# Immunotherapy Basic Principles

- Active vs Passive
- Specific vs Non-specific

# Passive Immunotherapy

- Monoclonal Antibody (passive, specific)
- Checkpoint blockers (active, nonspecific)
- Immunotoxin  (passive, specific)
- Interferon-alpha (non-specific)
- Interleukins (mixed)
- CAR T and Adoptive cell therapies (mixed)
- Vaccines (active; specific)

# Why Antibodies?

- Specificity
- Range of targets
- Versatility
- Half-life
- Production; storage
- Long track record; many approved

# Approved Anti-Cancer Antibody Drugs

| | | | |
|---|---|---|---|
| Daclizumab | Zenapax | (anti-IL2 receptor CD25) | 1997 ( FDA approval date) |
| Rituximab | Rituxan | (anti-CD20) | 1997* |
| Alemtuzumab | Campath | (anti-CD52) | 2001 |
| Traztuzumab | Herceptin | (anti-her2/neu) | 1998 |
| Gemtuzumab ozogomycin | Mylotarg | (anti-CD33) | 2000; withdrawn at 10 yrs |
| Ibritumomab tiux. | Zevalin | (Y-90-anti-CD20) | 2002 |
| Tositumomab | Bexxar | (I-131-anti-CD20) | 2003 |
| Cetuximab | Erbitux | (anti-EGFR) | 2004 |
| Bevacizumab | Avastin | (anti-VEGF) | 2004 |
| Panitumomab | Vectibix | (anti-EGFR) | 2006 |
| Ofatumumab | Arzerra | (anti-CD20) | 2009** |
| Catumaxamab | Removab | (anti-epcam Bisp) | 2009 *EMEA |
| Denosumab | Prolia | (rank ligand) | 2010 |
| Ipilimumab | Yervoy | (anti-CTLA-4) | 2011 |
| Brentuximab vedotin | Adcetris | (CD30-aurastatin) | 2011 |
| Obinutuzumab | Gazyva | (anti-CD20) | 2013* |
| Pertuzumab | Perjeta | (anti-her2/neu) | 2013 |
| Ado-trastuzumab emtansine | Kadcyla | (anti-her2/neu) | 2013 |

Conjugates Bispecifics Checkpoint blockade Receptors Also receptor function ** plus enhanced

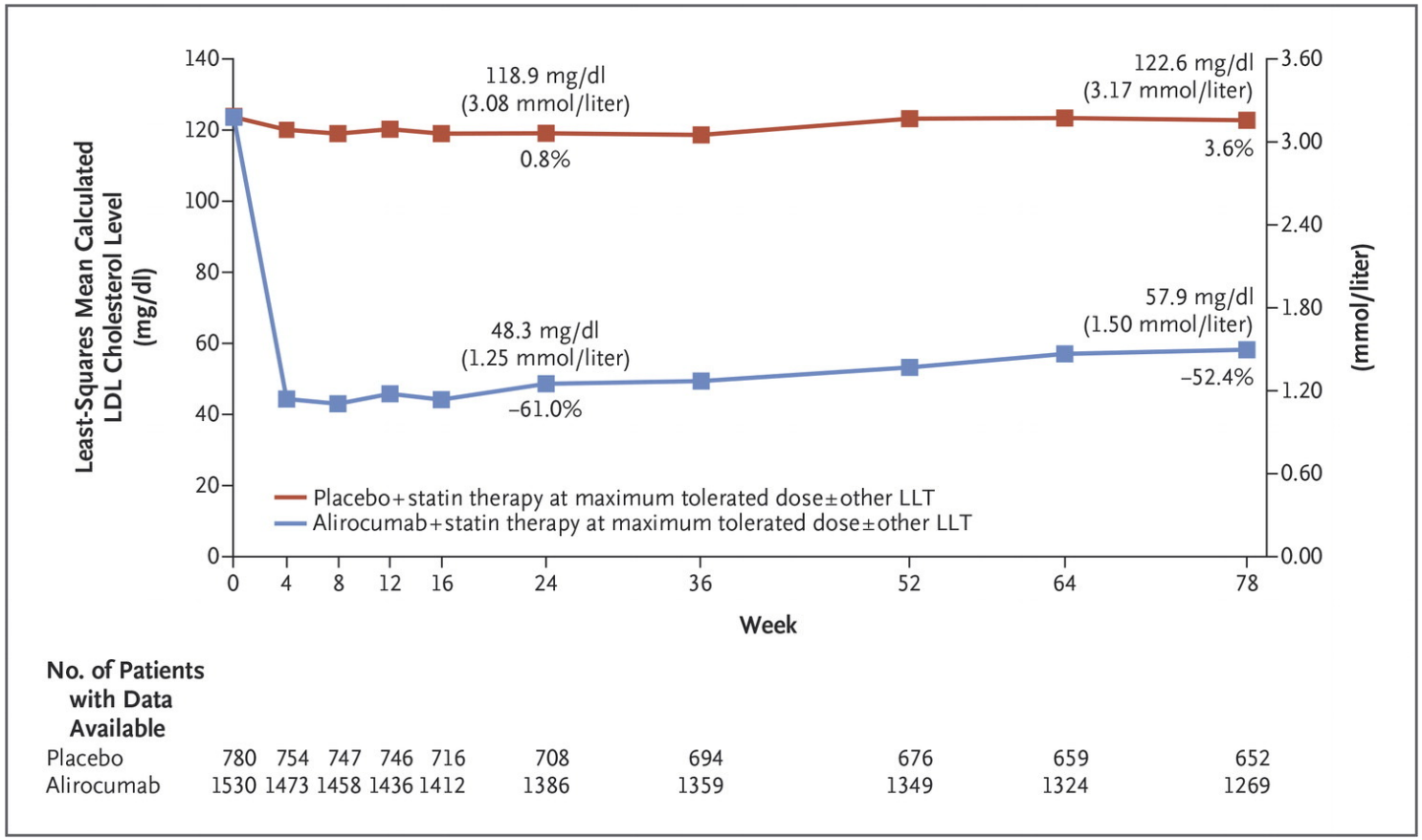
# Approved Anti-Cancer Antibody Drugs - 2

| | | | |
|---|---|---|---|
| Blinatumomab | Blincyto | (CD19-CD3 BiTE) | 2014 |
| Ramucirumab | Cyramza | (anti-VEGFR-2) | 2014 |
| Siltuximab | Sylvant | (anti-IL6) | 2014 |
| Nivolumab | Opdivo | (PD1) | 2014 |
| Pembrolizumab | Keytruda | (anti-PD1) | 2014 |
| Dinutuximab | Unituxan | (anti-GD2) | 2015 |
| Atezolizumab | Tecentriq | anti-PD-L1 | 2016 |
| Olaratumab | Lartruvo | PDGF-alpha | 2016 |
| Daratumumab | Darzalex | CD38 | 2016 |
| Avelumab | Bavencio | anti-PD-L1 | 2017 |
| Durvalumab | Imfinzi | anti-PD-L1 | 2017 |
| Bevacizumab-awwb | Mvasi | Biosimilar (VEGF) | 2017 |
| Cemiplimab-rwlc | Libtayo | (anti-PD1) | 2018 |
| Moxetumomab pasudotox-tdfk | Lumoxiti | CD22-P38 | 2018 |
| Mogamulizumab-kpkc | Poteligeo | CCR4 | 2018 |

- 13 mAb approved in first 15 years
- 15 approved in 2014-2018
- Only 3 naked cytotoxic mAb, not also working through a receptor
- Activity sometimes weak
- +25 more non-cancer mAb

