Immunotoxin Therapy of Cancer
Successes and Challenges

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Background

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.

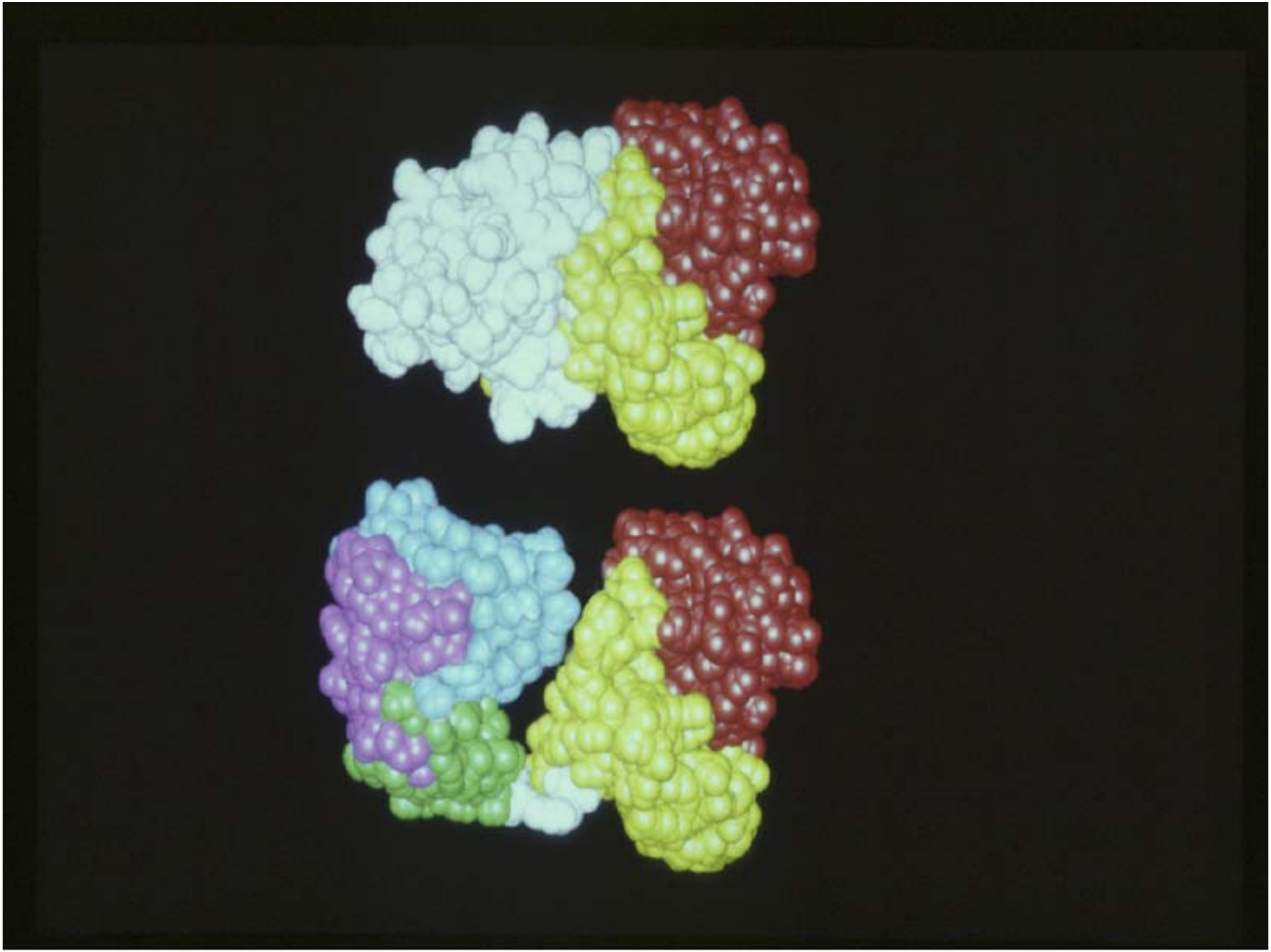
CD22 is an excellent example.

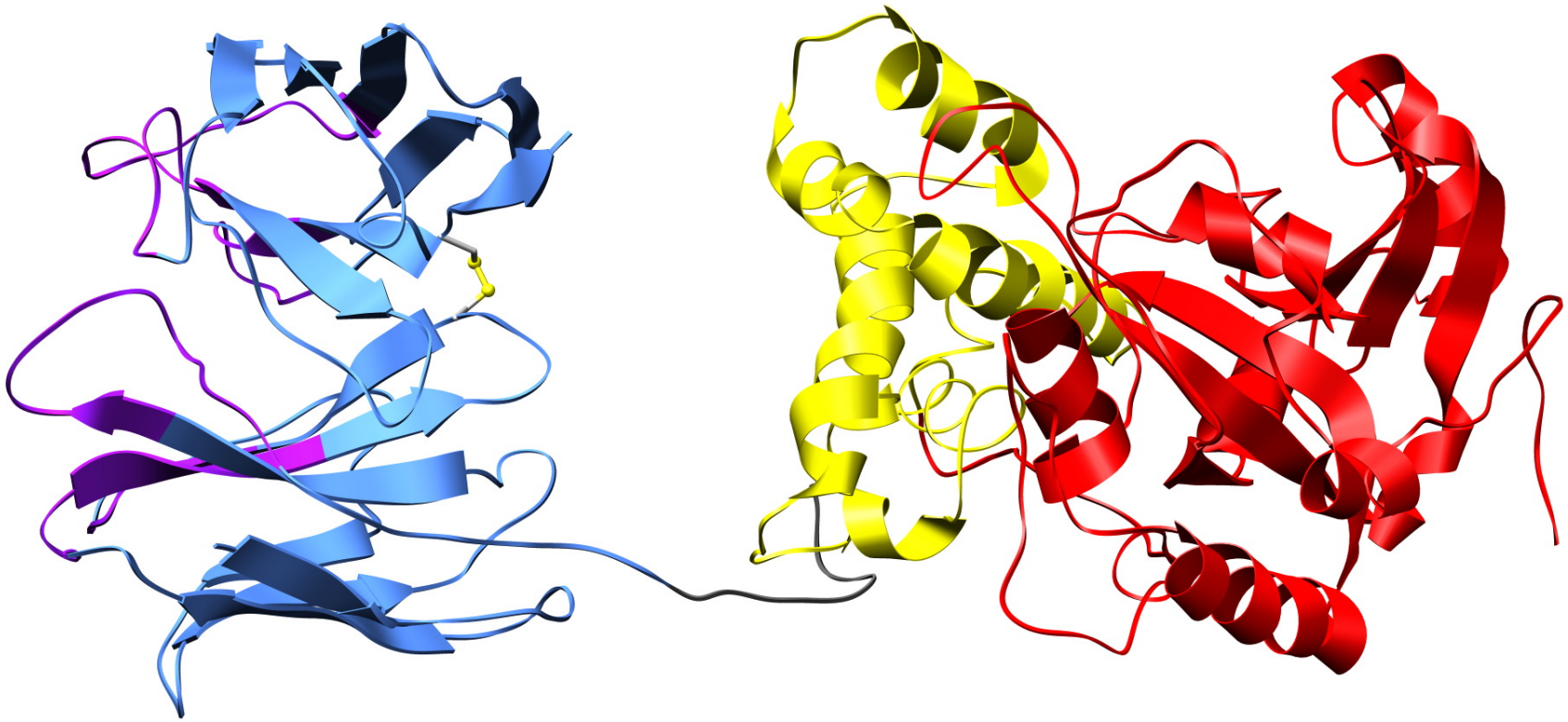
PROPERTIES OF TOXINS

- 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.**

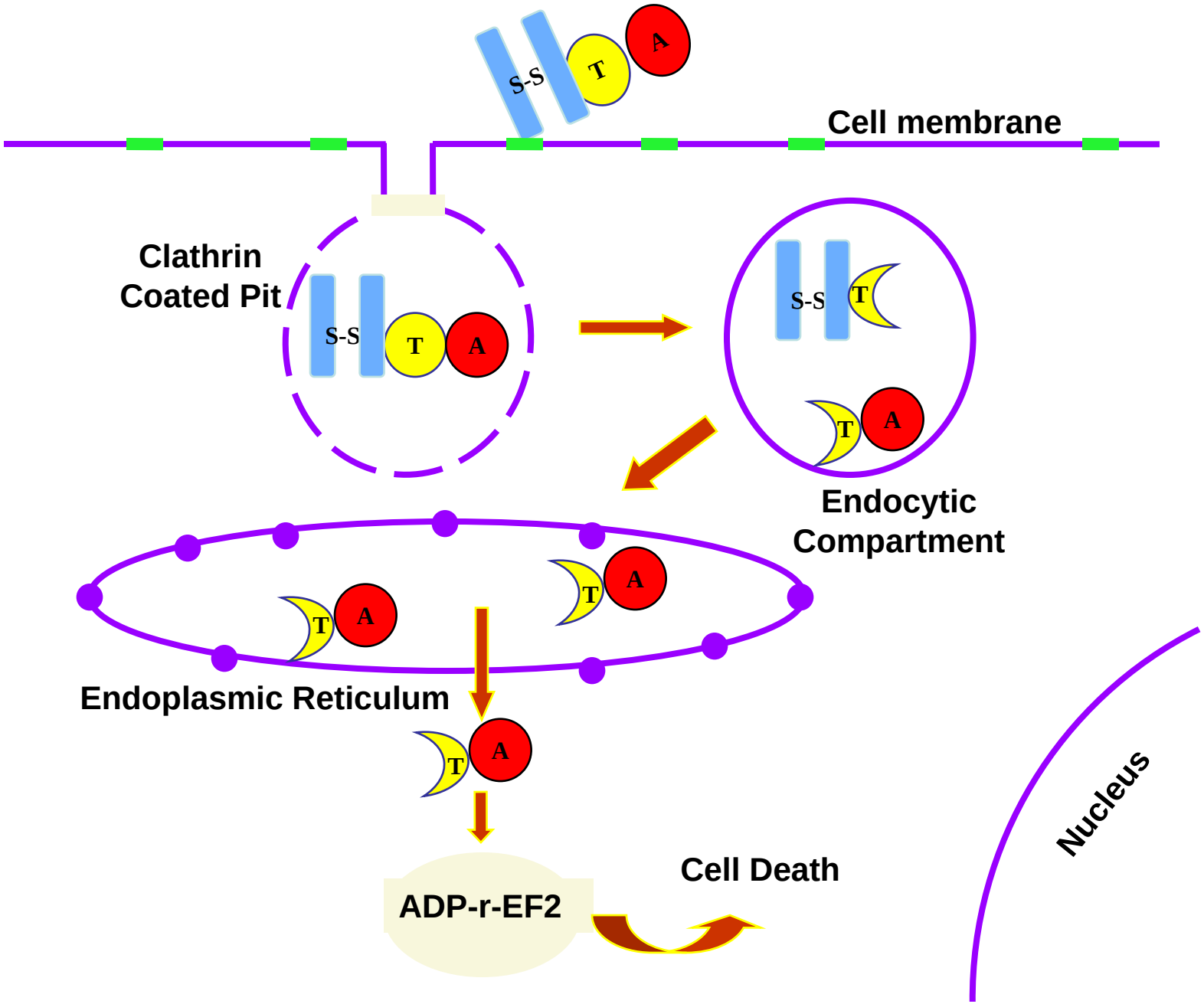




Fv

PE38

Mechanism of Immunotoxin Killing



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 - $4x10^4$ sites/cell**
- **99% of B-CLL - $1x10^3$ sites/cell**
- **70-85% of NHL - ?**
- **>90% of B-ALL - $5x10^3$ per cell**

HAIRY CELL LEUKEMIA

B-cell leukemia

2% of all Leukemias

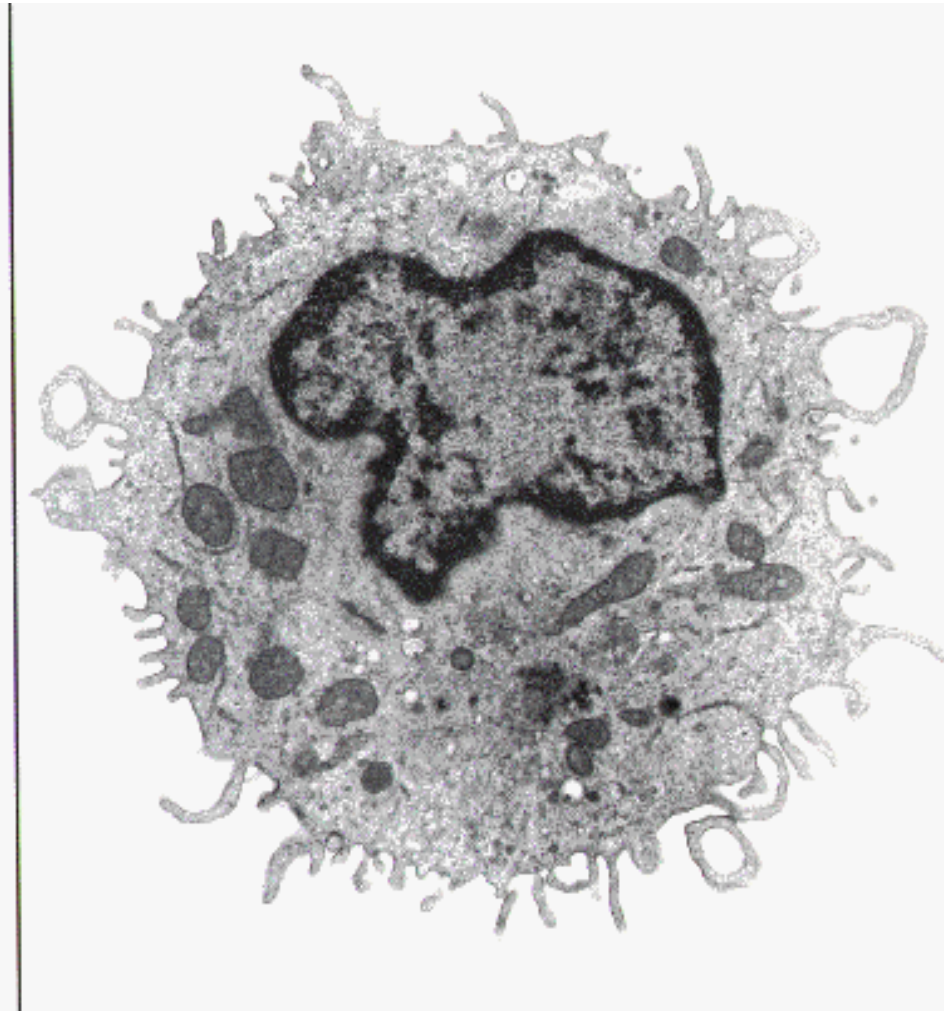
Pancytopenia, Splenomegaly

Purine analogs (CdA, DCF)

