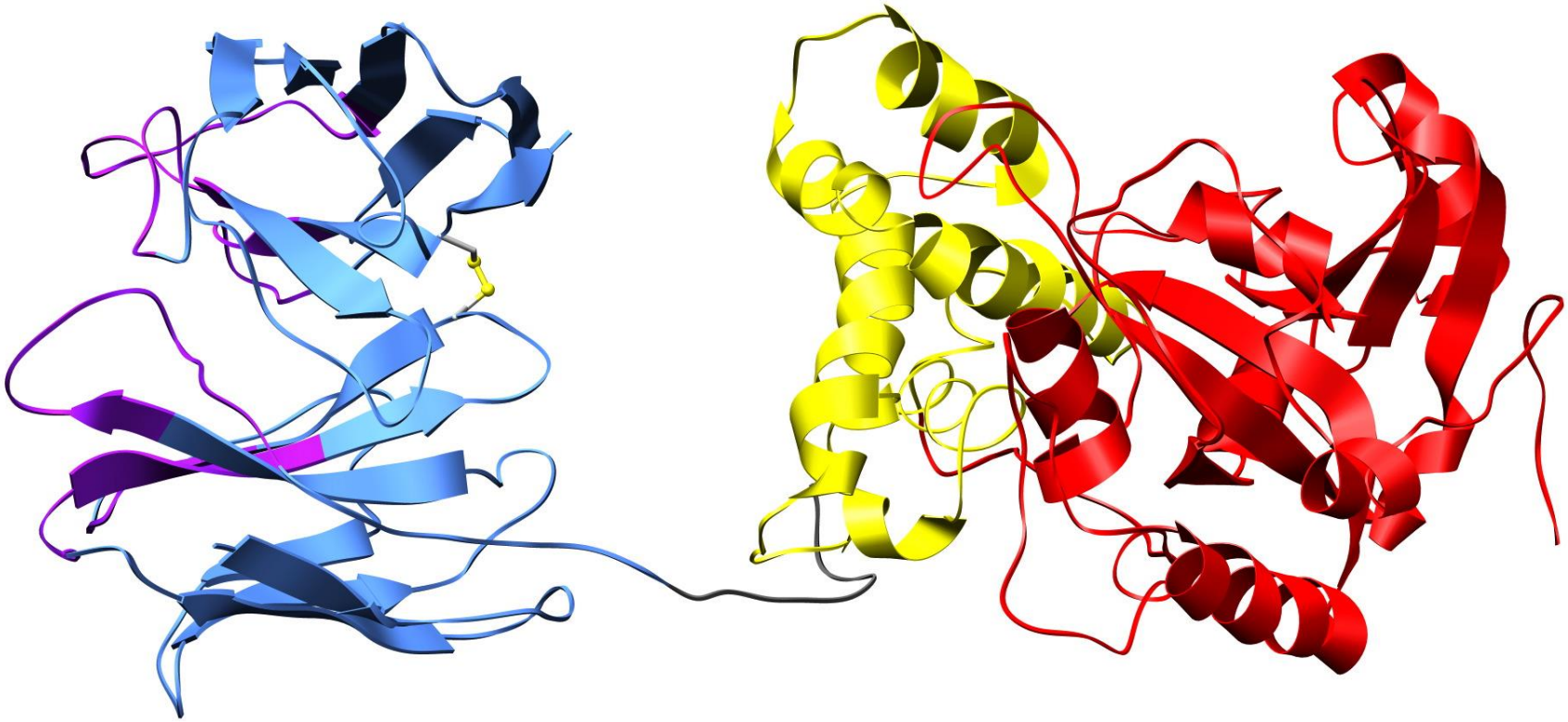
Pancreatic Cancer



Lung Cancer

Hassan et al. Clin. Cancer Res., 2004

SS1P Structure



Anti-Mesothelin

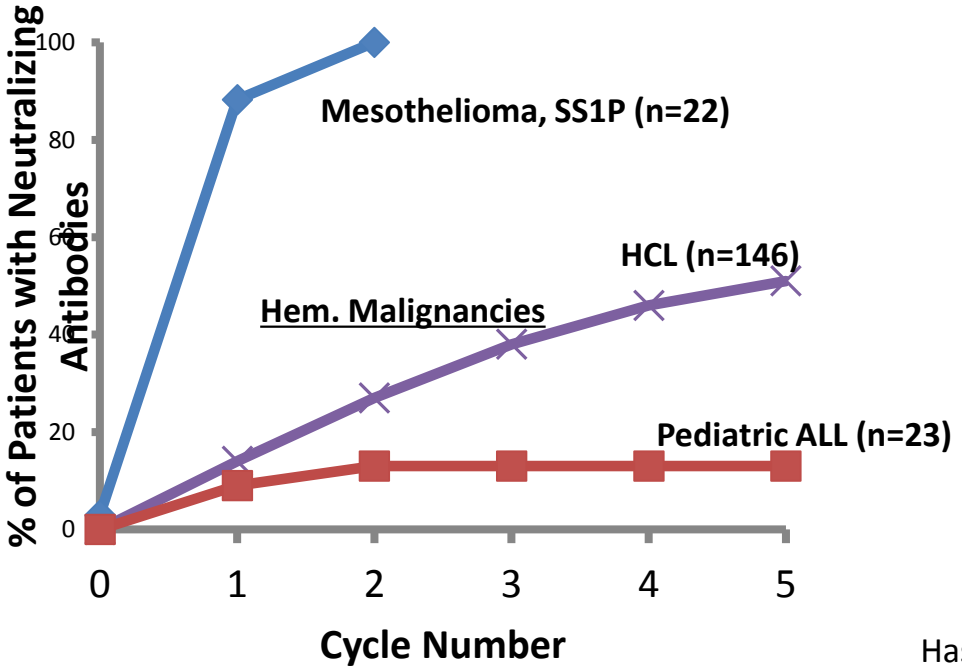
PE38

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

Hassan et al. Clin. Cancer Res., 2007

The Neutralizing Antibody Problem



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

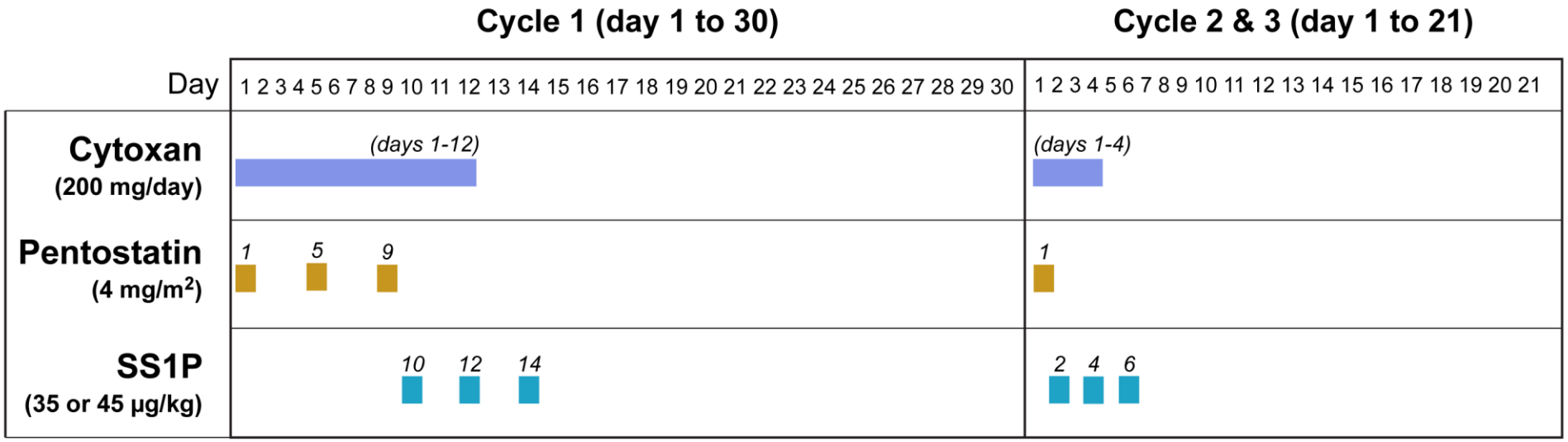
The Neutralizing Antibody problem

- 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.

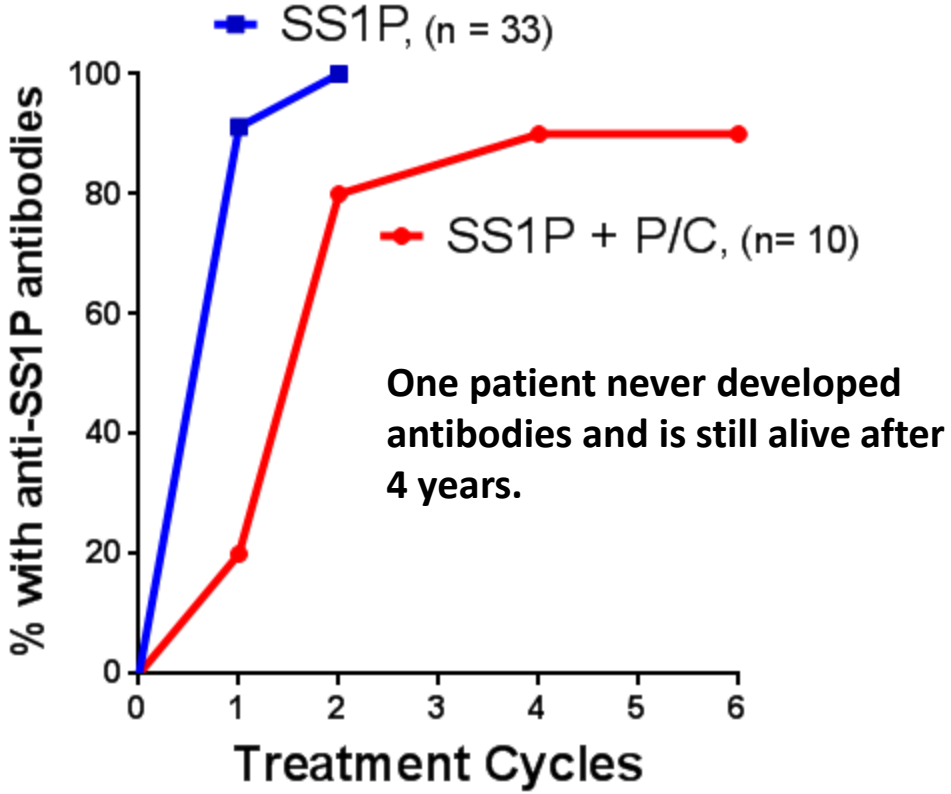
- Fowler et al found that a combination of **pentostatin and cytoxan** allowed bone marrow engraftment in the transplantation setting without myelosuppression.

