A Primer on

# Humoral Immunity, Antibody Constructs, and Applications to Cancer Immunotherapy

For
The International Society for Biological Therapy of Cancer

San Francisco CA
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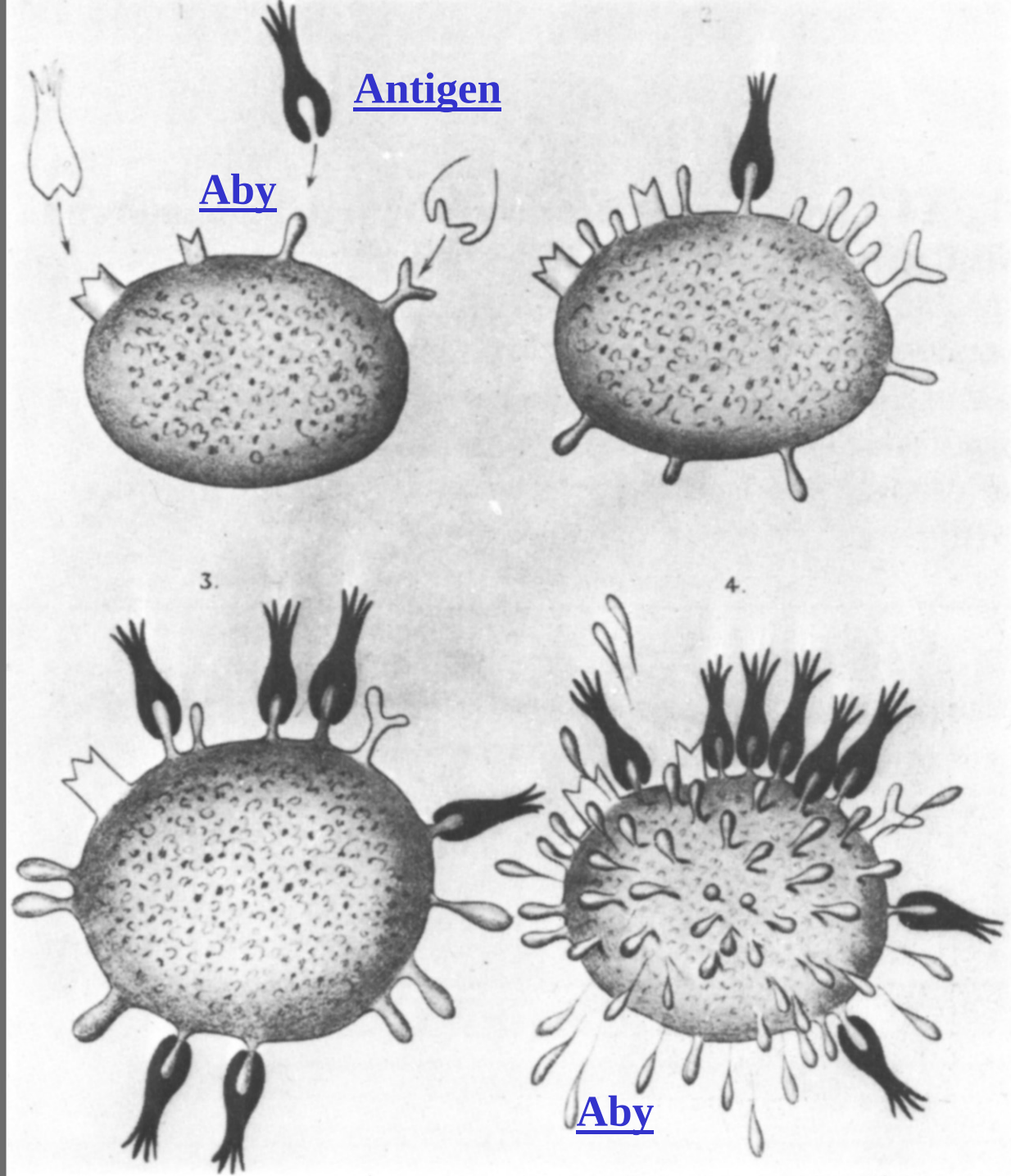
University of Wisconsin
Madison

# Humoral Immunity, Antibody Constructs and Applications to Cancer Immunotherapy

- What is Antibody (Ab)?
- Why do we have it?
- How and when is it made?
- How does it work?
- **CAN IT BE USED AGAINST CANCER?**



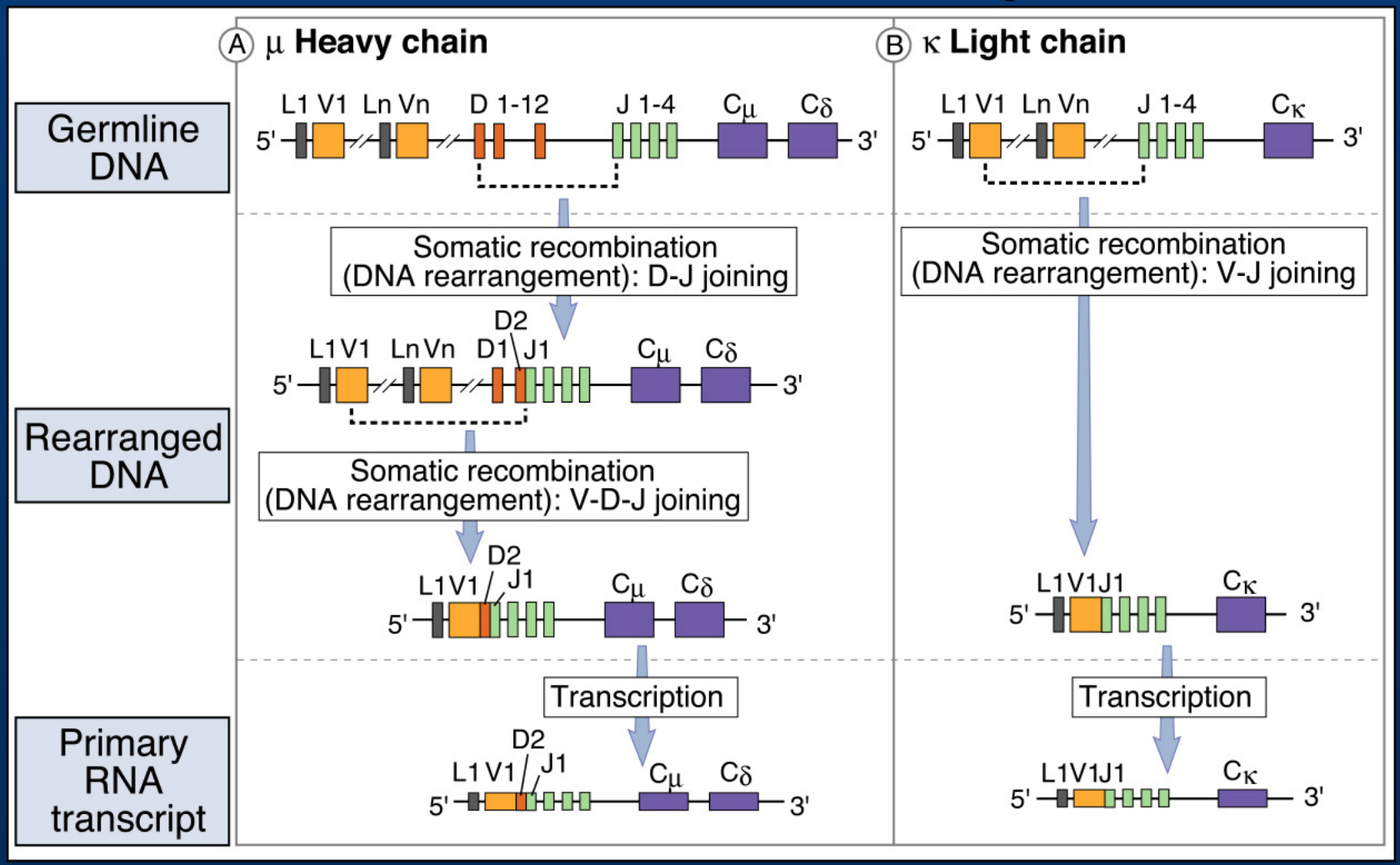

**Ehrlich's side chain theory**

**Roitt et al. 1985**

# Immunoglobulins (Antibodies)

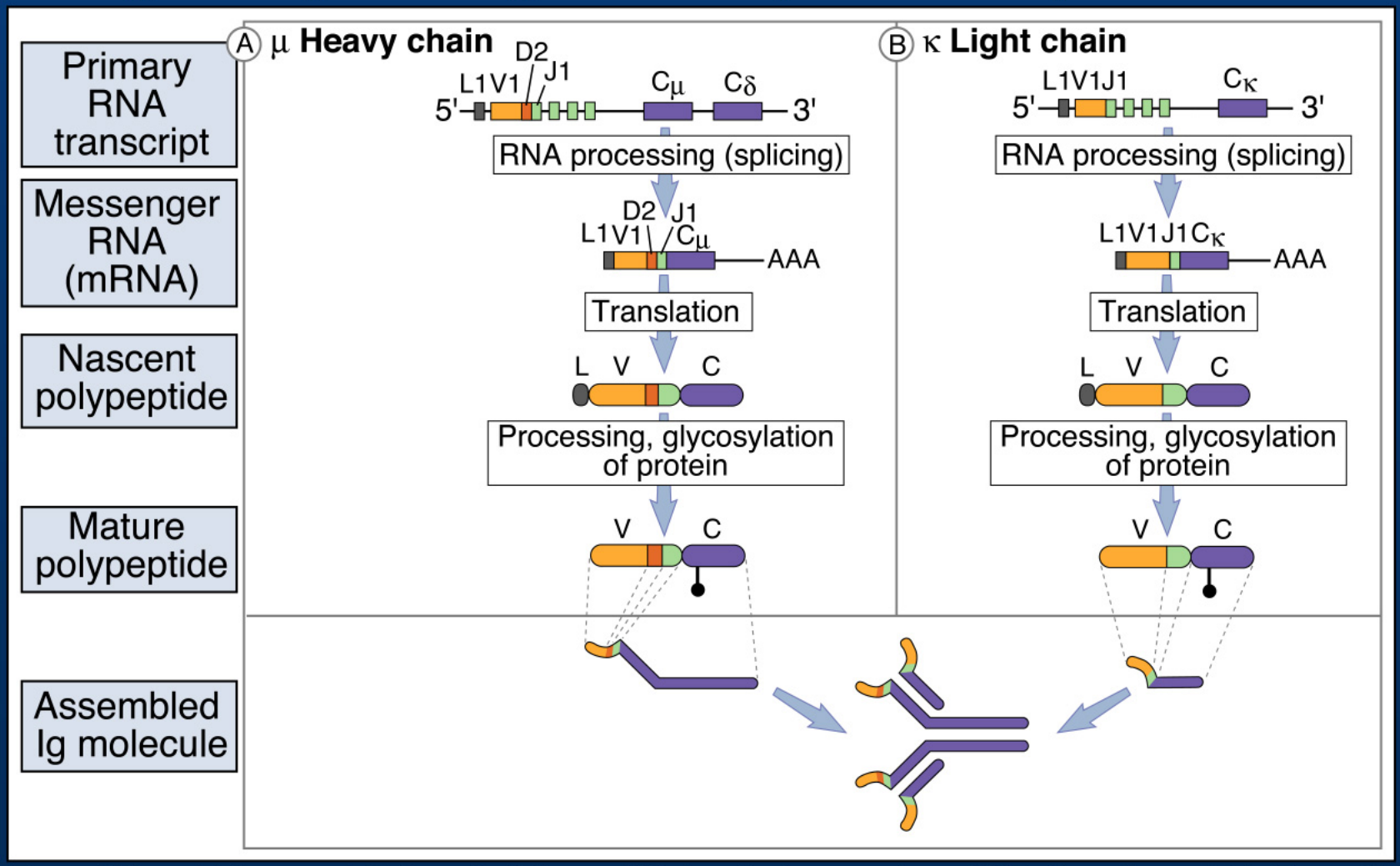
- Proteins found in plasma of all vertebrates
- Bind with high specificity to their molecular targets (antigens)
- Each individual has a broad spectrum of Aby to many, many antigens
- Provide protection against pathogens
- Demonstrate memory (better protection upon second exposure)