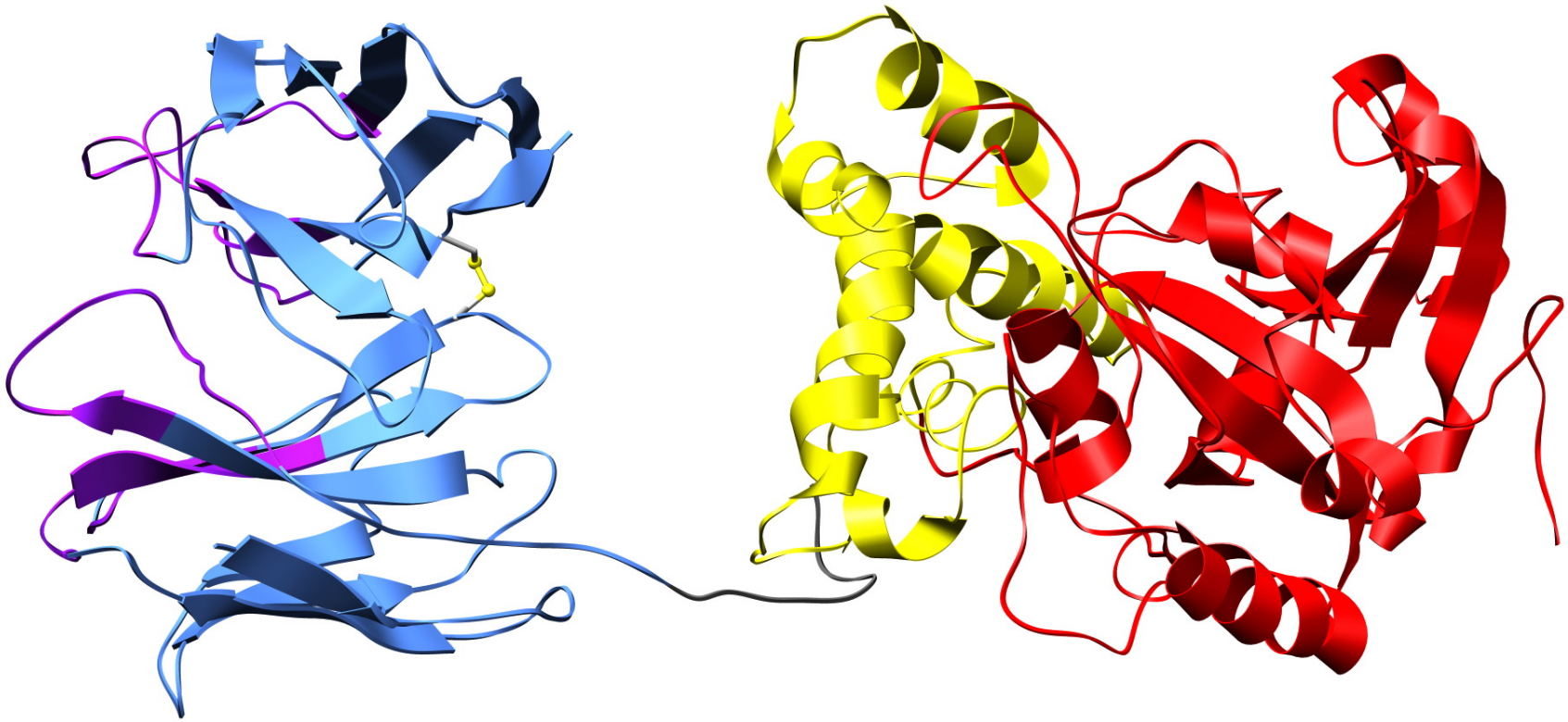
- **High response rates**
- **Not curative**
- **Patients become resistant**

HCLv (20%) primarily resistant

Very high CD22 expression



BL22 (CAT 3888) Structure



Anti-CD22

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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,

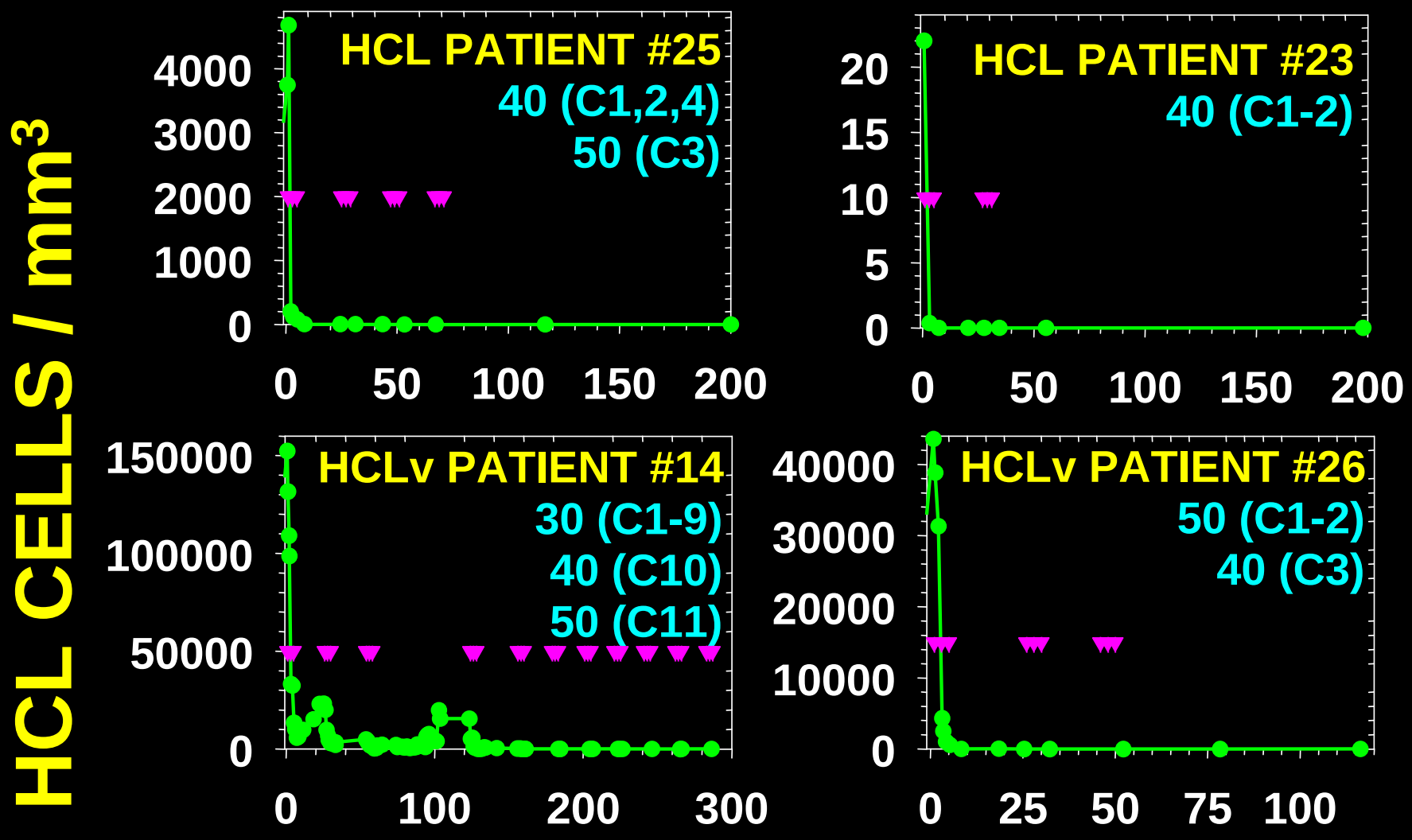
Retreat: **Every 21 days.**
No progressive disease
No antibodies to BL22

Patients Treated in Phase 1 Trial

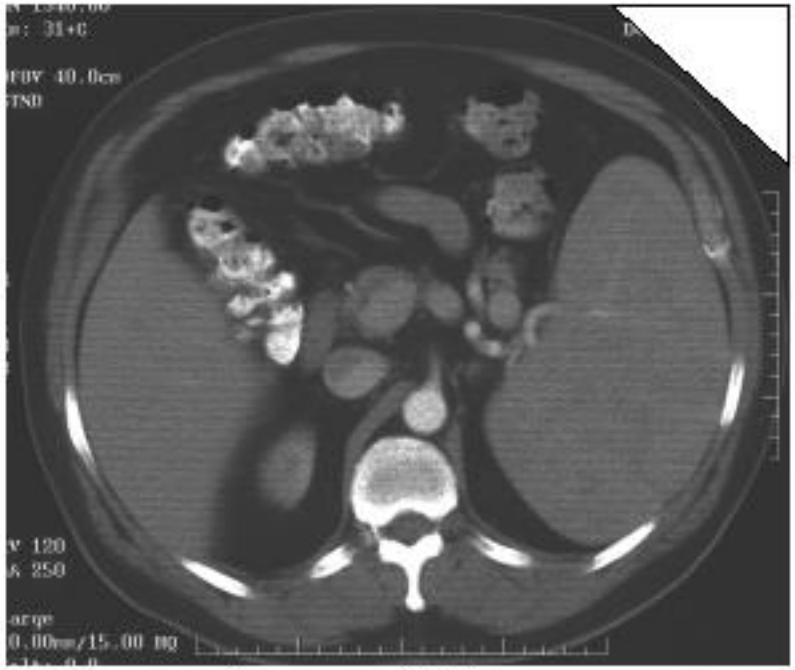
Non-Hodgkin's Lymphoma	**4**
Chronic Lymphocytic Leukemia	**11**
Hairy Cell Leukemia	**31**
Total Phase I Patients Treated	**46**

Total Cycles = 249

CR OF HCL OR HCL-v TO BL22



D-3



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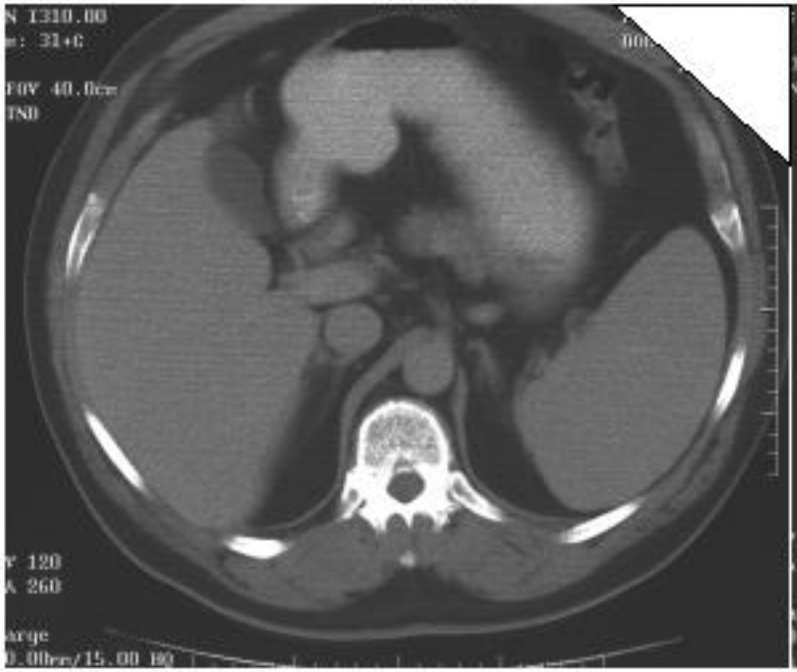

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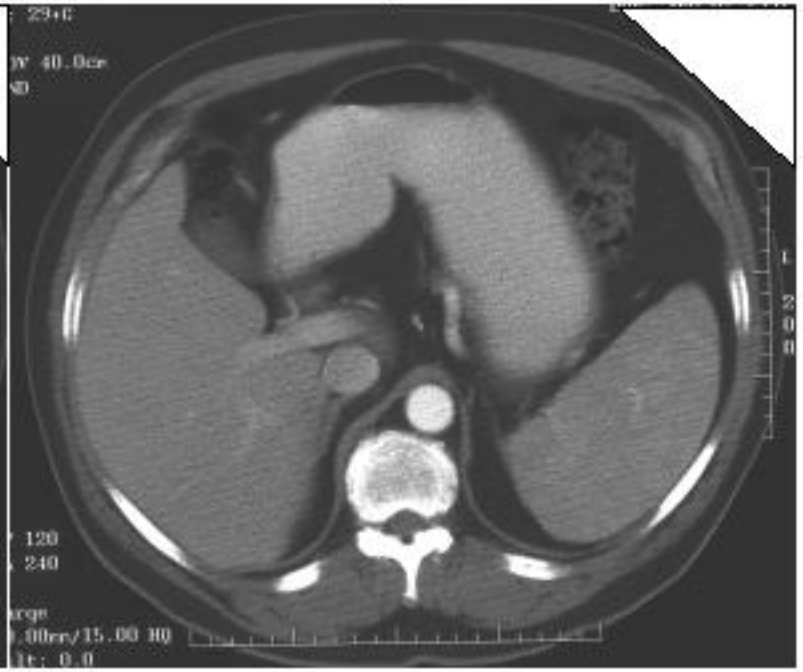
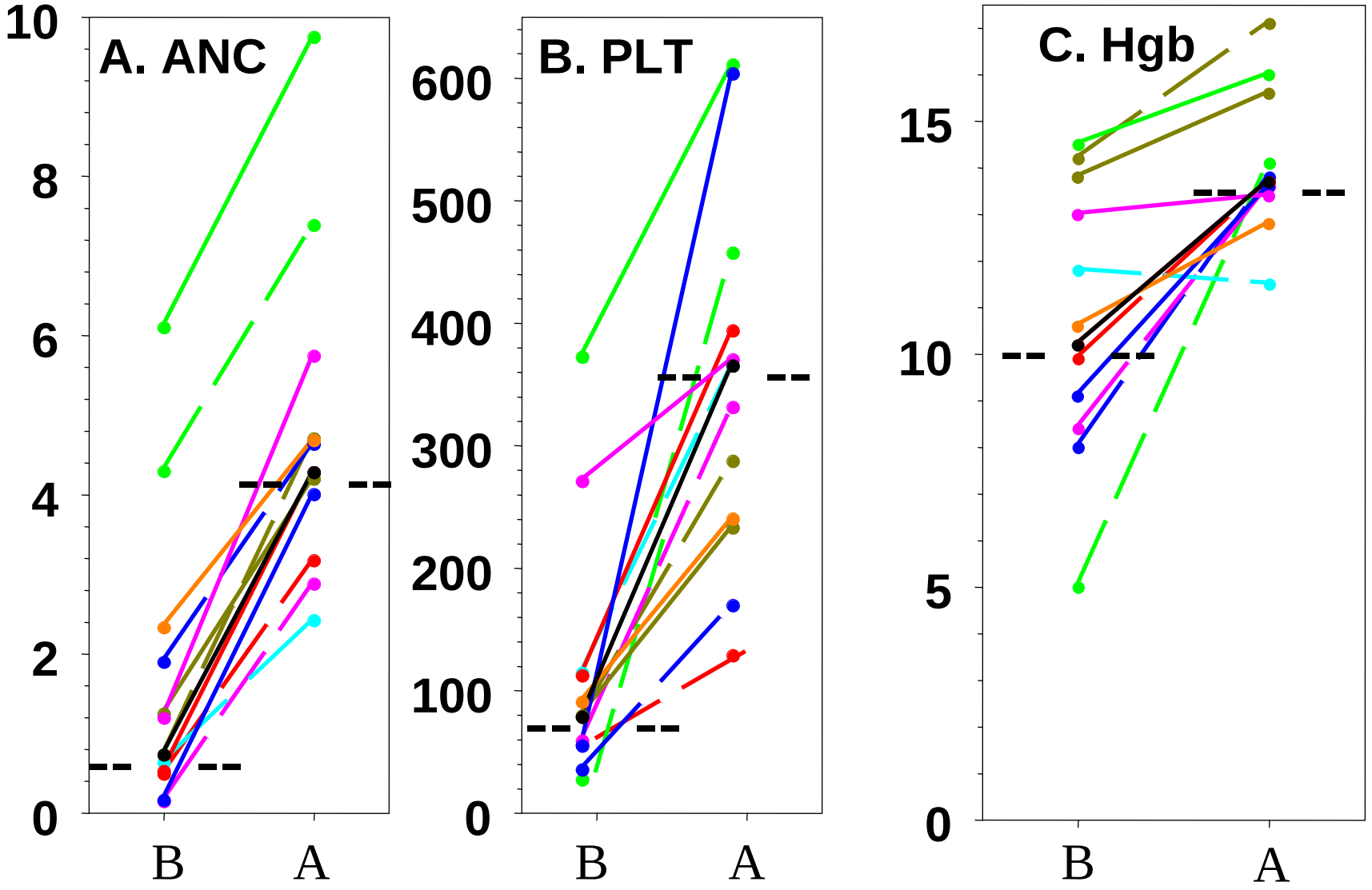