**Plus: > 12 biosimilar mAb's approved worldwide now**

# Efficacy and Safety of Alirocumab in Reducing Lipids

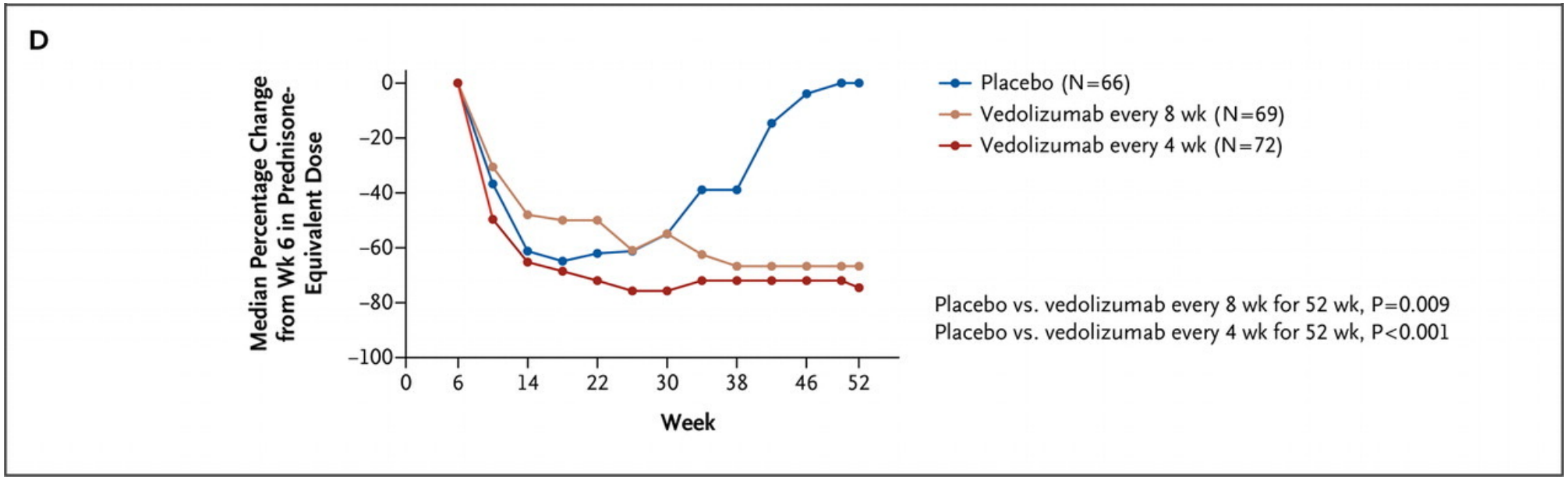



**Calculated LDL Cholesterol Levels over Time (Intention-to-Treat Analysis).**

Anti-PCSK9

*Robinson, et al; NEJM, 372(16), 2015*

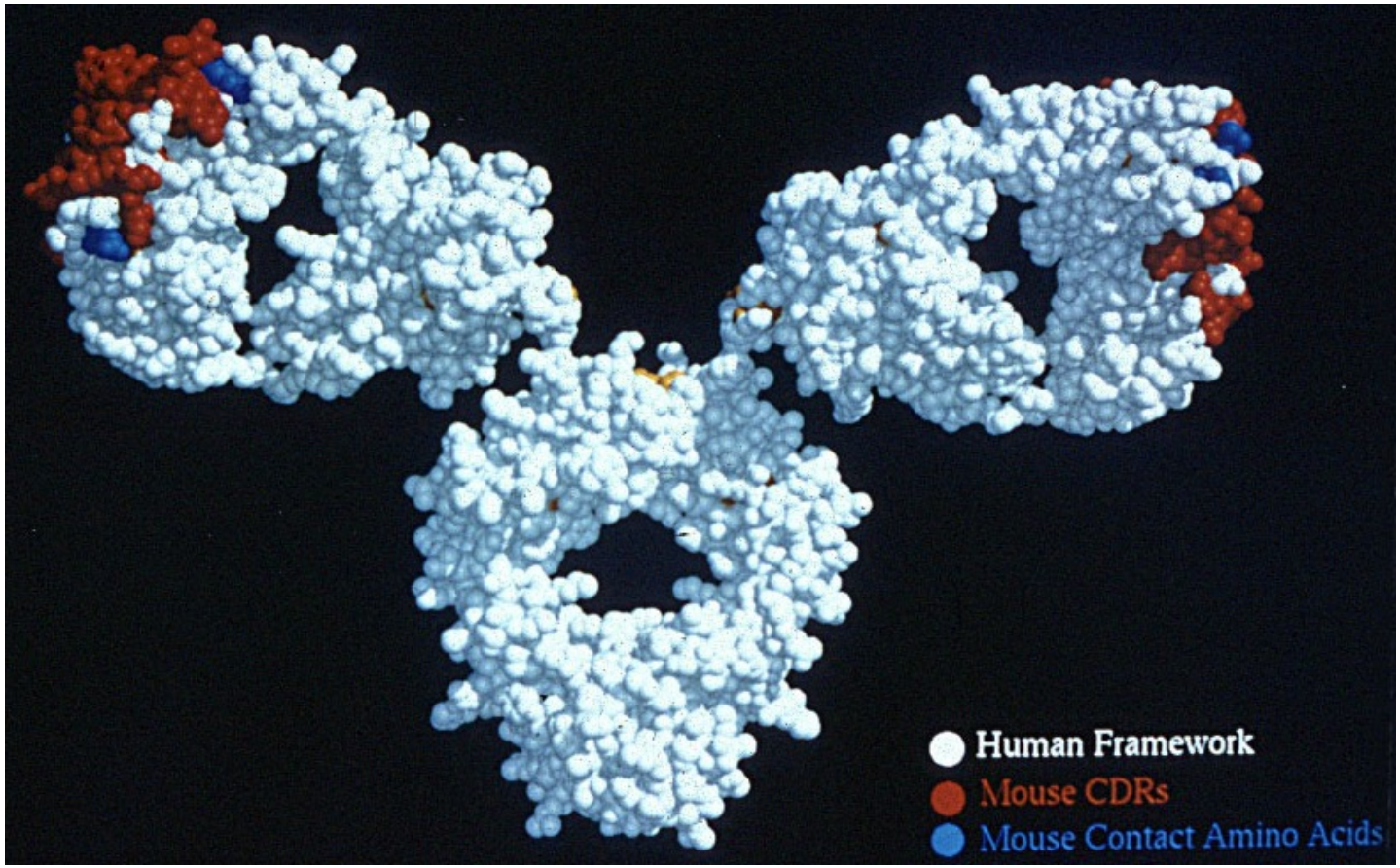
# Vedolizumab for Ulcerative Colitis



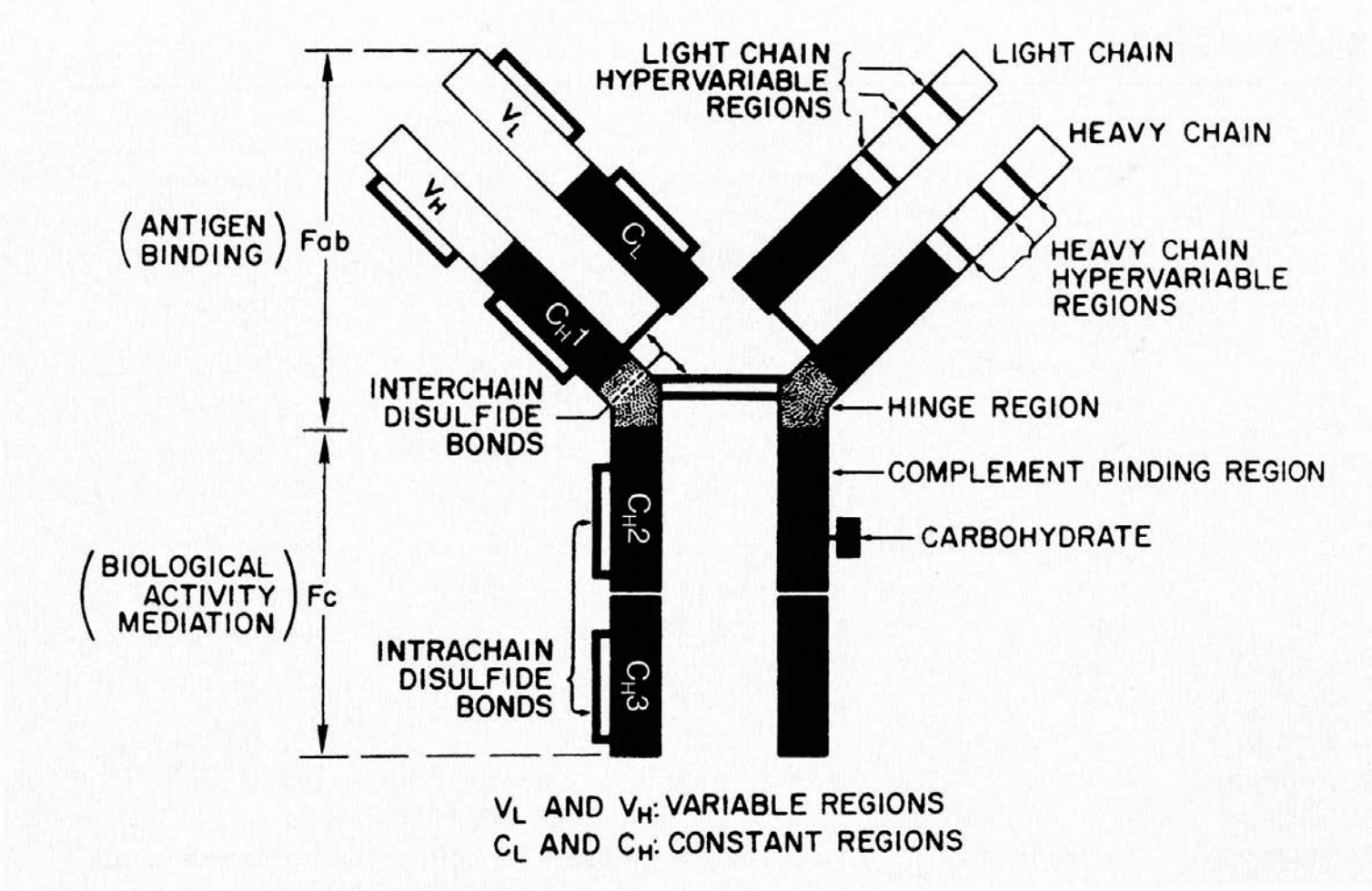

Anti-LPAM-1

*Feagan, et al; NEJM, 369(8), 2013*

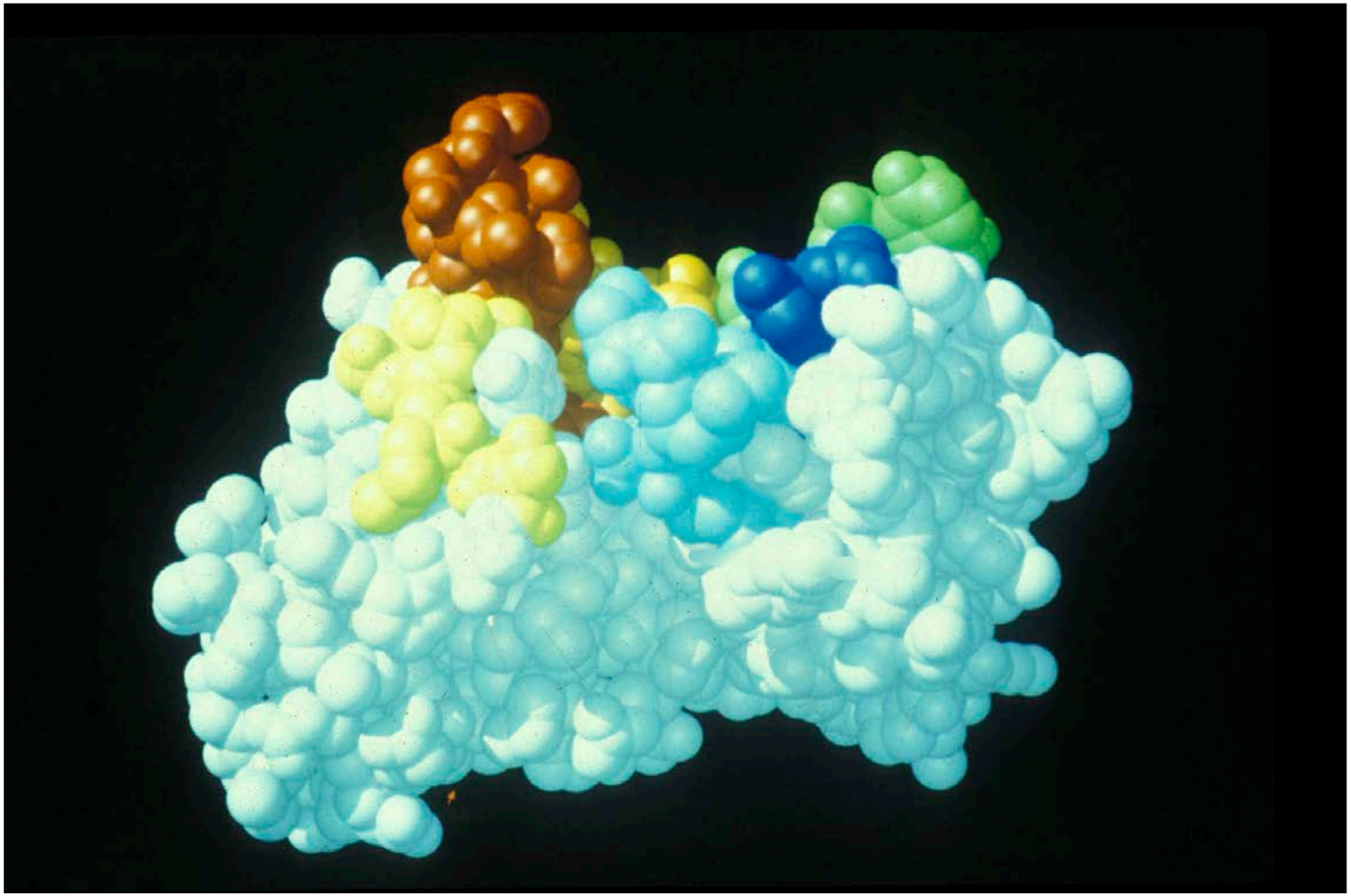
# Model of HuM195 – A Human IgG



# Schema of an IgG



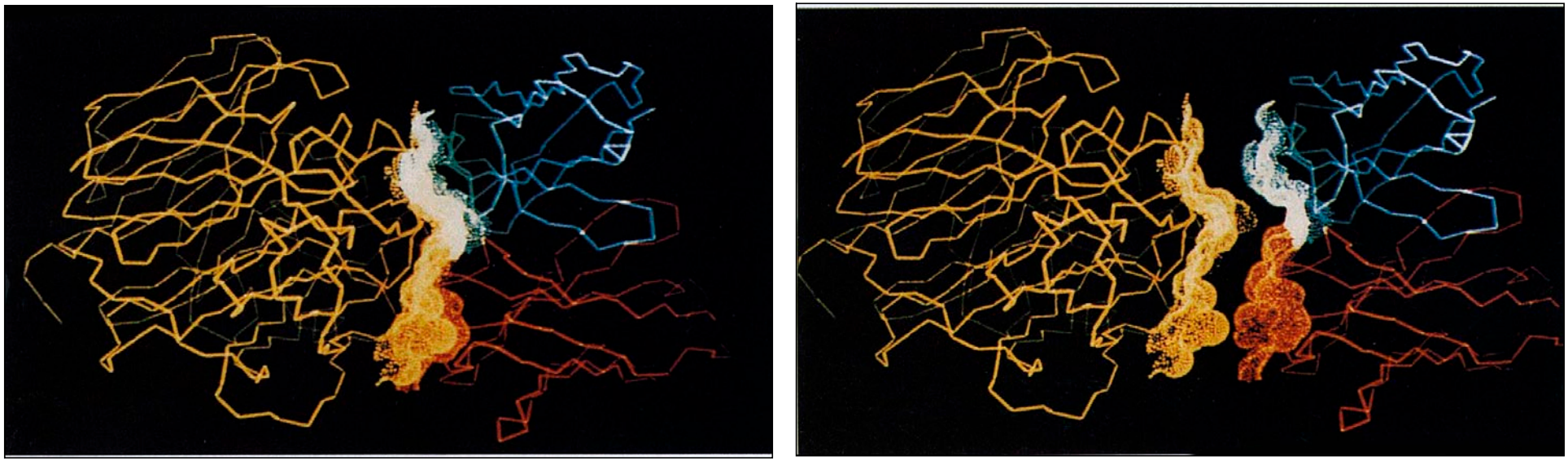
# Fab Binding Pocket



# Binding is over a large flat surface area

Cytochrome c

Fab



# Ig structures: G, A, M

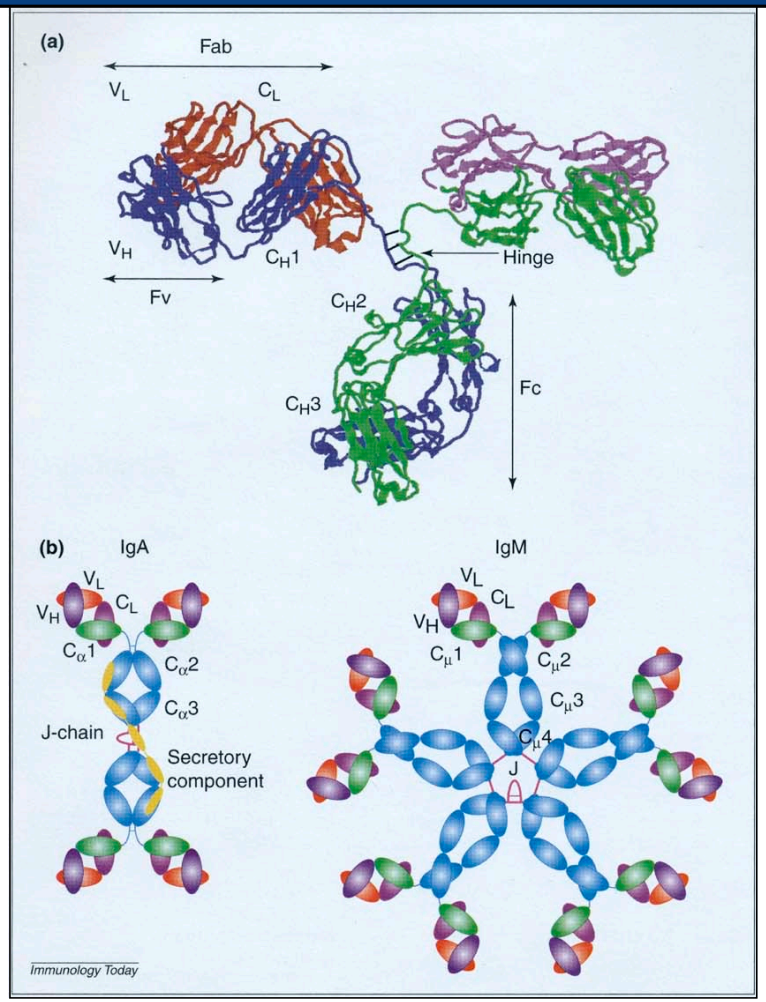
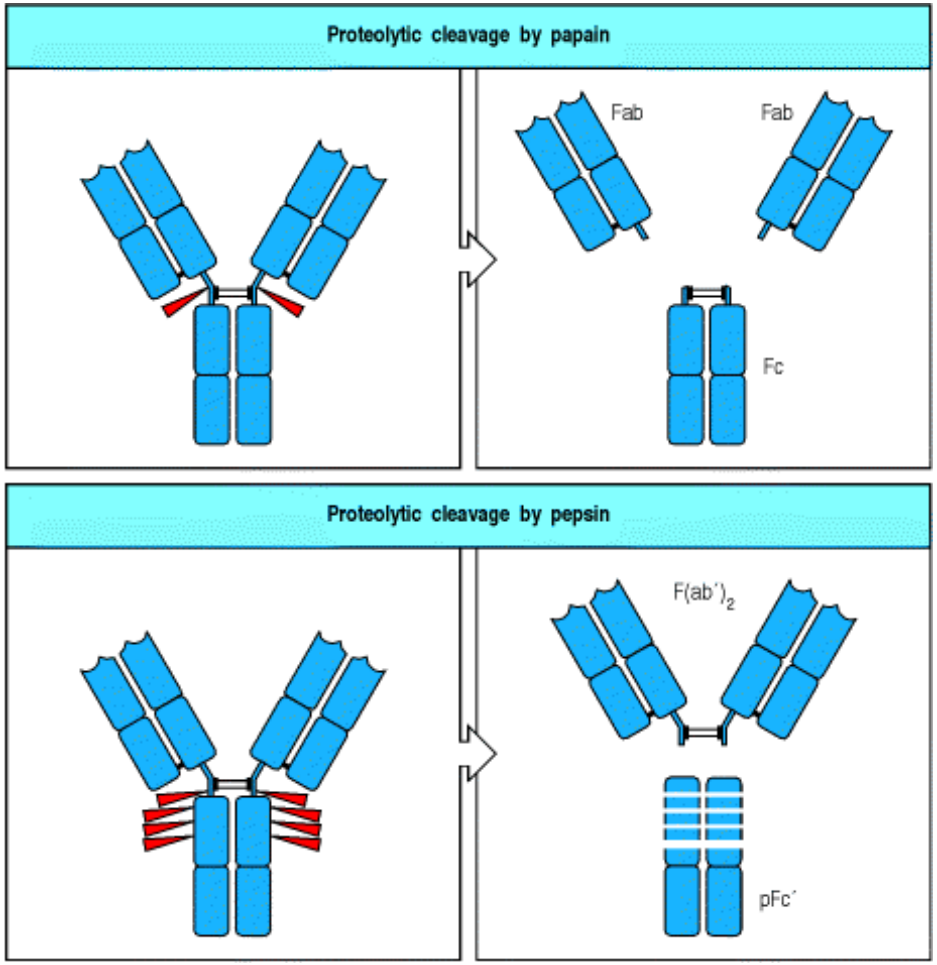



*Fig. 2. The antibody structure. (a) Domain organization of an IgG molecule. Antigen-binding surface is formed by variable domains of the heavy ($V_H$) and light ($V_L$) chains. Effector functions are determined by constant $C_H2$ and $C_H3$ domains. (b) The dimeric secretory IgA (SIgA) and pentameric IgM structures. In SIgA, secretory component is wound around the SIgA dimer and attached by disulphide bonds to the $C_\alpha2$ domain of each IgA monomer. The J-chain is required for joining the two subunits. The IgM heavy chains have five domains with disulphide bonds crosslinking adjacent $\mu_s$ chains. The IgM pentamer is the predominant polymeric form of IgM in vivo. Pentameric IgM normally contains a single additional polypeptide, the joining chain (J).*

# Enzymatic Cleavage of Ig

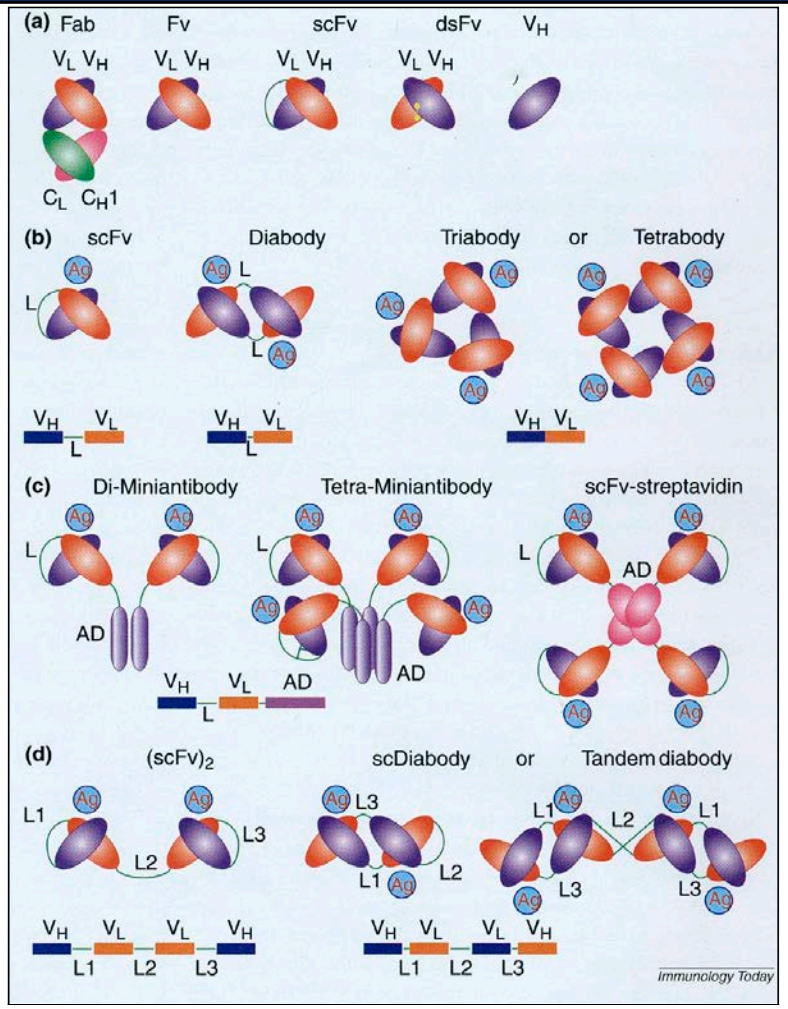
Independent domains are key to functions and engineering of mAb



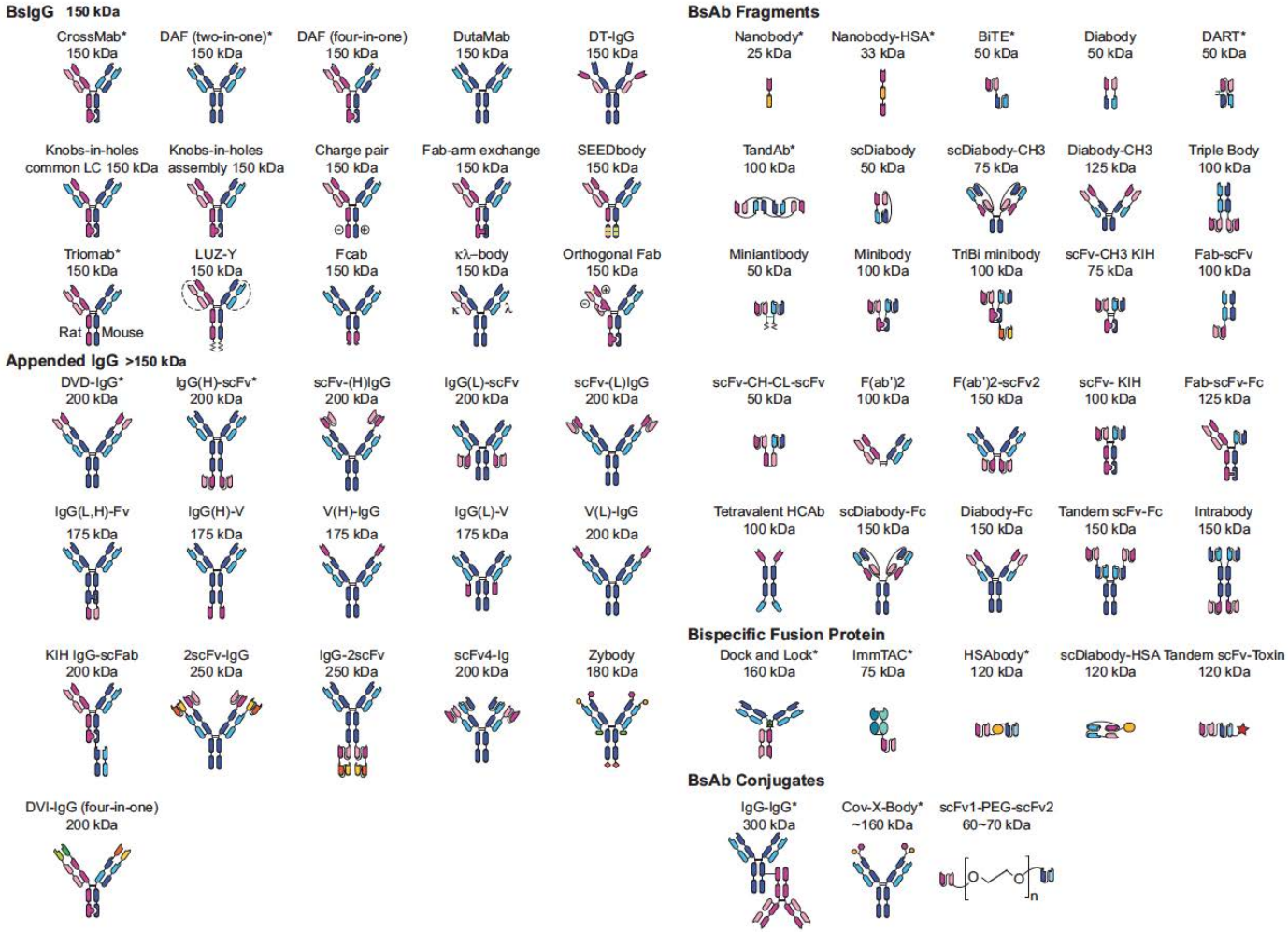

*Taken from Immunobiology, C.A. Janeway, Jr. and P. Travers, Garland Publishing Inc., NY*

# Rebuilding Ig functional domains

Mixing and matching
of domains
for new functions
and pharmacology



# Bi-Specific Antibody Formats



# Summary: Structure of Antibodies

- Bivalent/multivalent
- Large molecular mass
- Multifunctional
- Characterized by functional domains

# How to Make a Monoclonal Antibody?

Immunize: mice, rabbit, llama, human…..