# Ig heavy and light chain gene recombination and transcription

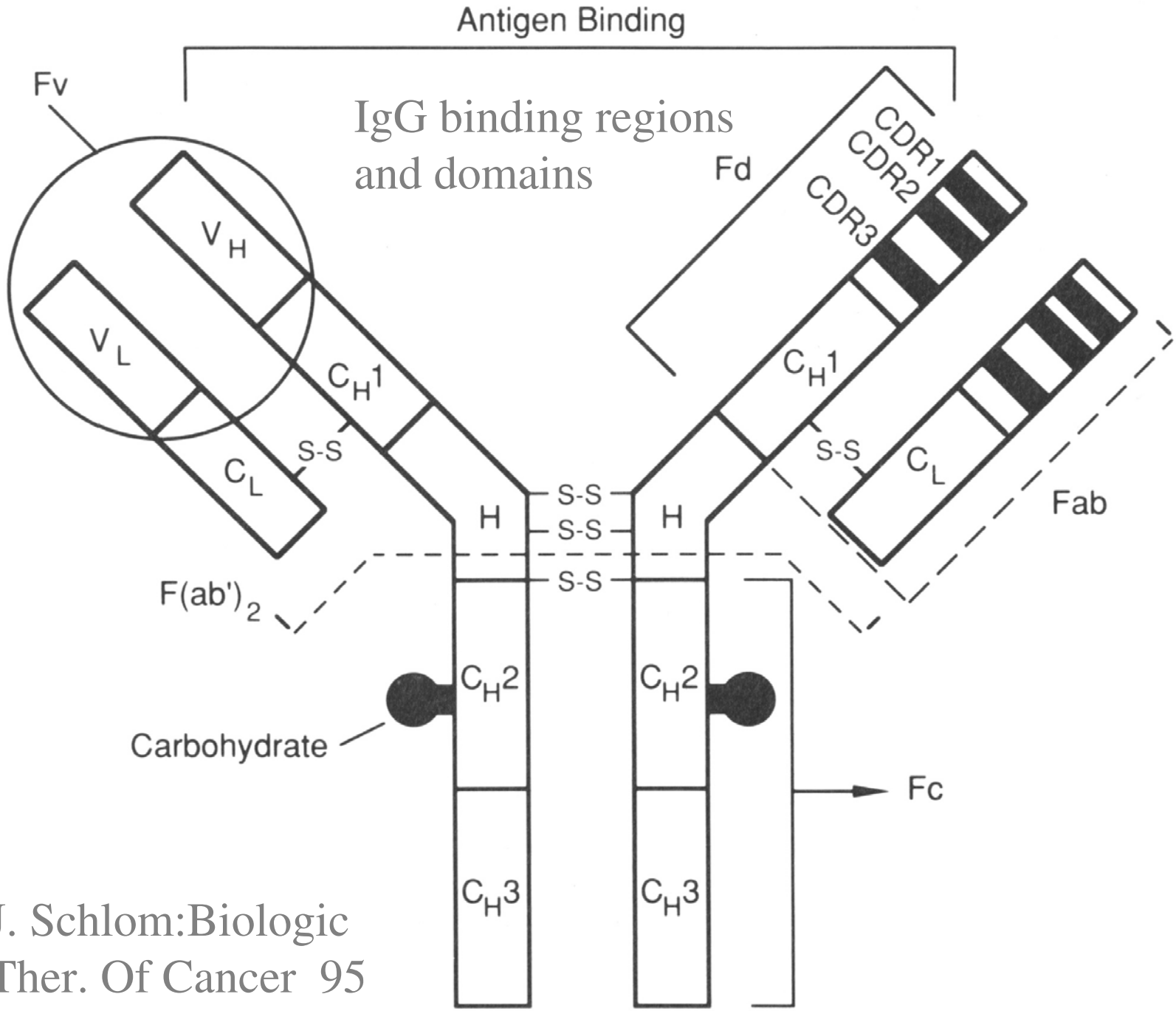



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-8a

# Ig heavy and light chain protein expression




From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-8b



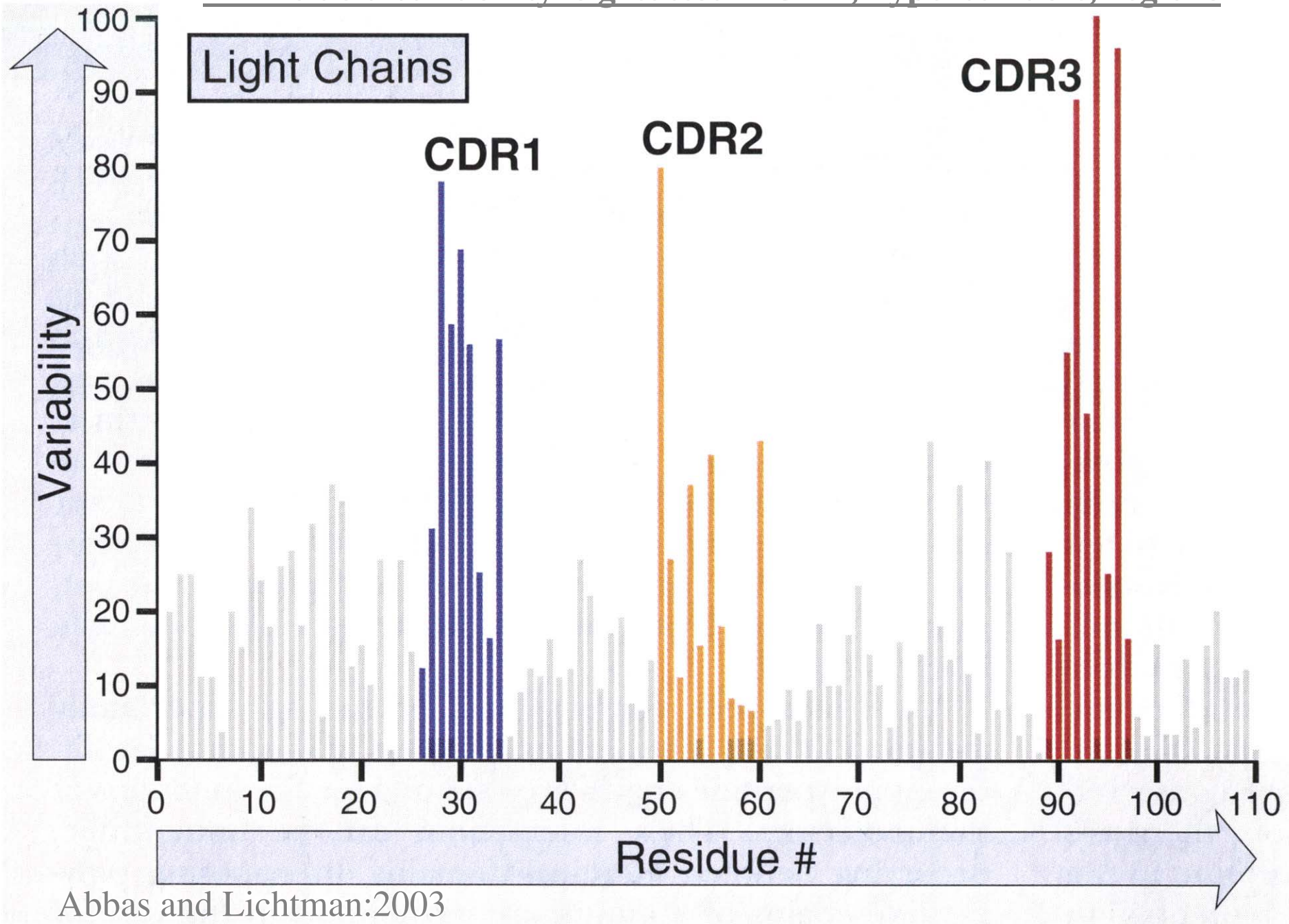

J. Schlom:Biologic
Ther. Of Cancer 95

# Immunoglobulins

- Multimeric proteins, made of heavy and light chains
- Formed by clonally distributed (~$10^9$) patterns of somatic gene rearrangements of V, D, J region genes