Blood Counts Before and After CAT-3888 Treatment

BL22 (CAT-3888) Phase I Study Summary

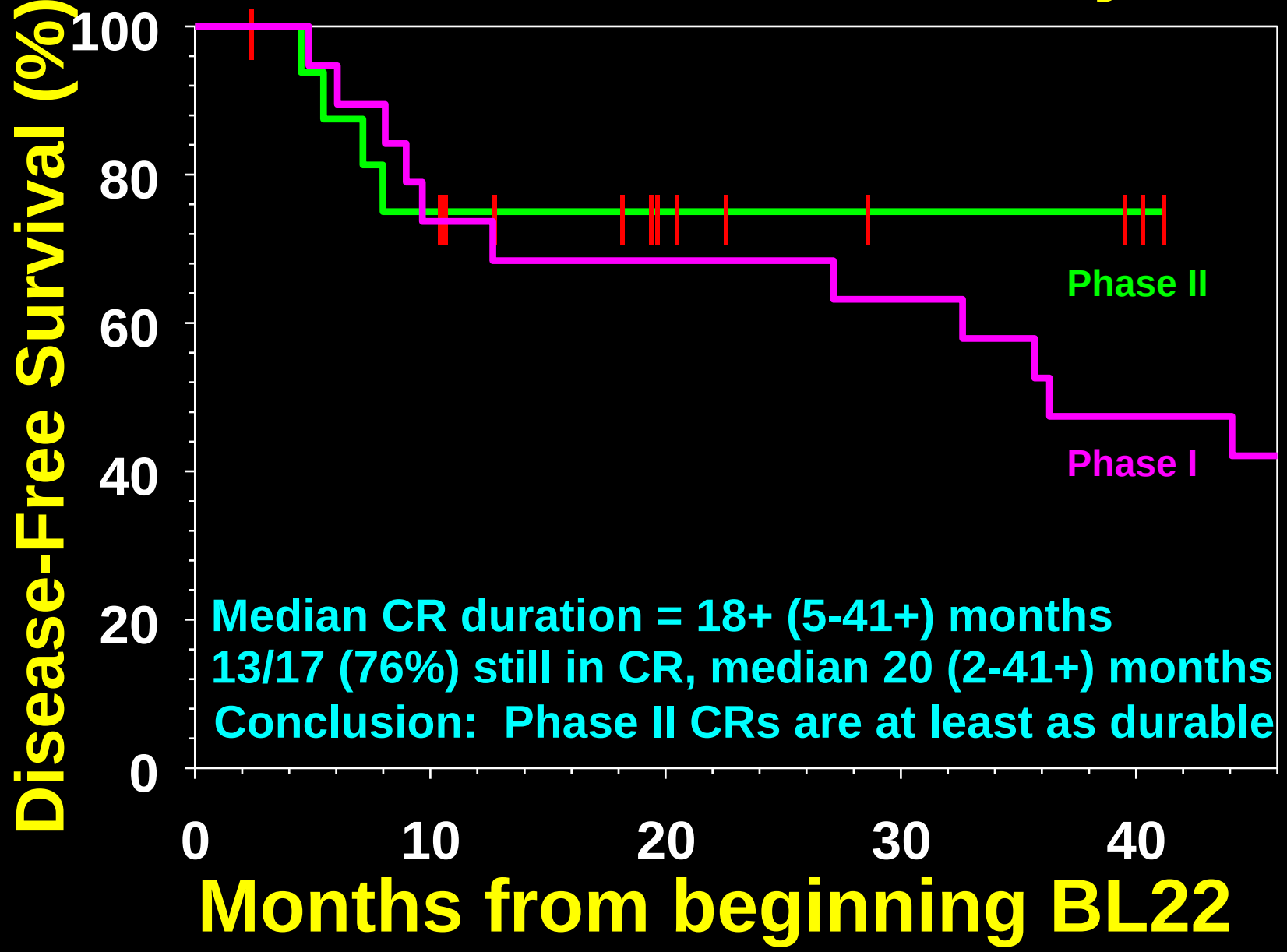
Tumor responses in HCL (n= 31 patients)

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**

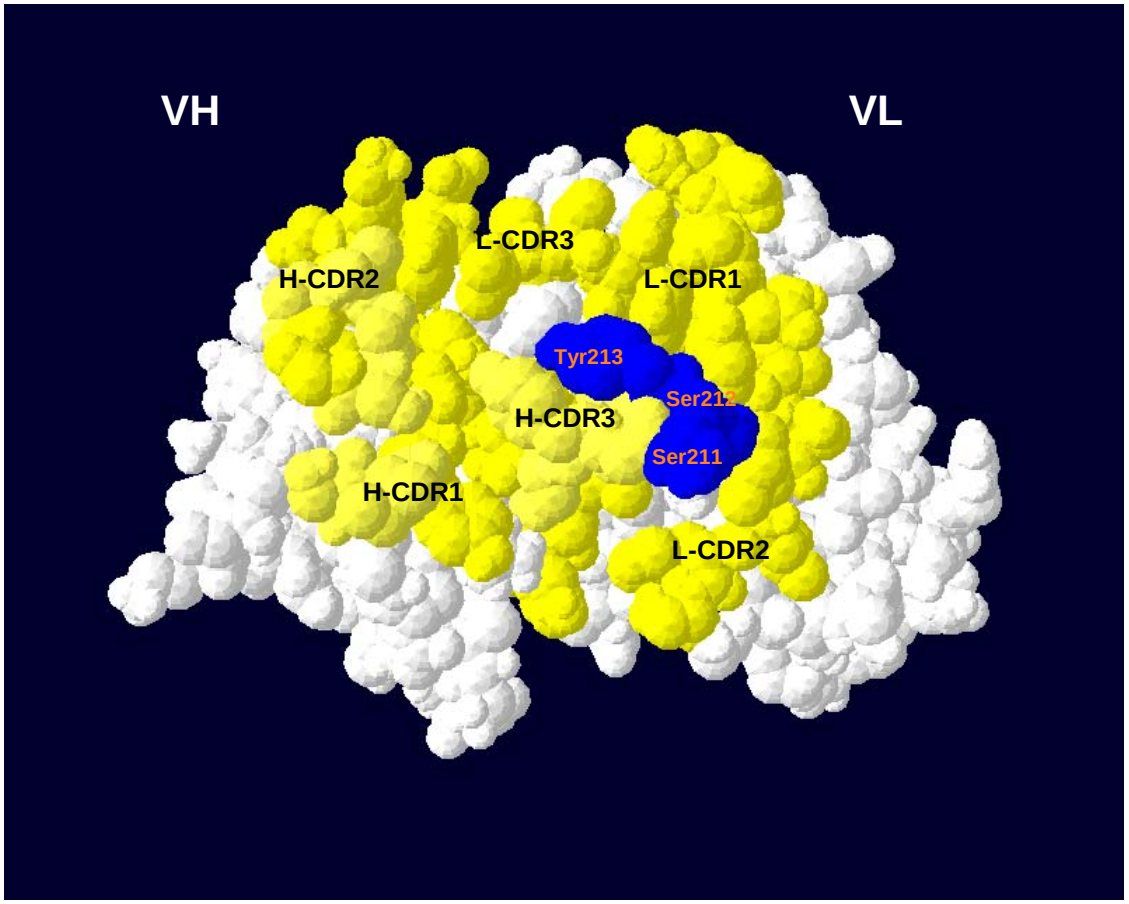
Phase II CR Durability



Reality Check

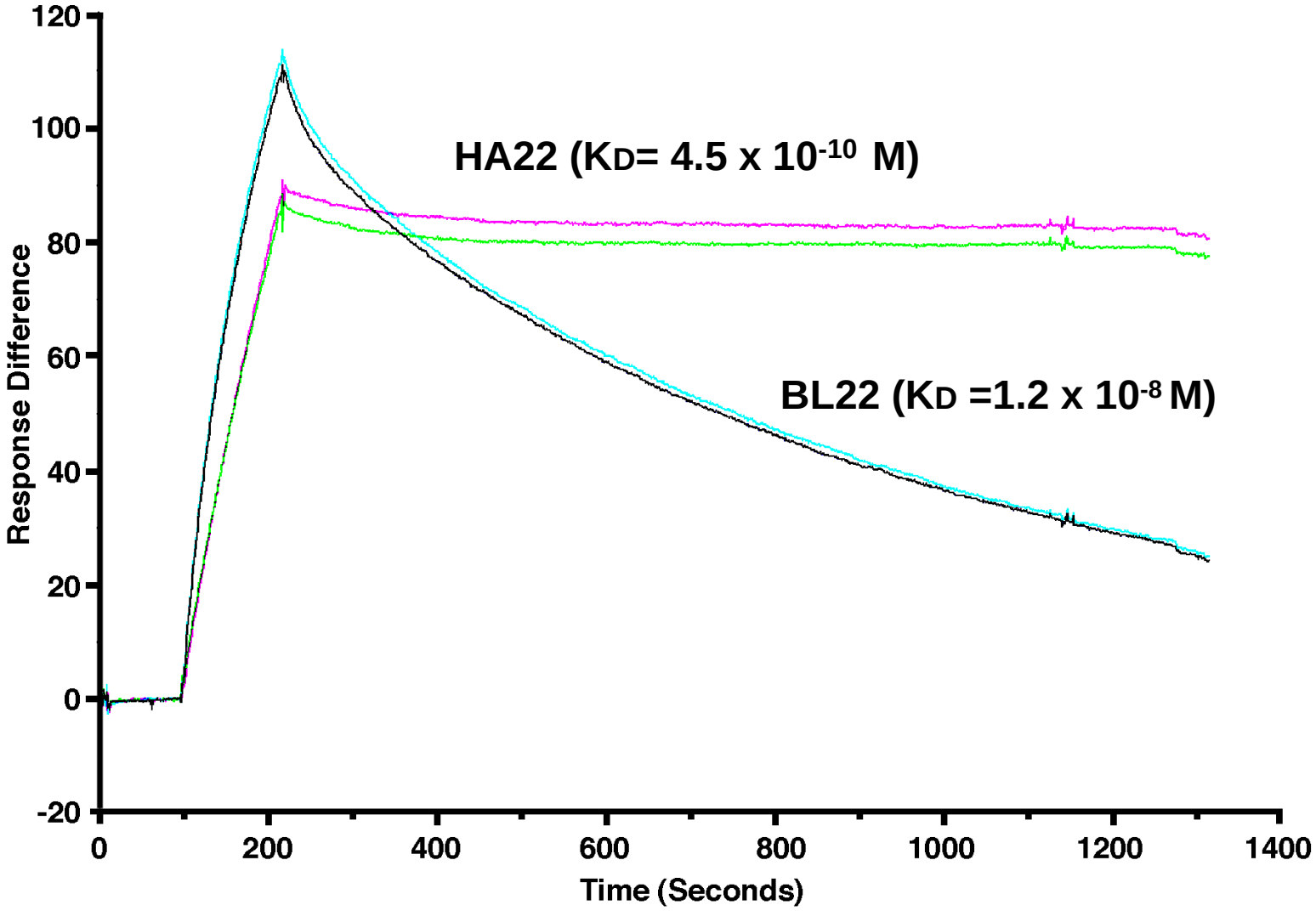
- HCL is a rare disease, 1000 new cases yearly.
- Market is small.
- CD22 also expressed on CLL, NHL, ALL.
- But these malignancies have lower CD22:
  HCL $4x10^4$, CLL $1\text{-}2x10^3$, ALL $5x10^3$, NHL?
  Need to increase activity.
- This can be done by increasing affinity.

Fv of BL22 showing CDRs in yellow and a hot spot in blue.

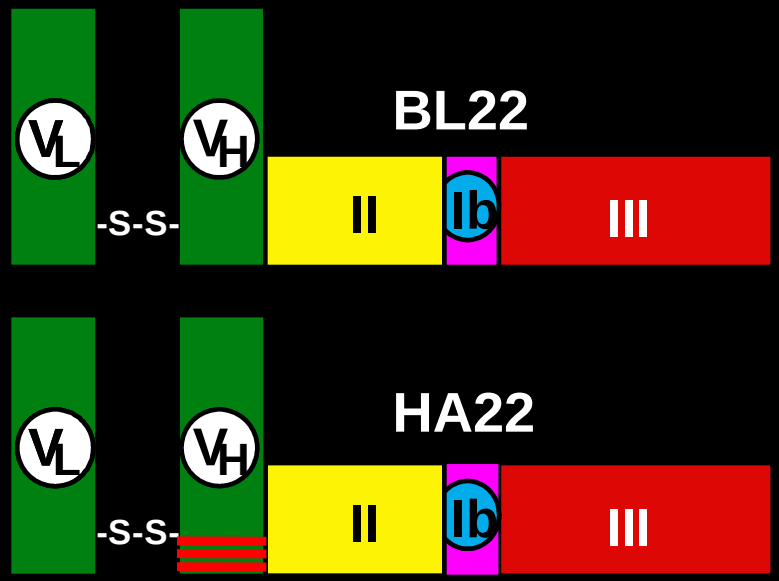


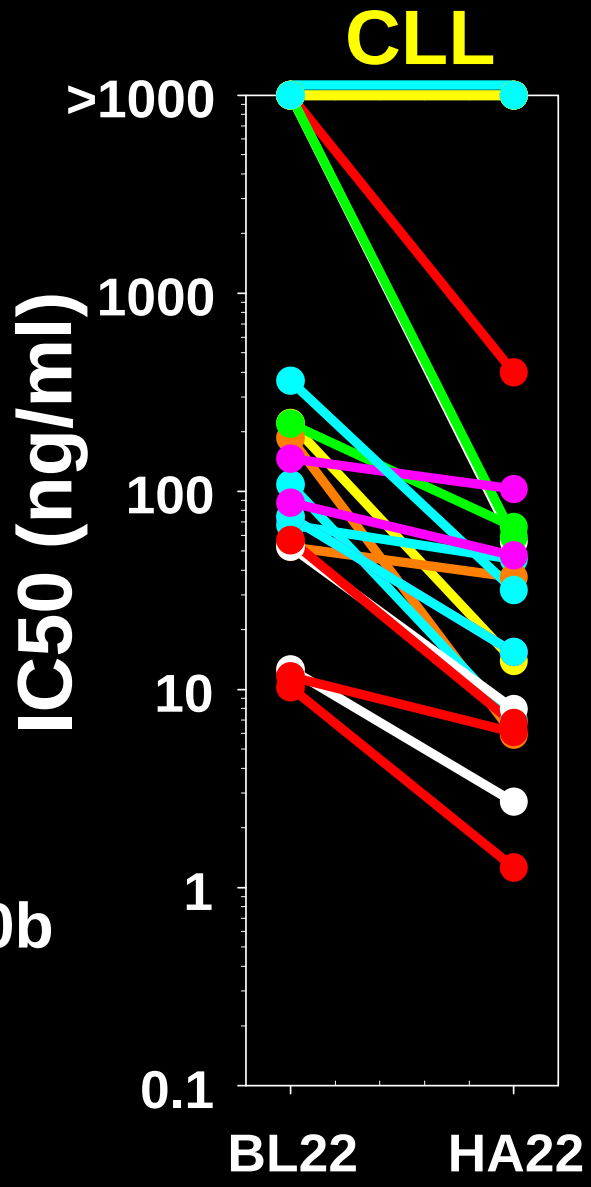
The SSY was mutated to THW and an immunotoxin made.