Pilot study of SS1P with pentostatin and cytoxan in advanced chemo-refractory mesothelioma

- **Previously treated patients with treatment refractory and progressive disease**



Pentostatin/Cytoxan delays antibody response

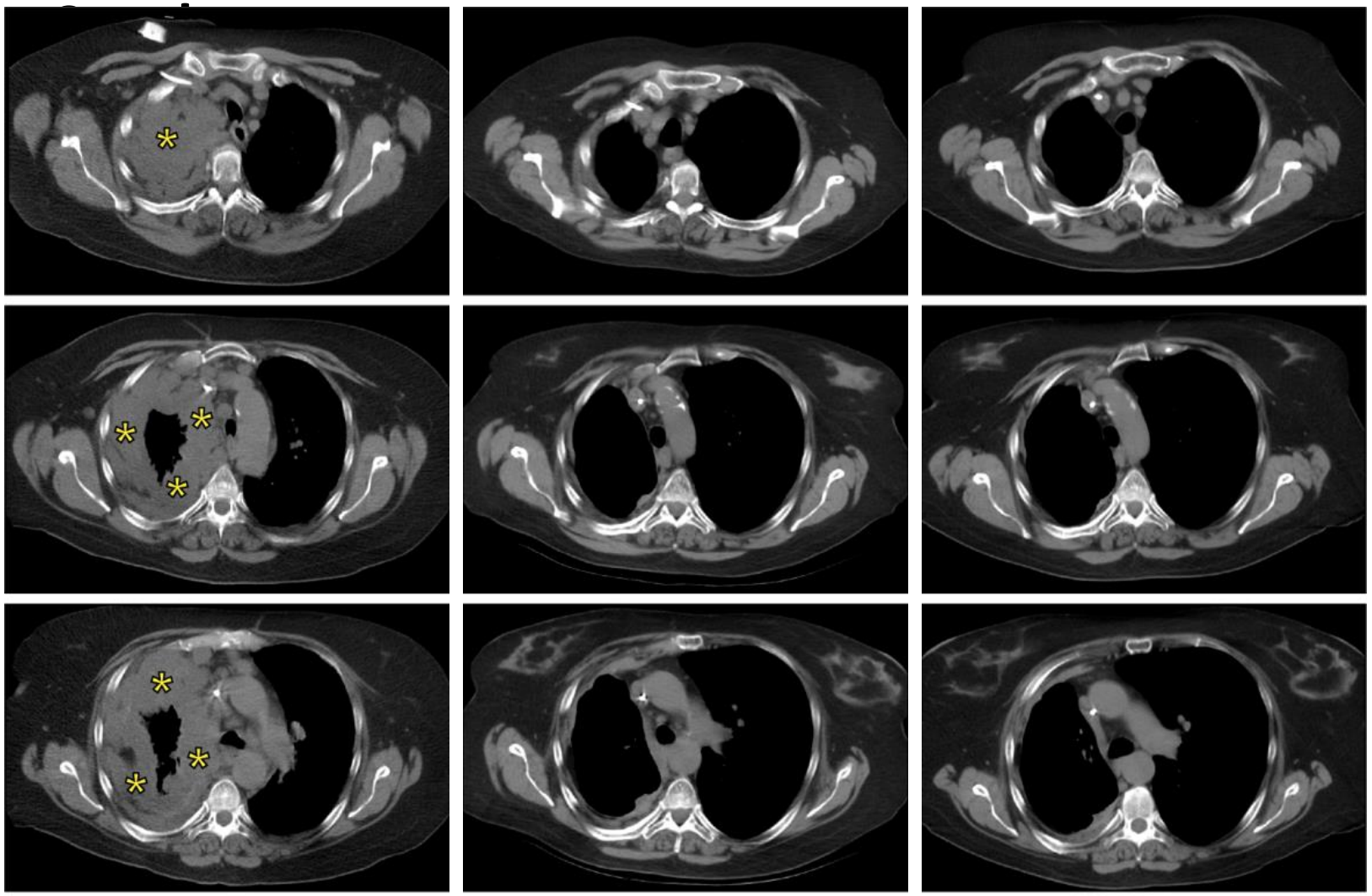


Delaying antibody responses is possible but it is complicated, inefficient and associated with toxicity.

Hassan, Pastan et al 2013

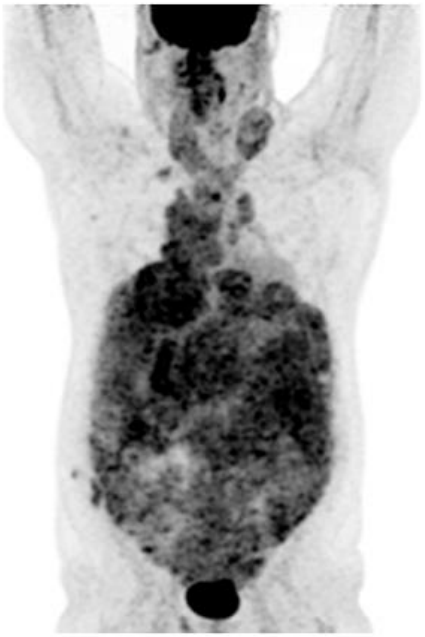
Serendipity

Patient 3 with extensive pleural mesothelioma:

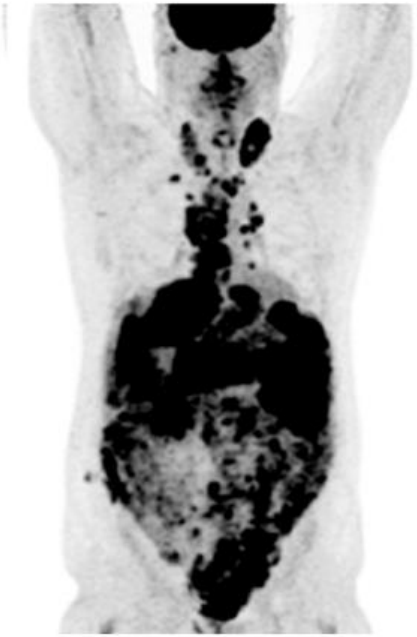


Day 12 3 months 15 months

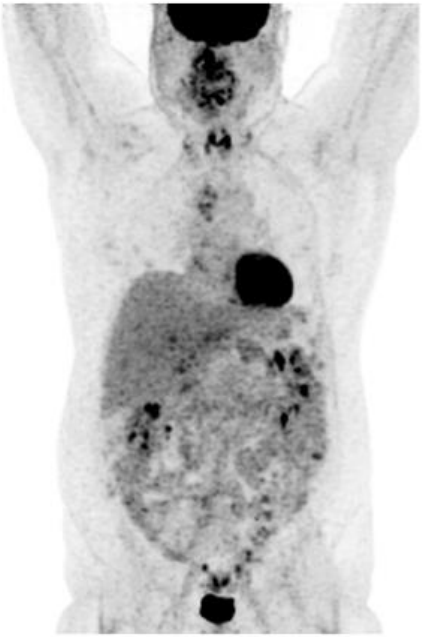
Patient 5 with widely metastatic peritoneal mesothelioma



Before treatment



1.6 months

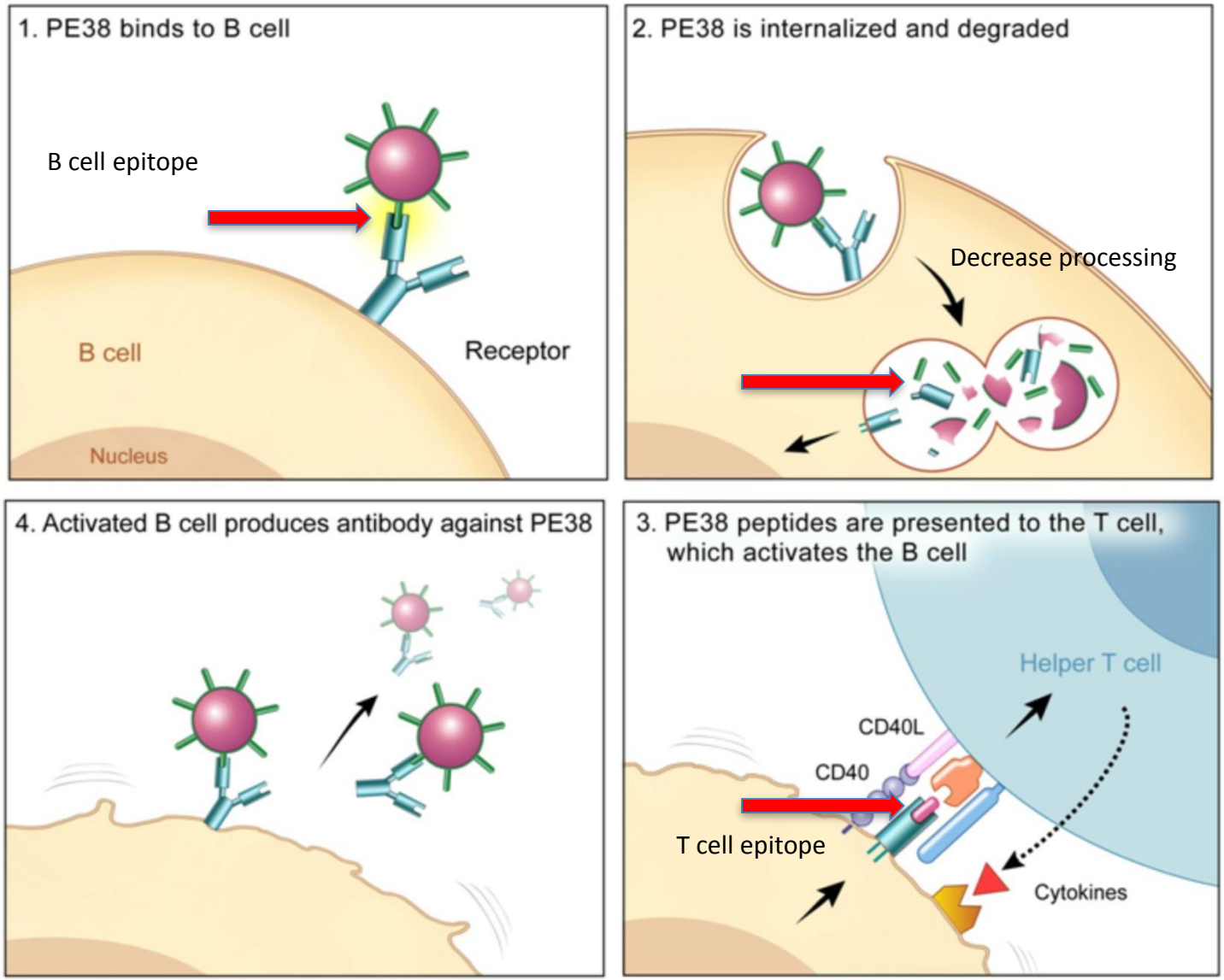


8 months

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

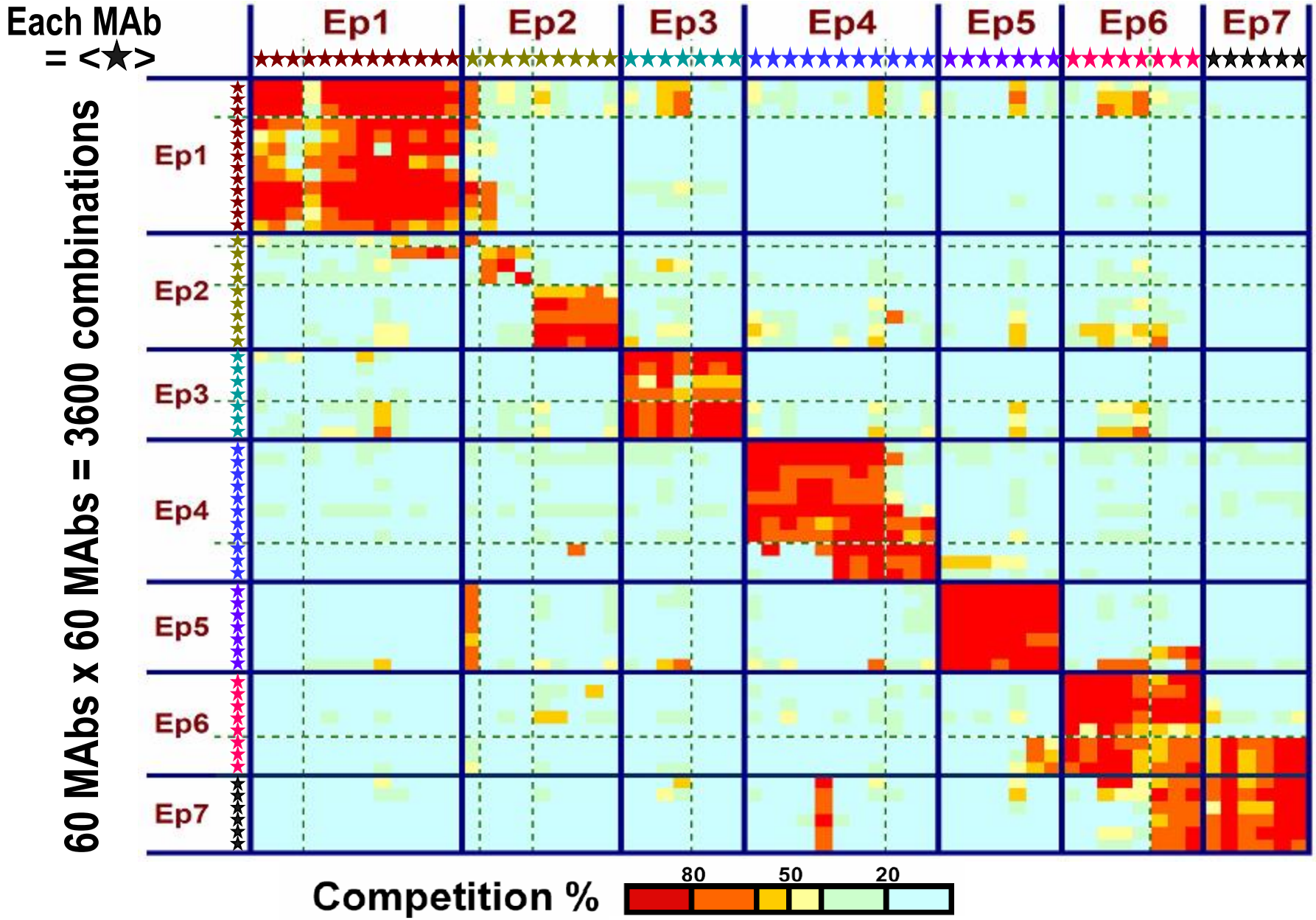


Strategy to decrease binding of toxin to receptor on B cells (Remove B cell Epitopes)