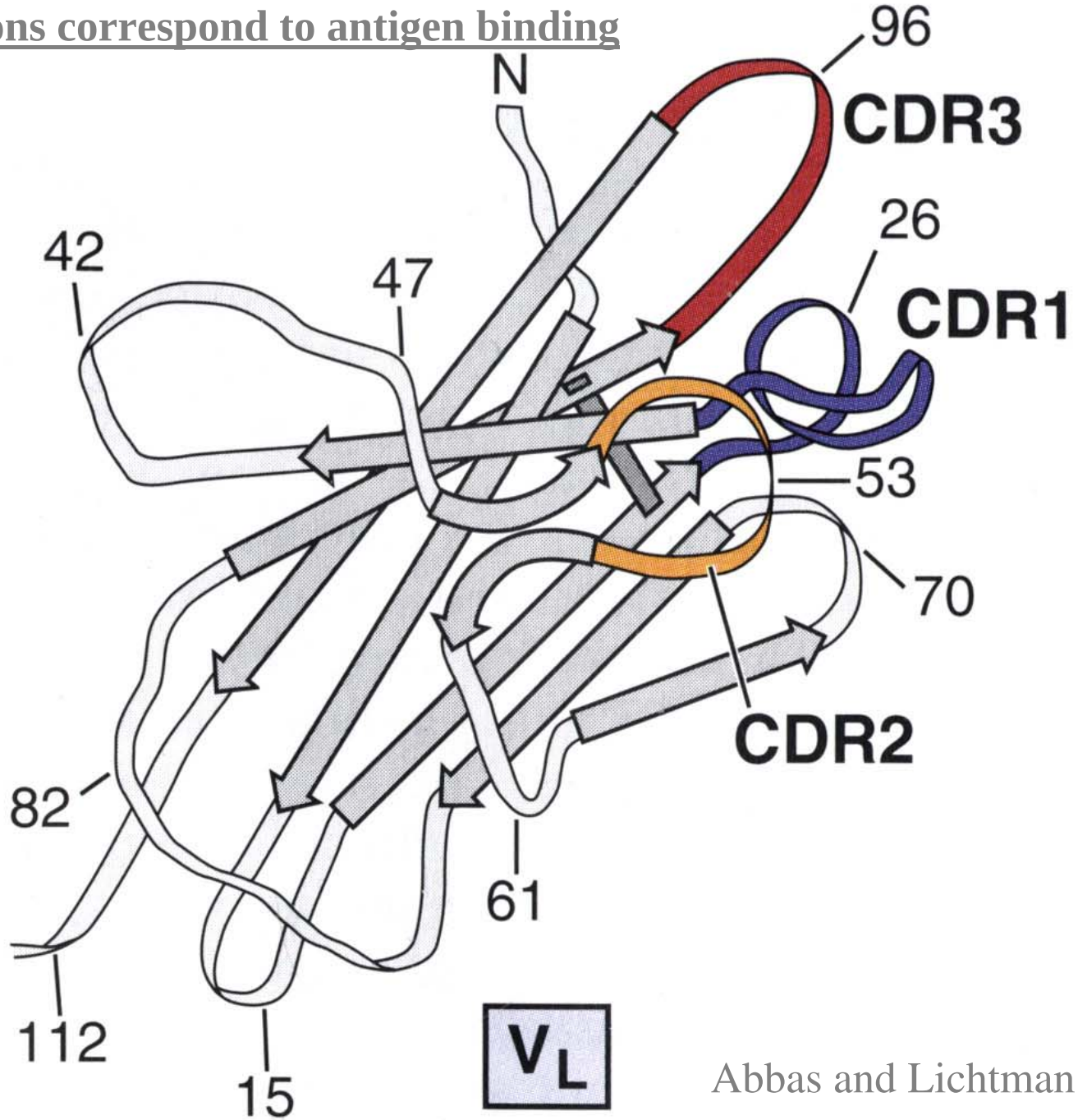
- HOW DO THEY BIND TO ANTIGEN?

# Amino acid variability is greatest in CDR, hypervariable, regions

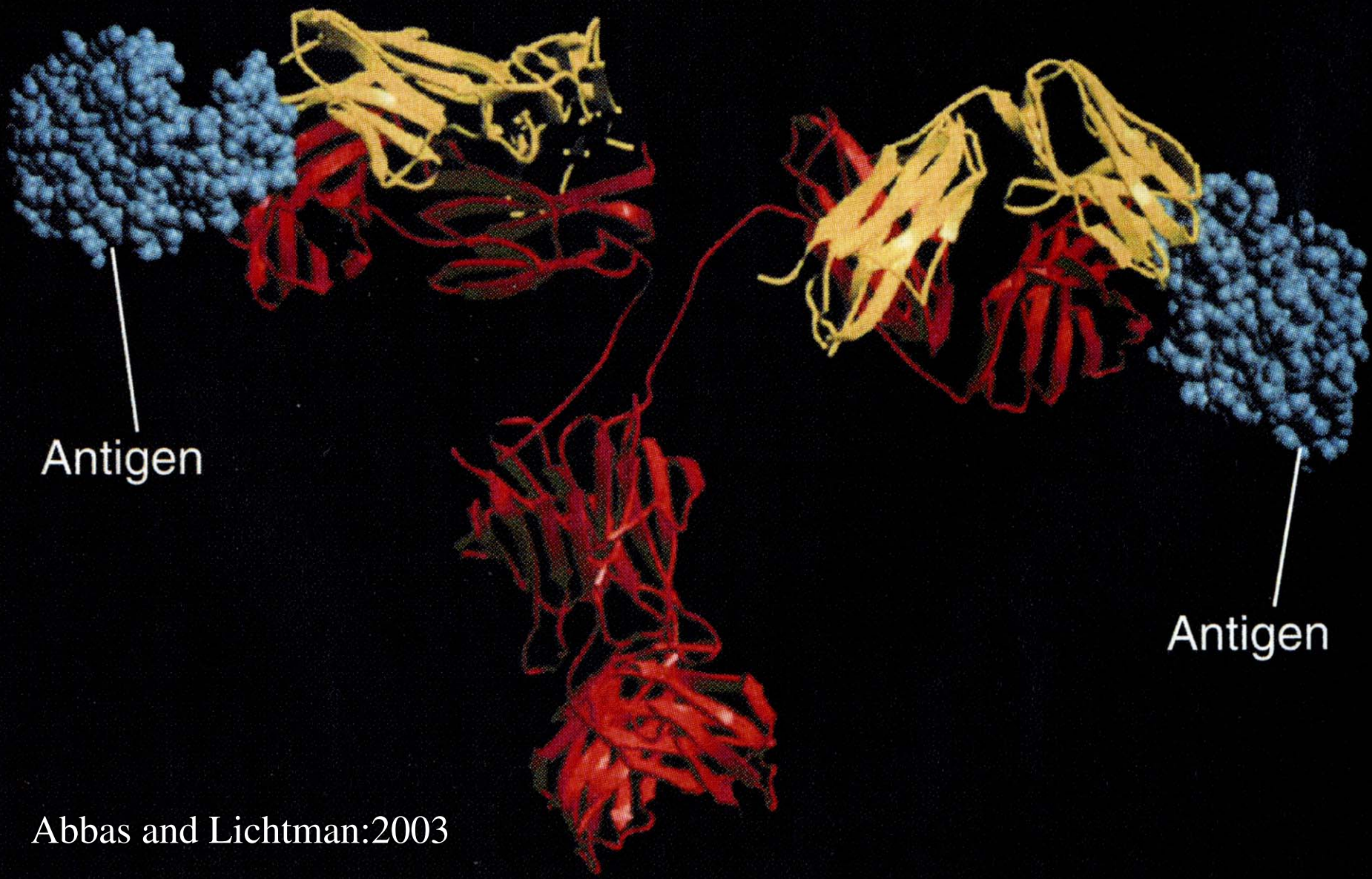



Abbas and Lichtman:2003

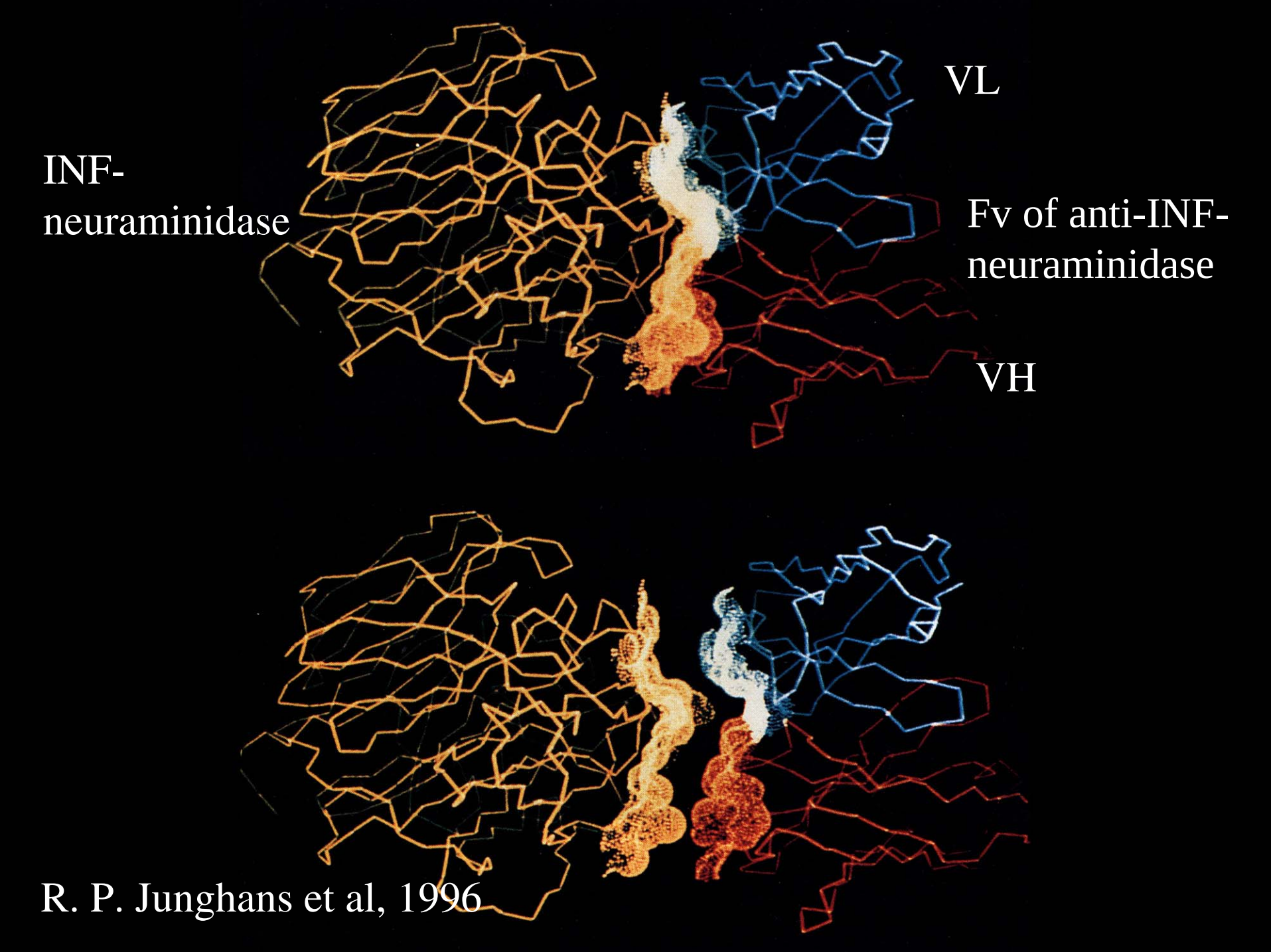
## CDR regions correspond to antigen binding