Increased Binding of Mutant immunotoxin HA22 (CAT-8015) to CD22-Ig Coated BIAcore Chips

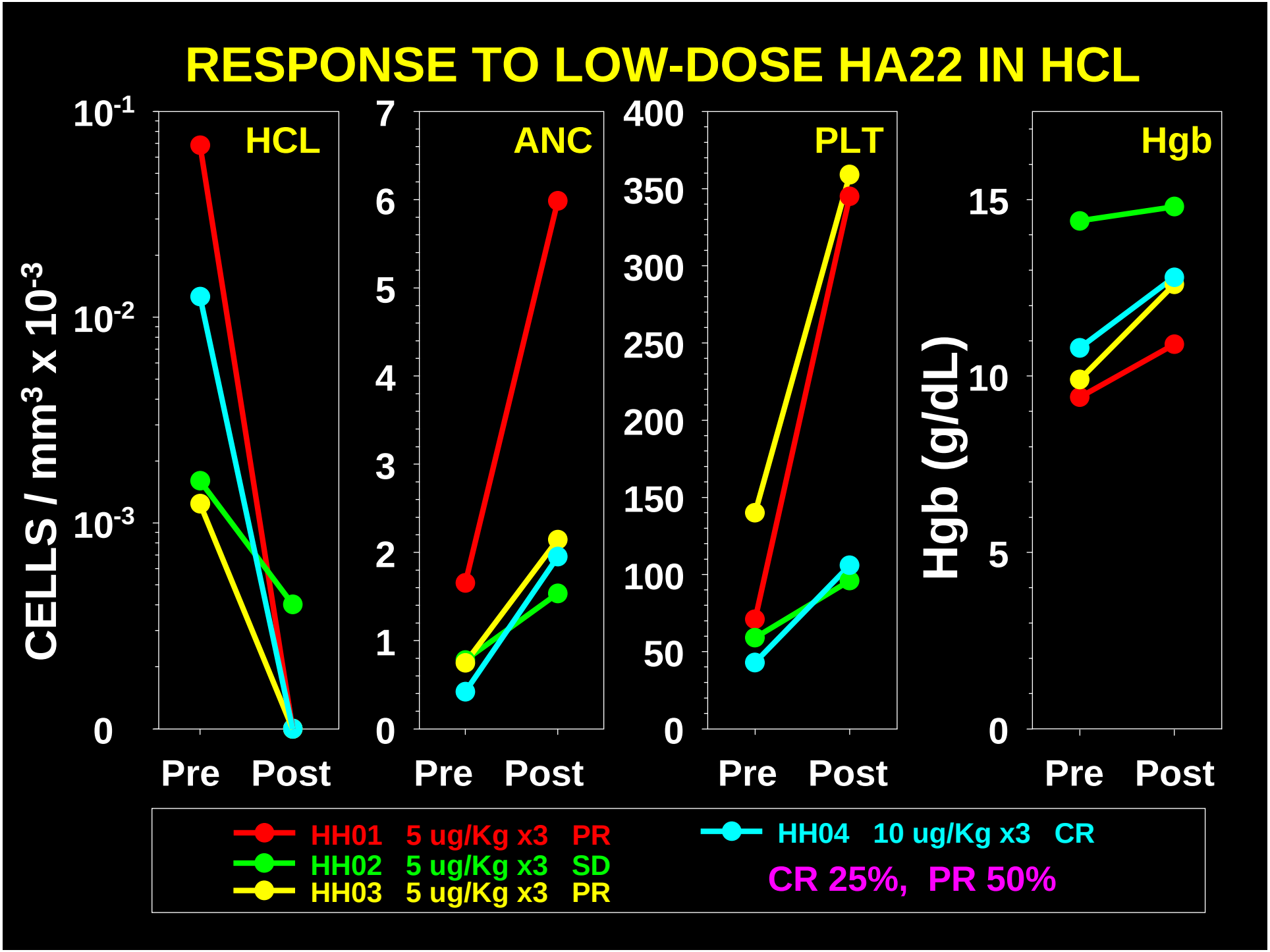


HA22 (CAT-8015): High affinity mutant of BL22

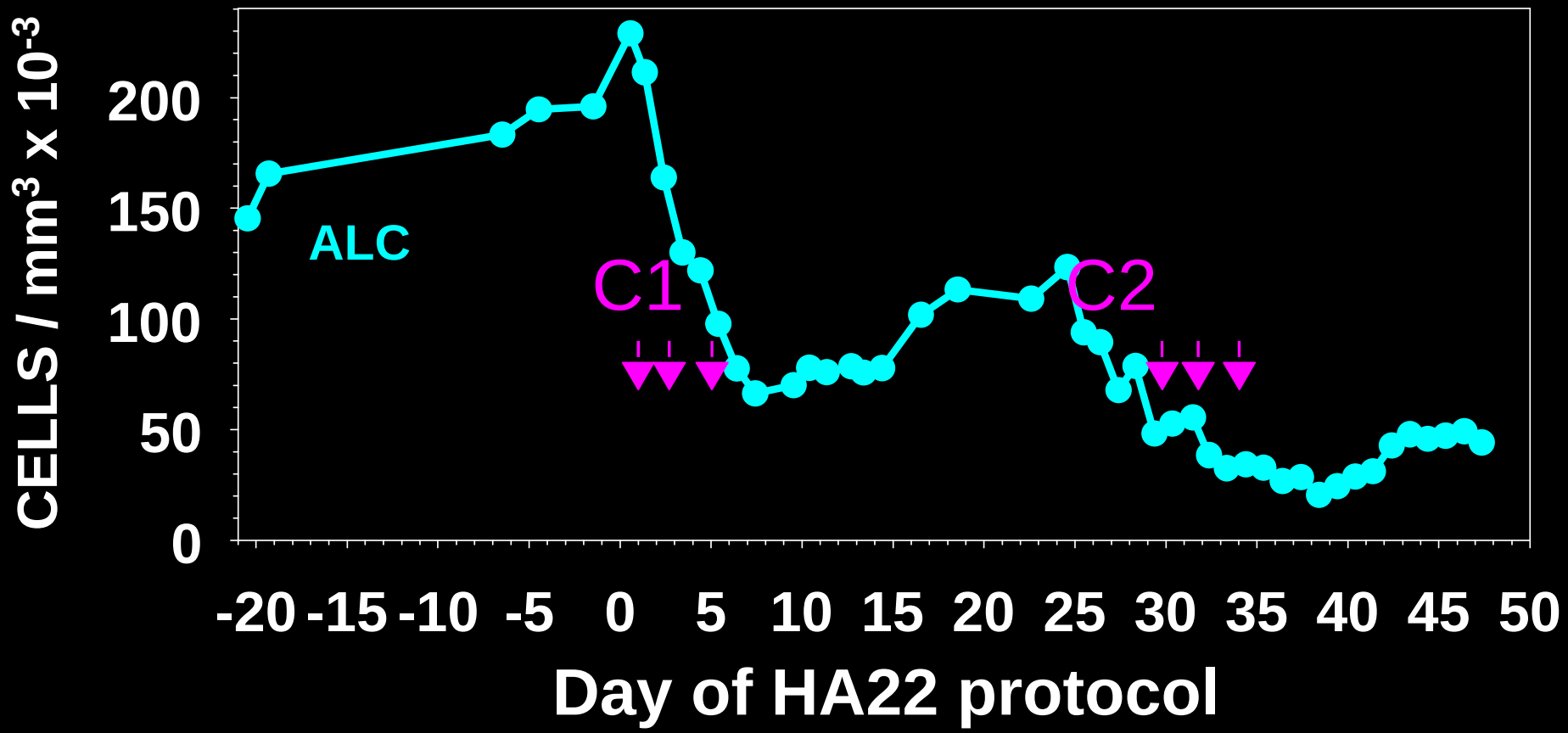




- Mutation: SSY → THW at 100, 100a, 100b
- Median cytotoxicity in CLL ↑ 5-fold
- Phase I Studies: CLL; HCL; NHL


RESPONSE TO LOW-DOSE HA22 IN HCL
HCL
ANC
PLT
Hgb
CELLS / mm3 x 10-3
Hgb (g/dL)
10-1
10-2
10-3
0
7
6
5
4
3
2
1
0
400
350
300
250
200
150
100
50
0
15
10
5
0
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
CR 25%, PR 50%

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 mutagenisis 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

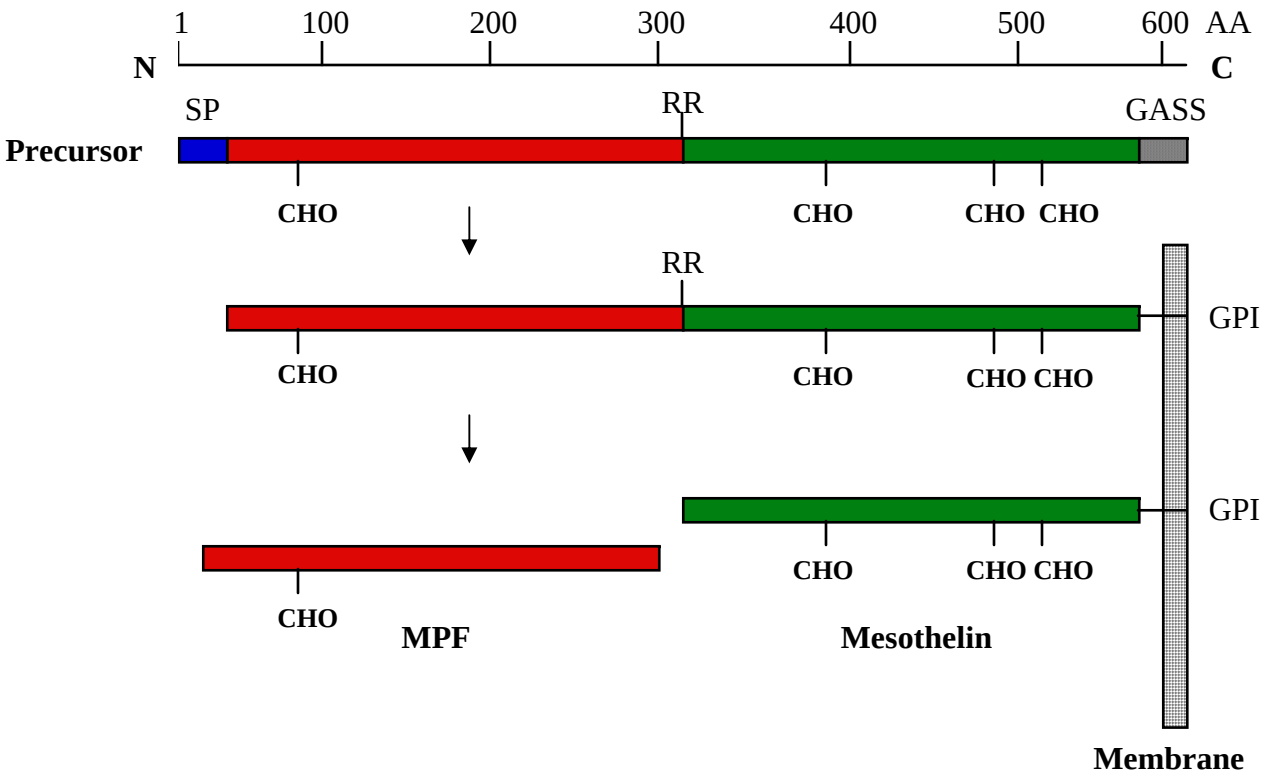
Targeted therapy of mesothelin expressing tumors using the anti-mesothelin immunotoxin