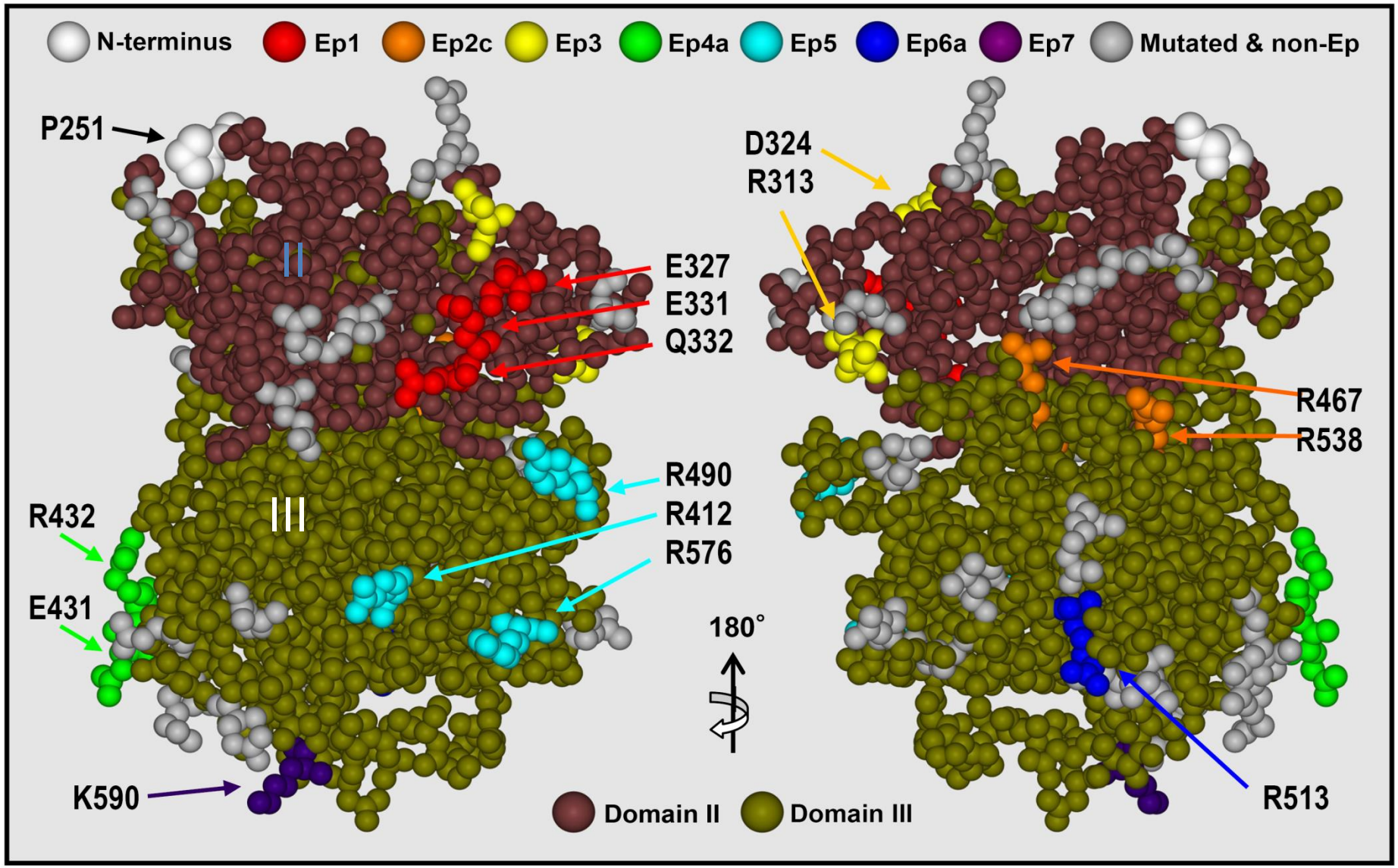
- Made large number of mabs in mice to PE38
- Mapped where they bound to the toxin
- Bulky amino acids like arg, gln glu
- Mutated to ala
- Showed mab binding was greatly decreased

Onda, Nagata et al

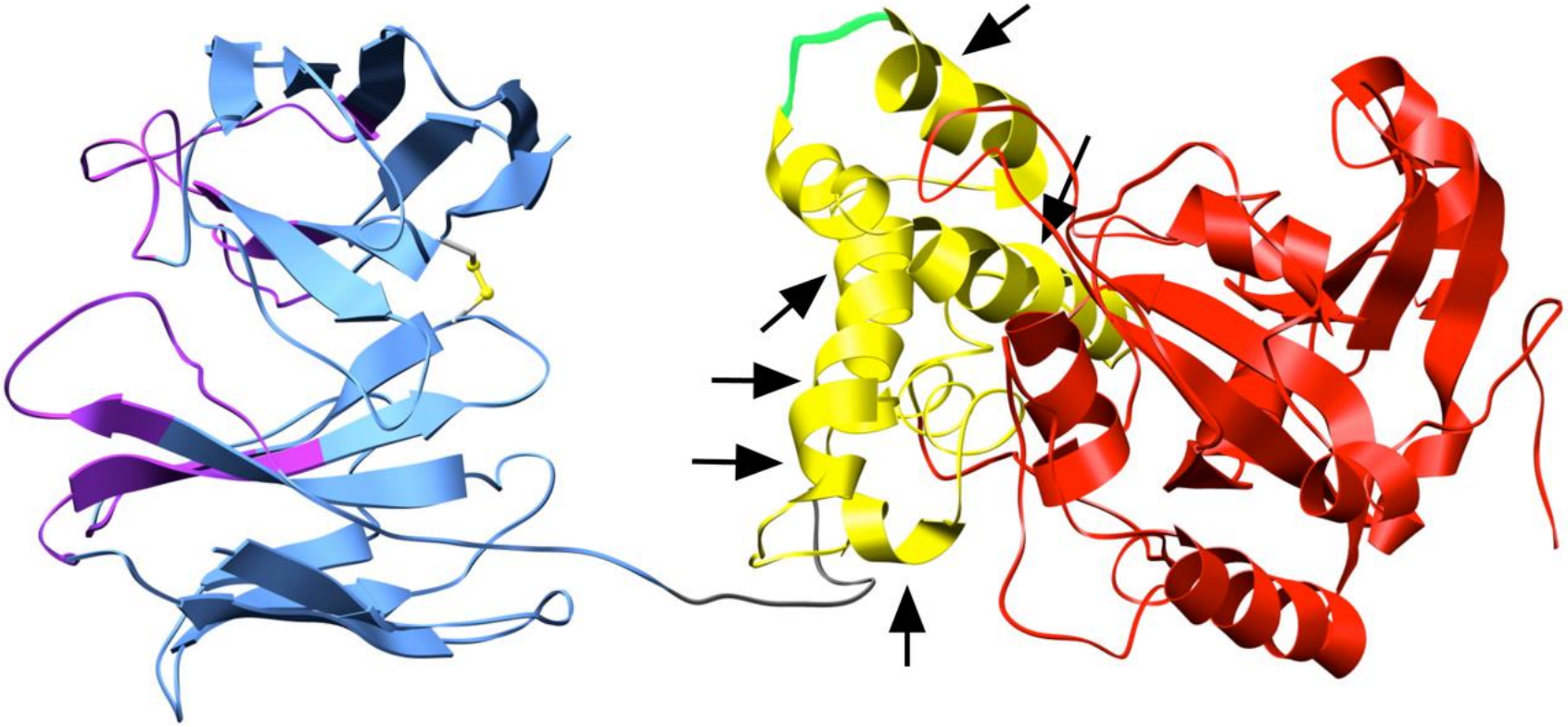
Identification of 7 major epitopes in PE38 by competition assay of 60 MAbs



Location of 7 major B cell epitopes in PE38



Domain II is very protease sensitive and can be deleted

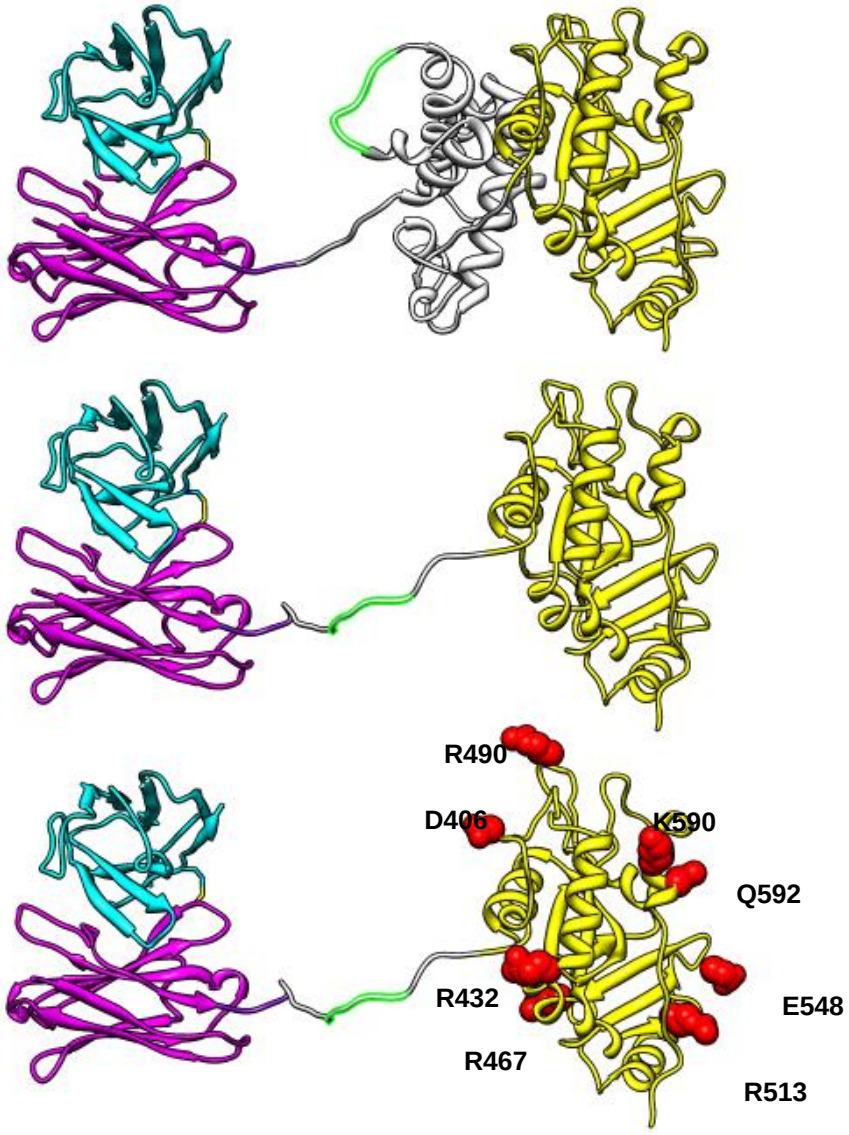


First tried to de-immunize for Mice by identifying and removing mouse B Cell Epitopes