Abbas and Lichtman:2003

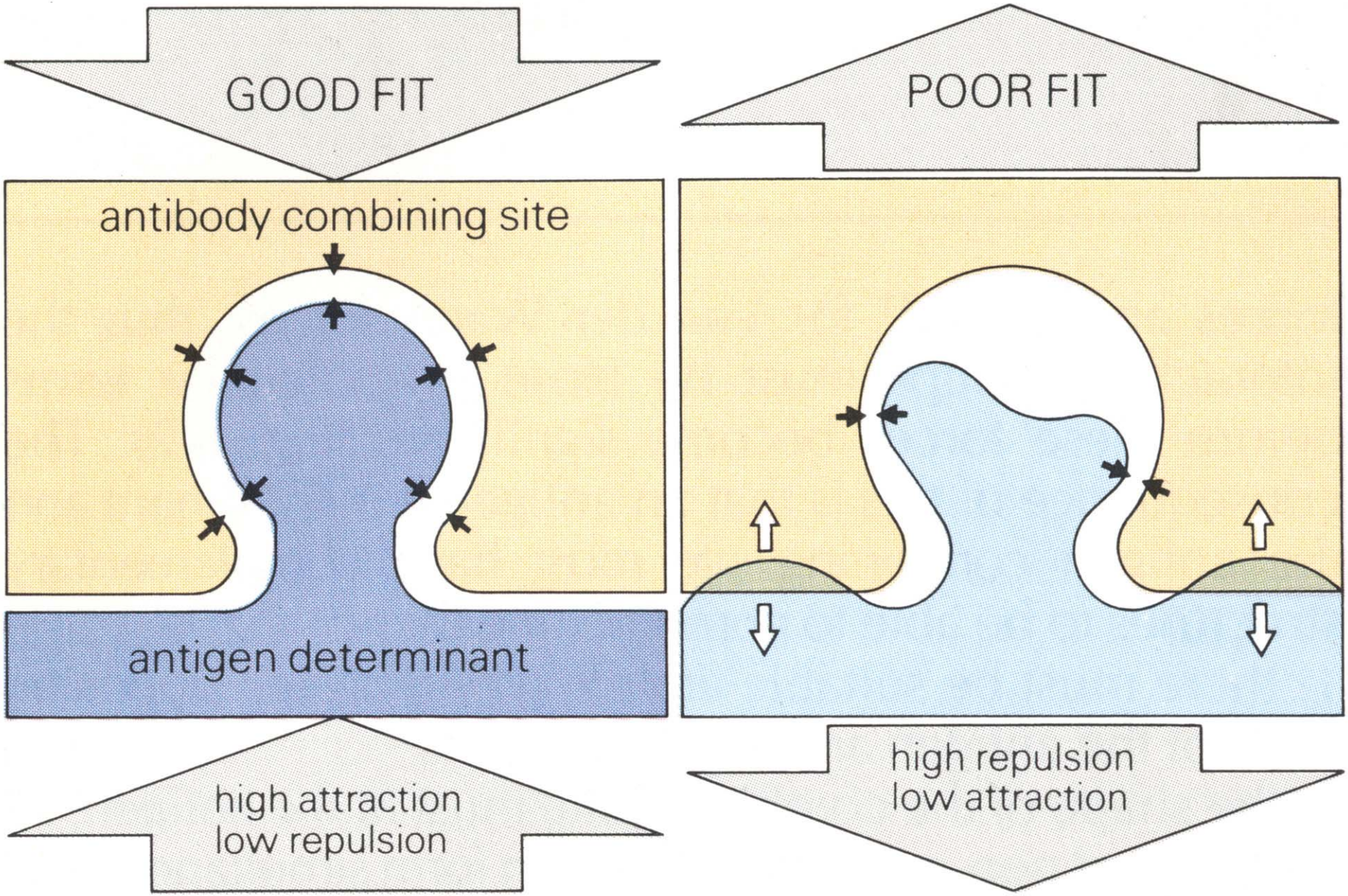



Abbas and Lichtman:2003



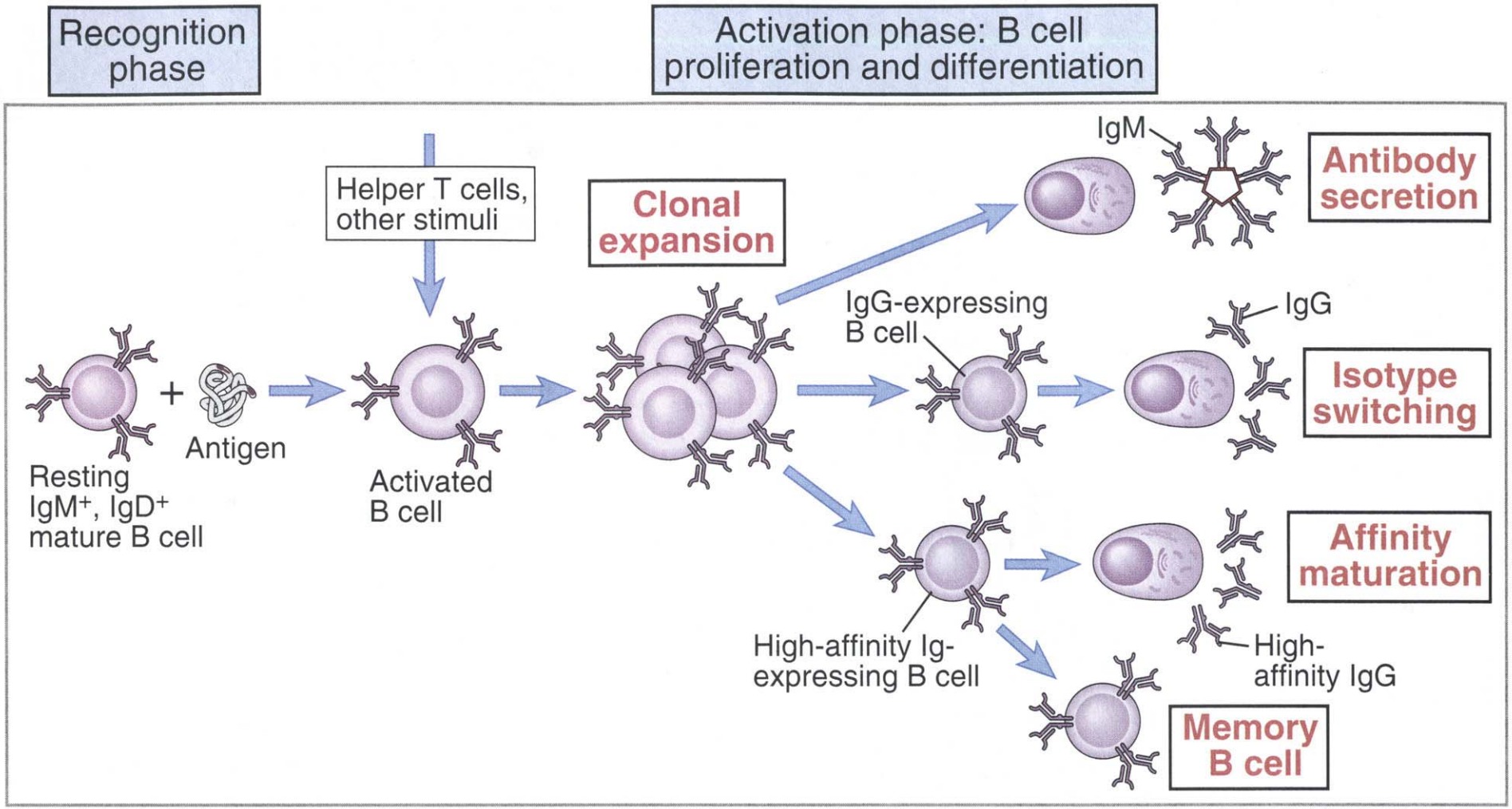

R. P. Junghans et al, 1996

# High Affinity Antibody: strong attractive and weak repulsive forces



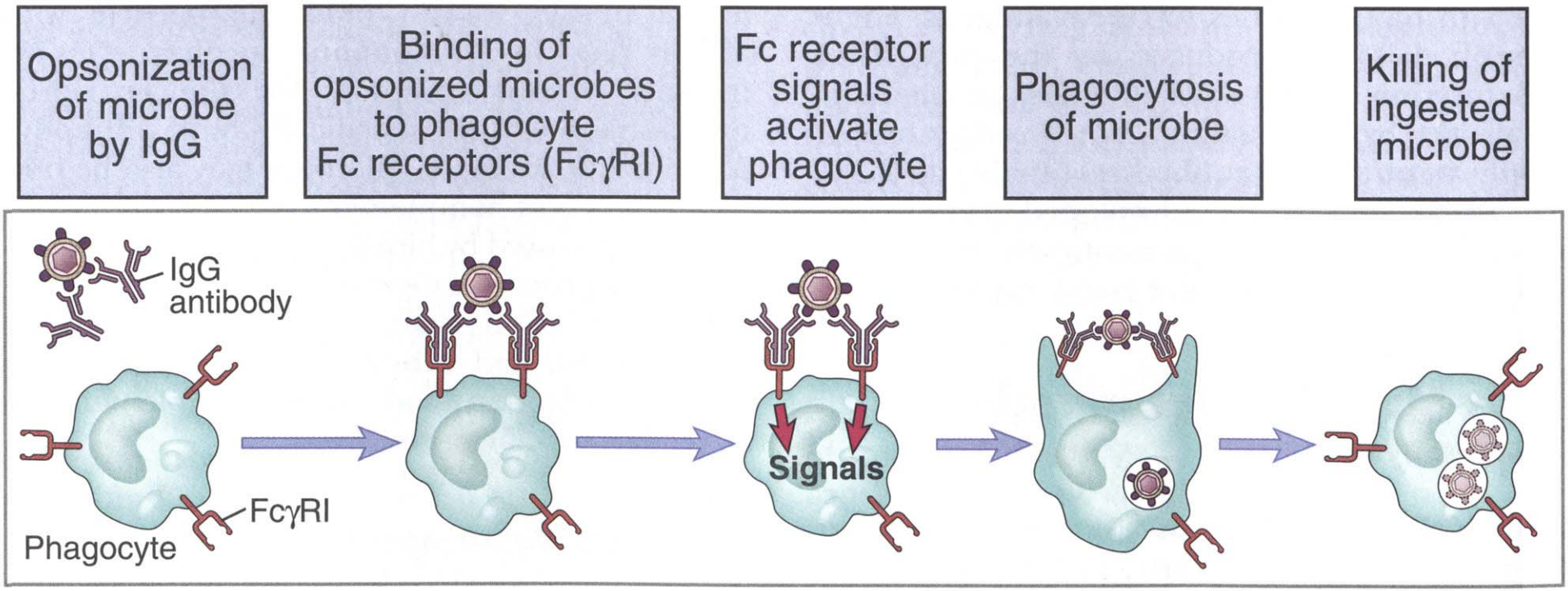

Roitt et al. 1985

# Phases of the humoral immune response



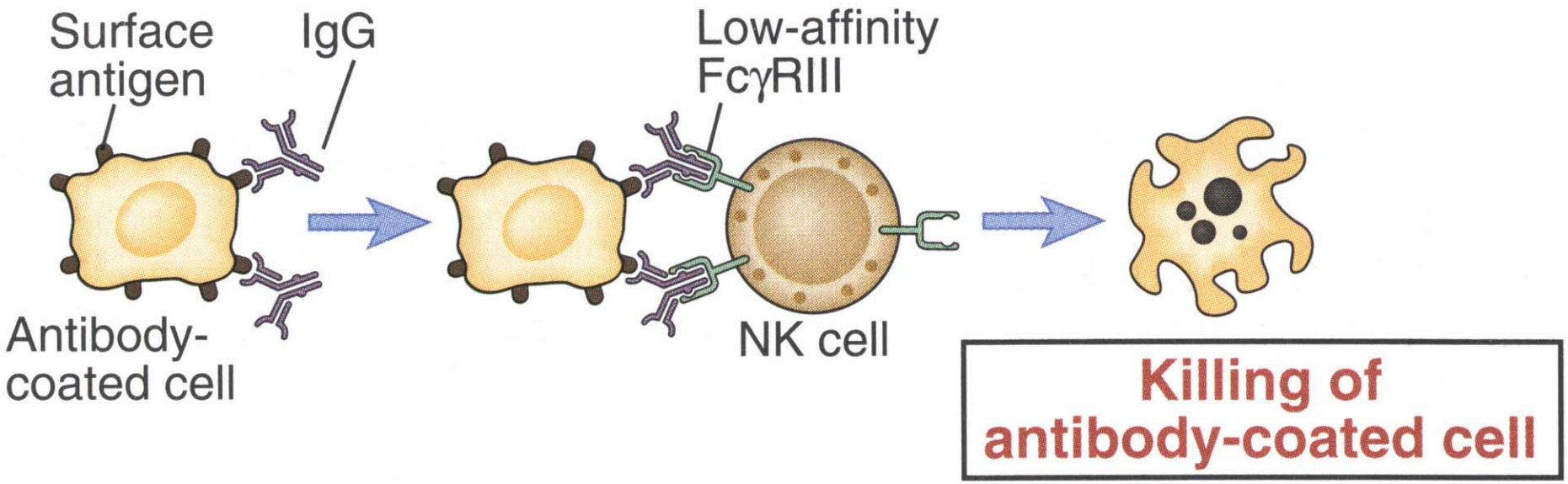

Abbas and Lichtman:2003