SS1P

Mesothelin

- **Is a 40 kDa PI linked cell surface glycoprotein.**

- **It is a differentiation antigen only present on normal mesothelial cells.**



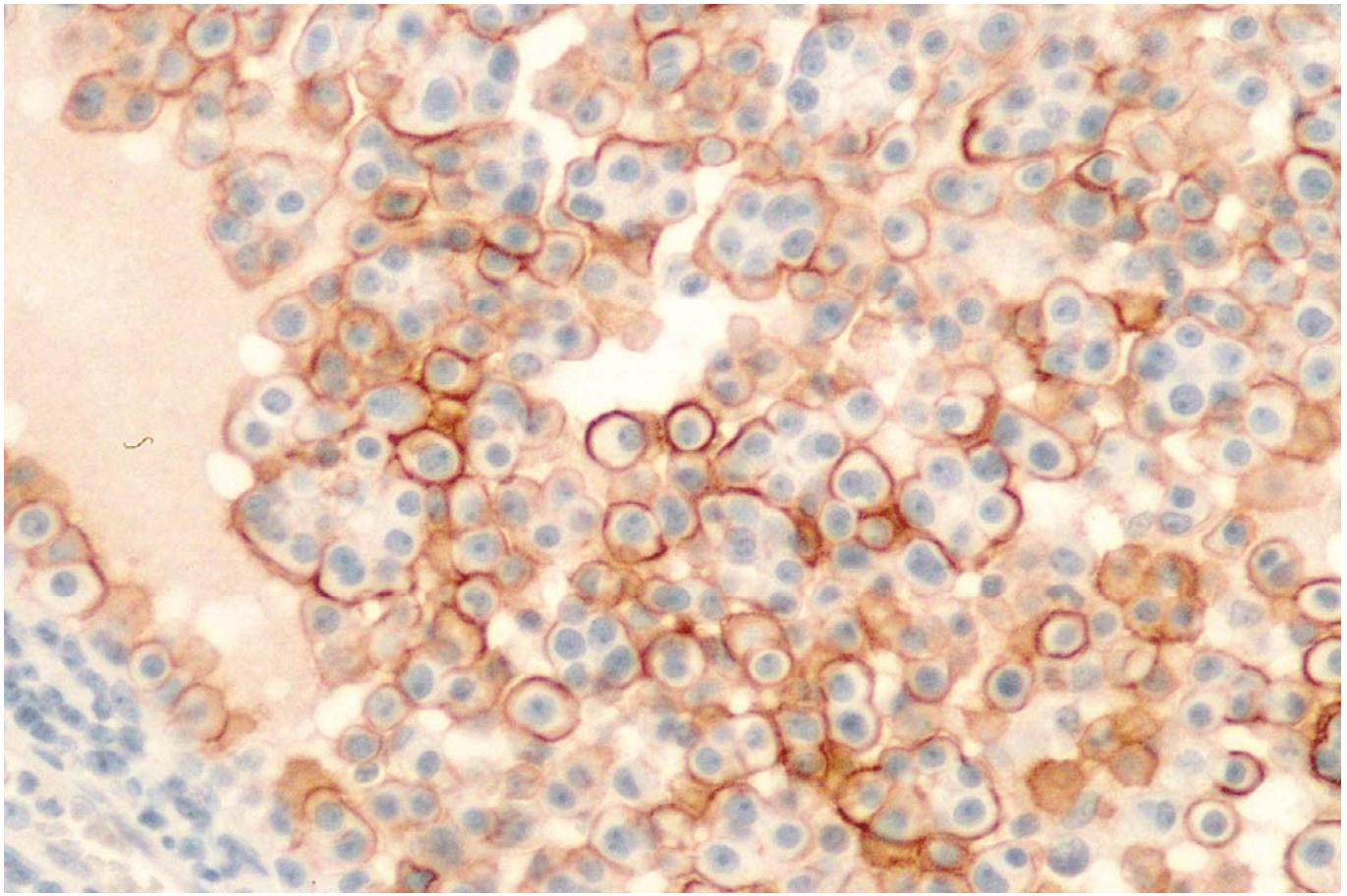
Mesothelin Expression

Normal tissues

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

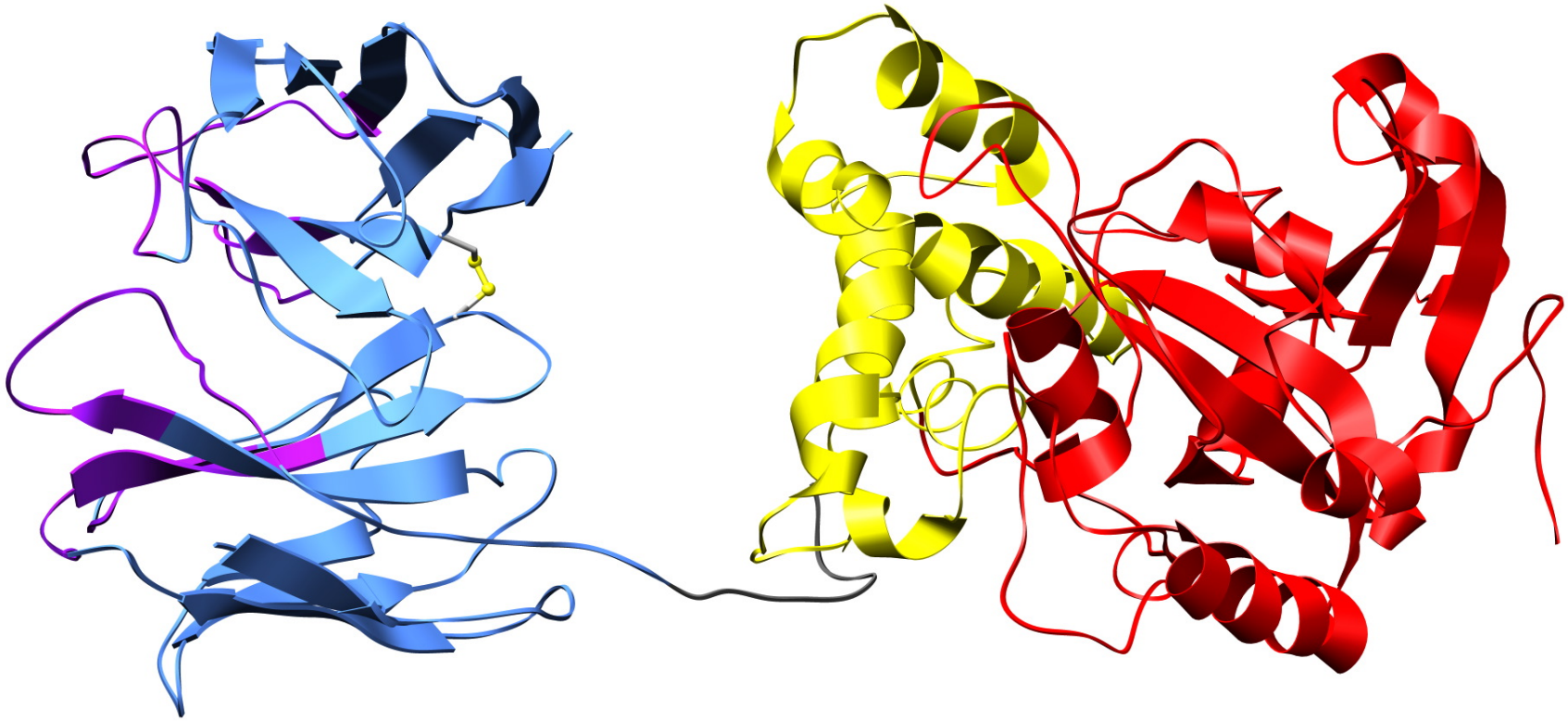
Human tumors

- **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.**

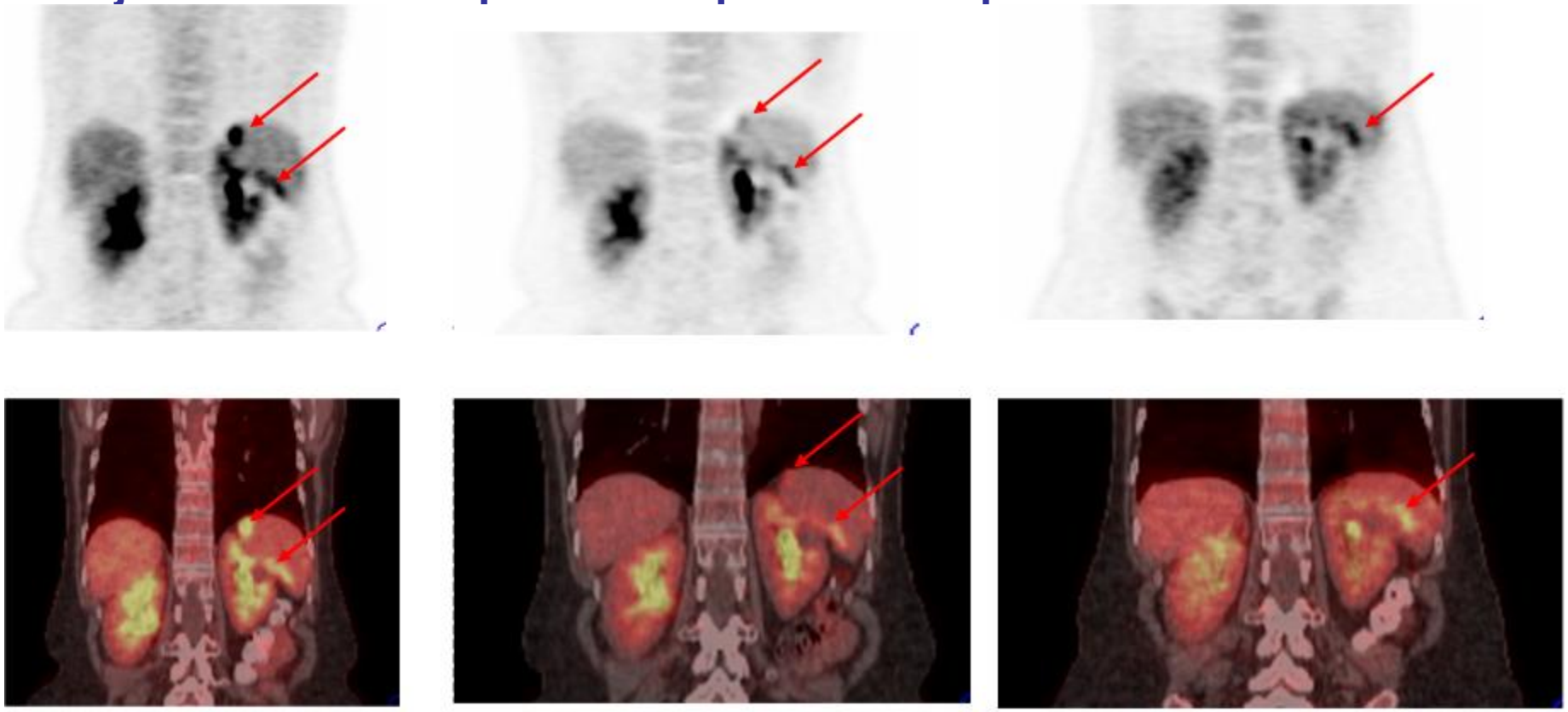
2. **No pericarditis**

SS1P : Tumor Response

Tumor response	Patients (n = 33)
Minor response	4
Resolution of ascites	2
Stable disease	16 (in many patients lasting several months)
Progressive disease	11

Patient 432

Objective tumor response in a patient with peritoneal mesothelioma



Baseline

Post Cycle 1

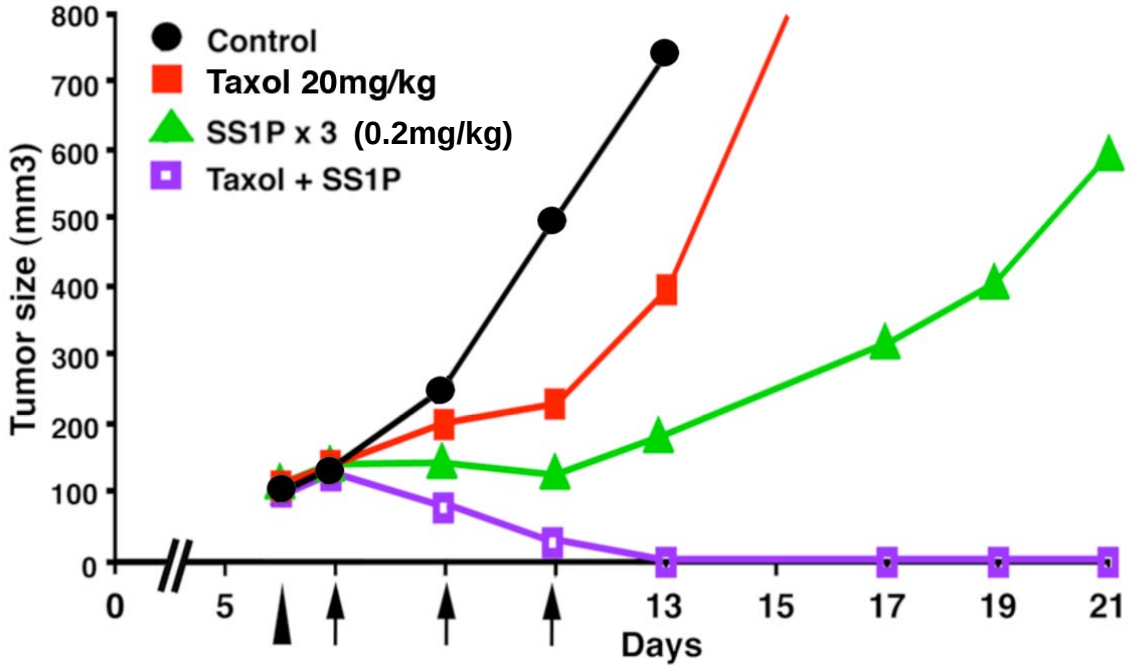
Post Cycle 2

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 ug/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

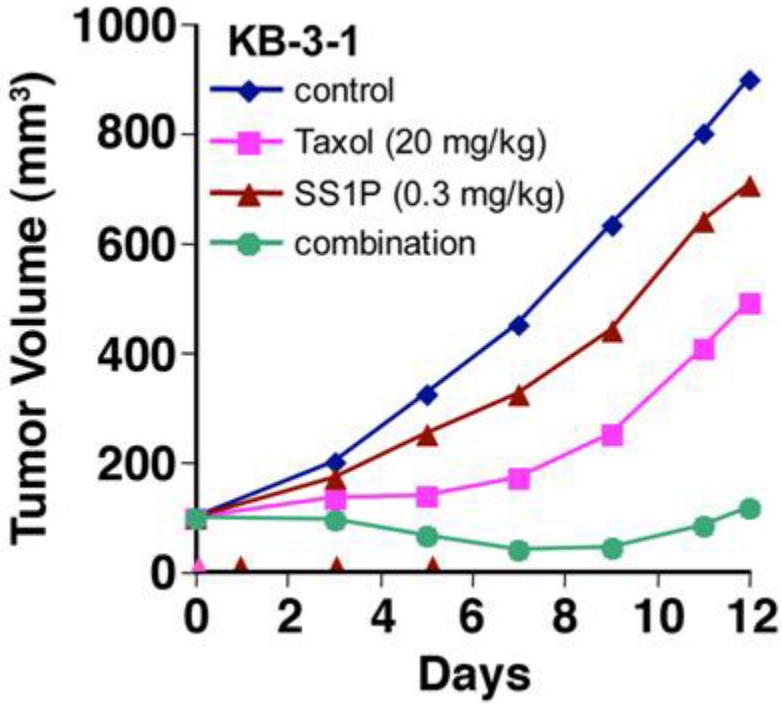
Anti-tumor Synergy when Taxol and Immunotoxin SS1P are Combined



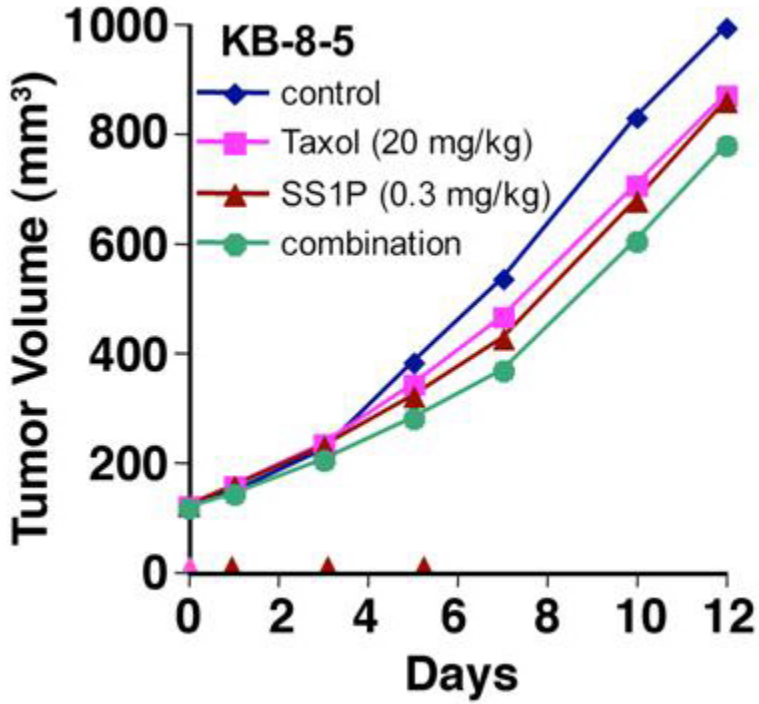
Summary of Synergy Results

Tumor	Immunotoxin	Target	Chemotherapy
A431/K5	SS1P	Mesothelin	Taxol
A431/K5	SS1P	Mesothelin	CDDP
A431/K5	SS1P	Mesothelin	Cytoxan
A431/K5	SS1P	Mesothelin	Gemcitabine
CA46	HA22	CD22	Taxol
CA46	HA22	CD22	Adriamcycin
KB (Hela)	HB21(Fv)PE40	TFR	Taxol
KB (Hela)	SS1P	Mesothelin	Taxol