Take B cells...immortalize.... Clone cell

Screen library: phage, yeast....

clone sequence

# Potential Cancer Targets of mAb

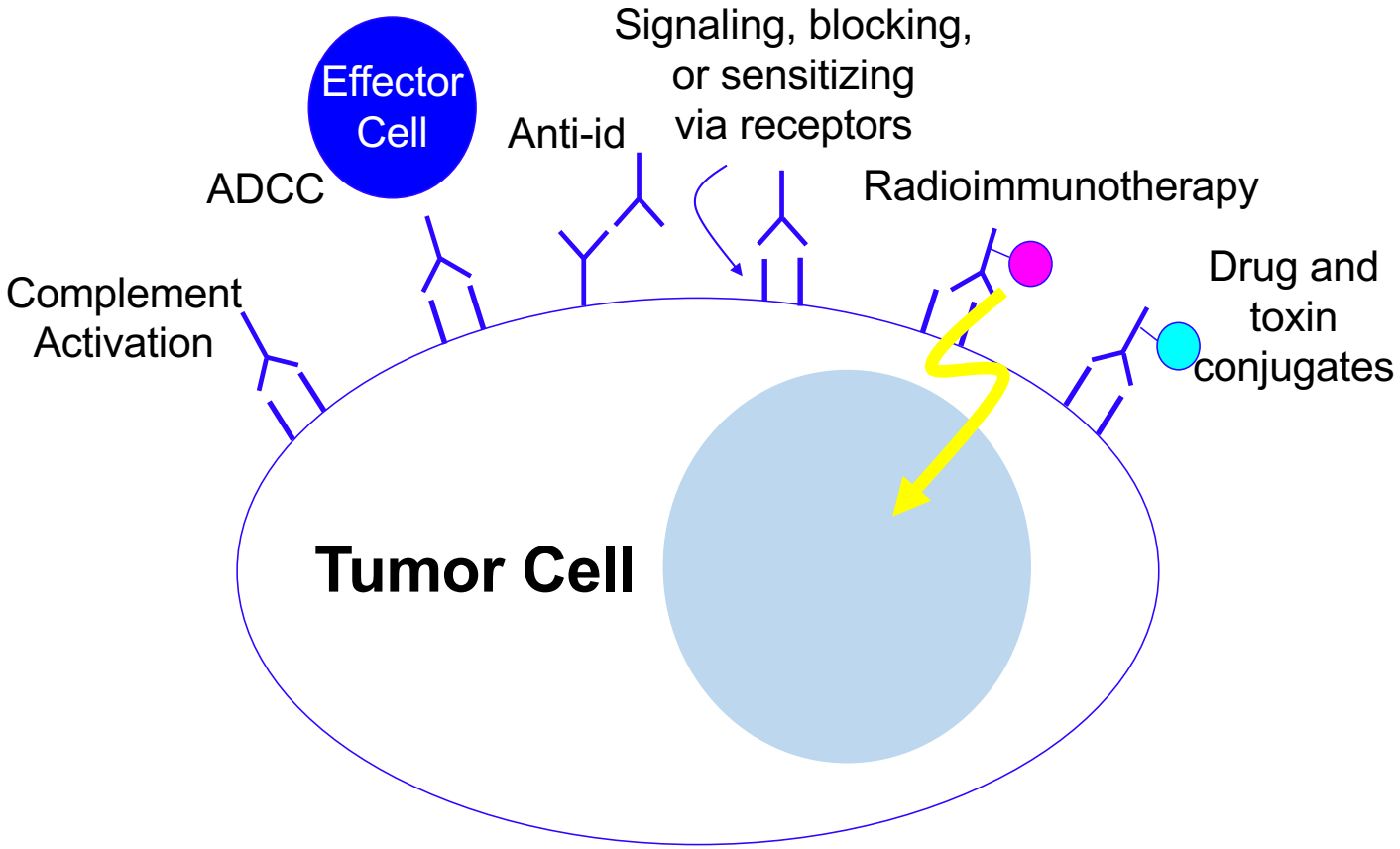
- Receptors: EGFR, IGFR, C-MET-r (HGF-r)
- Vascular antigens (not on cancer)
- Cell surface differentiation antigens
- Soluble growth factors and ligands
- Matrix integrins; mucins; glycospingolipids
- Apoptosis enhancers (Trail-R)
- Immune modulators (checkpoints- not on cancer)
- Internal antigens (after MHC presentation)
- Post-translational modifications

# Now: Targets are all Outside of Cell

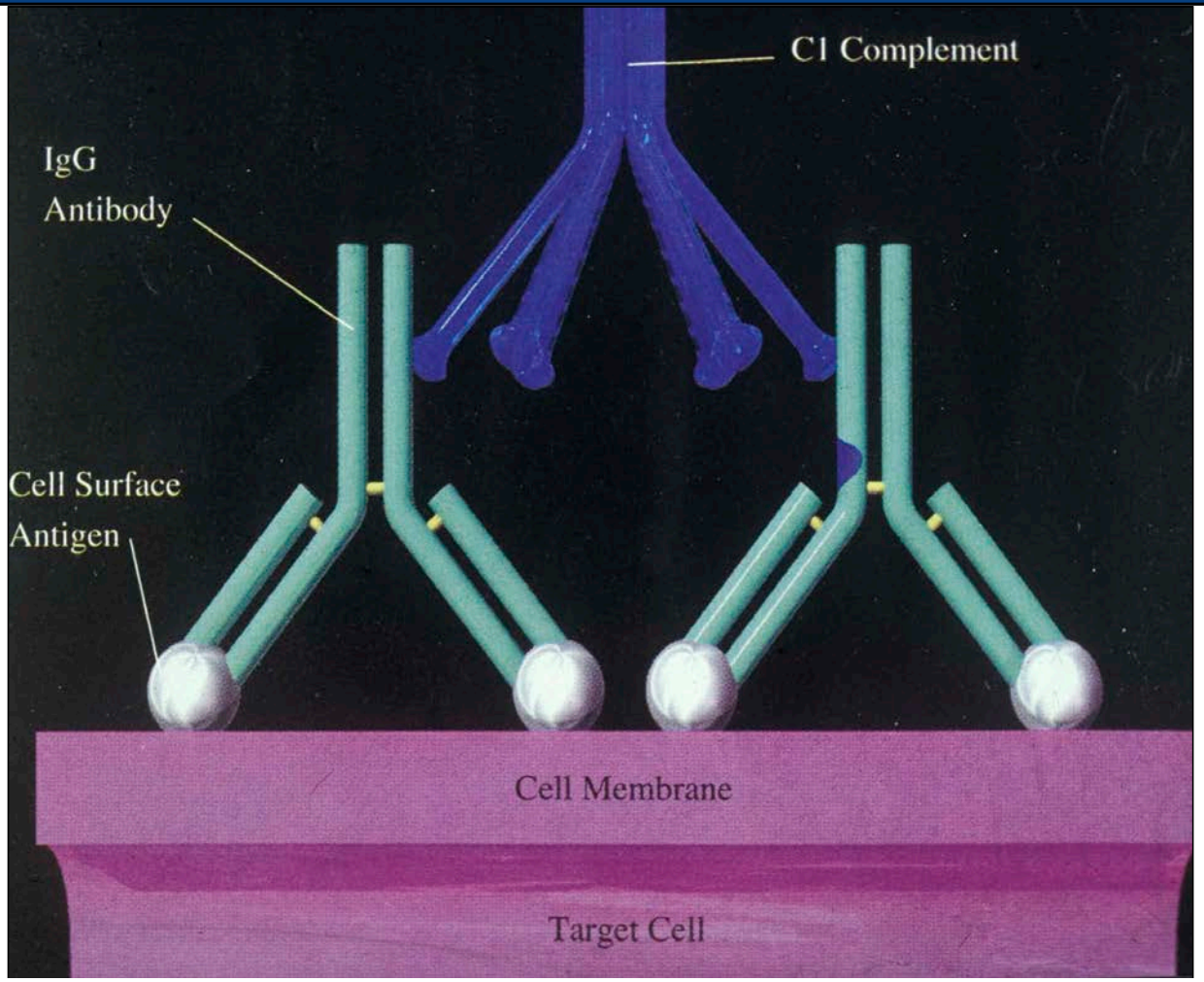
| Antigen category | Examples of antigens | Tumor types expressing antigen |
|---|---|---|
| **Cluster of differentiation (CD) antigens** | CD20 | non-Hodgkin lymphoma |
| | CD30 | Hodgkin lymphoma |
| | CD33 | Acute myelogenous leukemia |
| | CD52 | Chronic lymphocytic leukemia |
| **Glycoproteins** | EpCAM | Epithelial tumors (breast, colon, lung) |
| | CEA | Epithelial tumors (breast, colon, lung) |
| | gpA33 | Colorectal carcinoma |
| | Mucins | Epithelial tumors (breast, colon, lung, ovarian) |
| | TAG-72 | Epithelial tumors (breast, colon, lung) |
| | Carbonic anhydrase IX | Renal cell carcinoma |
| | PSMA | Prostate carcinoma |
| | Folate binding protein | Ovarian tumors |
| **Glycolipids** | Gangliosides (e.g., GD2, GD3, GM2) | Neuroectodermal tumors, some epithelial tumors |
| **Carbohydrates** | Lewis-$Y^2$ | Epithelial tumors (breast, colon, lung, prostate) |
| **Vascular targets** | VEGF | Tumor vasculature |
| | VEGFR | Epithelium-derived solid tumors |
| | $\alpha V\beta 3$ | Tumor vasculature |
| | $\alpha 5\beta 1$ | Tumor vasculature |
| **Growth factors** | ErbB1/EGFR | Glioma, lung, breast, colon, head and neck tumors |
| | ErbB2/HER2 | Breast, colon, lung, ovarian, prostate tumors |
| | ErbB3 | Breast, colon, lung, ovarian, prostate tumors |
| | c-MET | Epithelial tumors (breast, ovary, lung) |
| | IGF1R | Lung, breast, head and neck, prostate, thyroid, glioma |
| | EphA3 | Lung, kidney, colon, melanoma, glioma, hematological malignancies |
| | TRAIL-R1, TRAIL-R2 | Solid tumors (colon, lung, pancreas) and hematological malignancies |
| | RANKL | Prostate cancer and bone metastases |
| **Stromal and extracellular matrix antigens** | FAP | Epithelial tumors (colon, breast, lung, head and neck, pancreas) |
| | Tenascin | Glioma, epithelial tumors (breast, prostate) |