# Antibody mediated opsonization and phagocytosis of microbes



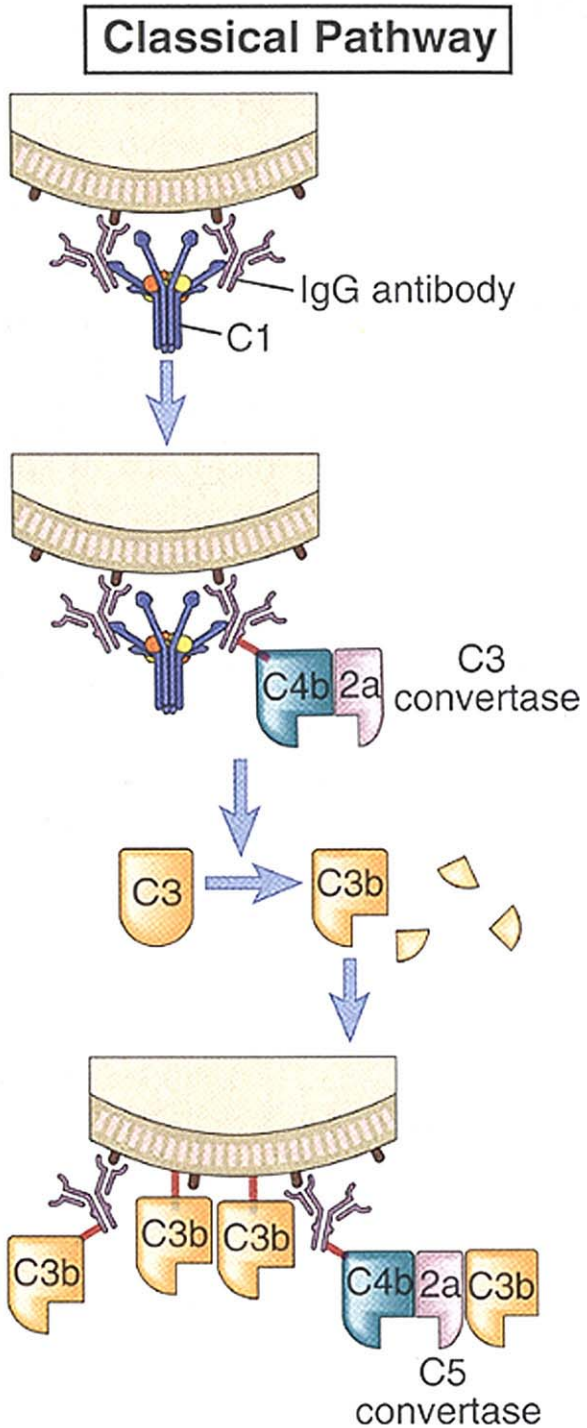

Abbas and Lichtman:2003

## Antibody Dependent Cell-mediated Cytotoxicity (ADCC)



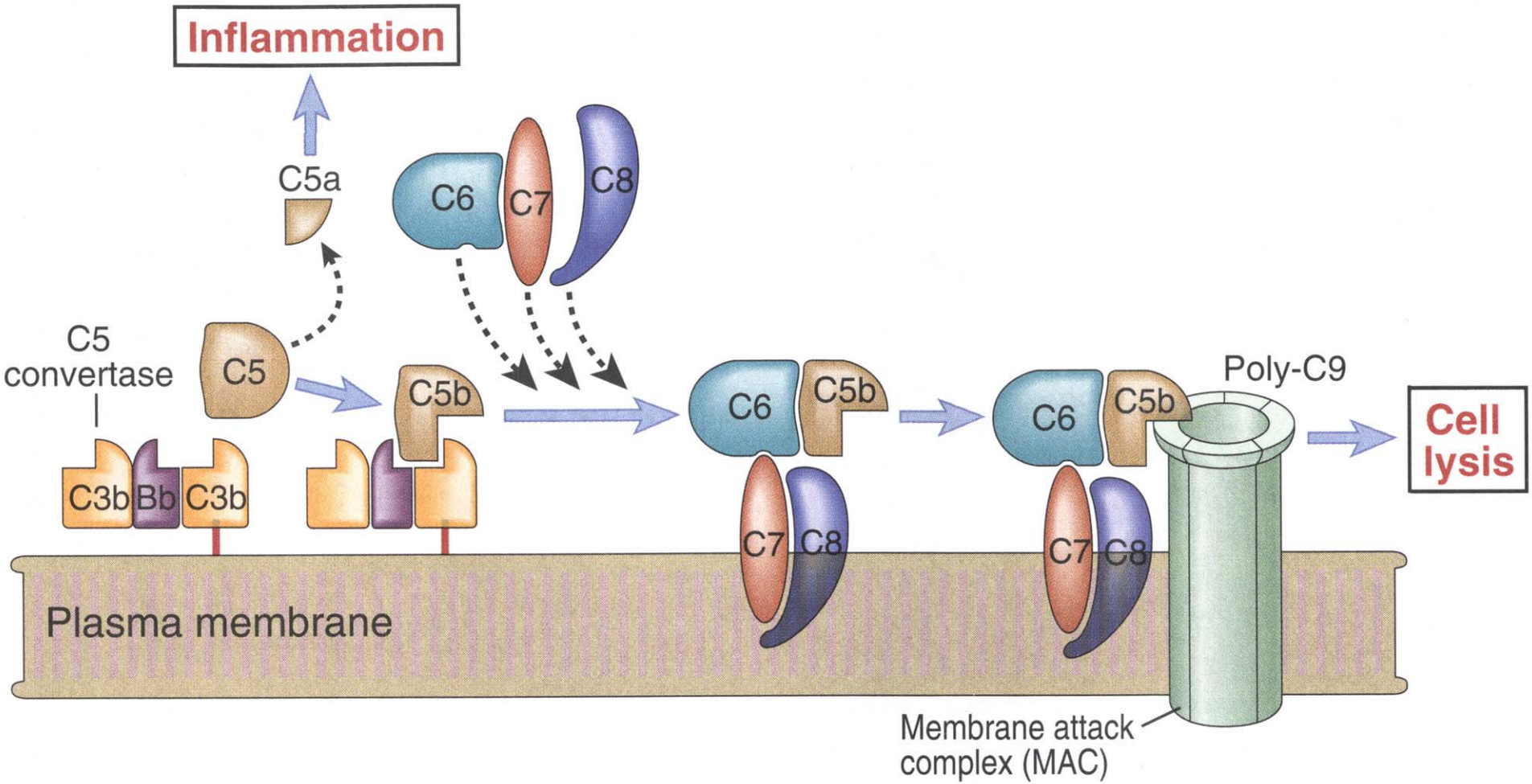

Abbas and Lichtman:2003

## Early steps in Complement activation



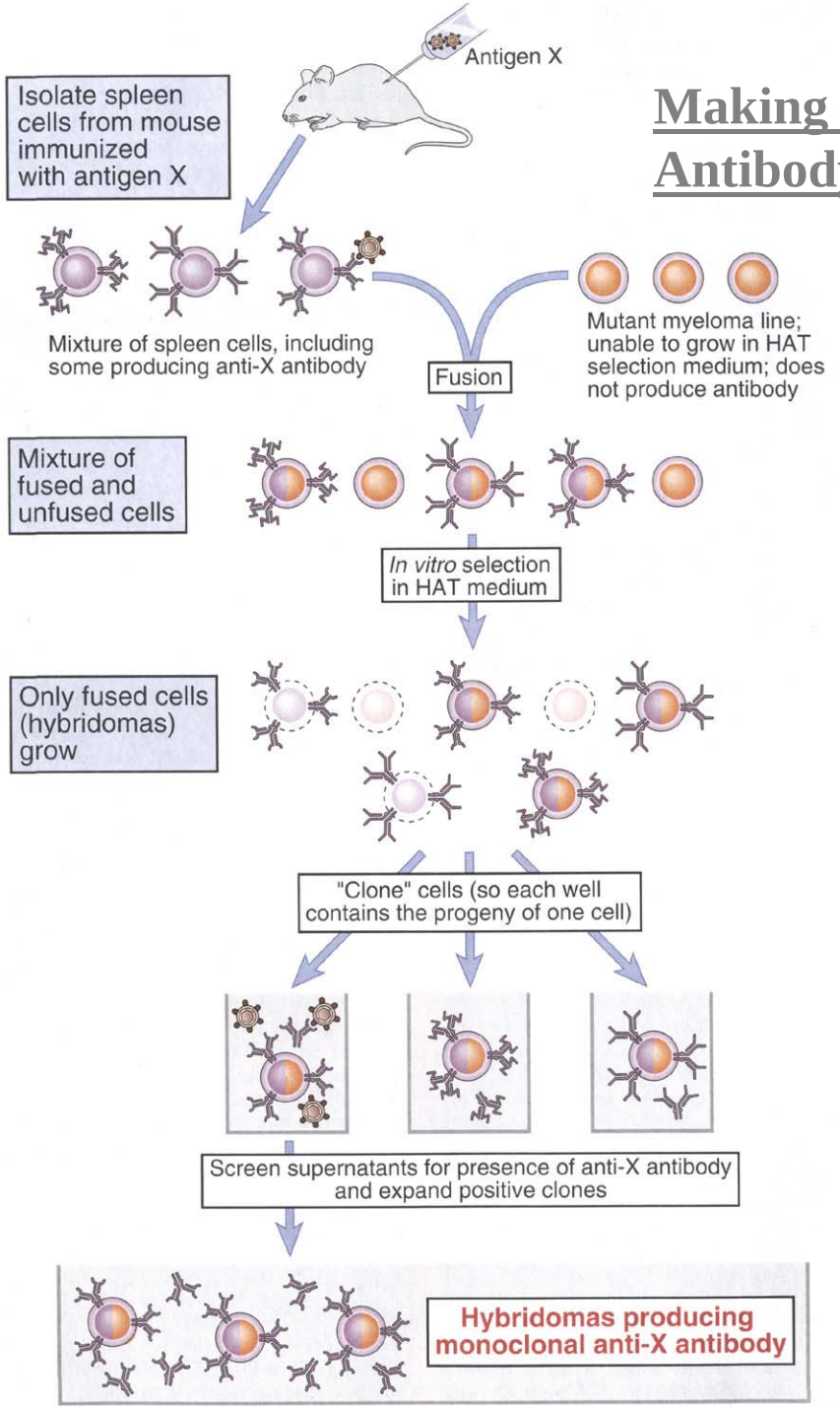

Abbas and Lichtman:2003

## Late steps in complement activation: formation of the membrane attack complex (MAC), resulting in osmotic lysis



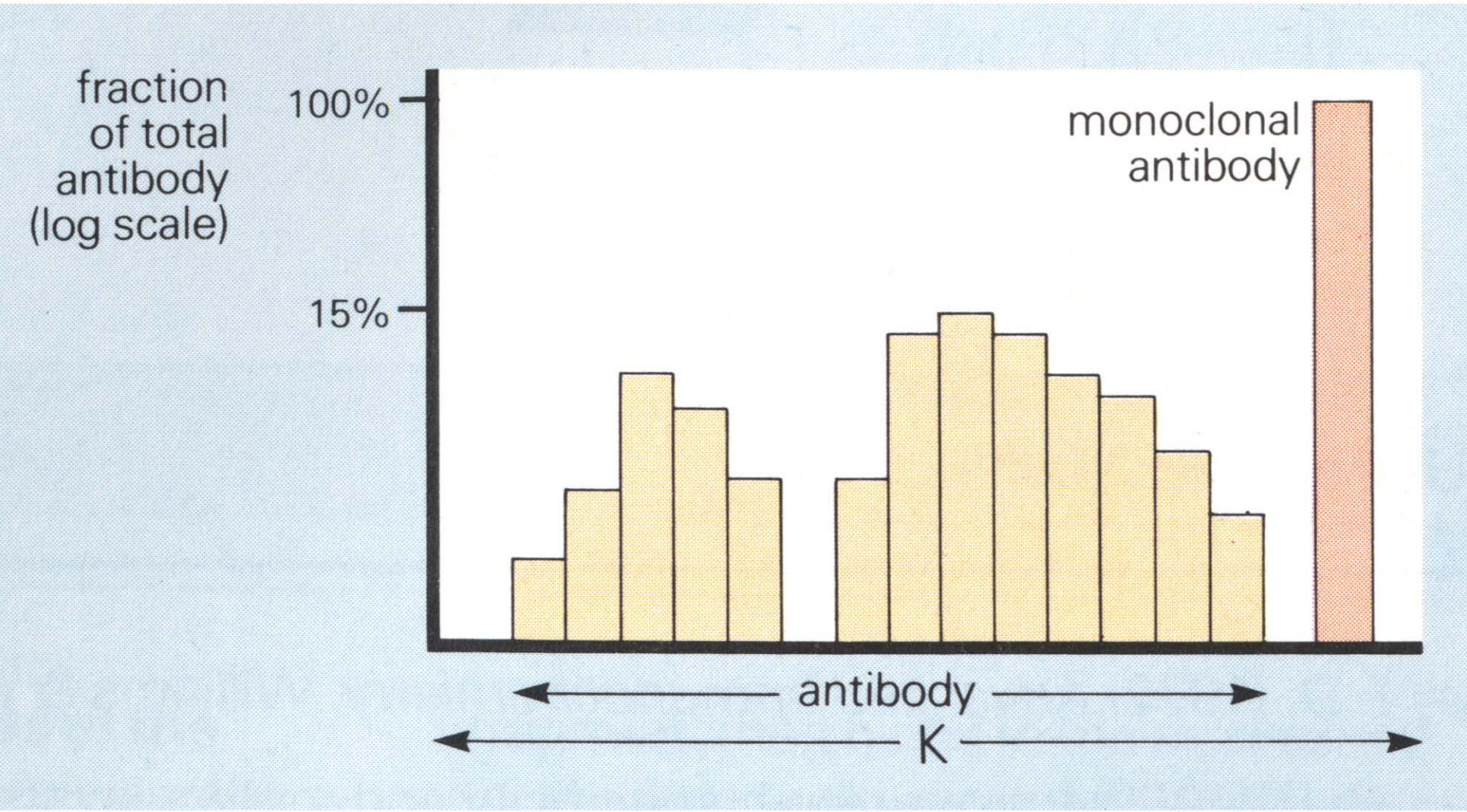