No Synergy with Taxol Resistant Tumor



Taxol sensitive



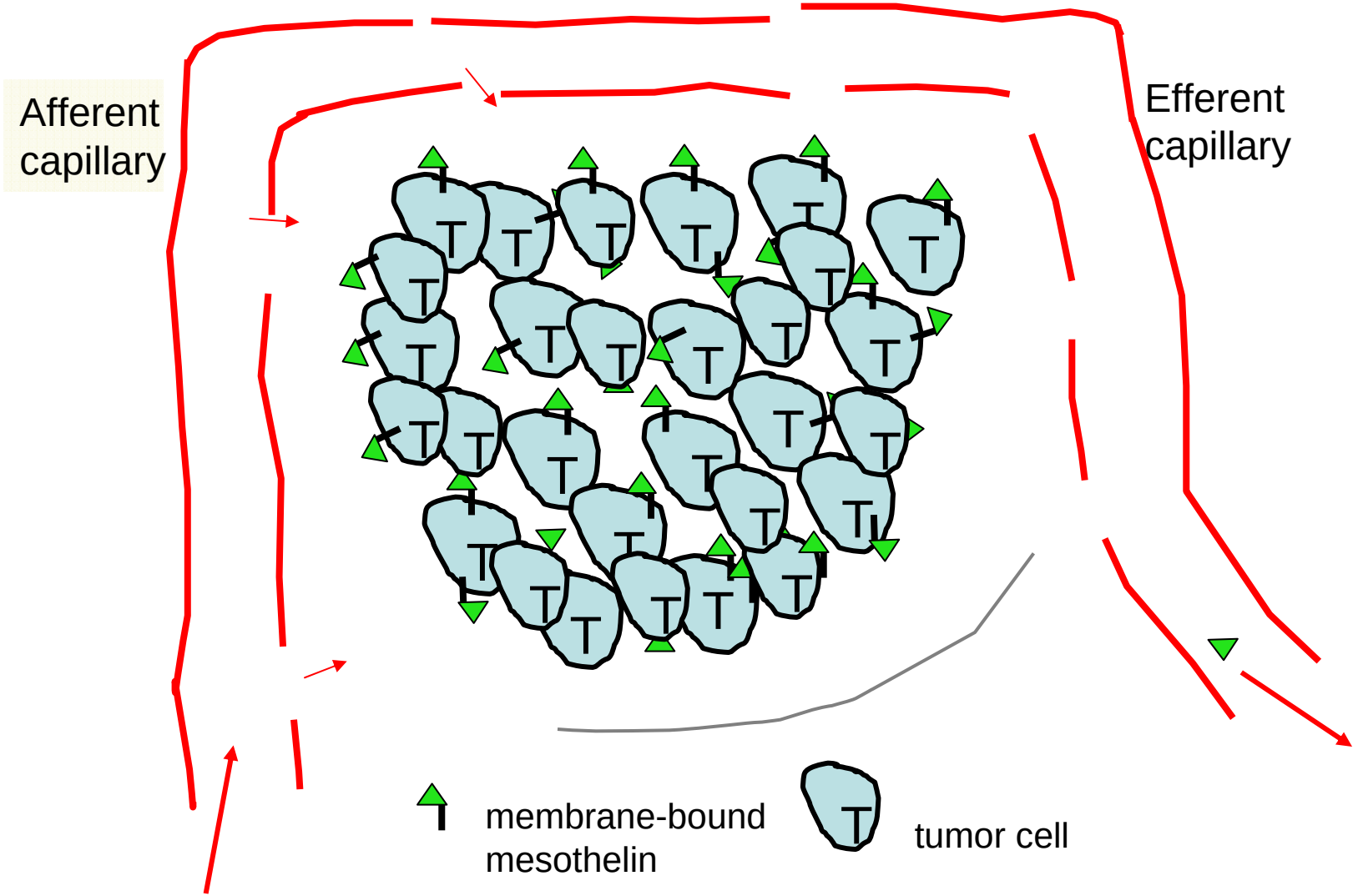
Taxol resistant

Zhang

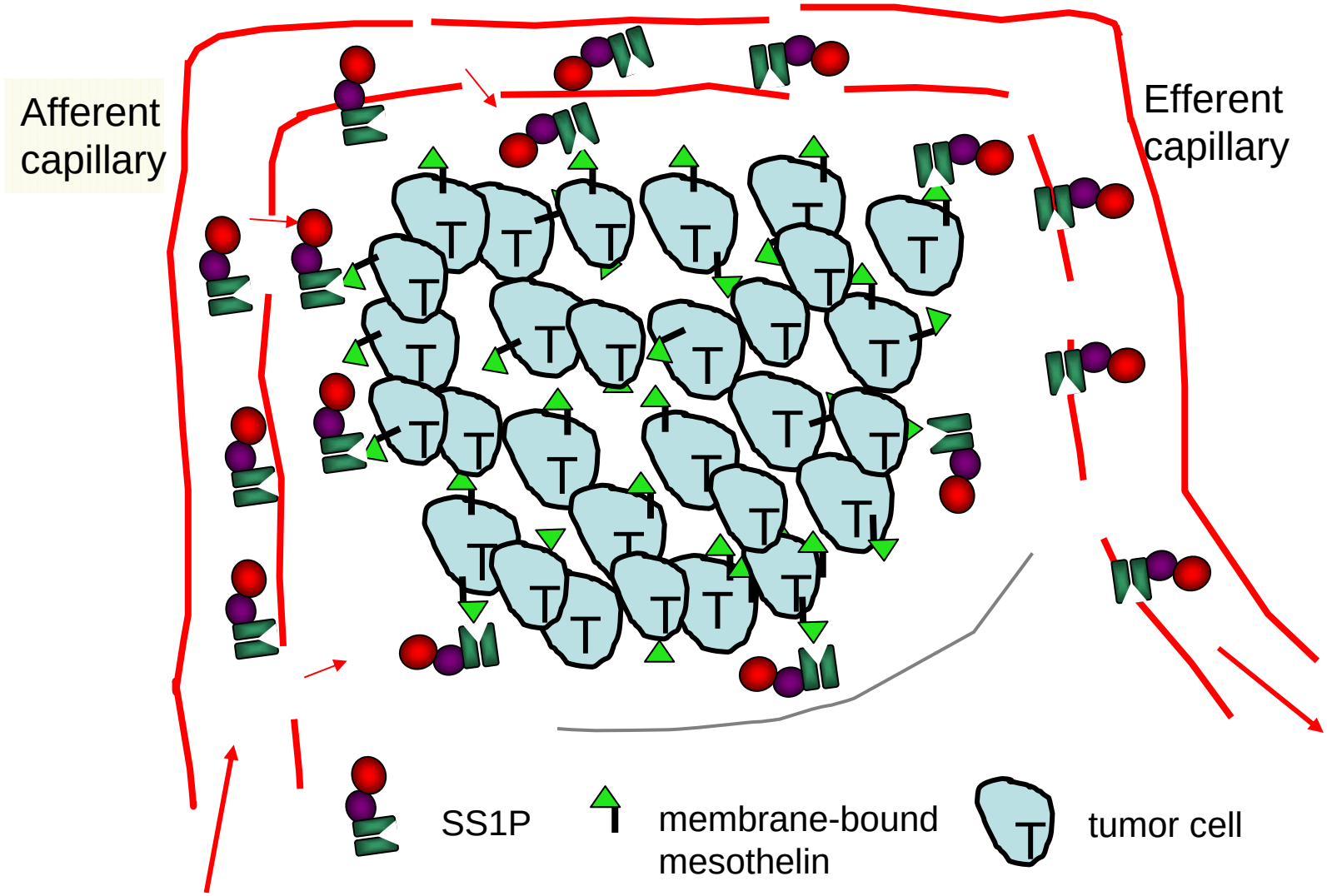
Conclusions

- 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.

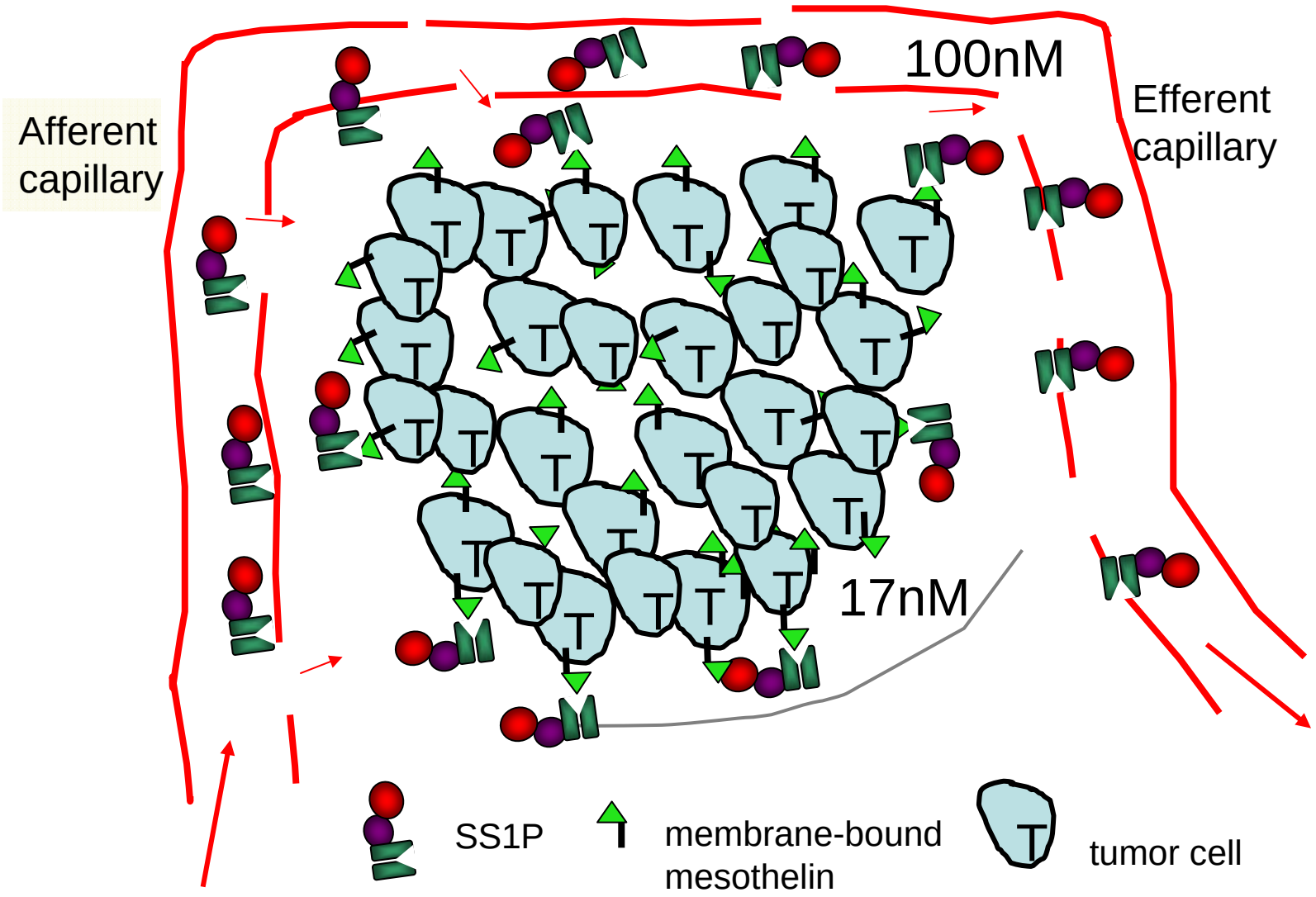
2003 model of solid tumor expressing mesothelin



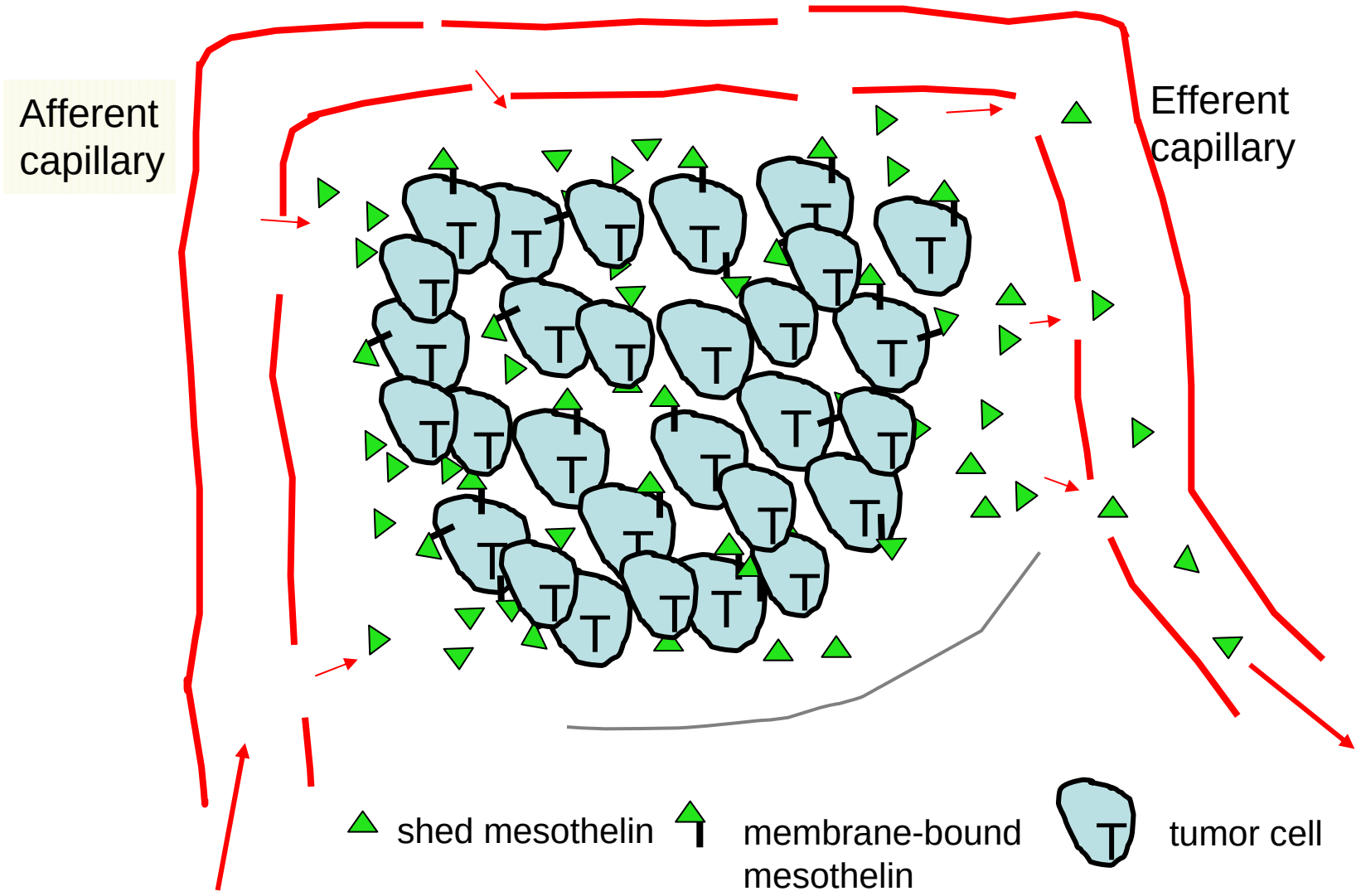
Model of Solid Tumor Expressing Mesothelin after SS1P Treatment – Weinstein Model