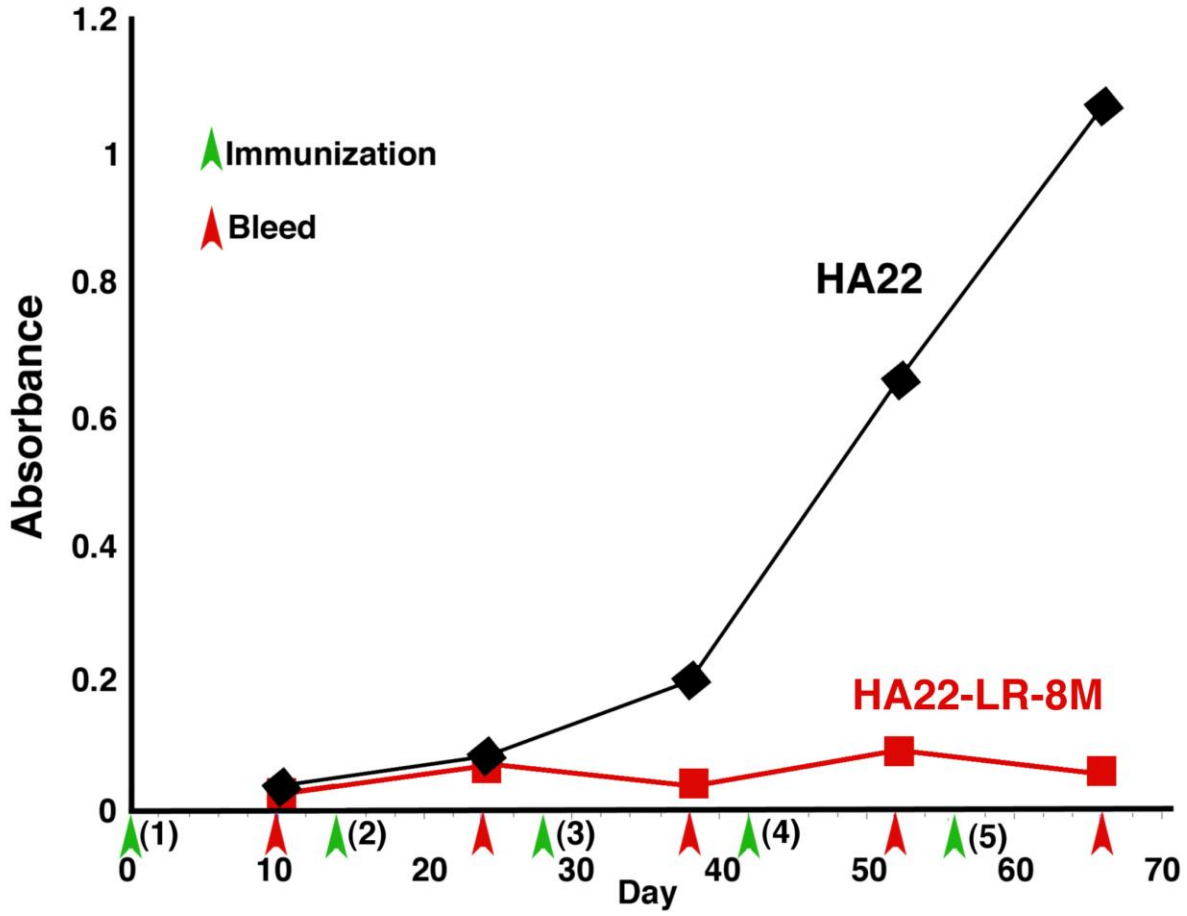
HA22

HA22-LR

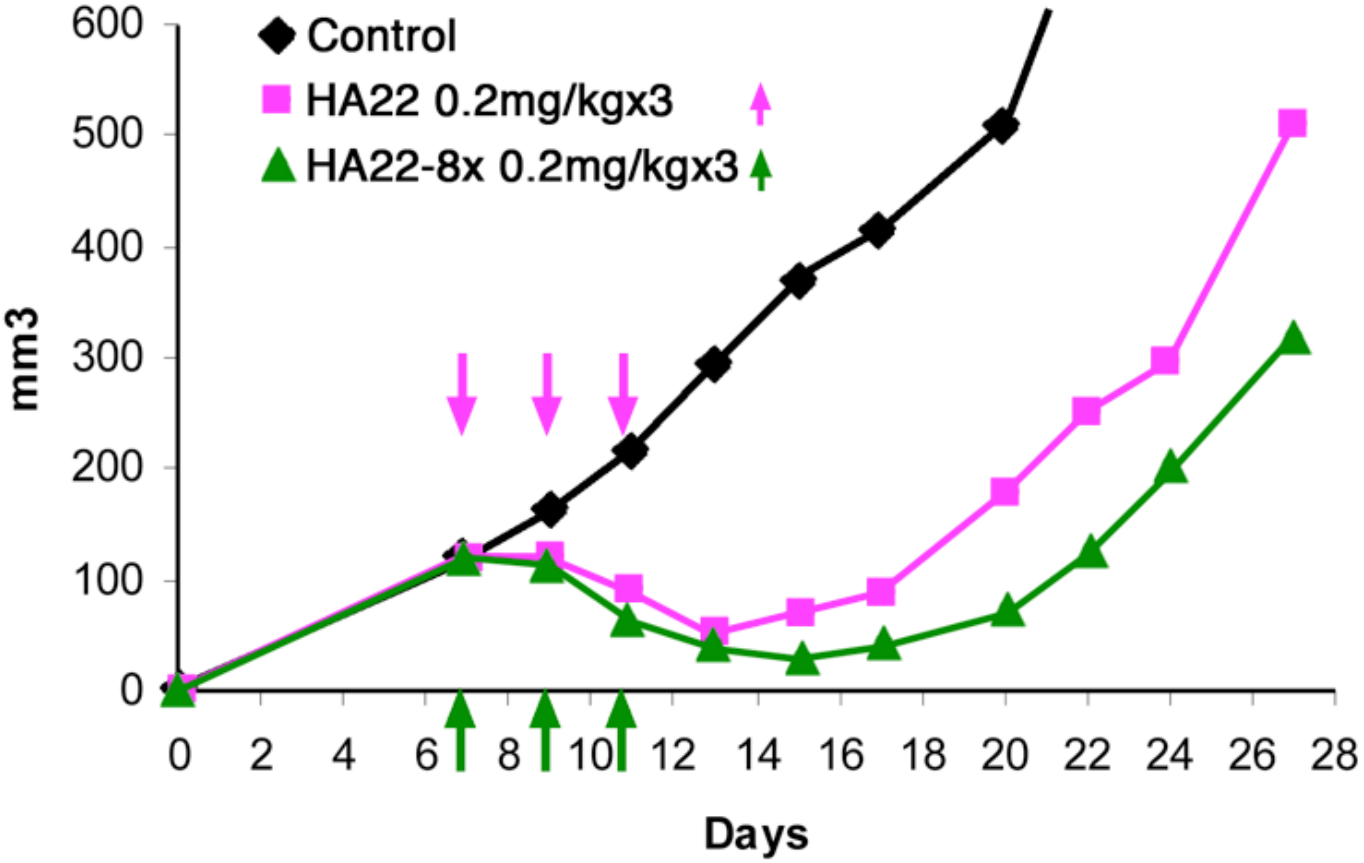
HA22-LR-8M



Decreased Immunogenicity of HA22-LR-8M in Mice



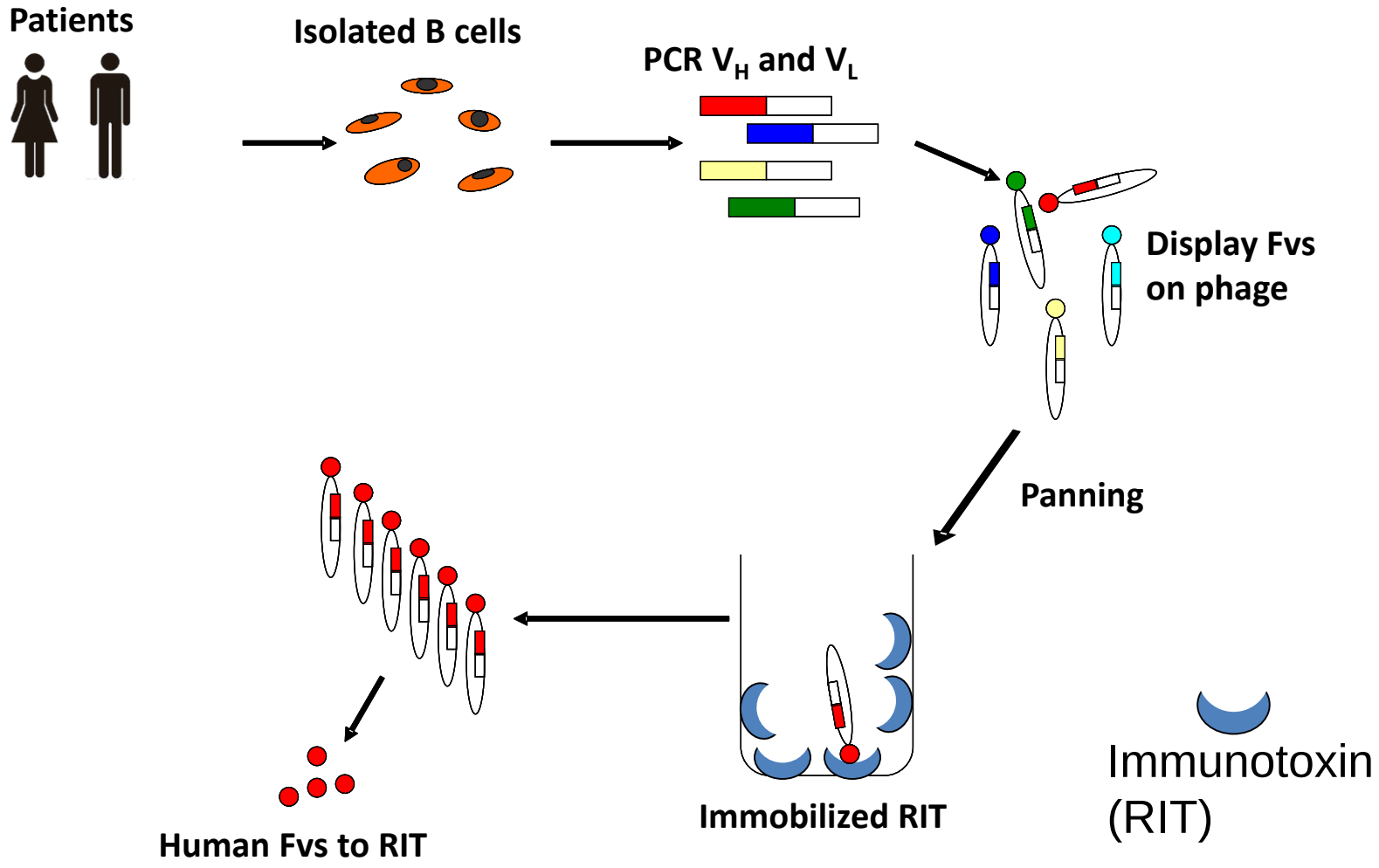
Anti-Tumor Activity of HA22 and HA22-8M



Laiman

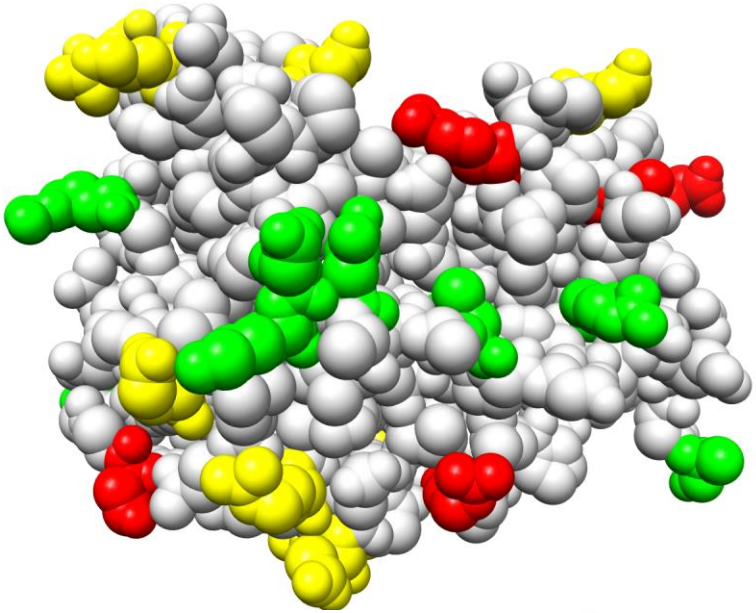
What about human B cell Epitopes?