*Scott, Allison, Wolchok. Cancer Immunology, 2012.*

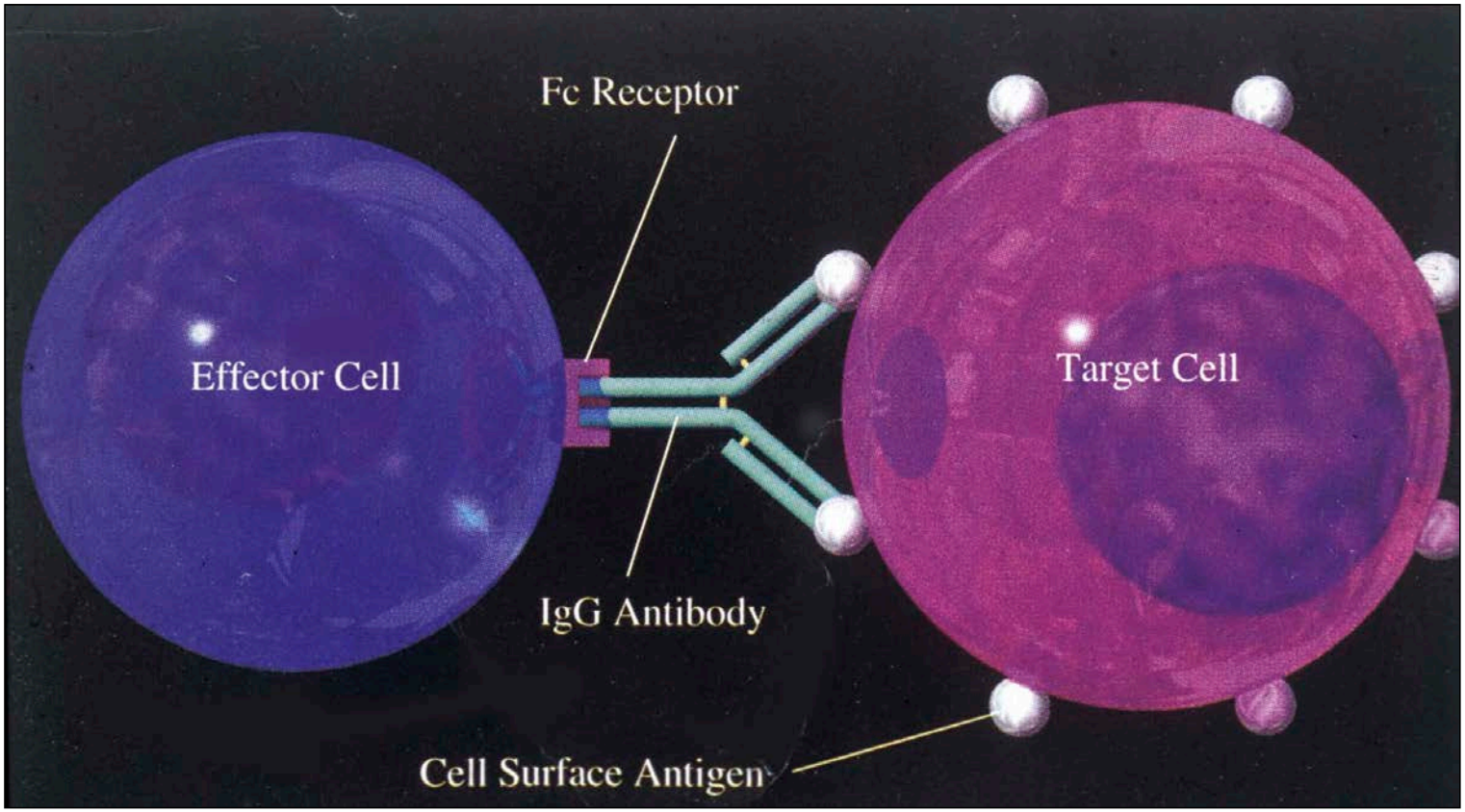
# Strategies for Antibody Therapy



# Complement Mediated Cytotoxicity: CMC

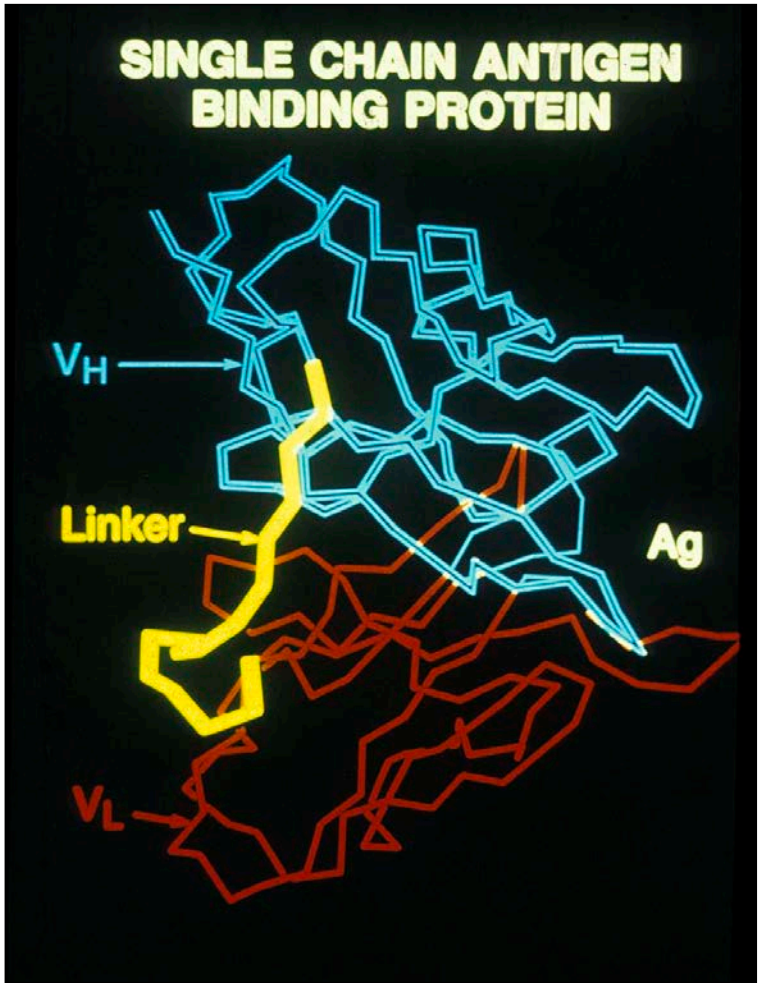


# Antibody Dependent Cellular Cytotoxicity: ADCC

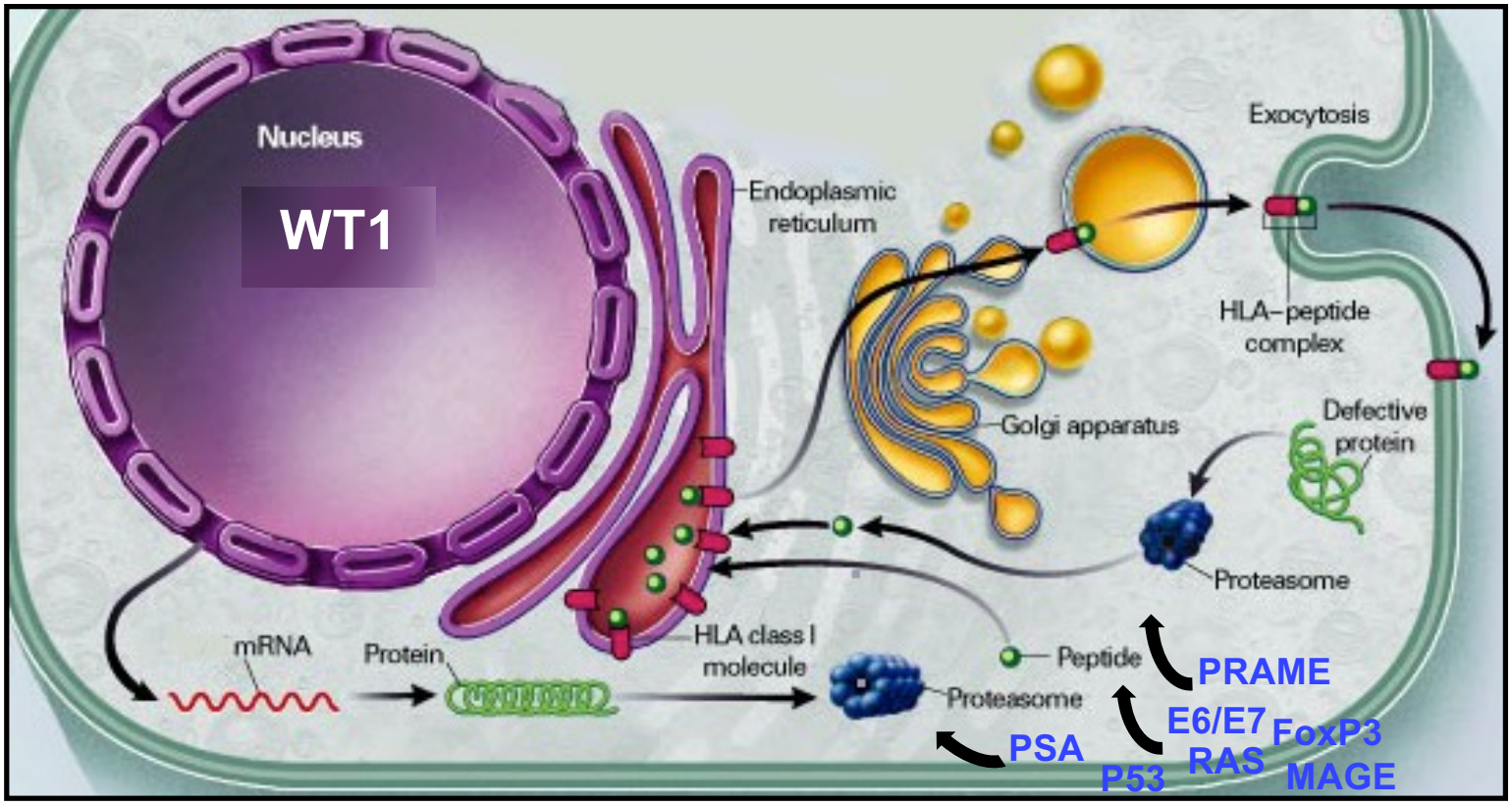


# Antibody Dependent Cellular Cytotoxicity: ADCC

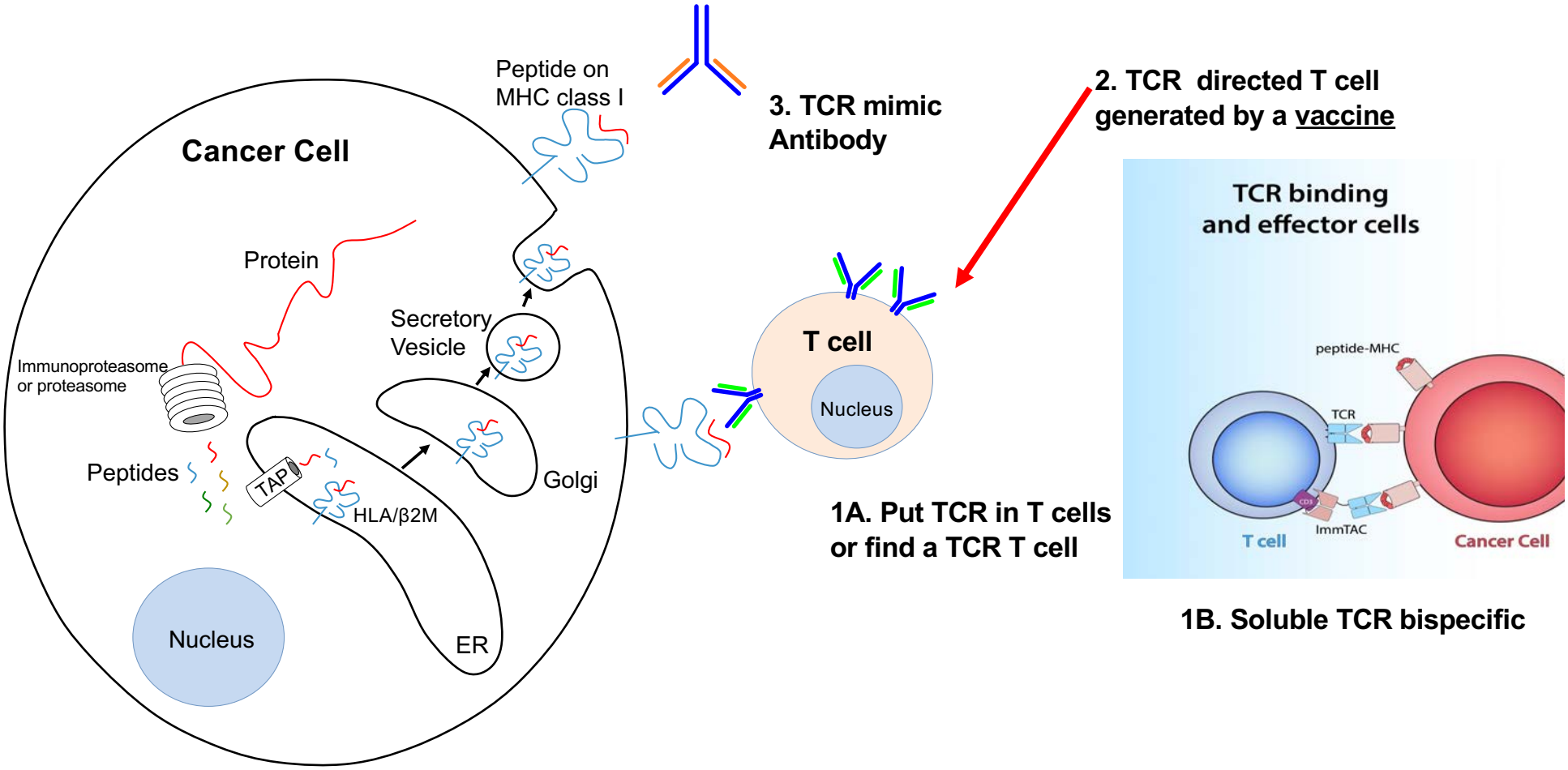
Bispecific mAb:
BiTEs



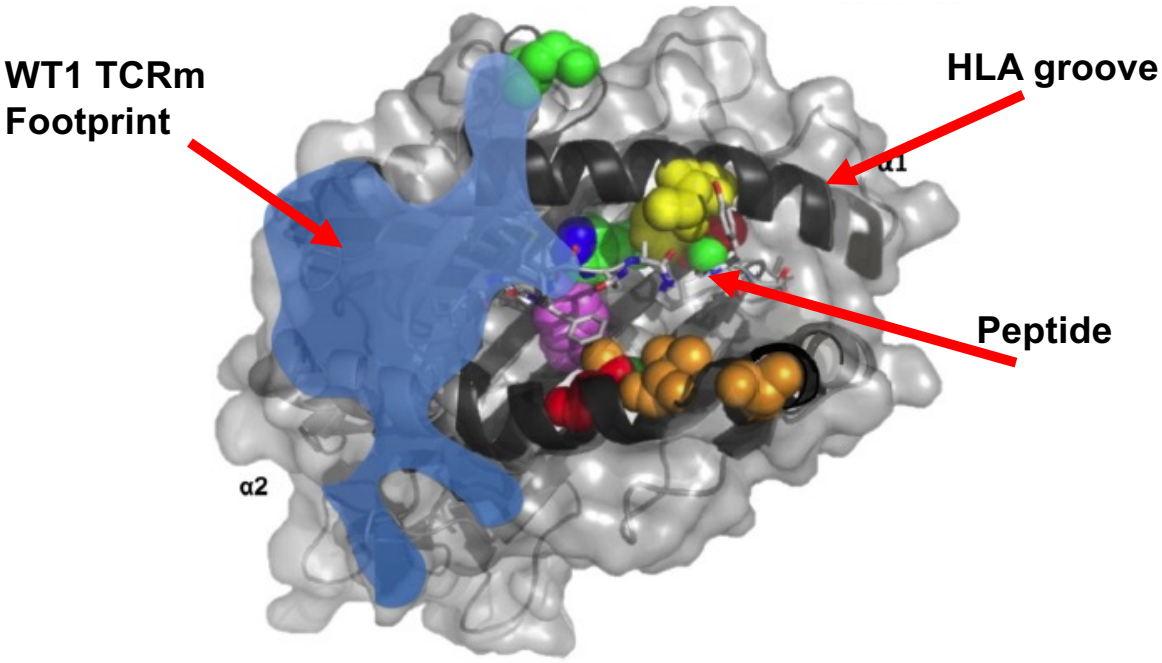
# Drugging Undruggable Targets via HLA Presentation

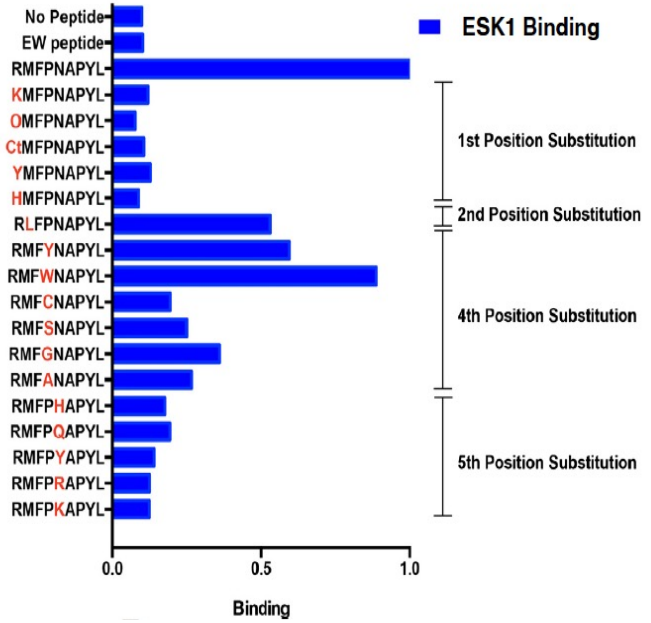


# Killing with Cells: TCR, ICB, Vaccines



# WT1 TCRm mAb Contact Region Allows Binding to Other A02 Subtypes and Other Proteomic Off-Targets



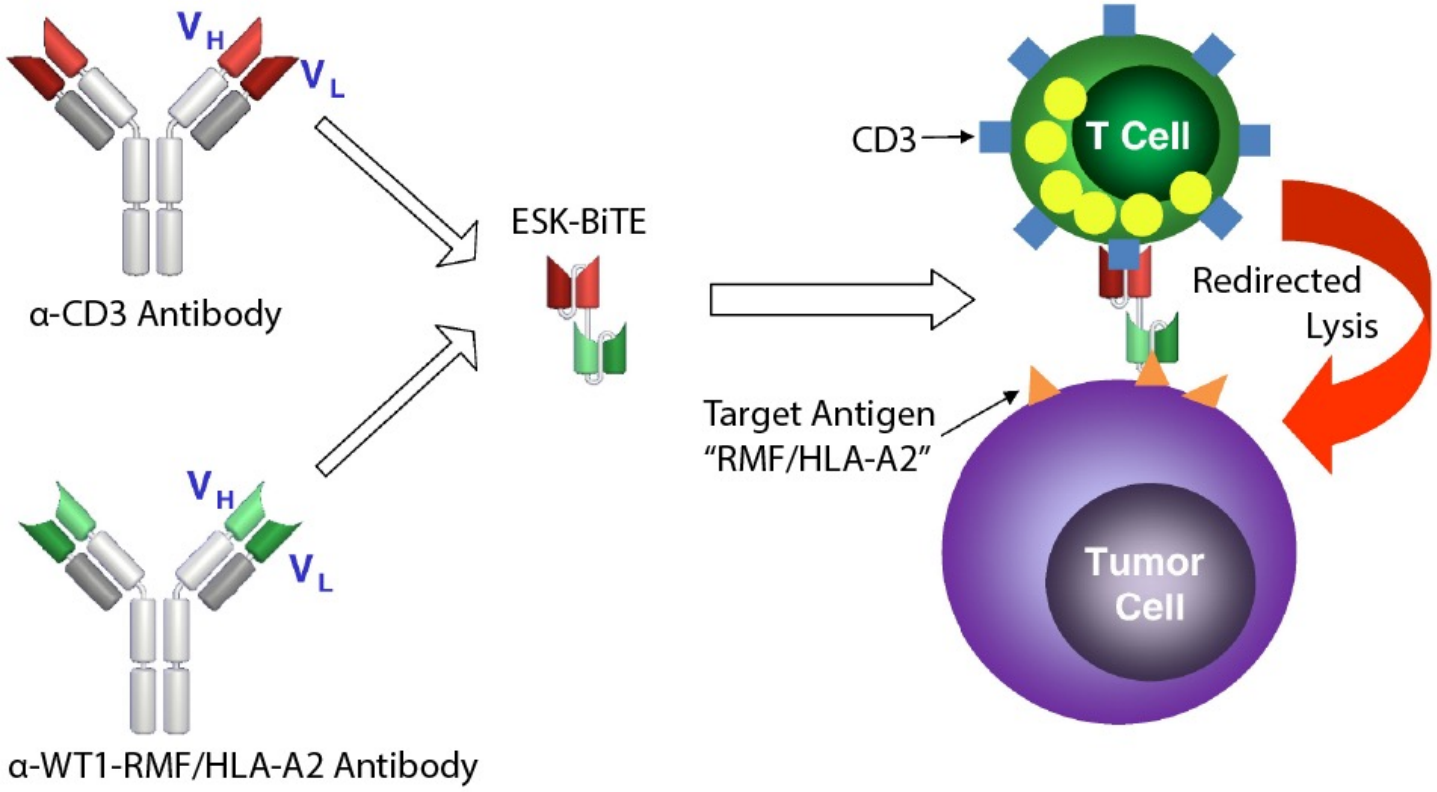

**Also mapped HLA-A*02-subtype differences:**

Positional differences between the HLA subtypes: A*02:02, A*02:03, A*02:05, A*02:06, A*02:07, and yellow: A*02:11..

None of the residues that vary between A*02 subtypes contacts ESK1.

*Ataie, JMB, 2015*

# Engineering the First BiTE Derived from TCR-mimic mAb, ESK1




*Modified from Exp. Cell Res. 317:1255, 2011*

# Mechanisms to Make Bispecifics

Strategies and mutations to overcome the BsIgG heavy chain-pairing problem.

| Company | Technology Name | Mutations in first heavy chain | Mutations in second heavy chain | Reference |
|---|---|---|---|---|
| Genentech | Knobs-into-holes | T366W | T366S, L368A, Y407V | Ridgway et al. (1996), Atwell et al. (1997) |
| Genmab | DuoBody | F405L | K409R | Labrijn et al. (2013) |
| Zymeworks | Azymetric | T350V, L351Y, F405A, Y407V | T350V, T366L, K392L, T394W | Von Kreudenstein et al. (2013) |
| Amgen | Charge pair | K409D, K392D | D399K, E356K | Gunasekaran et al. (2010) |
| Rinat-Pfizer | Charge pair | D221E, P228E, L368E | D221R, P228R, K409R | Strop et al. (2012) |
| Xencor | HA-TF | S364H, F405A | Y349T, T394F | Moore et al. (2011)) |
| EMD Serono | SEEDbody | IgG/A chimera | IgA/G chimera | Davis et al. (2010) |
| Regeneron | Differential protein A affinity | H435R | None | Davis et al. (2013) |

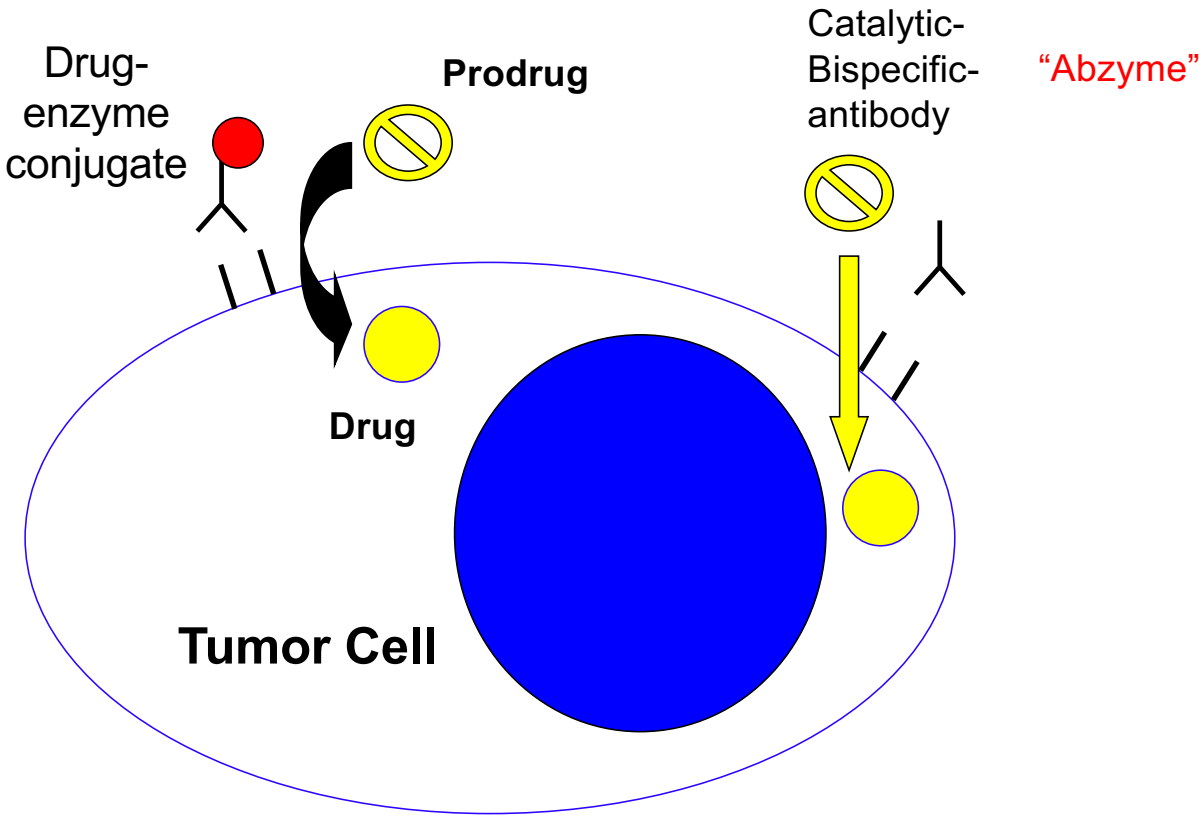
*Mol. Immunol, 2015*

# Bispecific Antibody Targets

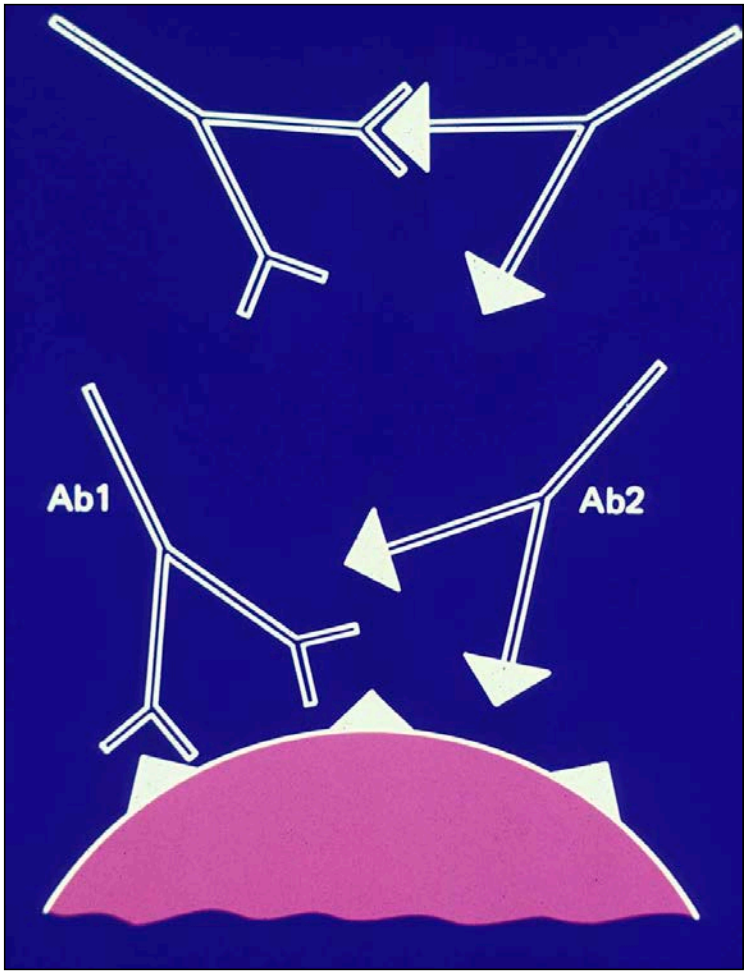
| Effector cell | Trigger molecule | Objective |
|---|---|---|
| T cells | TCR/CD3 | Tumor cytotoxicity |
| | CD2 | |
| NK cells | FcγRIIIa (CD16) | Tumor cytotoxicity |
| | CD38 | Kill virus-infected cells |
| | CD44 | |
| | CD56 | |
| | CD69 | |
| Granulocytes | FcγRI (CD64) (activated cells) | Tumor cytotoxicity |
| | FcαRI (CD89) | Kill pathogens |
| | CR3 (CD11b/CD18) | |
| Monocytes/ Macrophages | FcγRI (CD64) | Tumor cytotoxicity |
| | FcαRI (CD89) | Kill pathogens |
| | CR3 (CD11b/CD18) | ↑ Antigen presentation |
| | Mannose receptor | |
| Dendritic cells | FcγRI (CD64) | ↑ Antigen presentation |
| | Mannose receptor | |
| Erythrocytes | CR1 (CD35) | Clearance of pathogens/ Immune complexes |

Abbreviations: CR, complement receptor; FcR, Fc receptor; TCR, T-cell receptor.

# Strategies for Antibody Therapy: ADEPT and ADAPT



# Anti-Id Vaccine



# Multifunctional Antibodies – 1
## Rituximab anti-CD20

- ADCC
- CMC
- Decrease MAPK, AKT, IL10, BCL-2, P-Gp
- Increase ceramide signaling, apoptosis

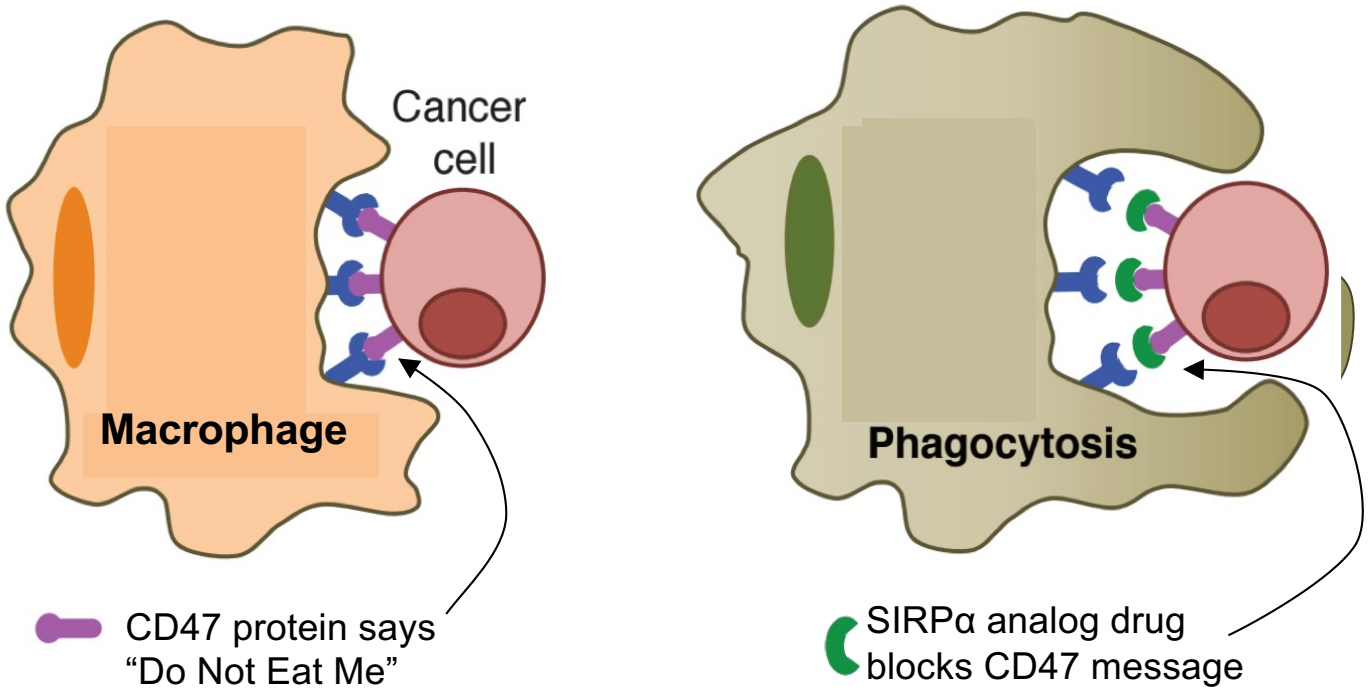
# **Multifunctional Antibodies – 2**
## Traztuzumab anti-her-2

- Blocks HER2 signaling (MAPK, PI3K)
- Increases HER2 degradation
- Results in cell cycle arrest, apoptosis
- ADCC
- Anti-angiogenic
- Decreased HER2 proteolysis & soluble form

# Antibodies as Immune Modulators

- Bound antigen presented by APC to T cells
- Activating FcR receptor
- Inactivating FcR receptors
- Use as Anti-idiotype vaccine
- Checkpoint Blockade: PD1, CTLA-4, Tim3, CD47/SIRP-a...