Abbas and Lichtman:2003

# Making Monoclonal Antibody (mAb)




Abbas and Lichtman:2003

# Affinity of polyclonal vs high affinity monoclonal antibody



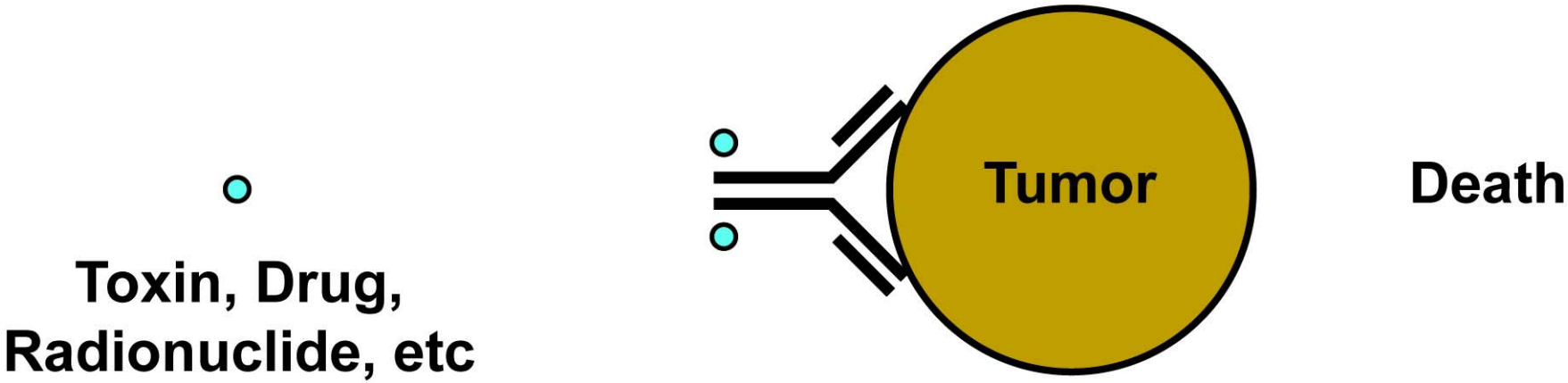

Roitt et al. 1985

# Clinically Relevant mAb target antigens

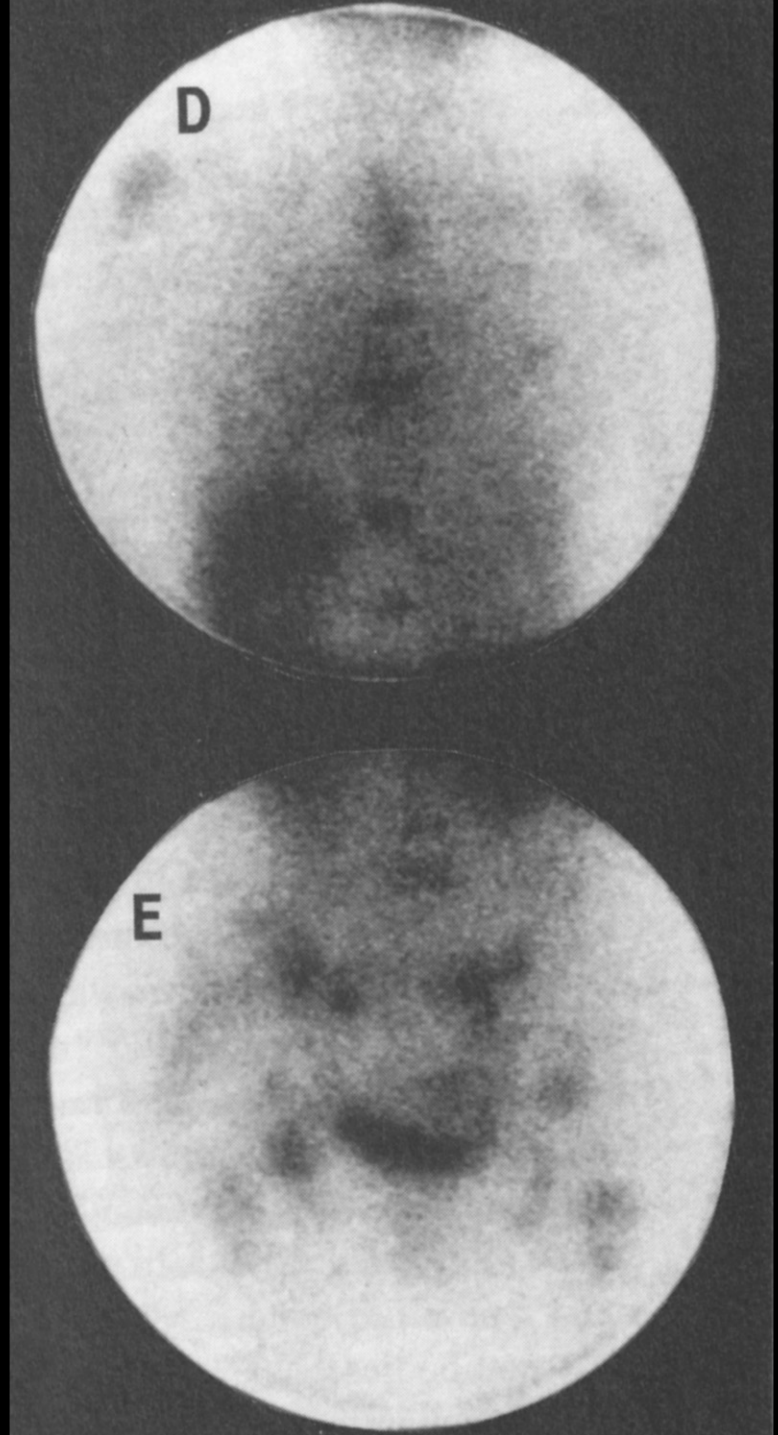
| LEUKEMIA | | SOLID TUMOR | |
|---|---|---|---|
| CD-20 | B | GD-2 | NBL/Mel |
| CD-19 | B | Her2 | Breast |
| CD-5 | T | EpCAM | AdenoCA |