Isolation of human antibodies to map the epitopes in RITs by Phage Display



Location of Epitopes in Domain III

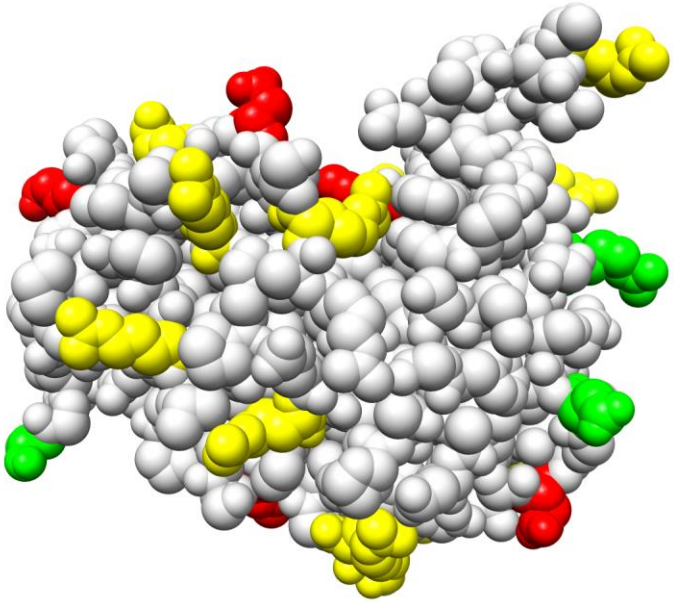
Front view



Green mouse

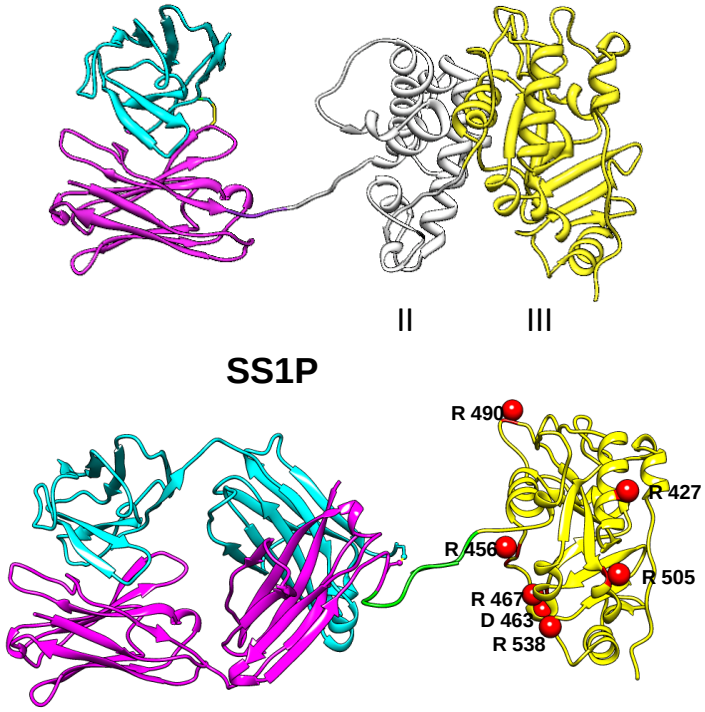
Red Human

Back View



Yellow both mouse and human

Collaboration with Roche to make a New RIT Targeting Mesothelin: RG7787/LMB-1OO

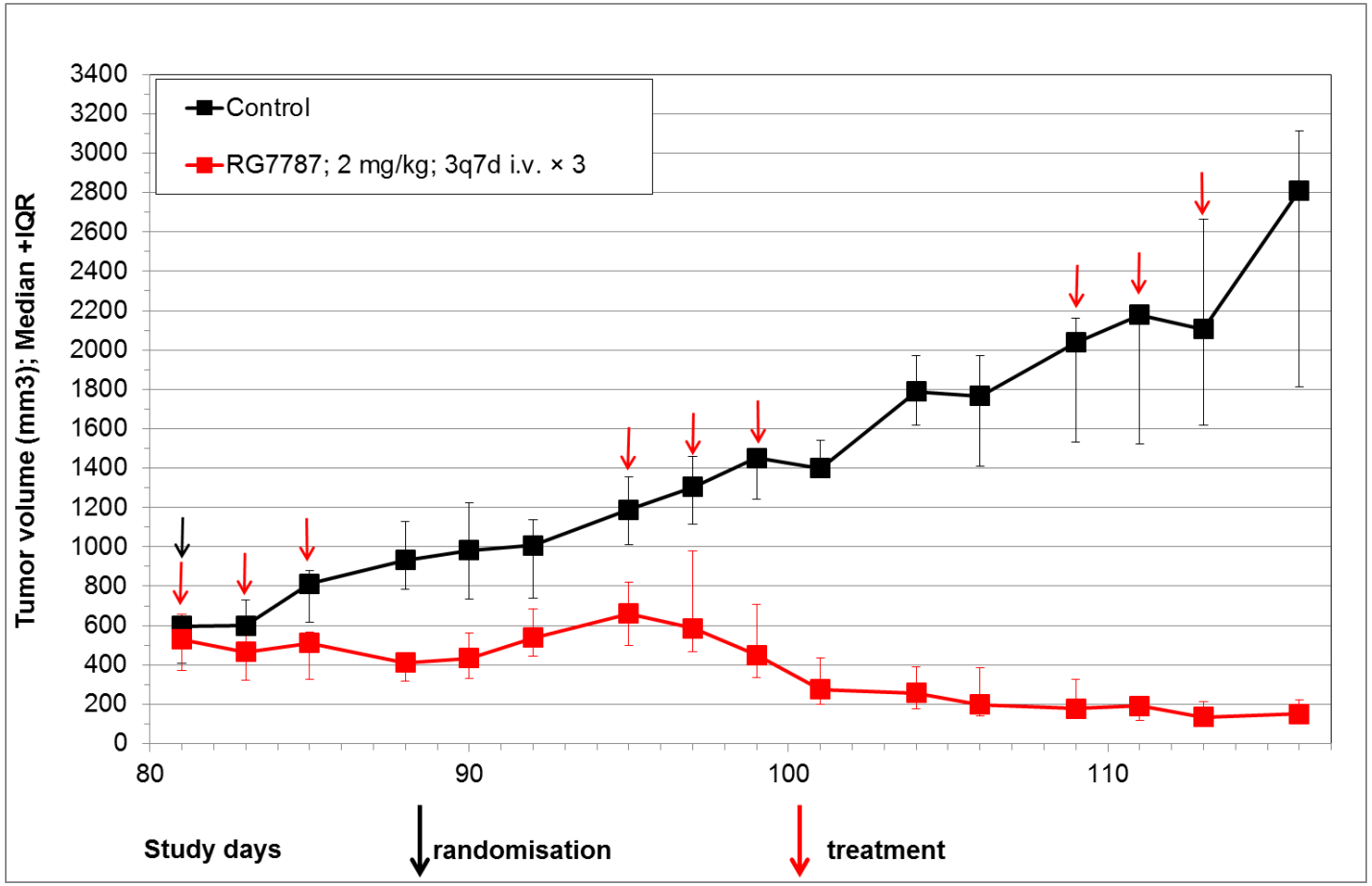


LMB-100

- 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 mm^3 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?

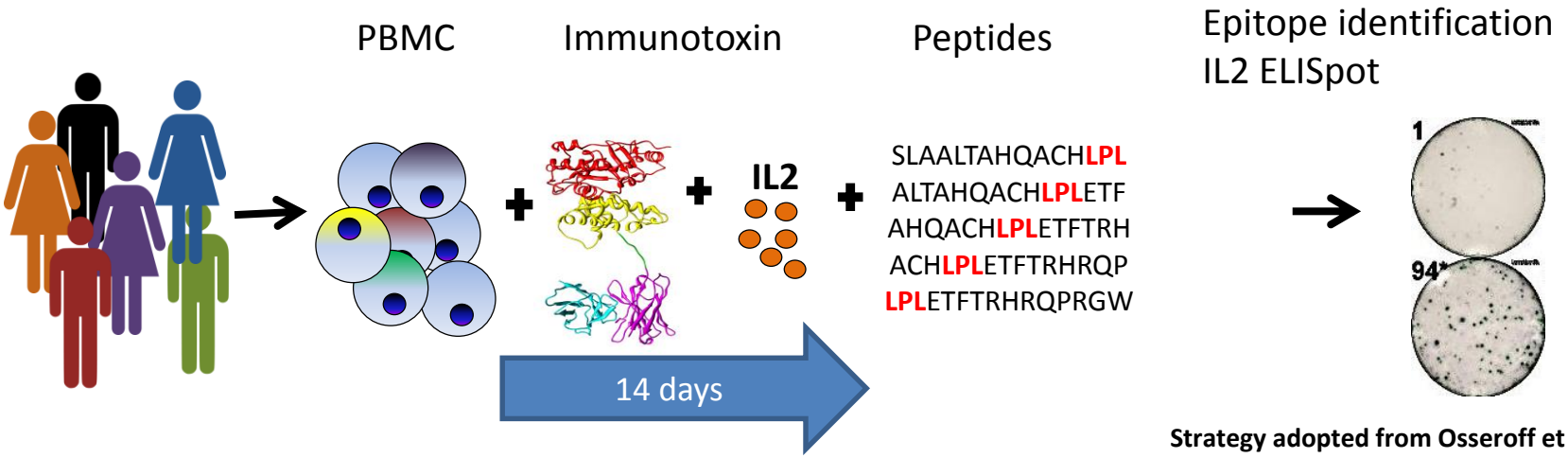
Approach described by Sette and colleagues

Project led by Ronit Mazor

PE38 peptide library

Peptide	Sequence	pool	Peptide	Sequence	pool	Peptide	Sequence	pool	Peptide	Sequence	pool
1	PEGGSLAALTAHQAC	1	29	ARLALTLAAAESERF	6	57	FVGYHGTFLEAAQSI	12	85	APEAAGEVERLIGHP	17
2	SLAALTAHQACH**LPL**	1	30	ALTLAAAESERFVRQ	6	58	YHGTFLEAAQSIVFG	12	86	AAGEVERLIGHPLPL	18
3	ALTAHQACH**LPL**ETF	1	31	LAAAESERFVRQGTG	7	59	TFLEAAQSIVFGGVR	12	87	EVERLIGHPLPLRLD	18
4	AHQACH**LPL**ETFTRH	1	32	AESERFVRQGTGNDE	7	60	EAAQSIVFGGVRARS	12	88	RLIGHPLPLRLDAIT	18
5	ACH**LPL**ETFTRHRQP	1	33	ERFVRQGTGNDEAGA	7	61	QSIVFGGVRARSQDL	13	89	GHPLPLRLDAITGPE	18
6	**LPL**ETFTRHRQPRGW	2	34	VRQGTGNDEAGAANG	7	62	VFGGVRARSQDLDAI	13	90	LPLRLDAITGPEEEG	18
7	ETFTRHRQPRGWEQL	2	35	GTGNDEAGAANGPAD	7	63	GVRARSQDLDAIWRG	13	91	RLDAITGPEEEGGRL	19
8	TRHRQPRGWEQLEQC	2	36	NDEAGAANGPADSGD	8	64	ARSQDLDAIWRGFYI	13	92	AITGPEEEGGRLETI	19
9	RQPRGWEQLEQCGYP	2	37	AGAANGPADSGDALL	8	65	QDLDAIWRGFYIAGD	13	93	GPEEEGGRLETILGW	19
10	RGWEQLEQCGYPVQR	2	38	ANGPADSGDALLERN	8	66	DAIWRGFYIAGDPAL	14	94	EEGGRLETILGWPLA	19
11	EQLEQCGYPVQRLVA	3	39	PADSGDALLERNYPT	8	67	WRGFYIAGDPALAYG	14	95	GRLETILGWPLAERT	19
12	EQCGYPVQRLVALYL	3	40	SGDALLERNYPTGAE	8	68	FYIAGDPALAYGYAQ	14	96	ETILGWPLAERTVVI	20
13	GYPVQRLVALYLAAR	3	41	ALLERNYPTGAEFLG	9	69	AGDPALAYGYAQDQE	14	97	LGWPLAERTVVIPSA	20
14	VQRLVALYLAARLSW	3	42	ERNYPTGAEFLGDGG	9	70	PALAYGYAQDQEPDA	14	98	PLAERTVVIPSAIPT	20
15	LVALYLAARLSWNQV	3	43	YPTGAEFLGDGGDVS	9	71	AYGYAQDQEPDARGR	15	99	ERTVVIPSAIPTDPR	20
16	LYLAARLSWNQVDQV	4	44	GAEFLGDGGDVSFST	9	72	YAQDQEPDARGRIRN	15	100	VVIPSAIPTDPRNVG	20
17	AARLSWNQVDQVIRN	4	45	FLGDGGDVSFSTRGT	9	73	DQEPDARGRIRNGAL	15	101	PSAIPTDPRNVGGDL	21
18	LSWNQVDQVIRNALA	4	46	DGGDVSFSTRGTQNW	10	74	PDARGRIRNGALLRV	15	102	IPTDPRNVGGDLDPS	21
19	NQVDQVIRNALASPG	4	47	DVSFSTRGTQNWTVE	10	75	RGRIRNGALLRVYVP	15	103	DPRNVGGDLDPSSIP	21
20	DQVIRNALASPGSGG	4	48	FSTRGTQNWTVERLL	10	76	IRNGALLRVYVPRSS	16	104	NVGGDLDPSSIPDKE	21
21	IRNALASPGSGGDLG	5	49	RGTQNWTVERLLQAH	10	77	GALLRVYVPRSSLPG	16	105	GDLDPSSIPDKEQAI	21
22	ALASPGSGGDLGEAI	5	50	QNWTVERLLQAHRQL	10	78	LRVYVPRSSLPGFYR	16	106	DPSSIPDKEQAISAL	22
23	SPGSGGDLGEAIREQ	5	51	TVERLLQAHRQLEER	11	79	YVPRSSLPGFYRTSL	16	107	SIPDKEQAISALPDY	22
24	SGGDLGEAIREQPEQ	5	52	RLLQAHRQLEERGYV	11	80	RSSLPGFYRTSLTLA	16	108	DKEQAISALPDYASQ	22
25	DLGEAIREQPEQARL	5	53	QAHRQLEERGYVFVG	11	81	LPGFYRTSLTLAAPE	17	109	QAISALPDYASQPGK	22
26	EAIREQPEQARLALT	6	54	RQLEERGYVFVGYHG	11	82	FYRTSLTLAAPEAAG	17	110	SALPDYASQPGKPPR	22
27	REQPEQARLALTLAA	6	55	EERGYVFVGYHGTFL	11	83	TSLTLAAPEAAGEVE	17	111	PDYASQPGKPPREDL	22
28	PEQARLALTLAAAES	6	56	GYVFVGYHGTFLEAA	12	84	TLAAPEAAGEVERLI	17			