# Cancer Cells Tell the Immune System: "Do Not Eat Me"



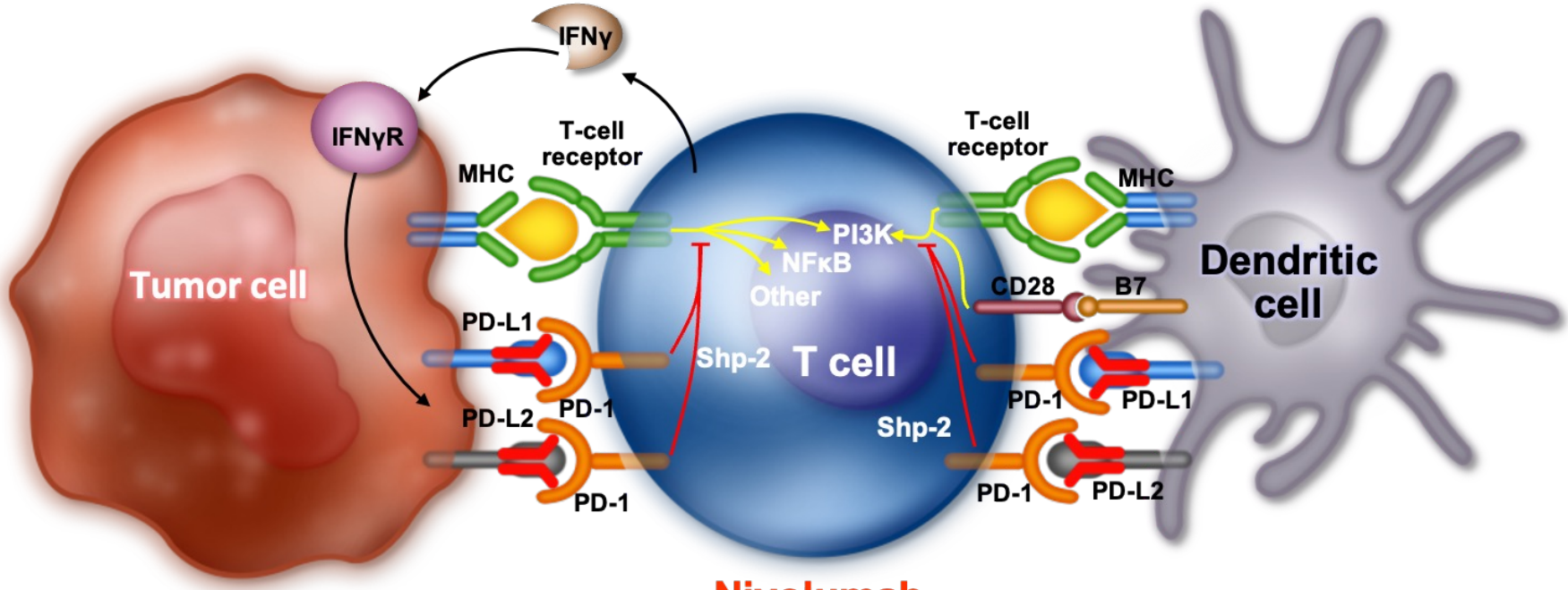

Macrophages might normally eat cancer cells, but cancer cells evade being eaten.

*Weiskopf et al, 2013 (Christopher Garcia lab)*

# Role of PD-1 Pathway in Suppressing Anti-Tumor Immunity

**Recognition of tumor by T cell through MHC/antigen interaction mediates IFNγ release and PD-L1/2 up-regulation on tumor**

**Priming and activation of T cells through MHC/antigen & CD28/B7 interactions with antigen-presenting cells**



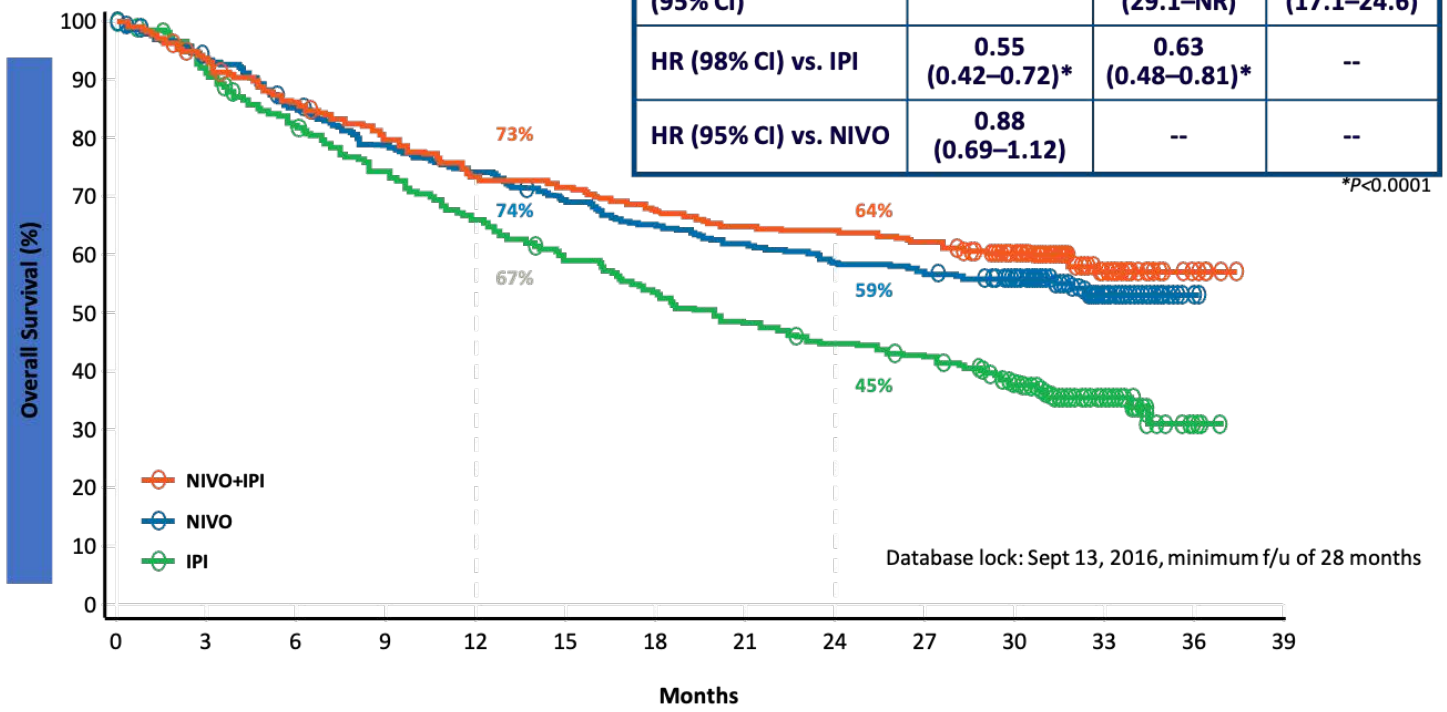

**Nivolumab**
**PD-1 Receptor Blocking Ab**

*Sznol et al., ASCO, 2013*

# Overall Survival in Melanoma

| | NIVO+IPI (N=314) | NIVO (N=316) | IPI (N=315) |
|---|---|---|---|
| Median OS, mo (95% CI) | NR | NR (29.1–NR) | 20.0 (17.1–24.6) |
| HR (98% CI) vs. IPI | 0.55 (0.42–0.72)* | 0.63 (0.48–0.81)* | -- |
| HR (95% CI) vs. NIVO | 0.88 (0.69–1.12) | -- | -- |

*$P<0.0001$




**Patients at risk:**

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NIVO+IPI | 314 | 292 | 265 | 247 | 226 | 221 | 209 | 200 | 198 | 192 | 170 | 49 | 7 | 0 |
| NIVO | 316 | 292 | 265 | 244 | 230 | 213 | 201 | 191 | 181 | 175 | 157 | 55 | 3 | 0 |
| IPI | 315 | 285 | 254 | 228 | 205 | 182 | 164 | 149 | 136 | 129 | 104 | 34 | 4 | 0 |

# Summary: Functions of Antibodies

- Binding
- Opsonization
- Complement activation
- FcR Binding  (ADCC)
- Crosslinking
- Signaling
- Antagonism of signaling
- Activation/inhibition of the immune system

# Antibodies Can Be Toxic

- Pancytopenia
- BM hypoplasia
- ITP. (platelet loss)
- AHA. (blood loss)
- Sepsis: bacterial, fungal, viral, protozoal
- Opportunistic infections
- Hyper-activation of immune system

# Form a Biotech Company: "GSK BioSciences"

Form 3 Teams: Design a Drug Platform

Challenge team 1: Your target is a cell surface protein found overexpressed on breast cancer cells (Her2), but also on some normal tissue.

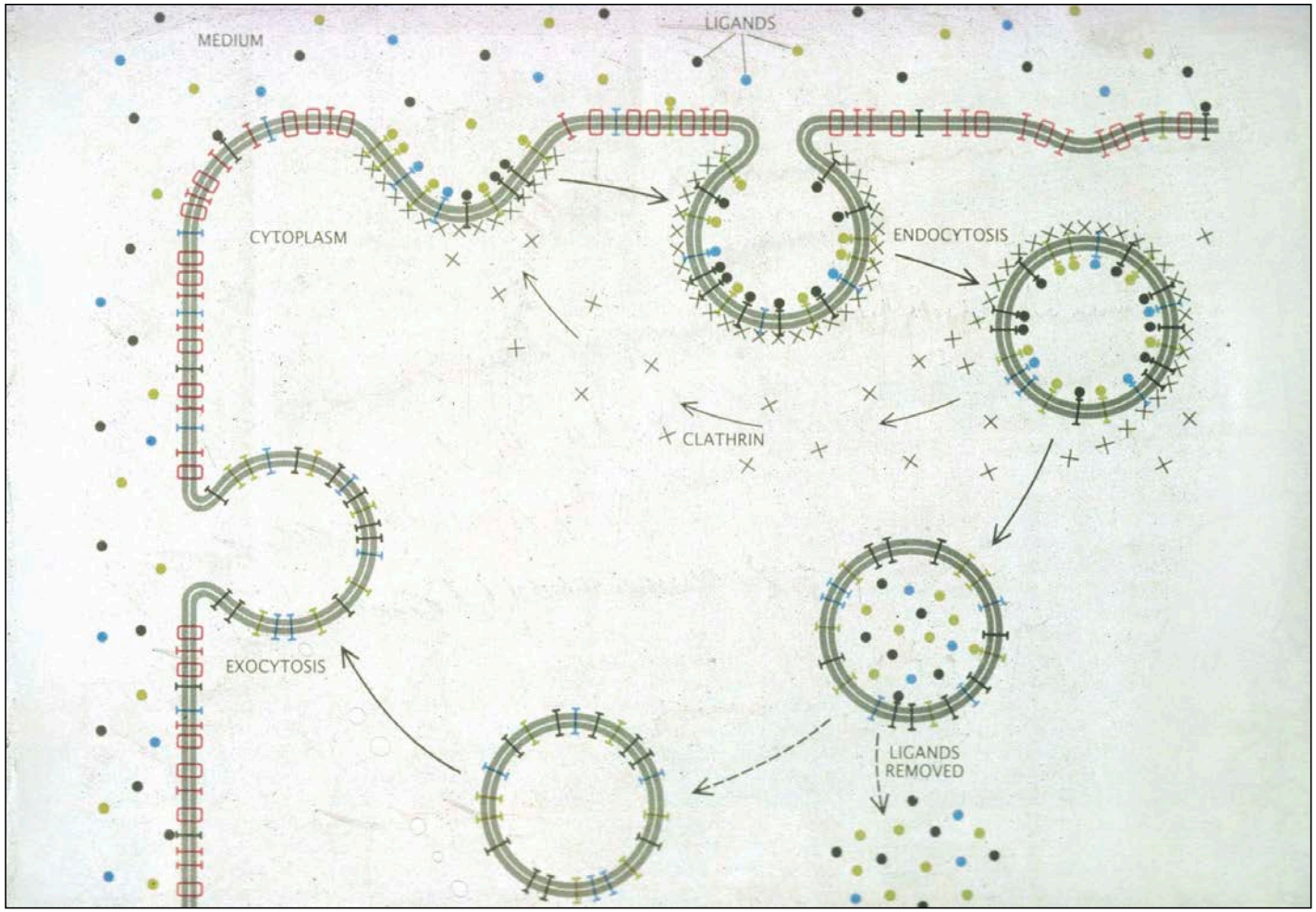
Challenge team 2:  Your target is a pancreas cancer protein inside the cell that is mutated ina single amino acid (KRAS).

Challenge team 3: Your target is on a tumor in the Brain (EGFRVIII).

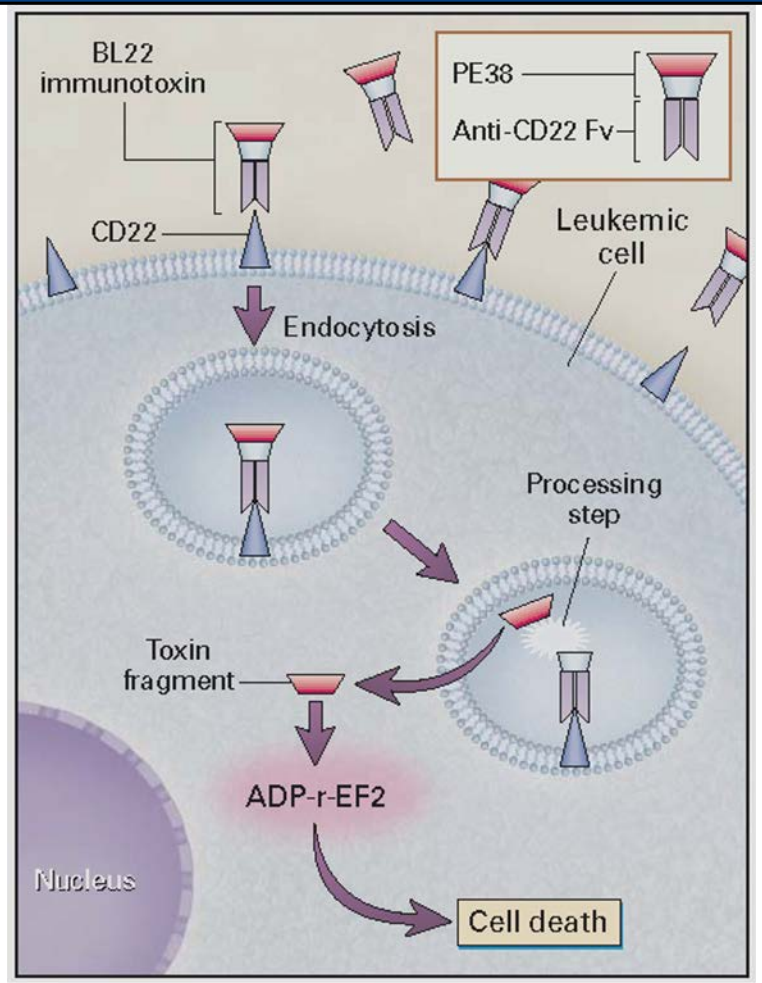
# Antibodies as Carriers

- Drugs
- Toxins
- Containers (e.g. liposomes)
- Isotopes
- Genes/viruses
- Enzymes (e.g. ADEPT)
- Cytokines
- Cells

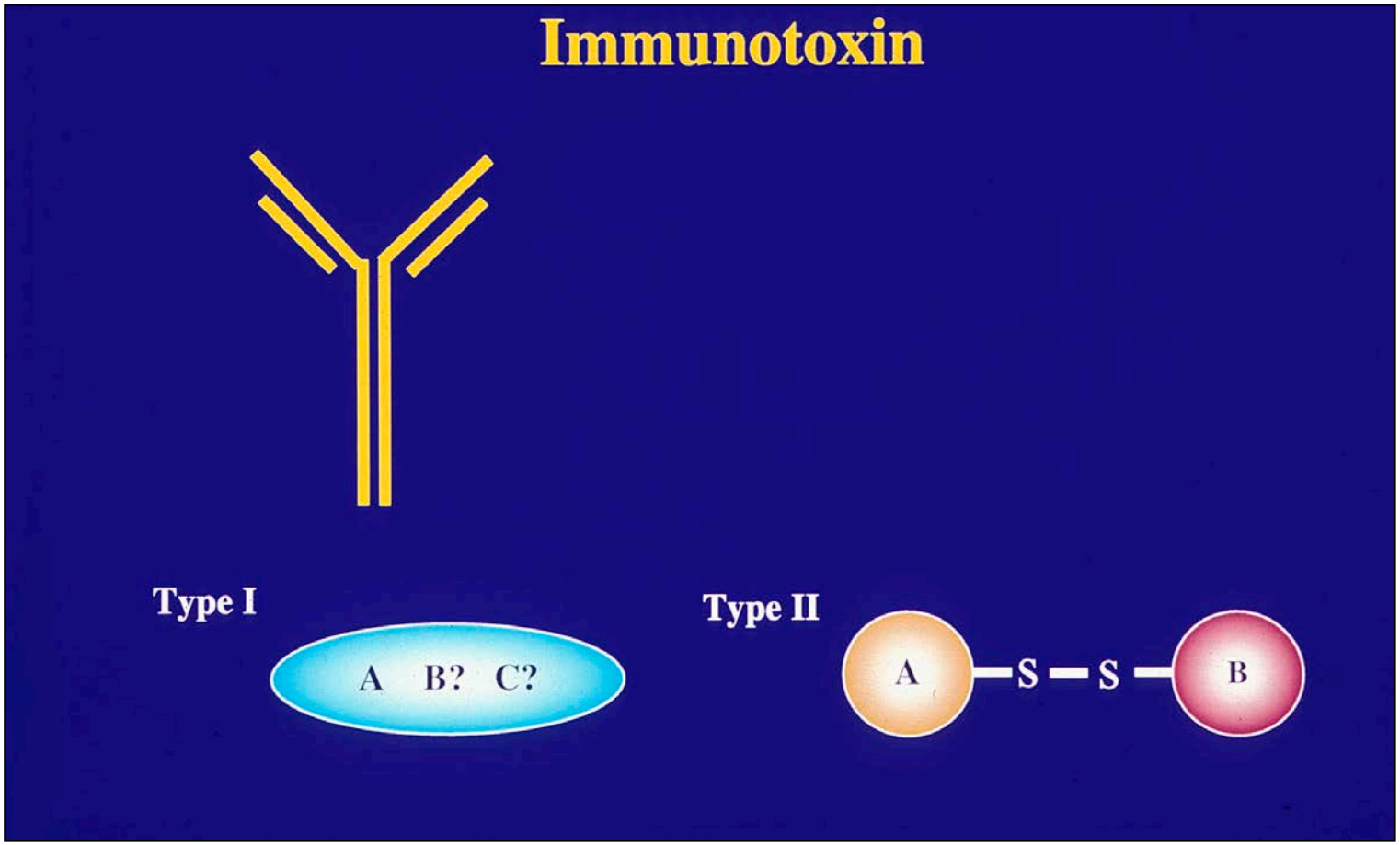
# Ig-Antigen complexes internalize



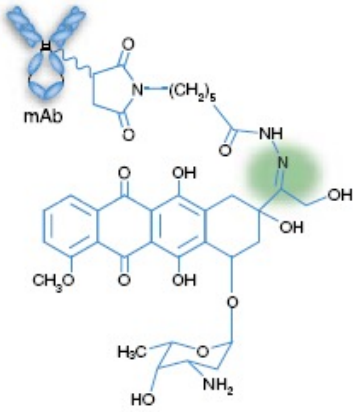
# Using Ig-Ag internalization to deliver toxins