# Mechanisms of mAb mediated anti-tumor effects

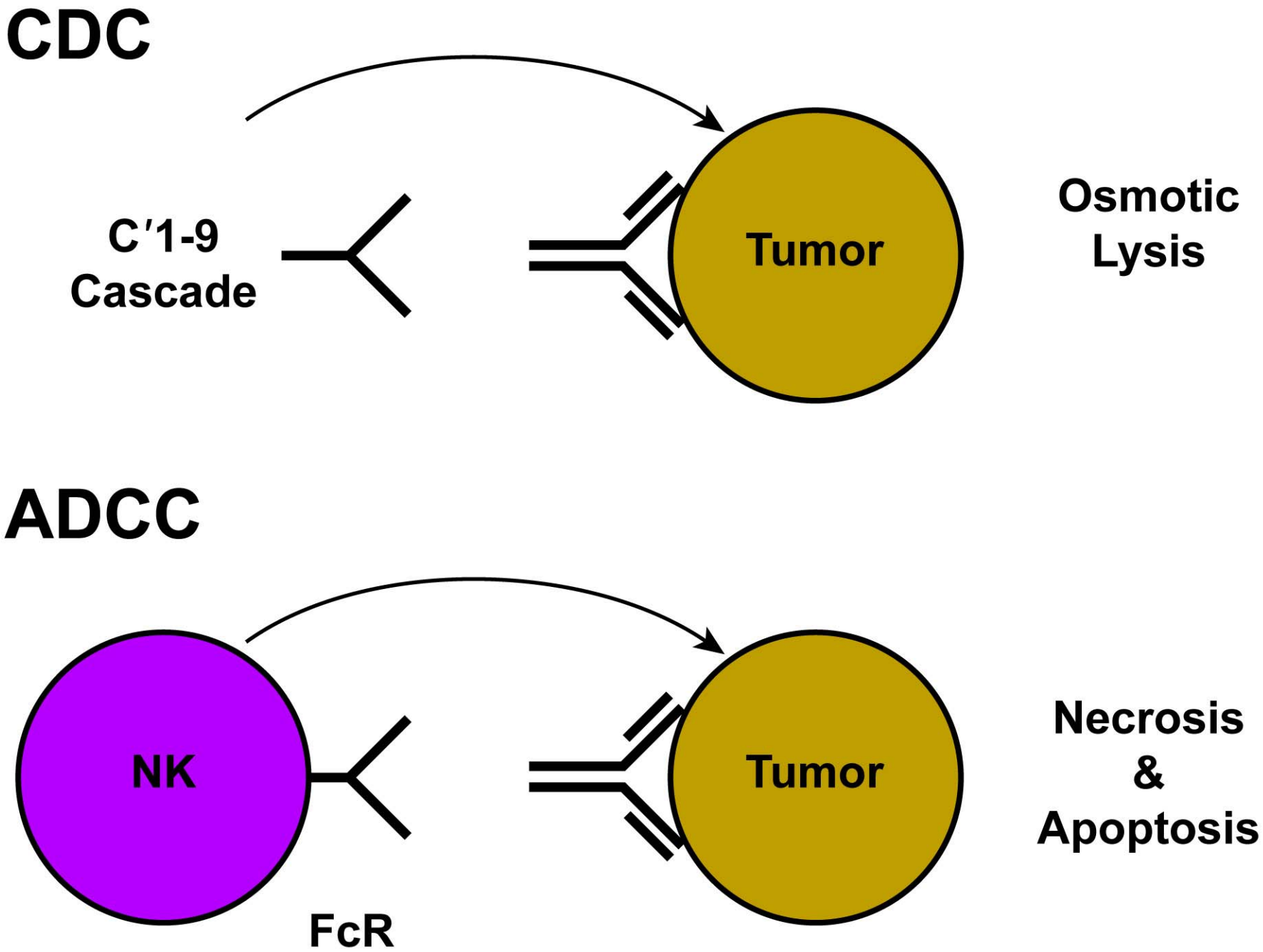
## Delivery of Toxic Agent

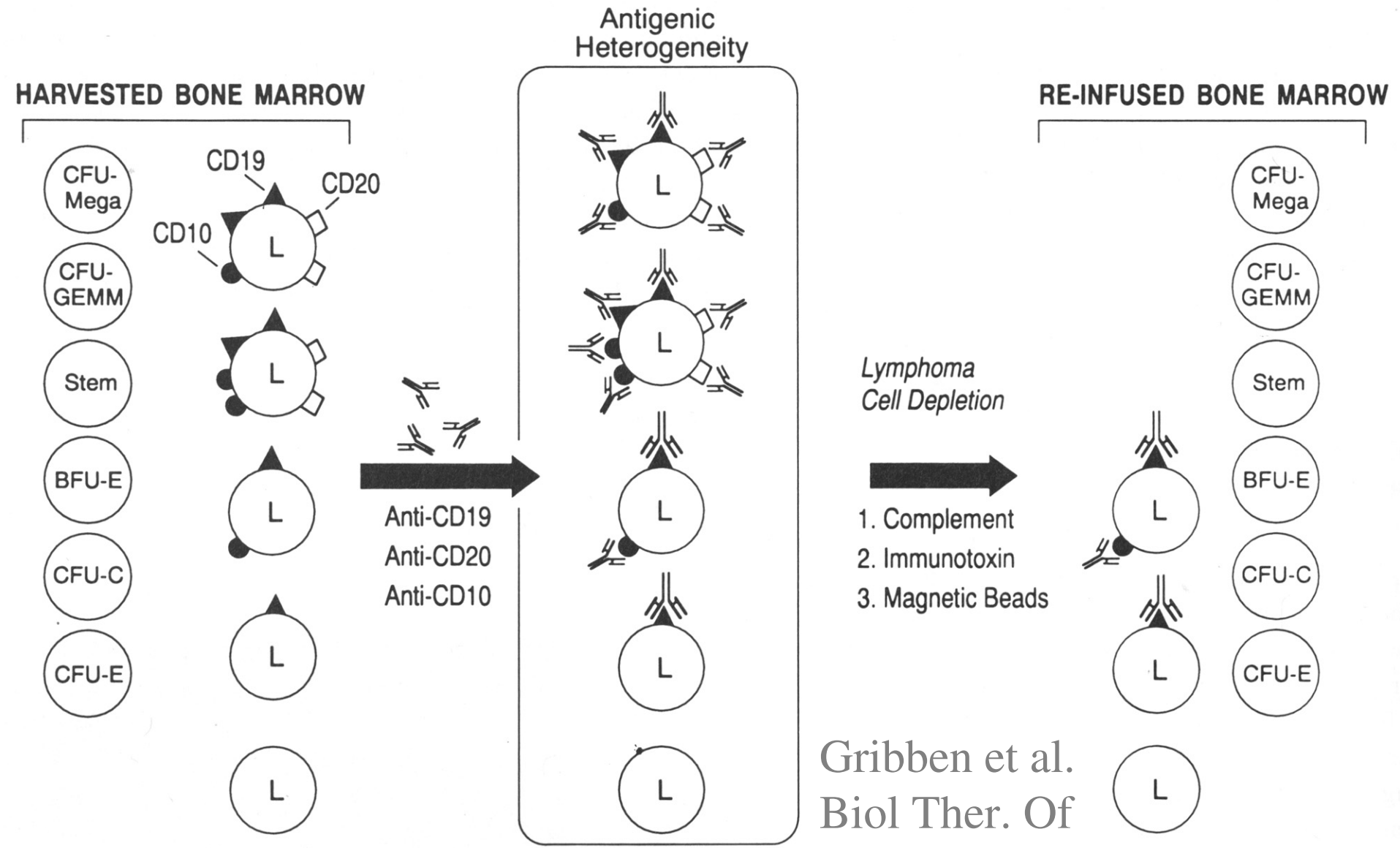


$^{131}$I-3F8 binding to melanoma



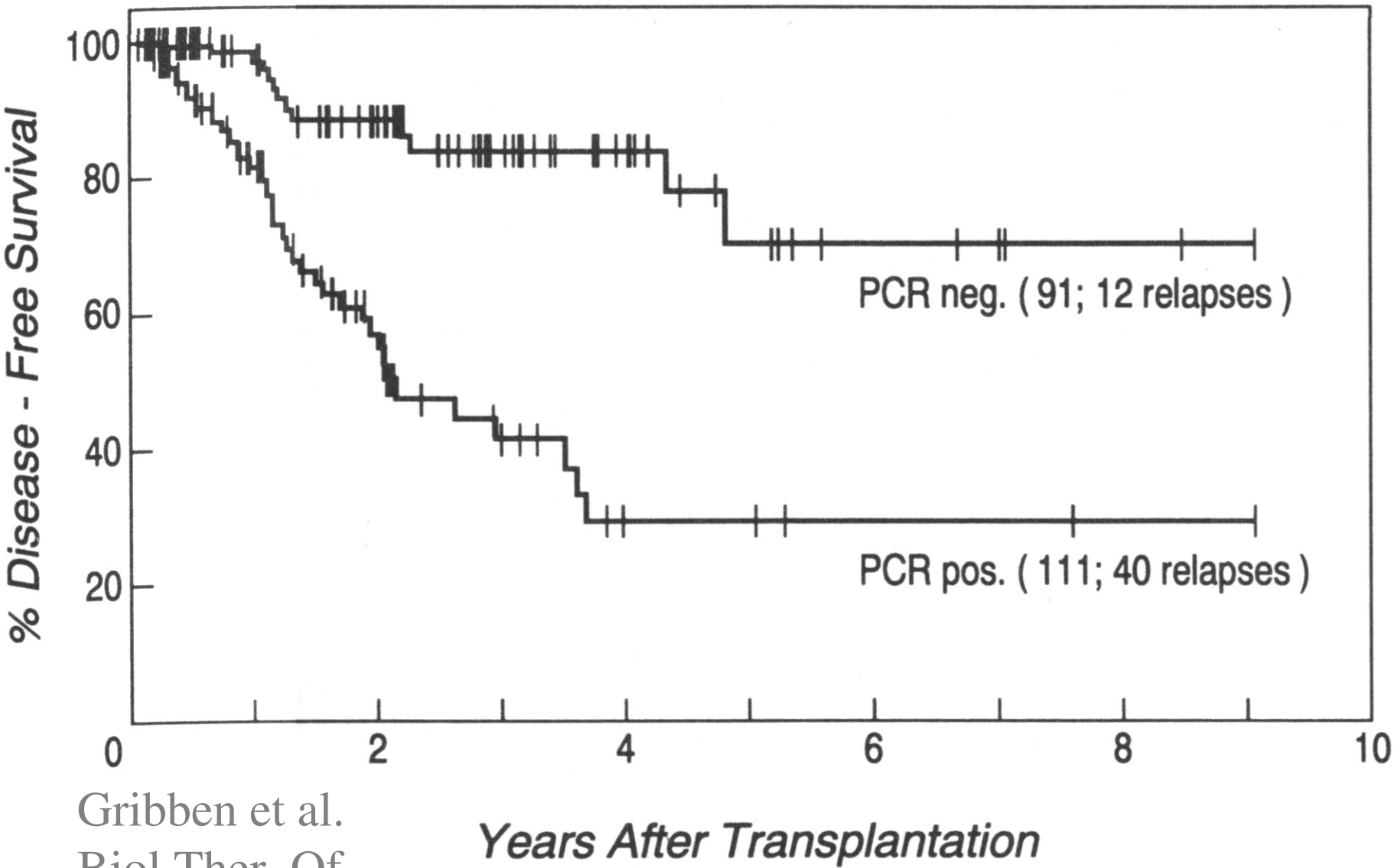

Cheung et al. Biol Ther. Of Cancer, 1995


CDC
C′1-9
Cascade
Tumor
Osmotic
Lysis
ADCC
NK
FcR
Tumor
Necrosis
&
Apoptosis