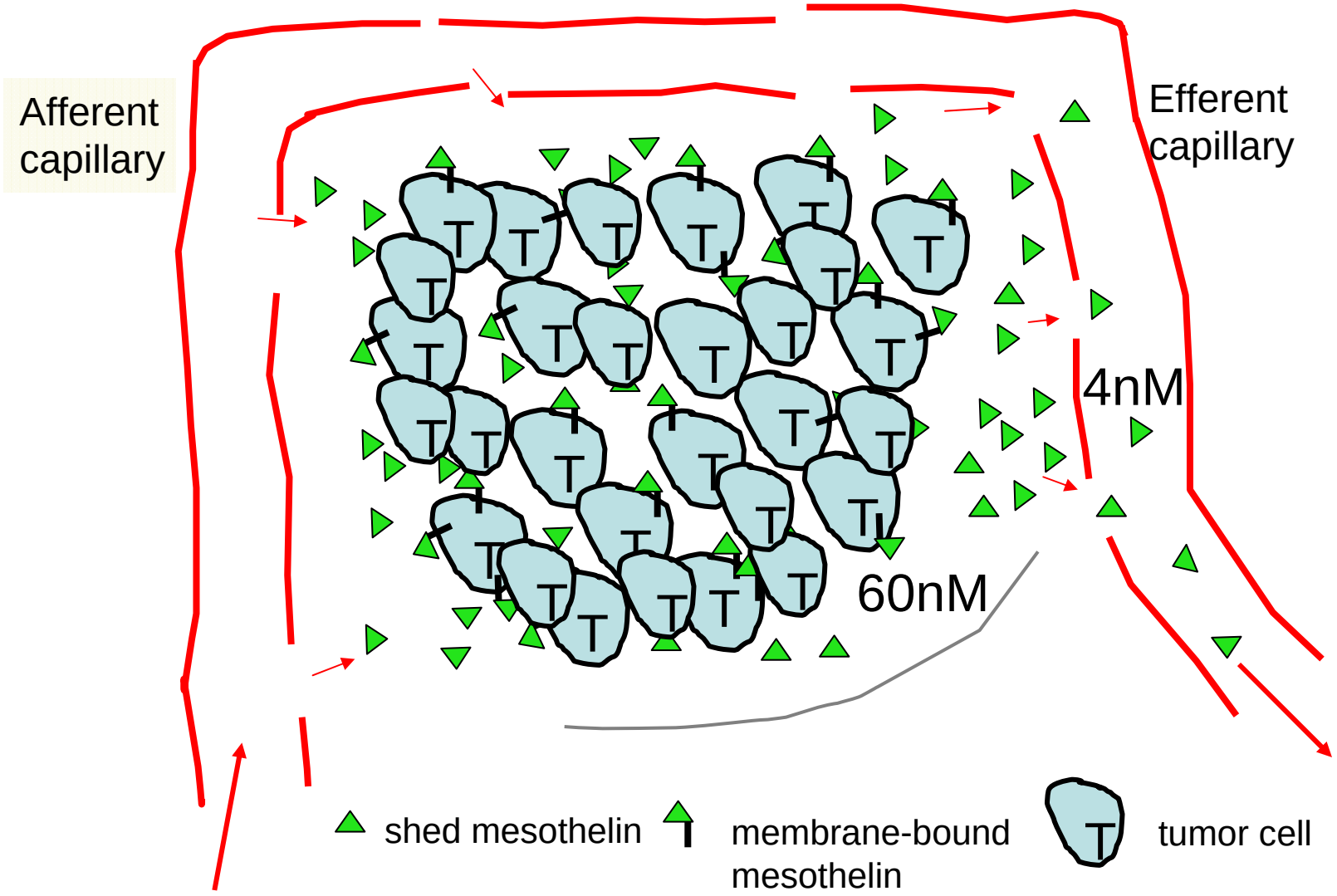
Levels of immunotoxin SS1P in Blood and Tumor



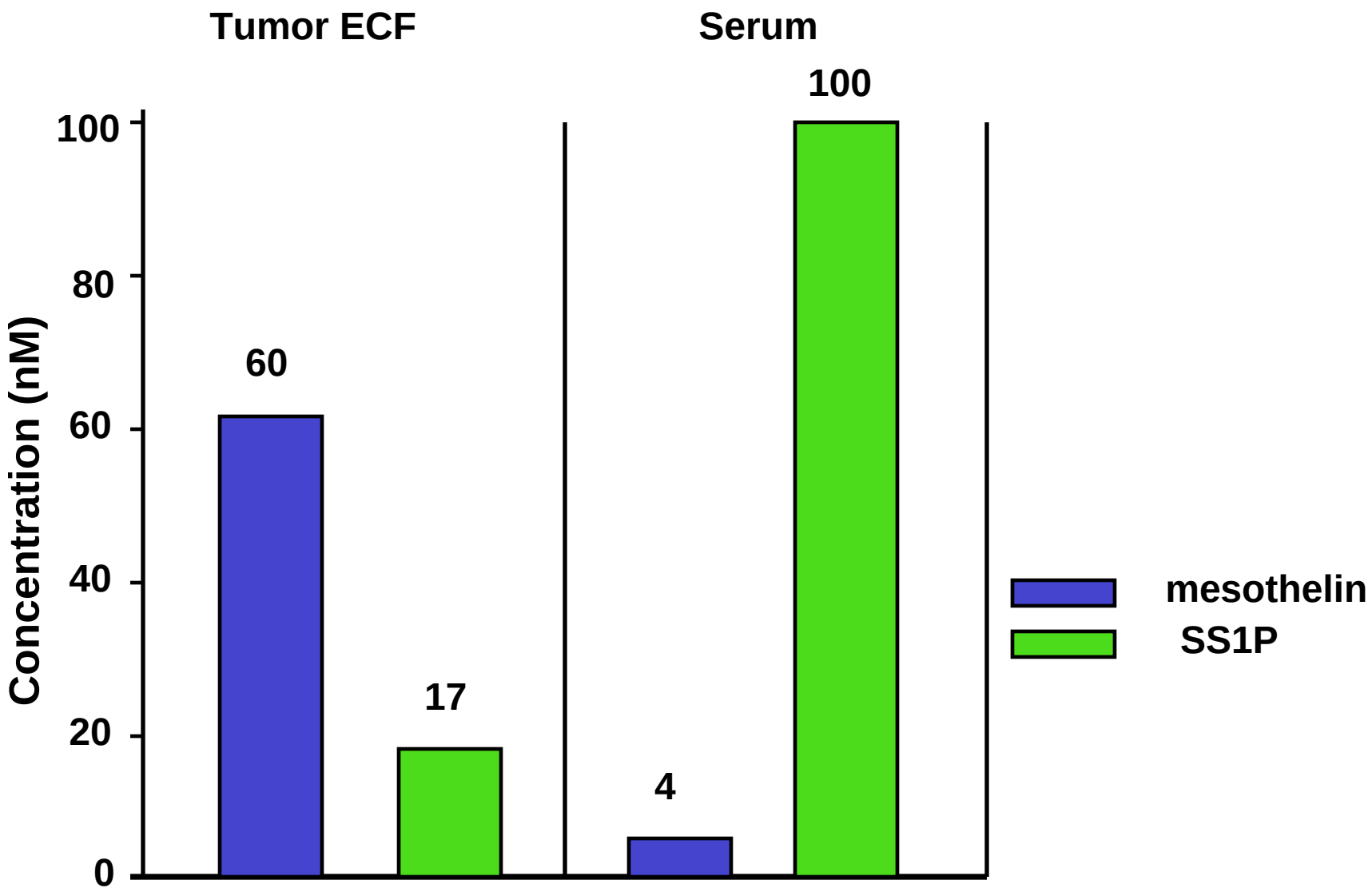
2007 Model of Solid Tumor in Which Mesothelin is Shed into the Blood



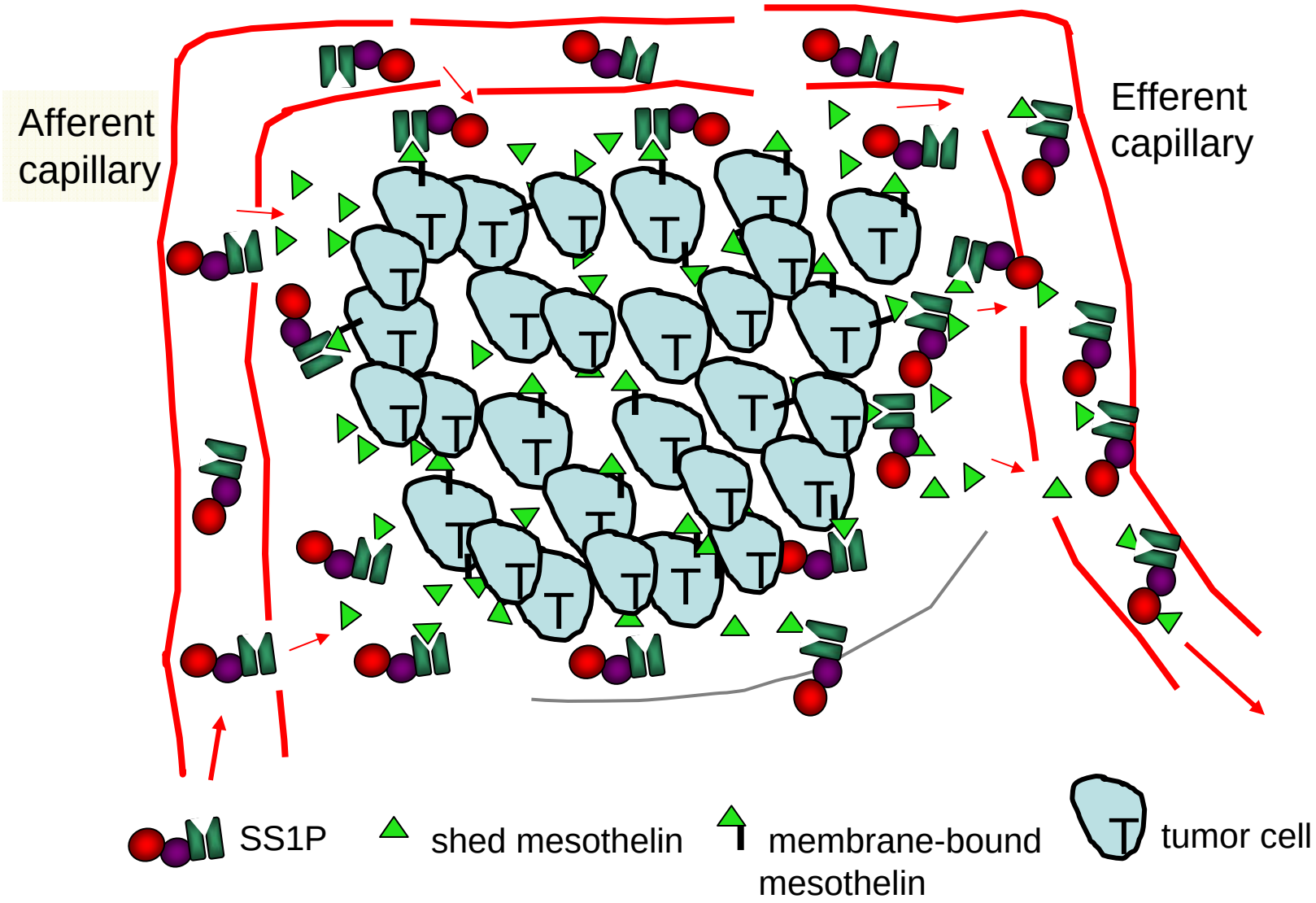
Concentrations of Shed Mesothelin in Blood and Tumor



Levels of Mesothelin and SS1P in Tumor Extracellular Fluid (ECF) and in Serum

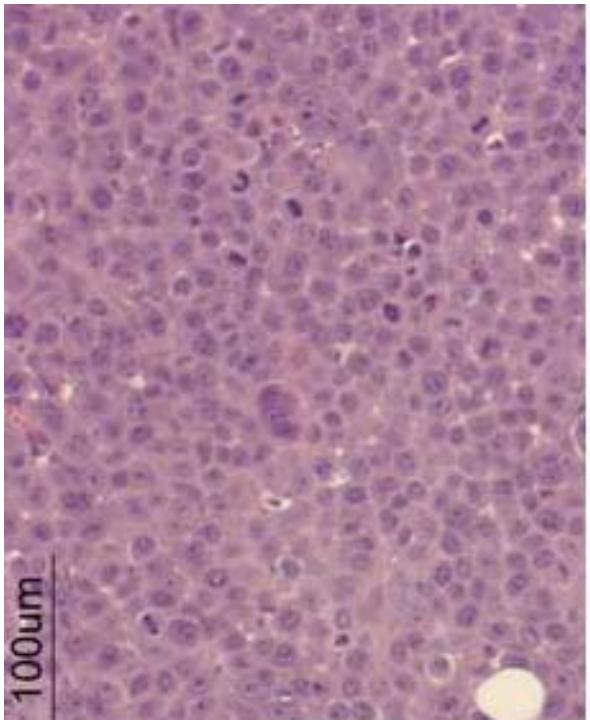


Immunotoxin molecules bind to outer cells of the tumor and also to shed mesothelin