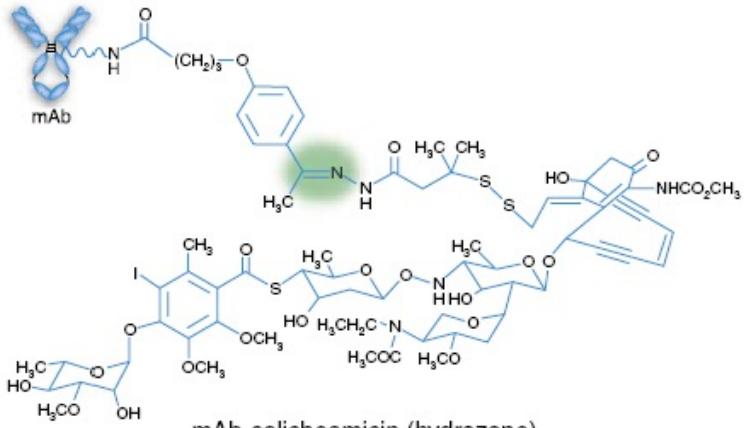
# Ig- plant hemi-toxins



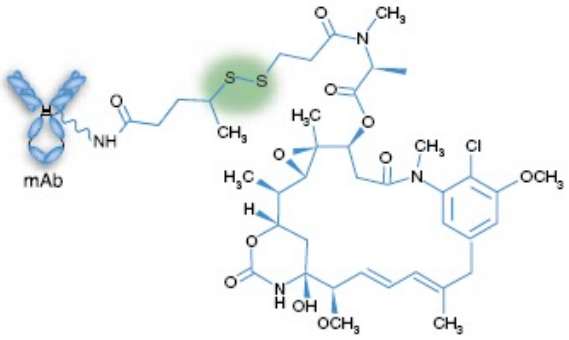
# Antibody-Drug Conjugates (ADC's)



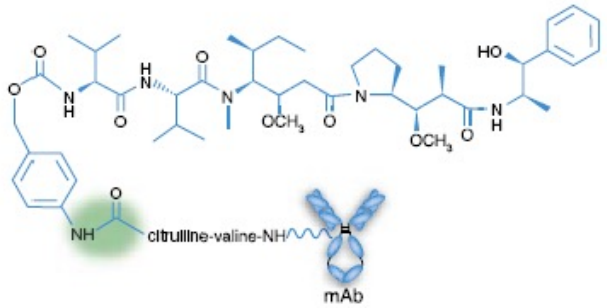

mAb-doxorubicin (hydrazone)




mAb-calicheamicin (hydrazone)




mAb-DM1 (disulfide)




mAb-auristatin (peptide)

*P. Senter*

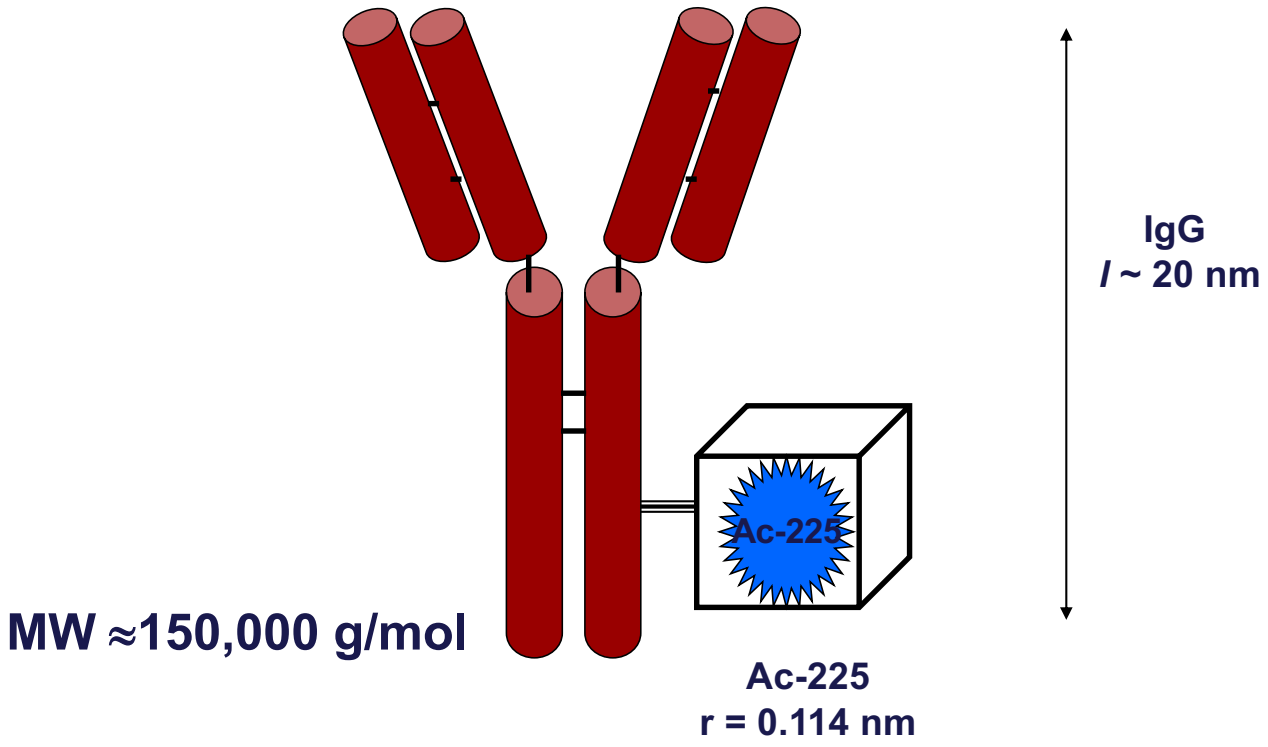
# Toxin-Antibody Conjugate Issues

- Potency
- Size
- Immunogenicity
- Internalization/ compartmentalization

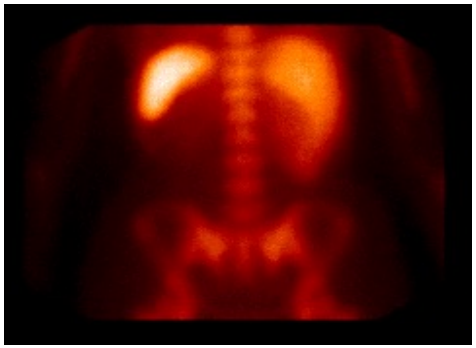
# Drug-Antibody Conjugate Issues

- Linkage and release or lack of release
- Potency
- Resistance
- Internalization/compartmentalization
- Specificity
- Clearance/metabolism
- Prodrugs

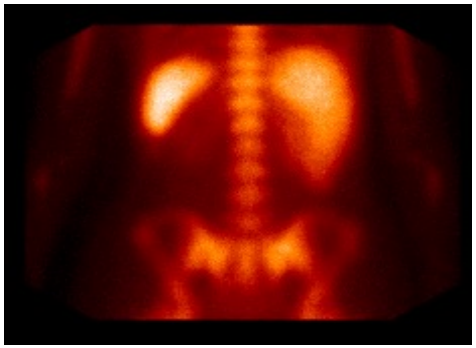
# Antibody-Chelate-Radionuclide Construct



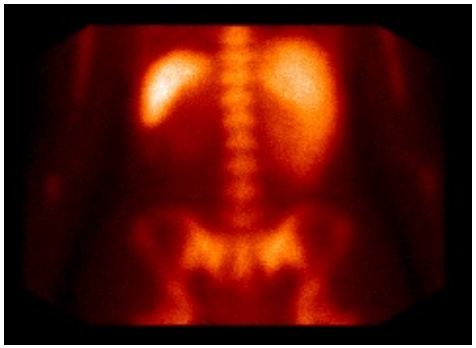
# Imaging of $^{111}$In-HuM195



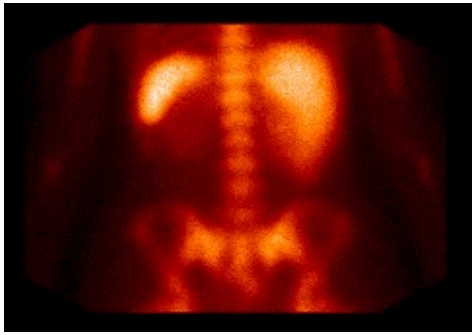
Day 0



Day 1

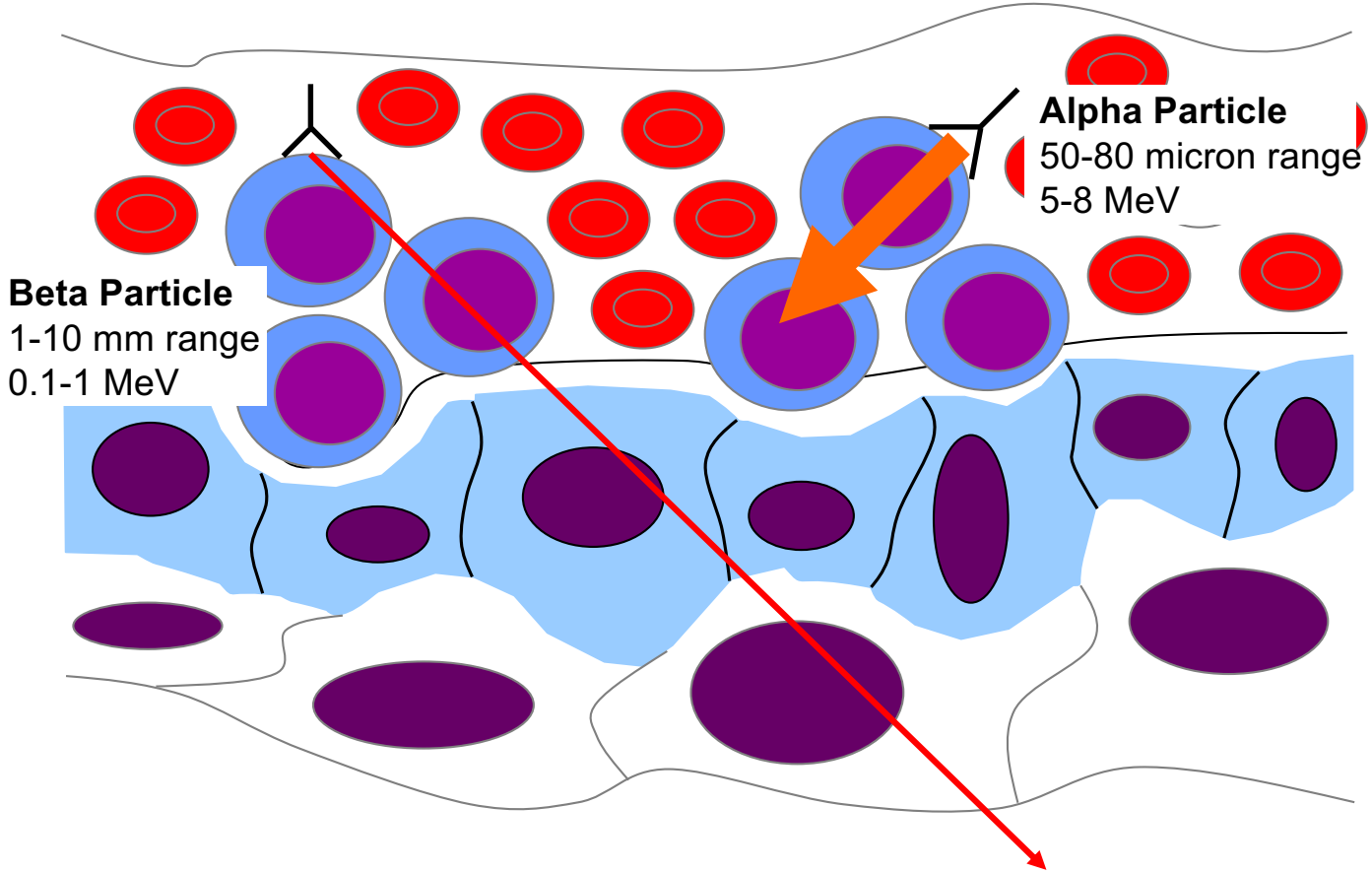


Day 2



Day 3

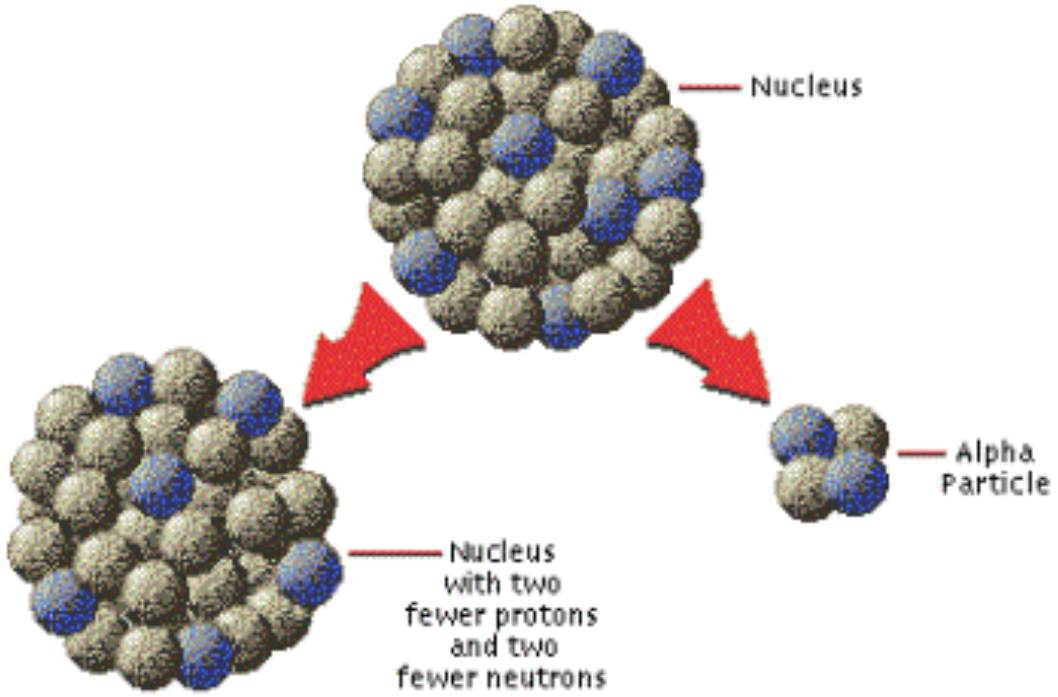
# Alpha and Beta Particle Radioimmunotherapy



# Comparison of $^{131}I$ and $^{90}Y$

| | Iodine-131 | Yttrium-90 |
|---|---|---|
| Particulate energy (keV) | 610 | 2280 |
| Mean path length (mm) | 0.8 | 5.3 |
| Half-life (days) | 8.1 | 2.7 |
| Gamma emissions | Yes | No |
| Retention after internalization | +/- | ++ |

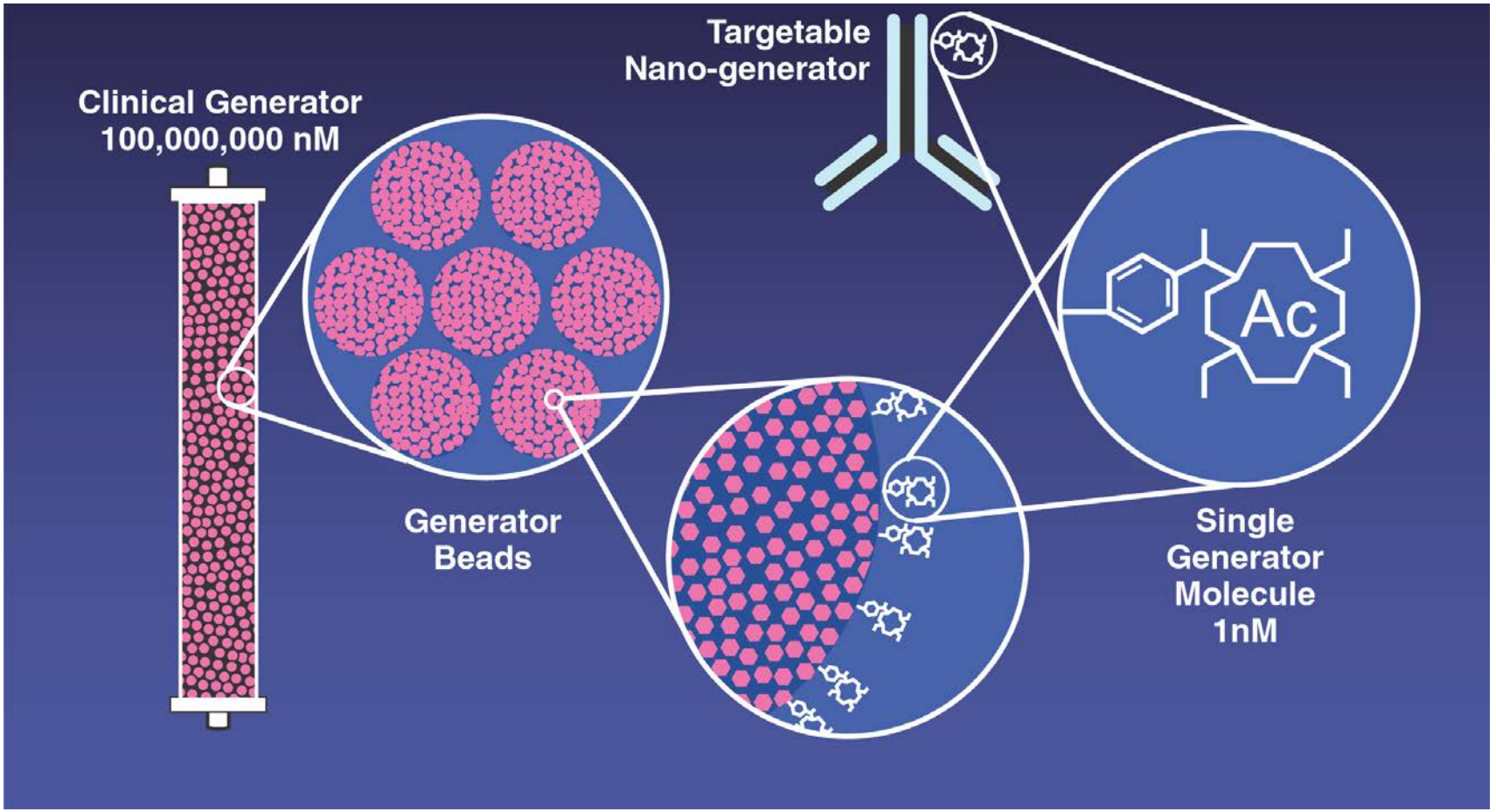
# Alpha Particle Emission




Alpha decay. The nucleus breaks down to form an atom with two fewer neutrons and two fewer protons. Also given off is an alpha particle, which is effectively a helium atom.