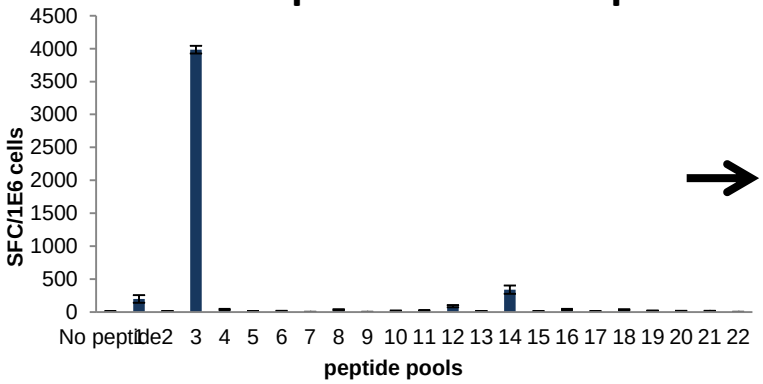
Identification and elimination of T cell epitopes

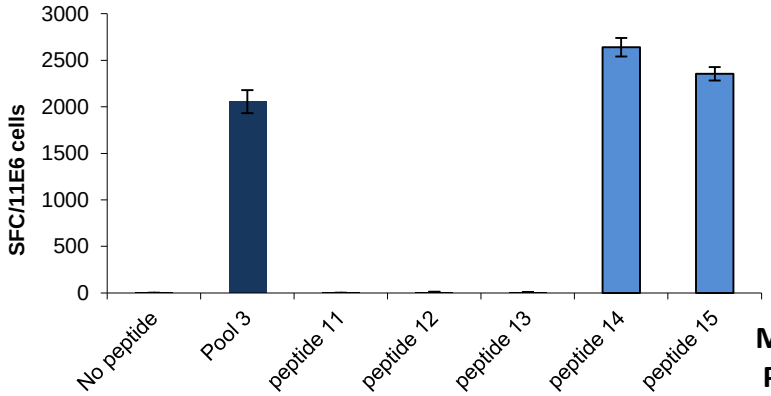


Strategy adopted from Osseroff et al. 2010

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

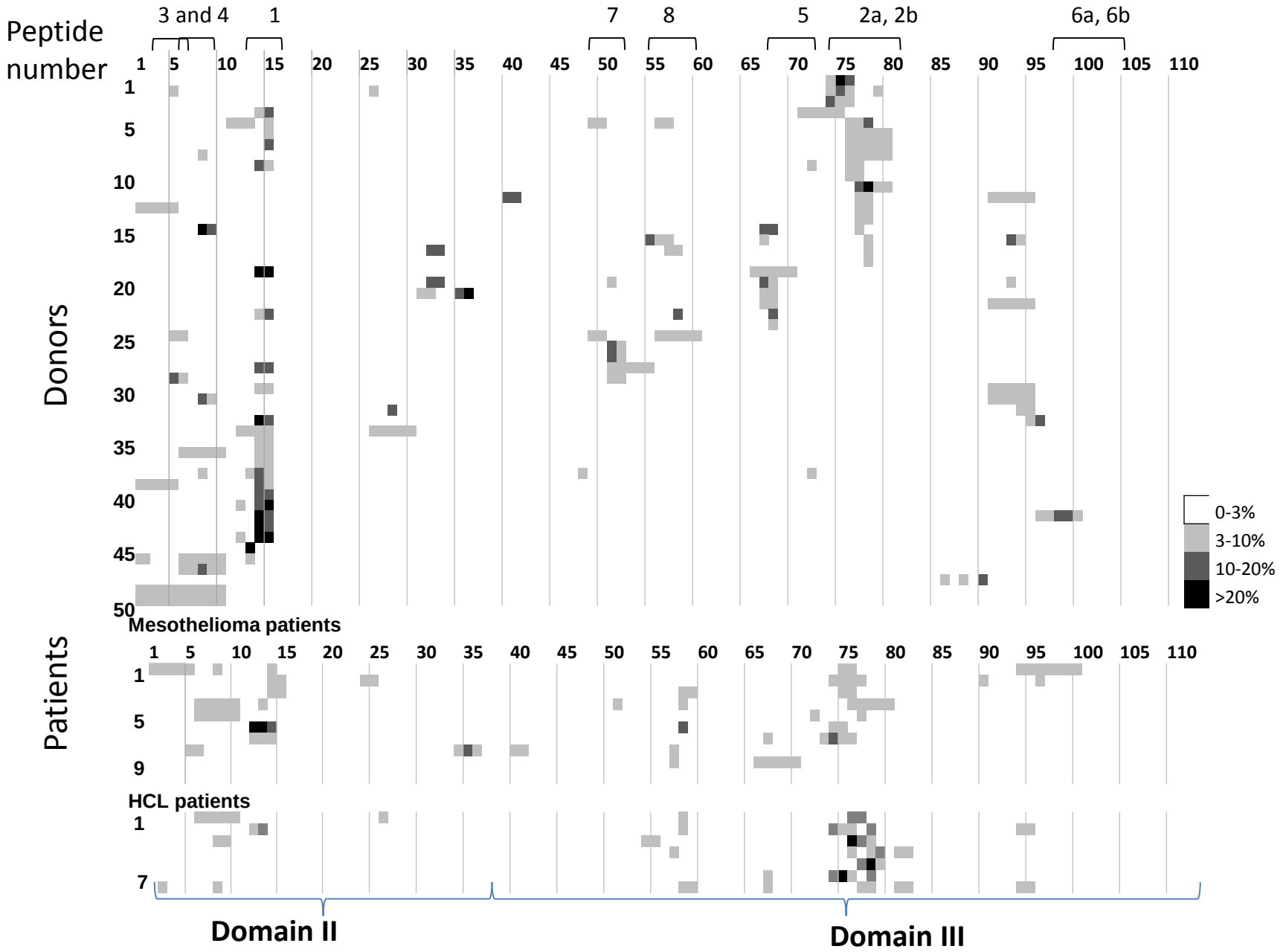
Representative response





Mazor et al. PNAS 2012

Summary of ELISpot responses after *In Vitro* Expansion (n=50)



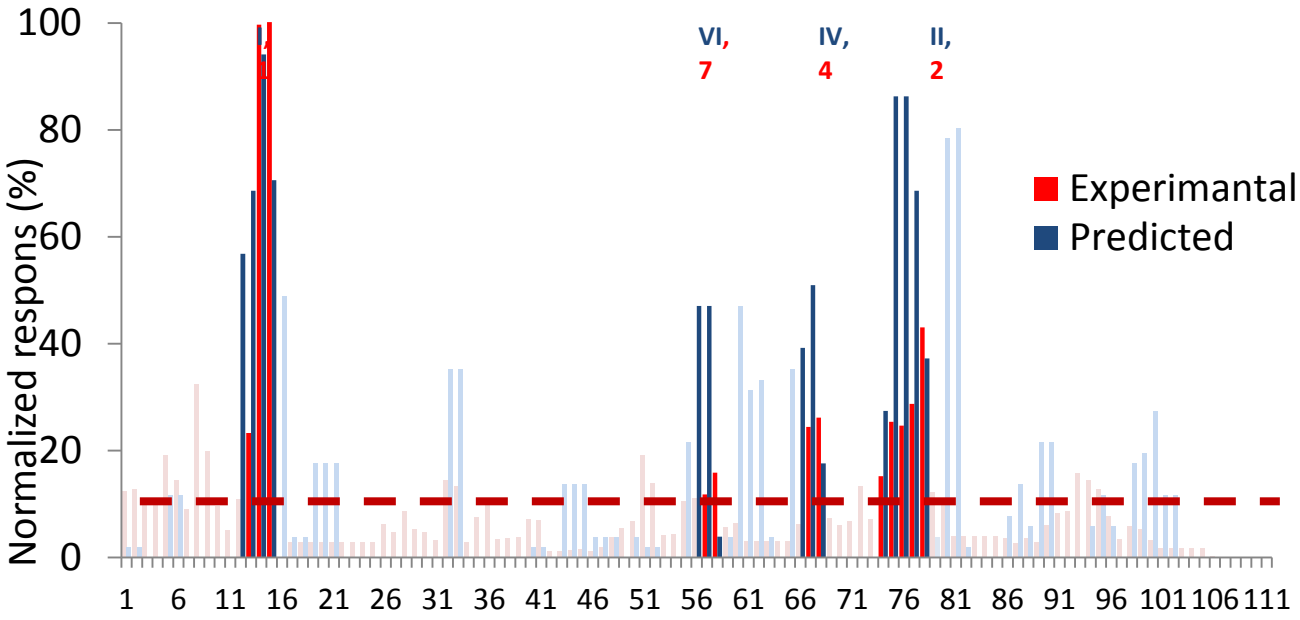
Epitopes summary

Epitope ranking	Peptide #	Sequence	Responses		
			Donors (n=50)	Mesothelioma (n=9)	HCL (n=7)
1	**13-15**	**LVALYLAARLSWNQV**	**21**	**6**	**1**
2 A	77-78	GALLRVYVPRSSLPG	14[a]	3[a]	6[a]
2 B	74-76	IRNGALLRVYVPRSS	10[a]	6[a]	5[a]
3	8-9	RQPRGWEQLEQCGYP	9	3	3
4	5-6	LPLETFTRHRQPRGW	10	2	0
5	67-68	WRGFYIAGDPALAYG	8	2	2
6 A+B	93-96	GPEEEGGRLETILGWPLA	8	1	2
7	51-52	TVERLLQAHRQLEER	5	1	0
8A+B	56-59	FVGYHGTFLEAAQSIVFG	5	5	4

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

[a] donors and patients that responded to epitope 2A overlap with the patients and donors that responded to 2B.