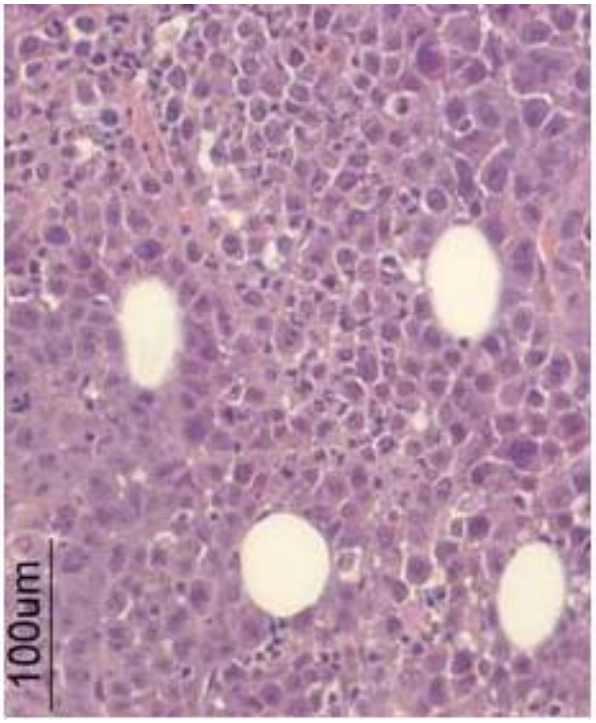


Effect of Taxol on KB Tumor Morphology

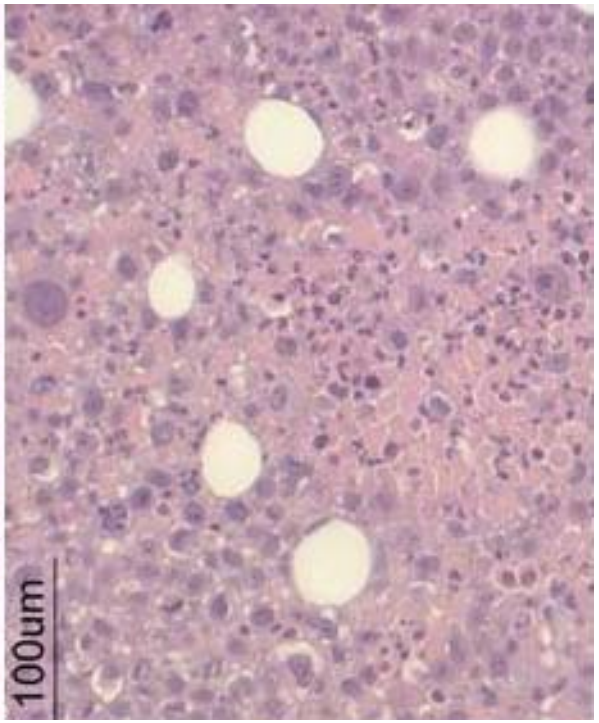
Untreated



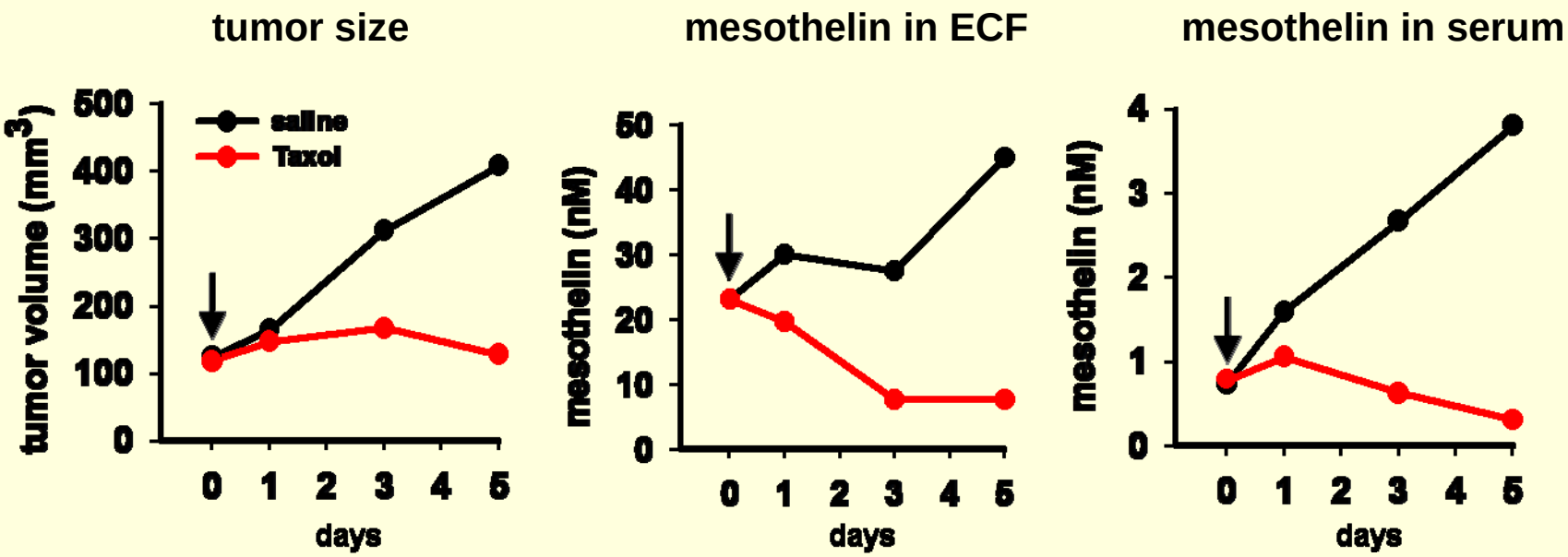
Taxol Day 2



Taxol Day 3

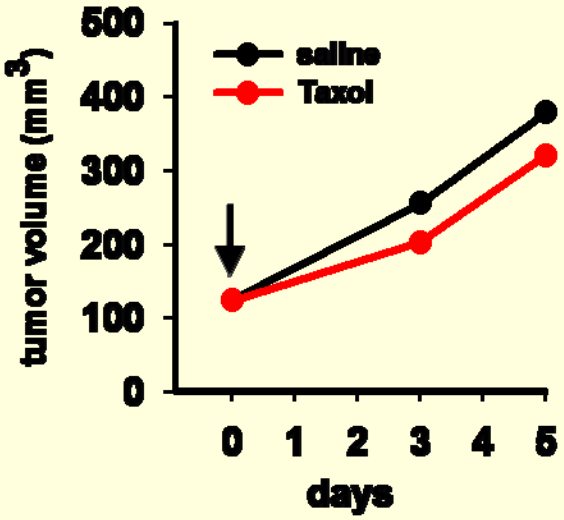


Taxol Arrests Growth and Lowers Mesothelin Levels in KB Tumors and in Blood

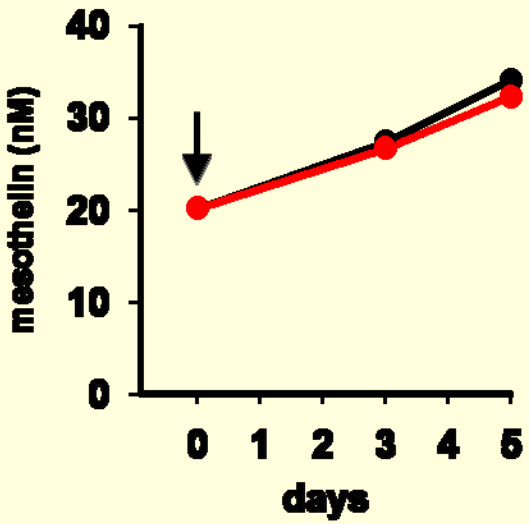


Taxol fails to arrest growth or decrease mesothelin levels in Taxol resistant KB-8-5 tumors

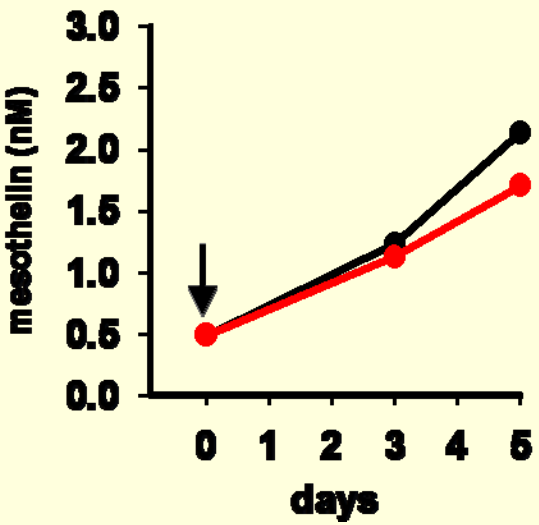
tumor size



mesothelin in ECF

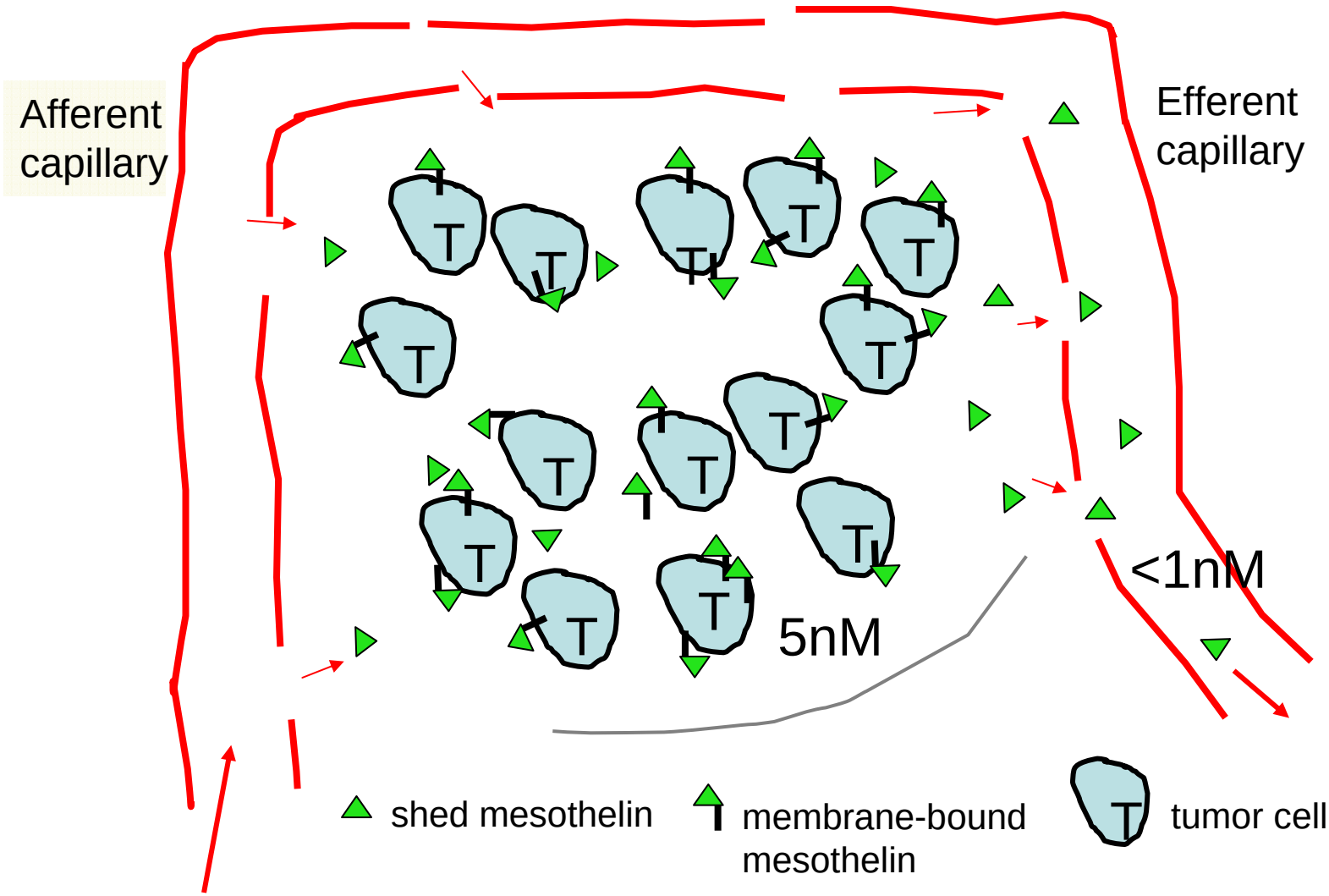


mesothelin in serum

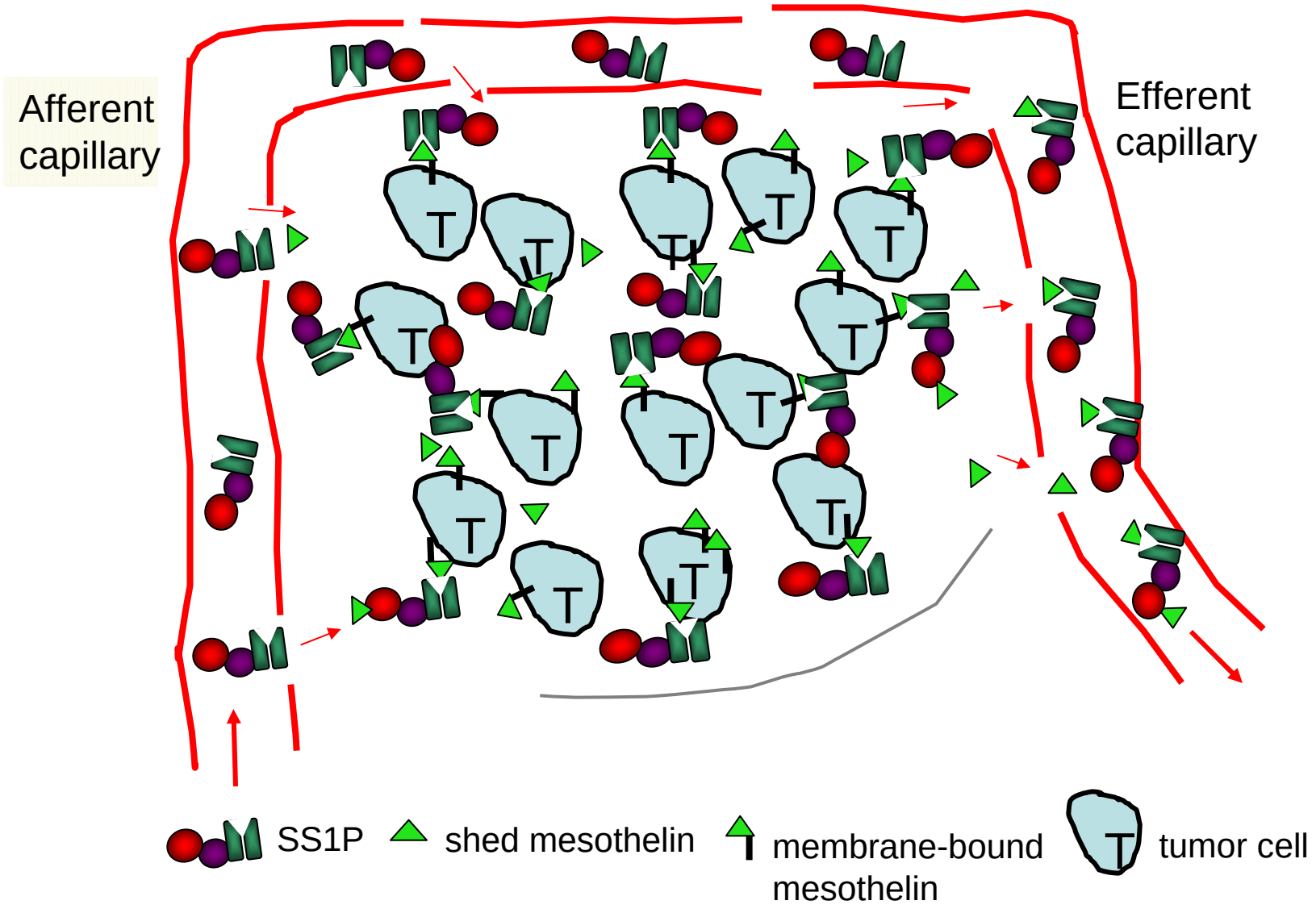


Yujian Zhang

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