*epswww.unm.edu/facstaff/zsharp/ 106/atoms_files/image006.gif*

# Targetable Atomic Nano-Generator



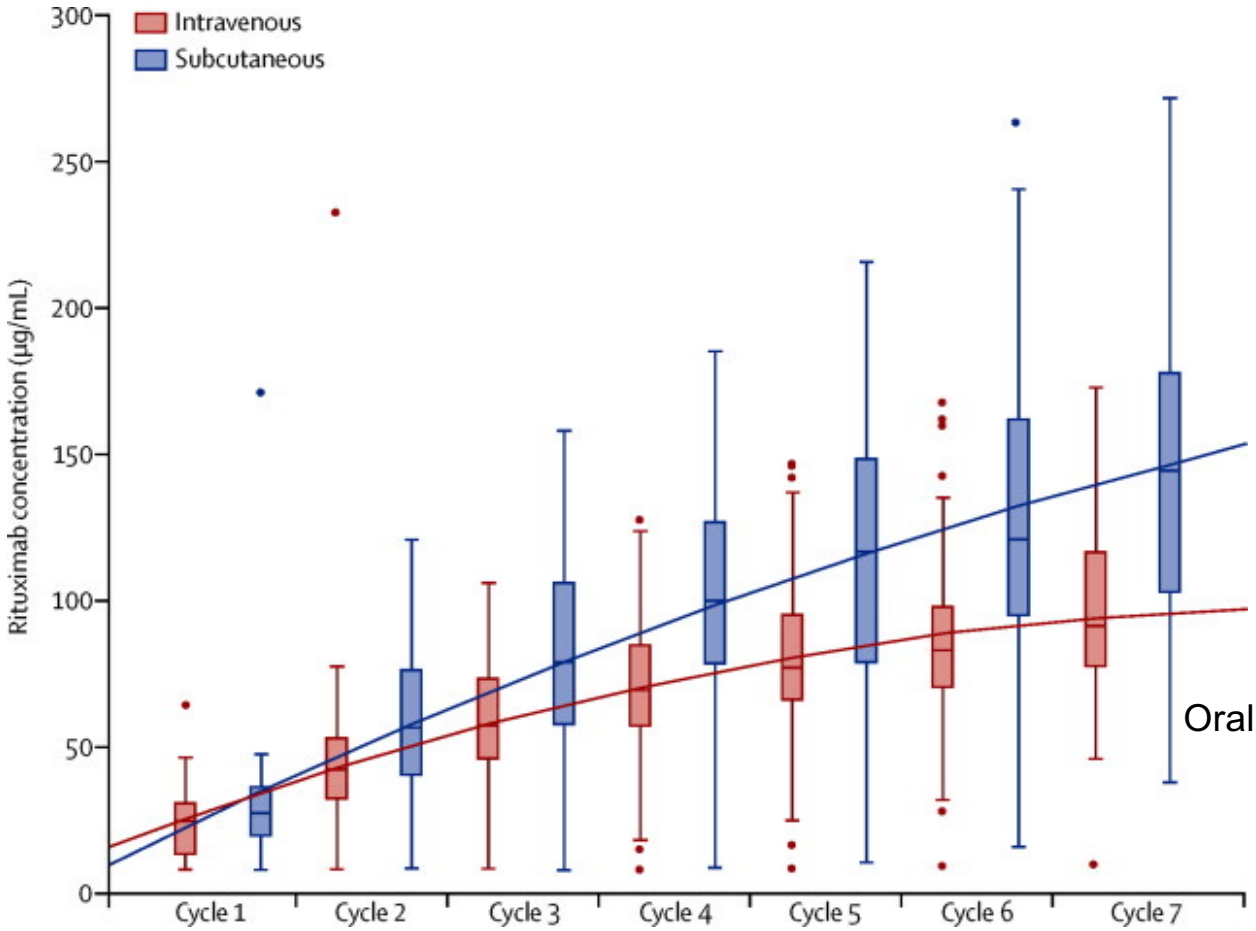
# Isotope Drug Conjugate Issues

- Emission Characteristics and complexity
- Alpha vs Beta vs Gamma
- Half-life
- Stability
- Metabolism/ daughters/ clearance

# Why Not Antibodies?

- Not oral agents
- Immunogenicity
- Pharmacokinetics/diffusion
- Half-life
- Antigen Heterogeneity/selection
- Resistance

# Easier Routes of Administration of mAb



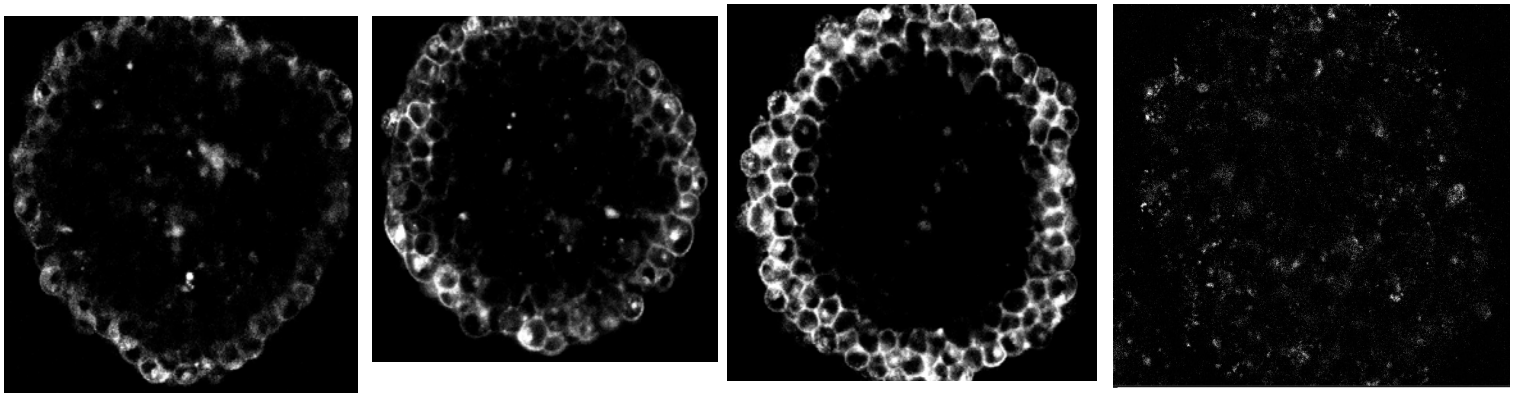

Oral next??

**Rituximab serum through concentrations by induction treatment cycle**

*Davies, et al; Lancet Oncology, 15(3), 2014*

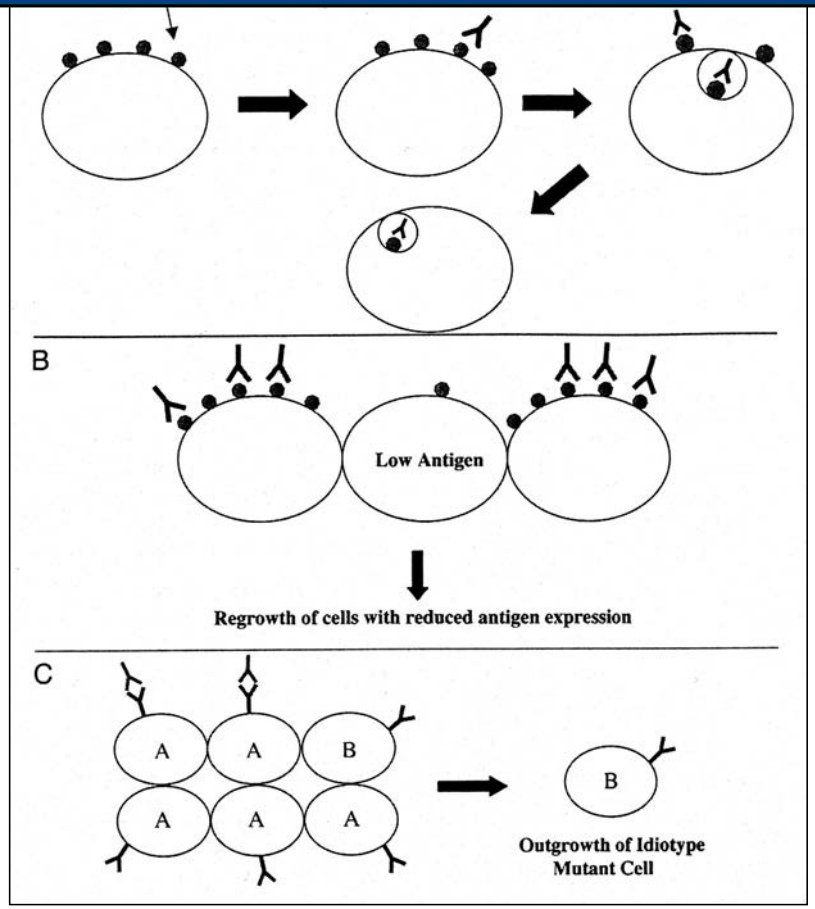
# Antibody Penetration is Slow

Confocal microscopy slices through the equator of LNCaP spheroids following incubation with 10 μg/ml FITC-J591 and wash (3x).

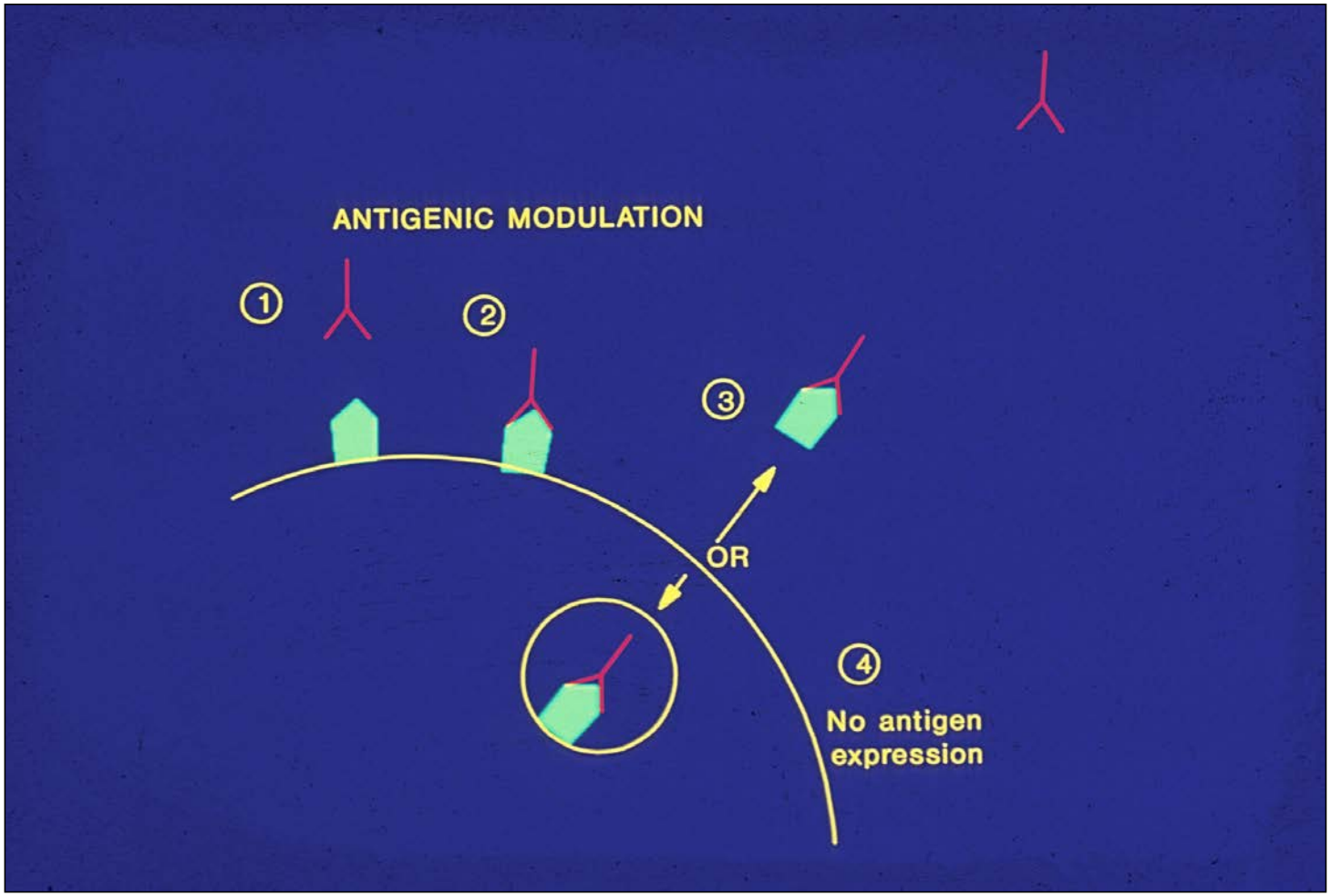


| Incubation duration (min): | | | |
|---|---|---|---|
| 15 | 60 | 120 | 120 |
| **Ab penetration (cell diameters):** | | | |
| 1 | 2 | 3 | Control: (10 μg/ml FITC-HuM195) |

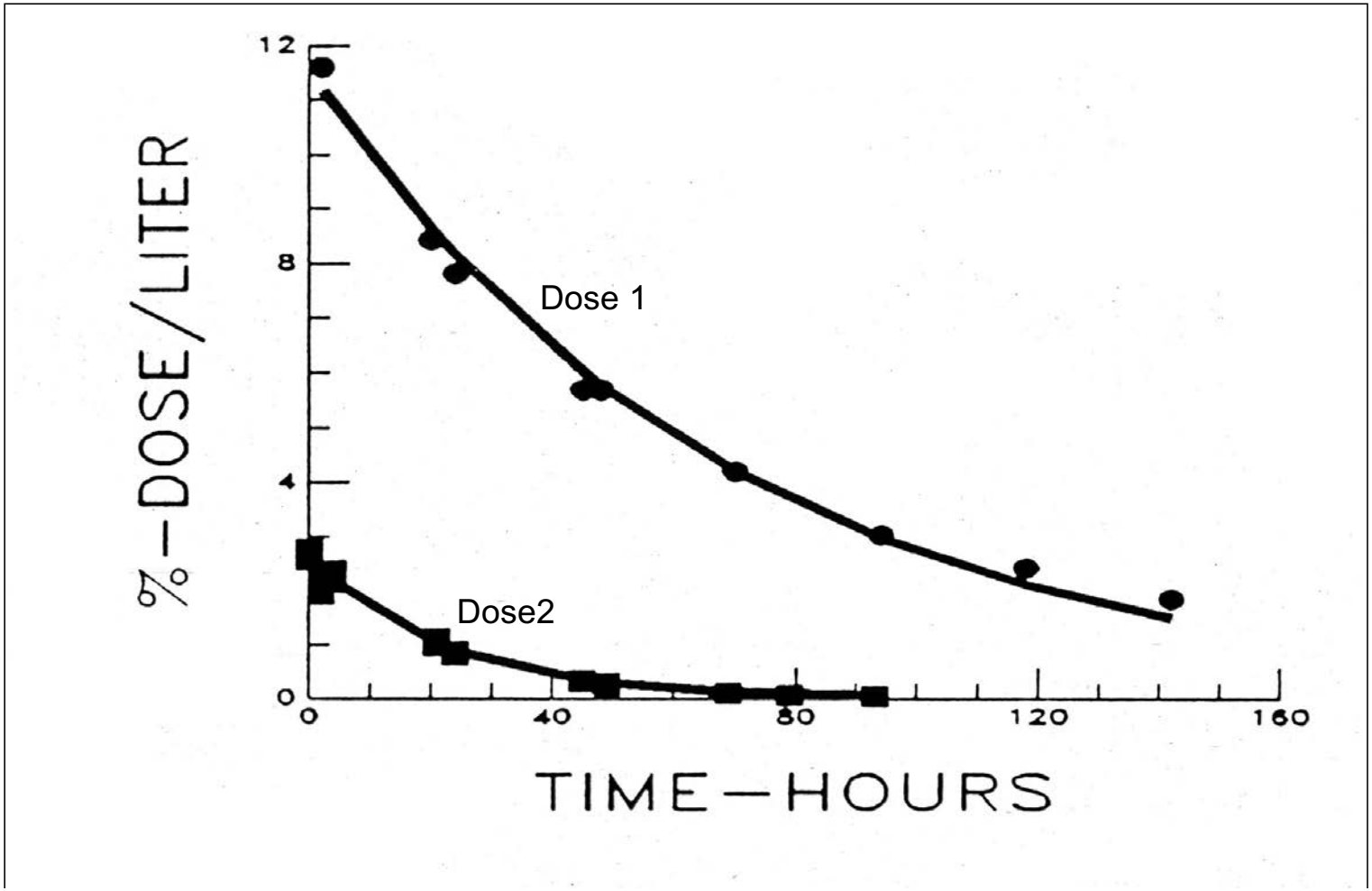
# Resistance to Antibody Therapy (downregulation and heterogeneity)