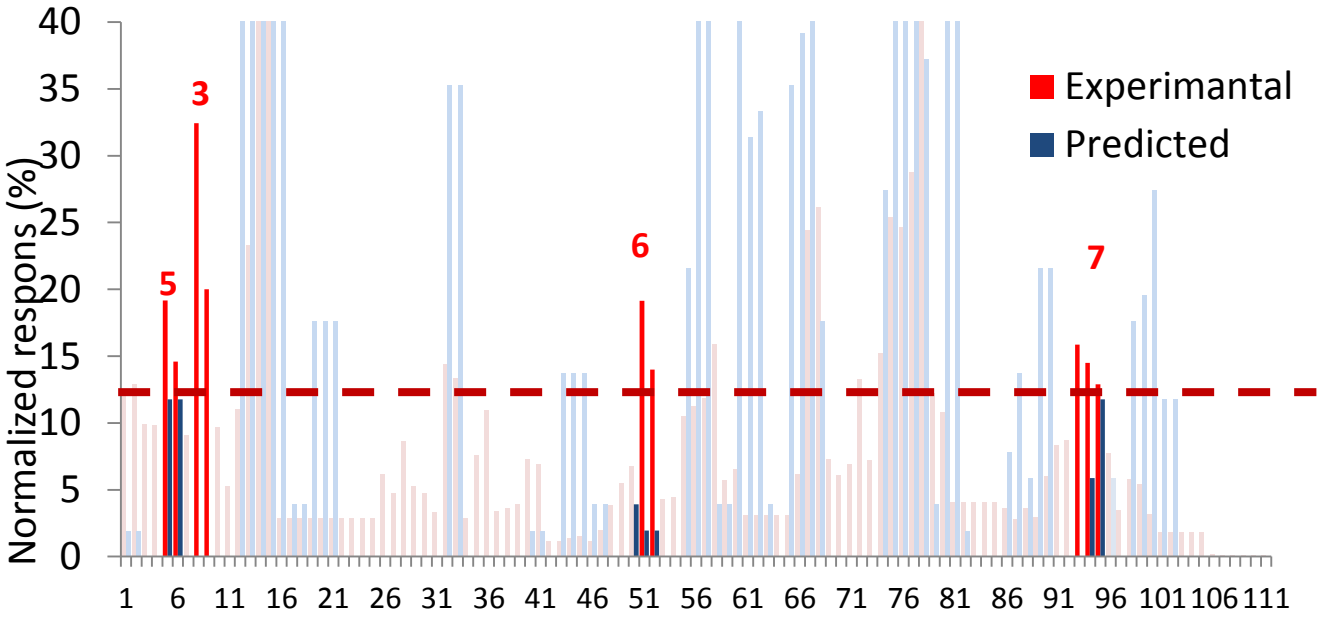
4/8 epitopes were <u>successfully</u> predicted by the algorithm



4/8 False positive in this threshold

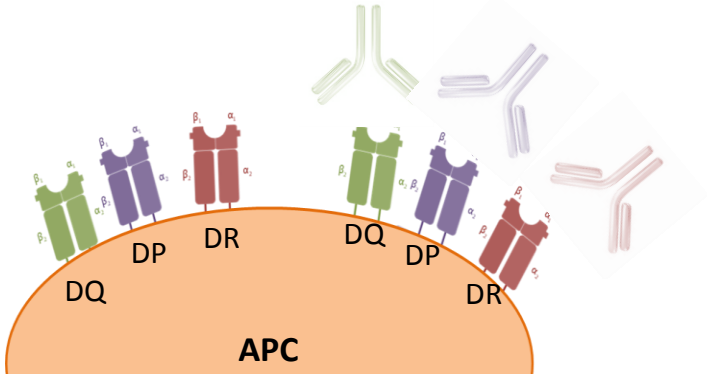
Mazor and Tai et al.
JIM 2015

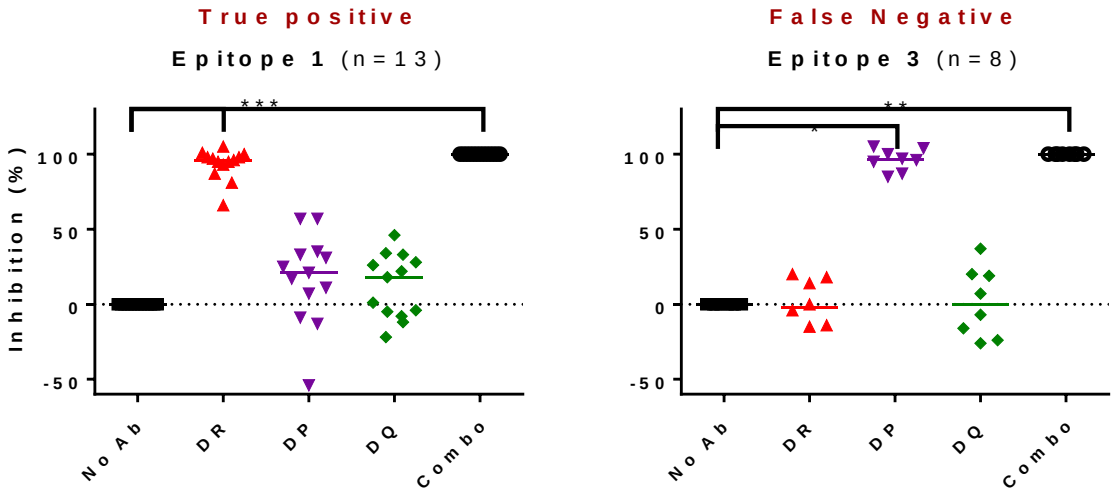
4/8 epitopes were <u>missed</u> by the algorithm



Mazor and Tai et al.
JIM 2015

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)

		Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8	Donor 9	Donor 10	Average (n=21)
no peptide		1%	13%	2%	4%	3%	3%	1%	3%	1%	5%	**6%**
wt 15	LVALYLAARLSWNQV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**
L294A	AVALYLAARLSWNQV	6%	4%	8%	9%	4%	25%	3%	7%	19%	27%	**14%**
V295A	LAALYLAARLSWNQV	5%	103%	2%	27%	19%	13%	63%	14%	1%	5%	**22%**
A496G	LVGLYLAARLSWNQV	11%	118%	10%	24%	16%	19%	57%	27%	31%	34%	**30%**
L297A	LVAAYLAARLSWNQV	6%	2%	8%	8%	4%	10%	6%	19%	19%	17%	**10%**
Y298A	LVALALAARLSWNQV	6%	2%	5%	9%	6%	16%	29%	14%	8%	15%	**8%**
L299A	LVALYAAARLSWNQV	49%	11%	21%	11%	13%	3%	17%	19%	39%	22%	**22%**
A300G	LVALYLGARLSWNQV	89%	64%	35%	82%	91%	16%	135%	32%	223%	72%	**73%**
A301G	LVALYLAGRLSWNQV	65%	87%	54%	67%	142%	6%	107%	53%	167%	88%	**75%**
R302A	LVALYLAAALSWNQV	20%	49%	0	22%	22%	2%	31%	86%	5%	18%	**17%**
L303A	LVALYLAARASWNQV	96%	74%	49%	108%	108%	79%	66%	78%	209%	77%	**78%**
S304A	LVALYLAARLAWNQV	101%	109%	83%	106%	92%	121%	92%	68%	233%	99%	**97%**
W305A	LVALYLAARLSANQV	70%	114%	72%	102%	124%	54%	116%	59%	305%	178%	**106%**

Gray represents <10%

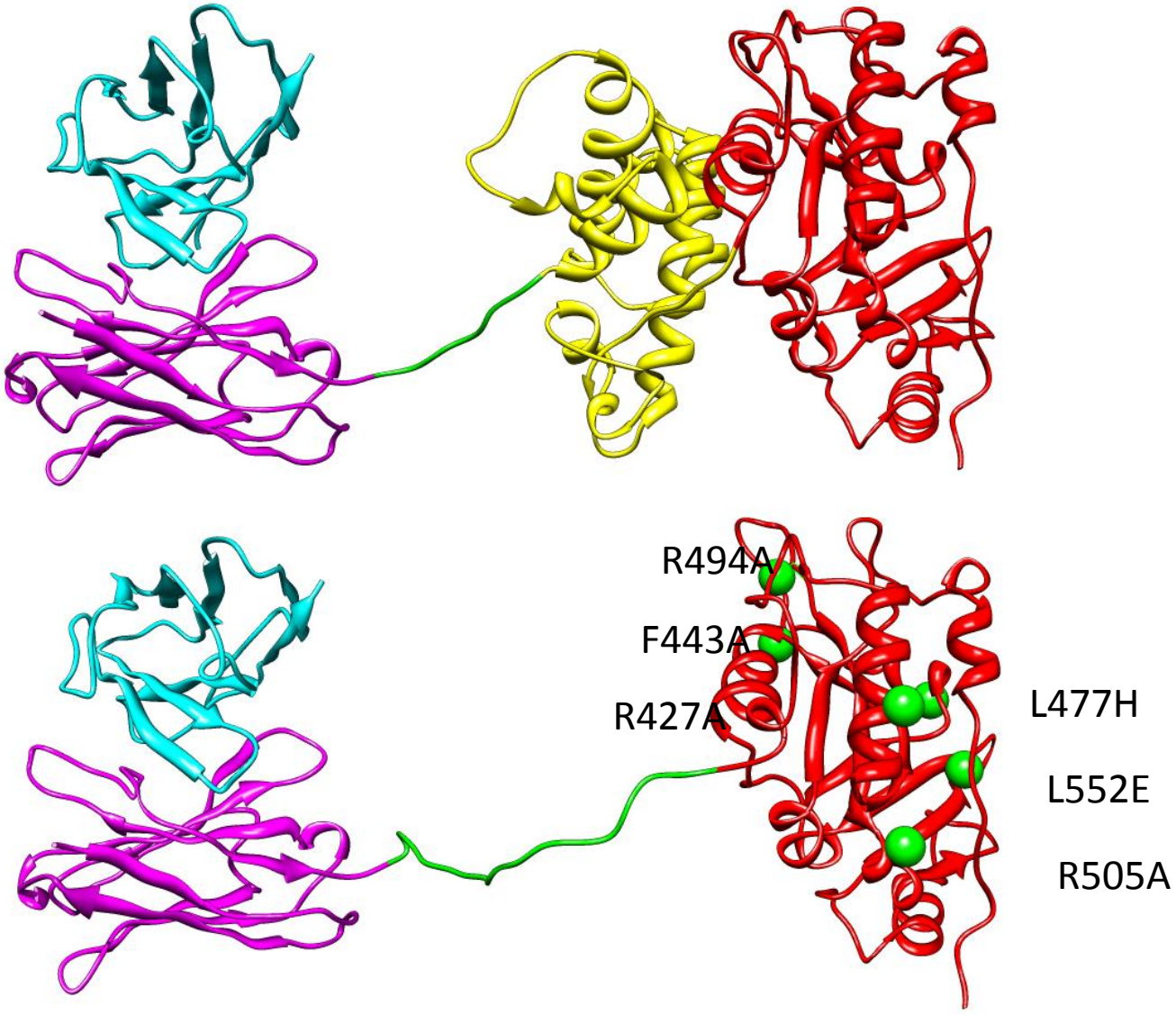
A few different single mutations diminish the response to peptide15

Mazor et al. 2012

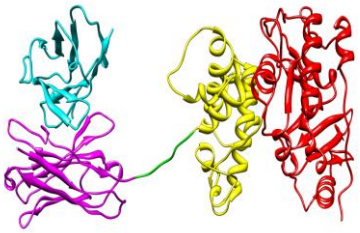
Structures of SS1P and LMB-T20 targeting Mesothelin