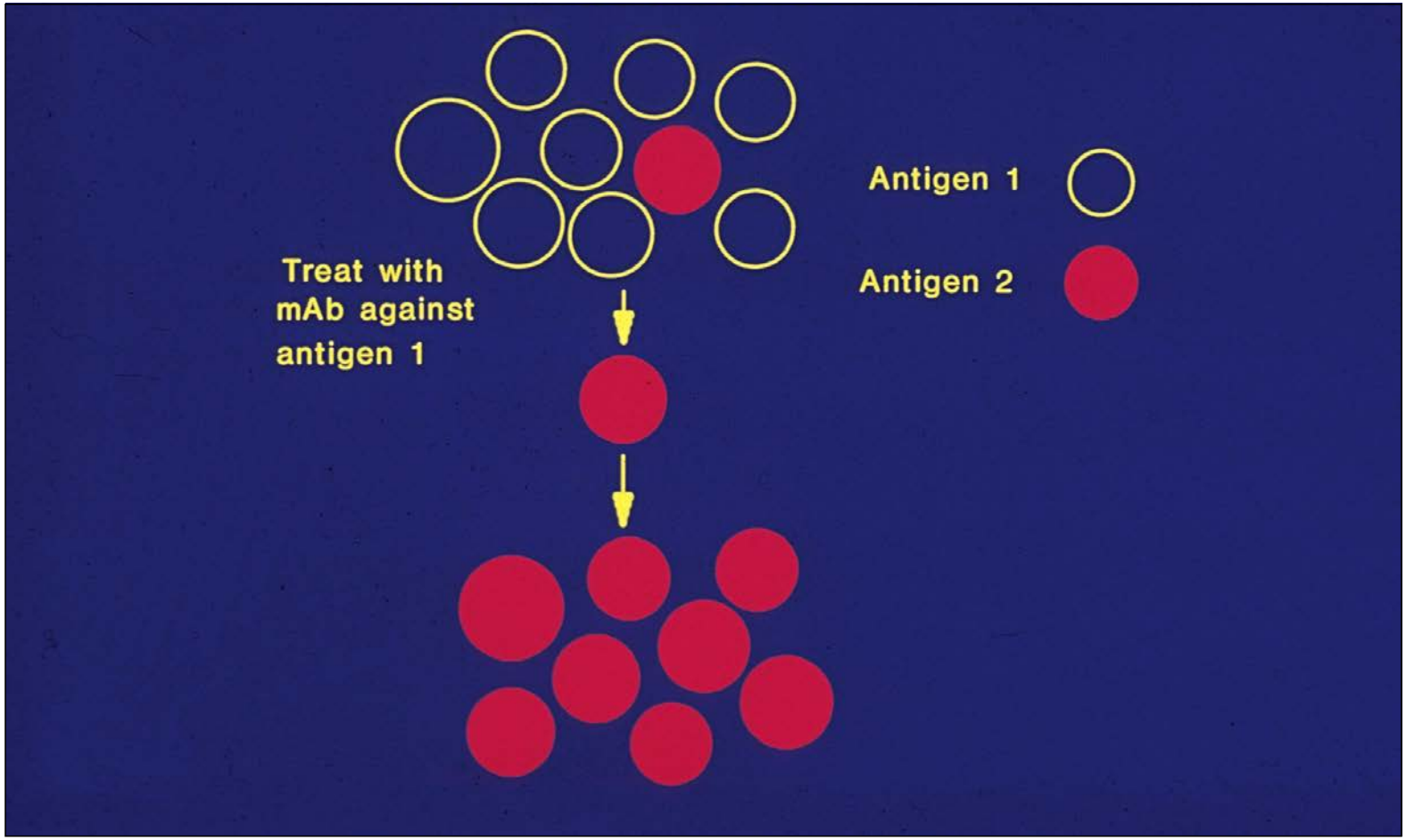
# Ag down regulation leads to escape



# HAMA, HAHA, HACA… In Treated Humans



# Immunoselection leads to escape



# Summary: mAb Functionality

## Monoclonal Antibody

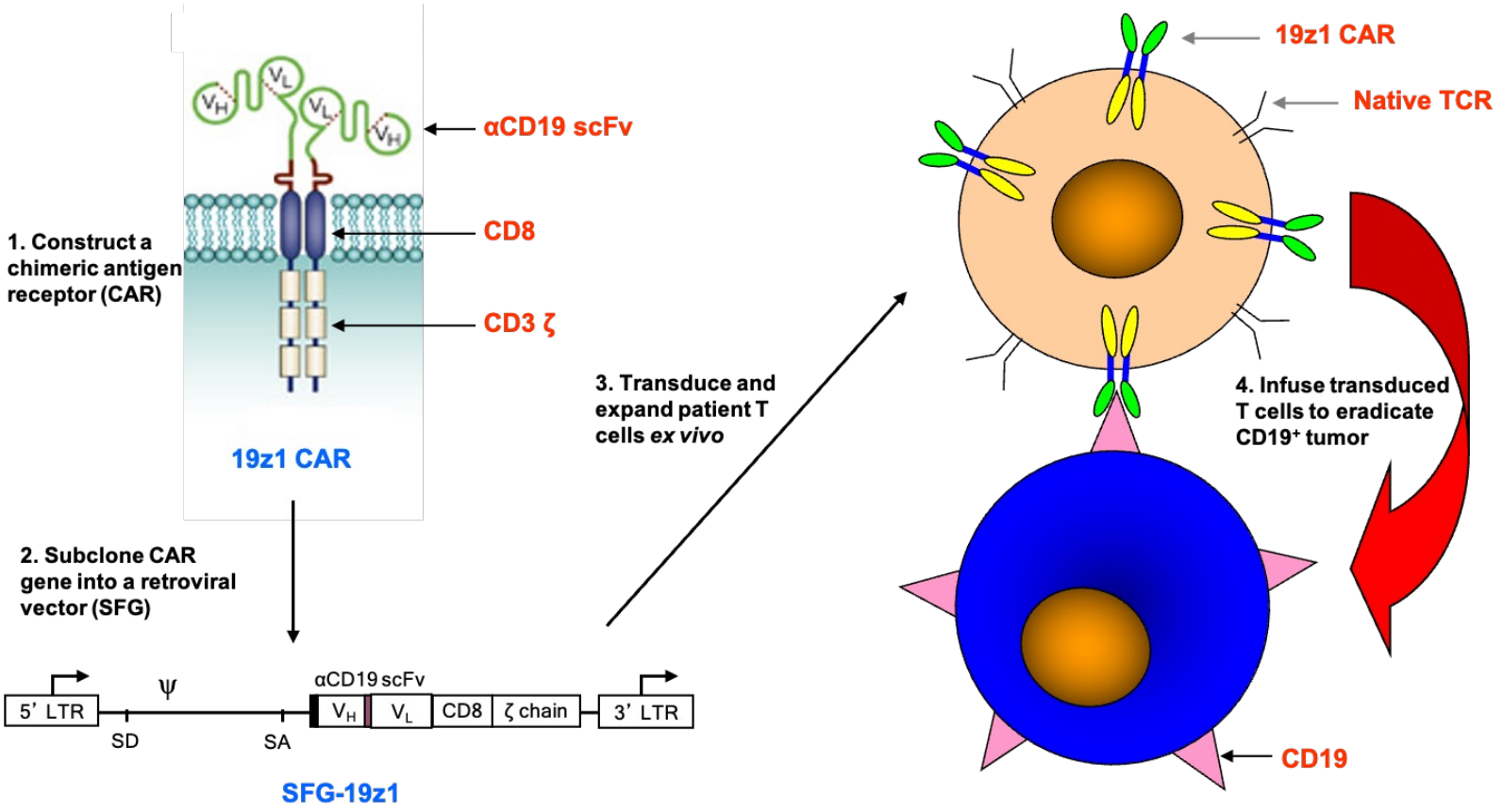
bind, block, activate, downregulate, crosslink, recruit effectors, recruit $C^1$, opsonize, sequester, immunize

carry: drugs, toxins, isotopes, imaging agents

## Small molecules

bind, block, activate, down regulate, penetrate

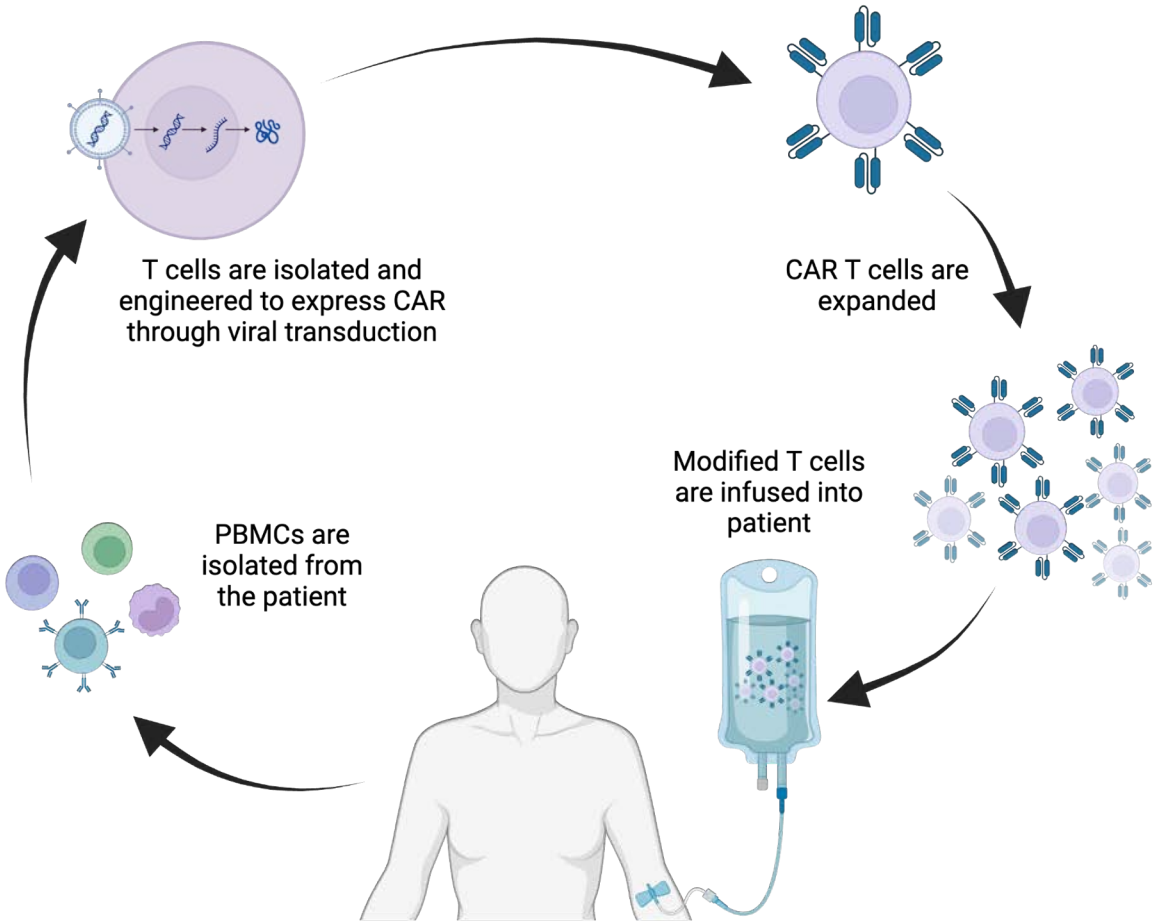
# Generation of CD19-Targeted T cells for Treatment of B cell Malignancies



# CAR T Cell Therapy Production in a Clinical Setting

**CAR =**
**Chimeric Antigen Receptor**

**“Synthetic Immunology”**

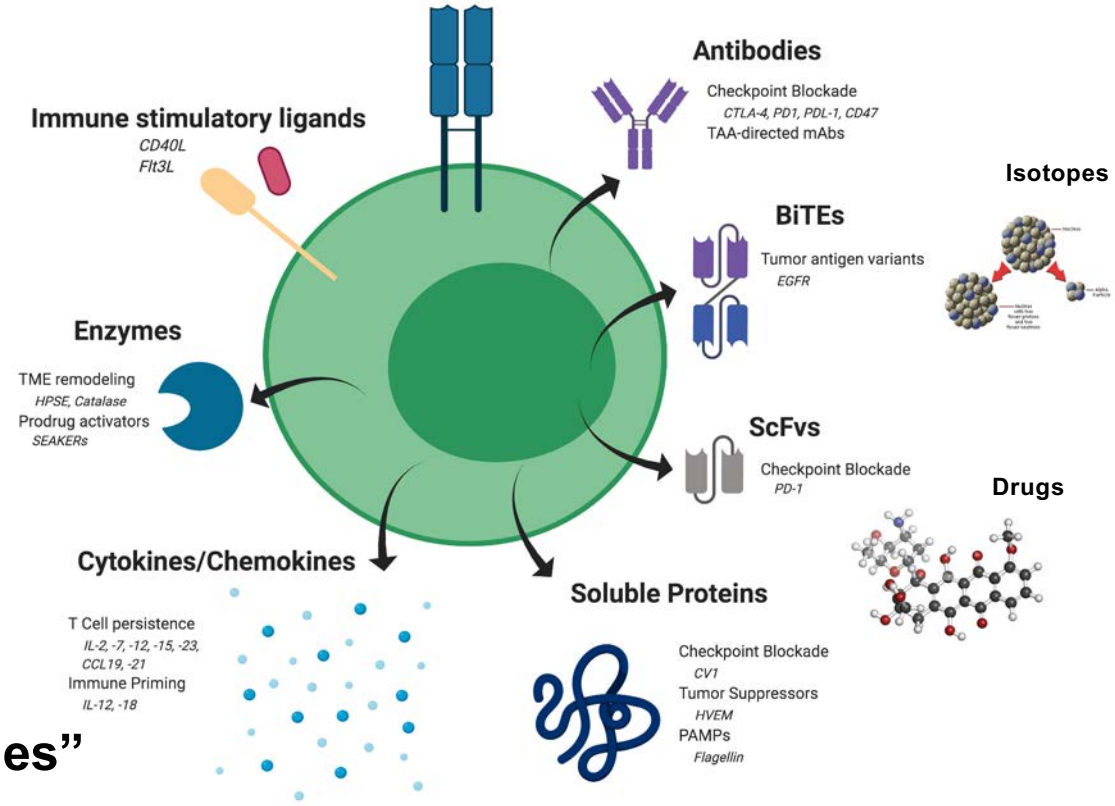



*Guzman, G. et al. (2023)*

# Specificity Localized with Synthetic Cells

**Can we increase specificity by using a cell to localize drug delivery?**

**Synthetic Biology Engineering for Cargo Delivery**

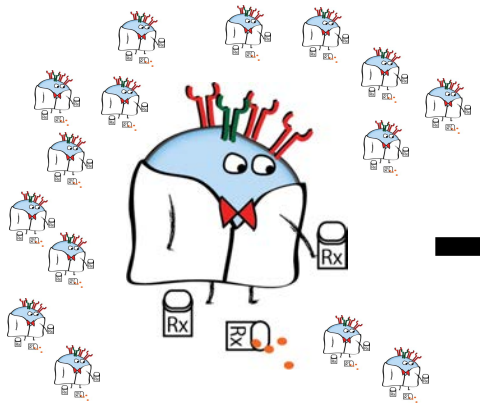



**"Targeted Cellular Micropharmacies"**

# SEAKER Cell: A Cancer-Targeted Micropharmacy

**CAR T cells are engineered to generate small molecule cancer drugs locally at the cancer**

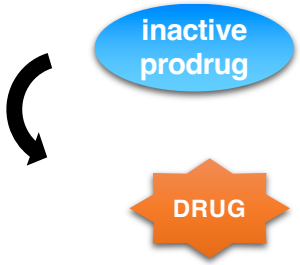
**SEAKER Cells**
**(Synthetic Enzyme-Armed KillER)**



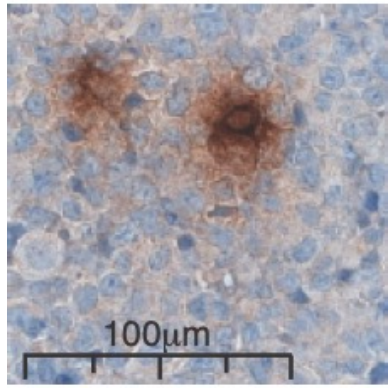

***recognizes tumor antigen and proliferates locally***


***SEAKER cell releases enzyme***

***non-toxic, inert prodrug administered systemically***




***SEAKER-expressed enzyme catalytically converts prodrug to active drug locally at tumor site***



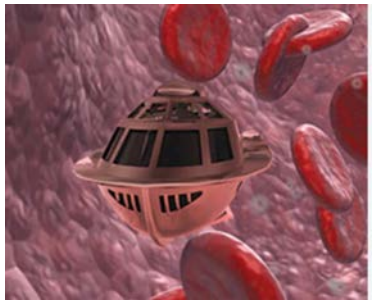

**SEAKER in Tumor Releasing Enzyme**

*Tan & Scheinberg labs:*
*Gardner, Lee, Bourne, Nature Chem Biol 2021*
*Lee, Org Lett, 2023; Bourne CIR, 2023; Corless, ACS Chem Biol,2023*

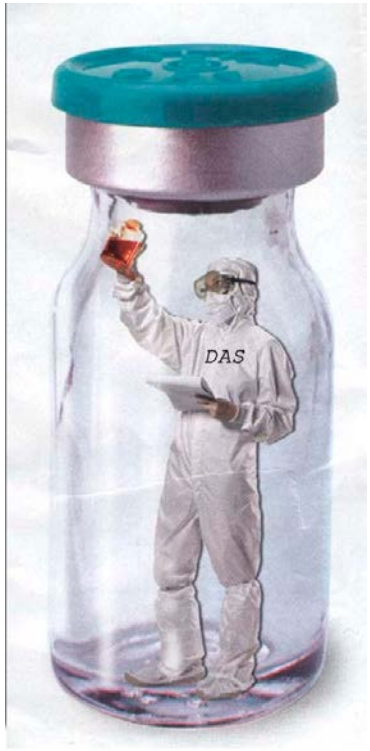
# Cancer Specificity through Drug Delivery Decisions

## Allowing cell-based drugs to make smart decisions.

**These decision gates can be applied to the Targeted Cellular Micropharmacies, yielding smarter, Targeted Cellular Micropharmacists**

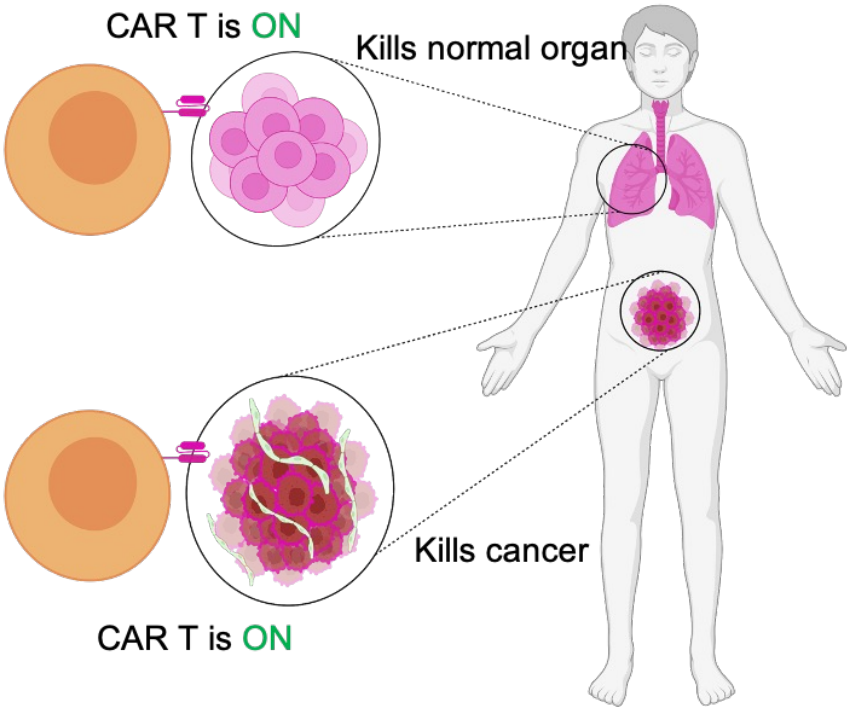


1966: Fantastic Voyage

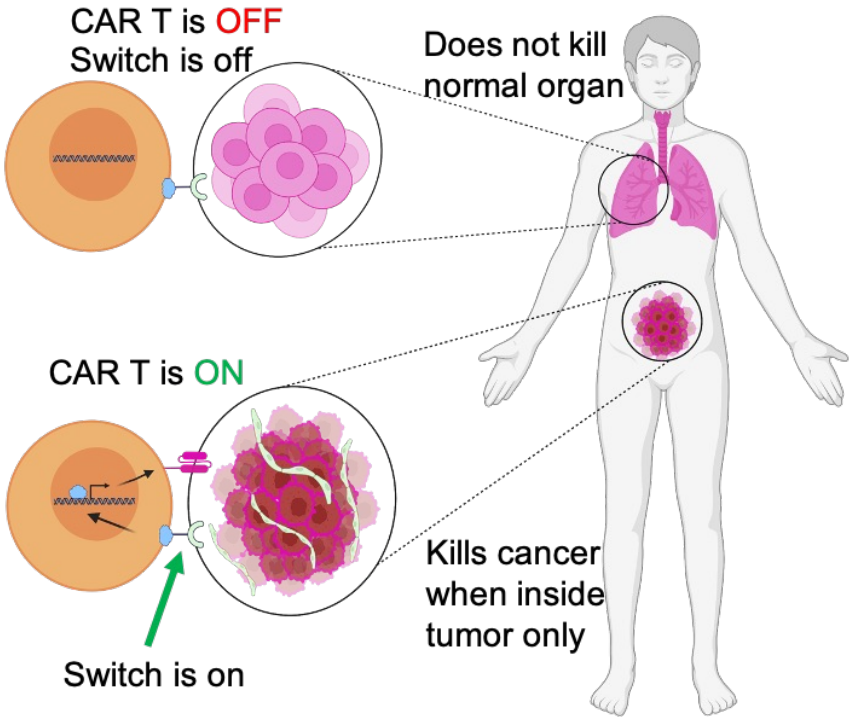


# Allowing CAR T Cells to Make Logical Decisions on When to Act

**Traditional CAR T cell: *always ON***




**"Gated" CAR T cell: *uses an ON-switch***




*SynNotch system first described by Lim*

# mAb Development Issues

## Monoclonal Antibodies

- Easy to make to any target quickly
- Predictable PK
- Predictable, usually minimal, toxicity
- Few off target effects
- Clear pathway to market
- Fastest growing class of cancer drugs
- CAR T are very expensive & patient specific
- BiTes has short T1/2 in vivo

## Small Molecules

- Usually far cheaper to manufacture
- Usually more stable
- Structure better definable
- IP simpler, but generic possible
- PO, IV, SC available

# mAb Pharmacology

| | Monoclonal Antibody | Small Molecules |
|---|---|---|
| **Kinetics** | long (weeks) | short (min – hours) |
| **Bioavailability** | Oral: very poor | varies |
| **PK analysis** | can be imaged | sampling |
| **Immunogenicity** | occasional | rare with drugs |
| **MDR/ Cross-resistance** | unlikely | yes |
| **Cross-toxicity** | less often | yes |

# mAb Biodistribution & Availability

- Small molecules far better
- Monoclonal antibody
  - not orally available
  - do not penetrate CNS well
  - enter tissues and tumors slowly
    (poor penetration may reduce toxicity)

# Form a Biotech Company: "GSK Microdelivery Systems"

Form 2 Teams: Design a drug and a back up drug

Challenge Team 1: Your target is ganglioside GD2 on lung cancer cell's surface.

Challenge Team 2: Your target is a lineage marker protein CD33 on leukemia cells that rapidly internalizes into the cell.