SS1P

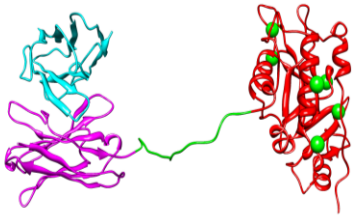
LMB-T20



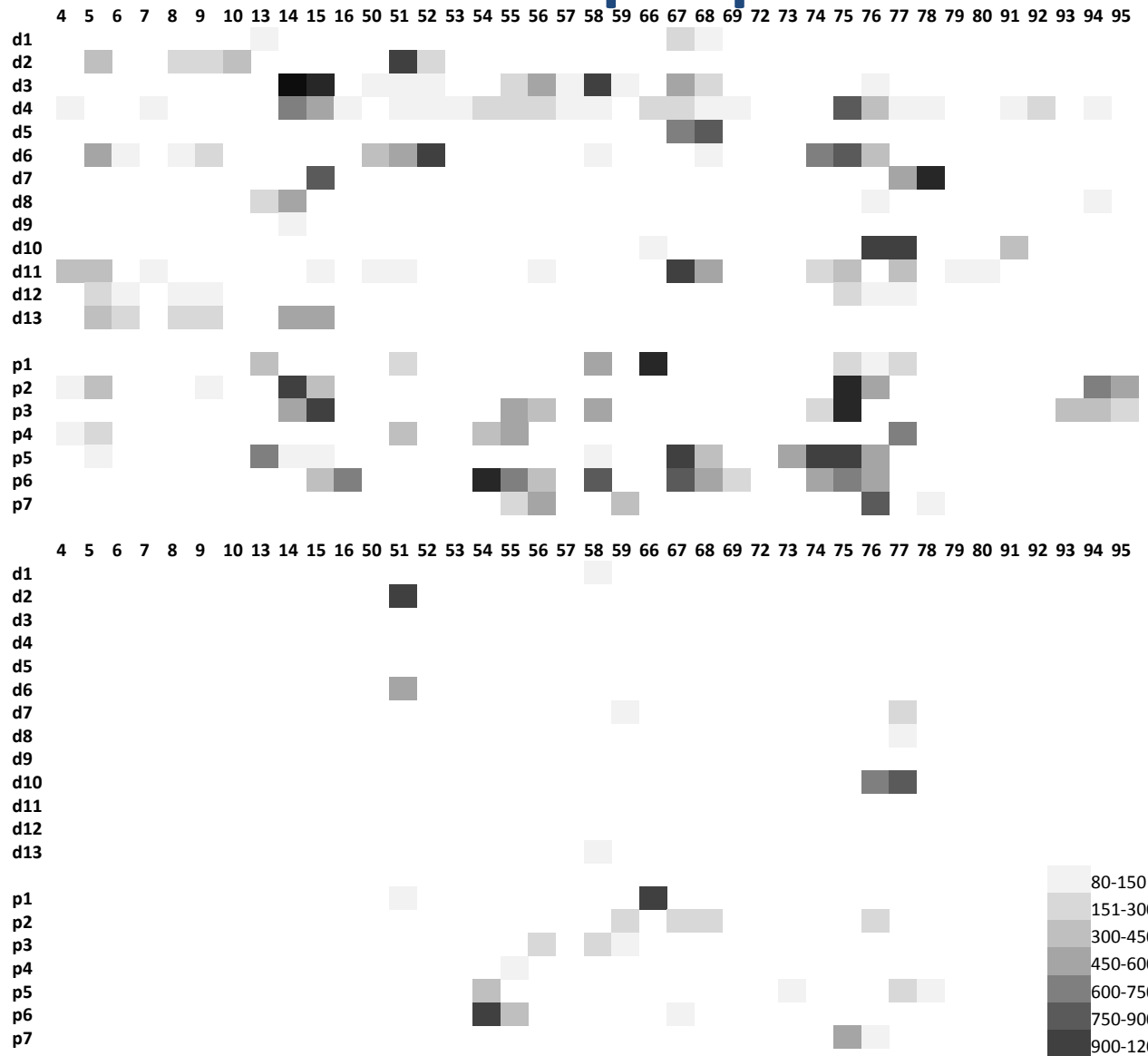
T20 has greatly diminished T-cell activation and no new T-cell epitopes



MP



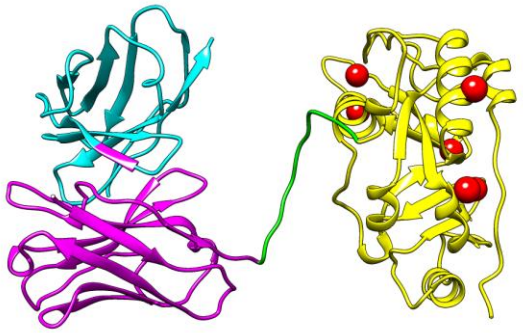
T20



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

LMB-T20 (anti- Mesothelin)



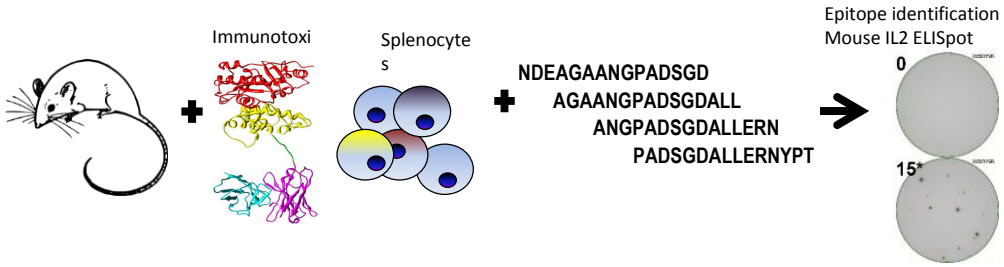
Immunotoxin Cell line	Cancer type	IC_{50} (pM) SS1P	LMB-T20
HAY	Mesothelioma	13	2
Patient RH29	Mesothelioma	635	4
KLM1	Pancreas	62	10
MKN 28	Stomach	21	12

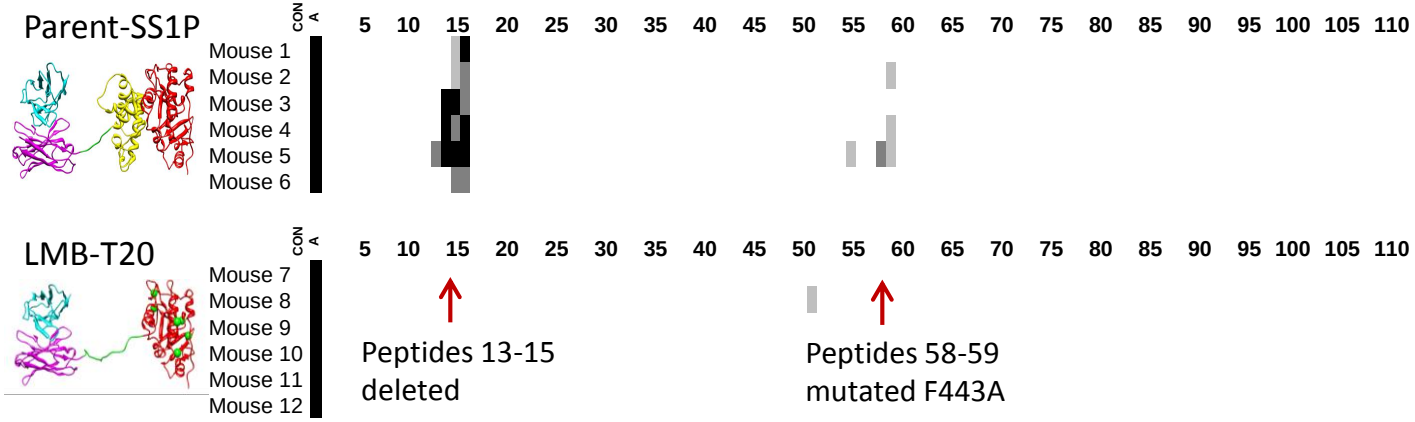
Mazor et al. 2015 MCT

Proof of Concept

Is the elimination of T cell epitopes sufficient to prevent ADA in a mouse?

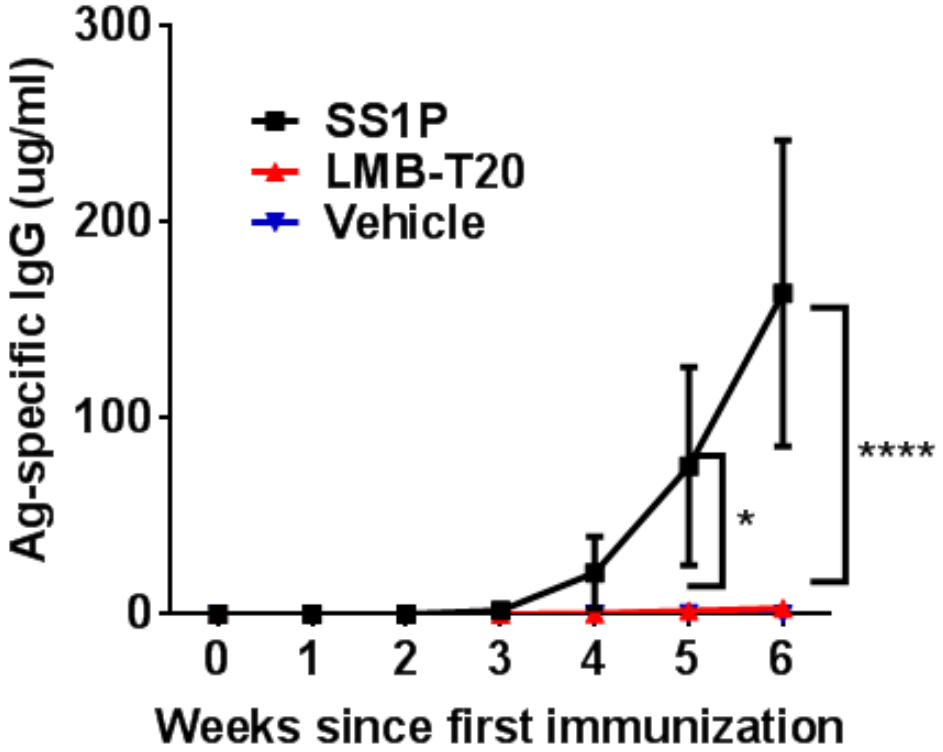
T cell epitope mapping of PE38 peptides in BALB/c identifies two epitopes





Mazor et al. 2015 Cell Mol Immunol

Immunization with LMB-T20 does not induce formation of Ag-specific IgG

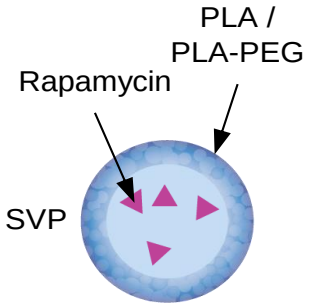


Not Yet In the Clinic

Collaboration with Selecta Bioscience

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

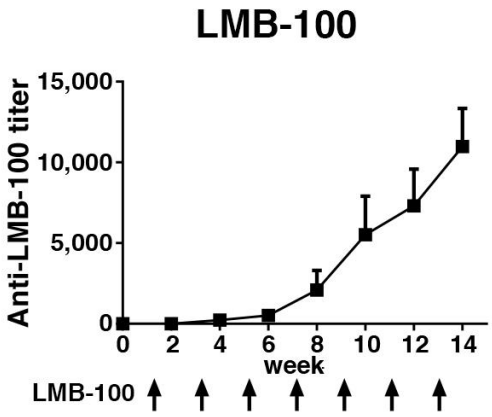
- poly(lactidecoglycolide) (PLGA)
- Taken up by APC
- ContainRapamycin
- Successfully prevented immunogenicity of: Factor VIII, pegsiticase, and adalimumab in mice, rats and monkeys
- SVP-Rapamycin + pegsiticase currently in Phase 2 clinical trials

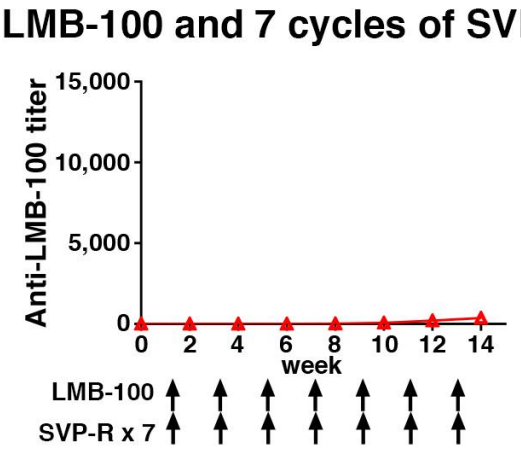


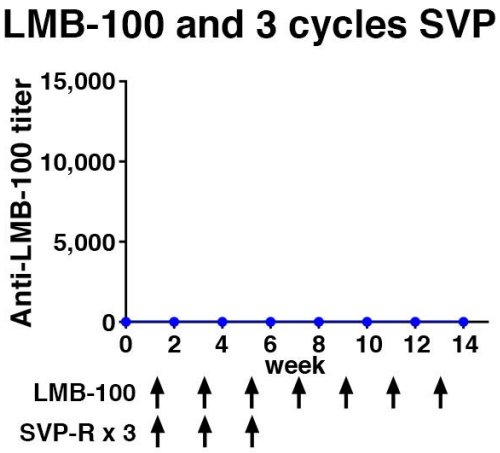
Clinical Trial

- 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