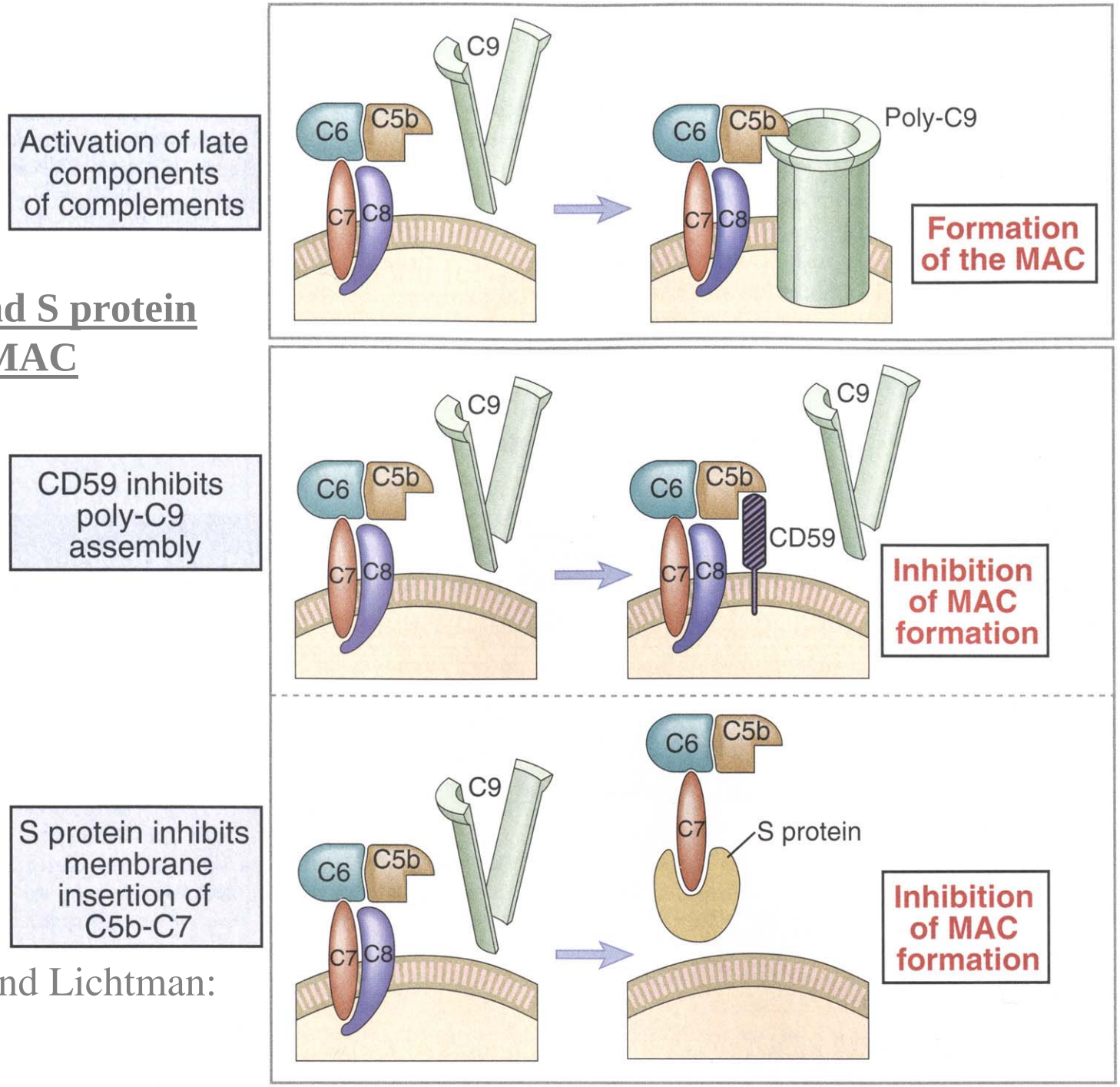

Gribben et al. Biol Ther. Of Cancer, 1995

# ABMT for B-cell NHL: Infusion of PCR+ vs. PCR- marrow




Gribben et al.
Biol Ther. Of
Cancer, 1995

## CD59 and S protein Inhibit MAC




Abbas and Lichtman: 2003

# CD59, but not CD55 or CD46, regulates Complement mediated killing of NHL lines by Rituxan in vitro

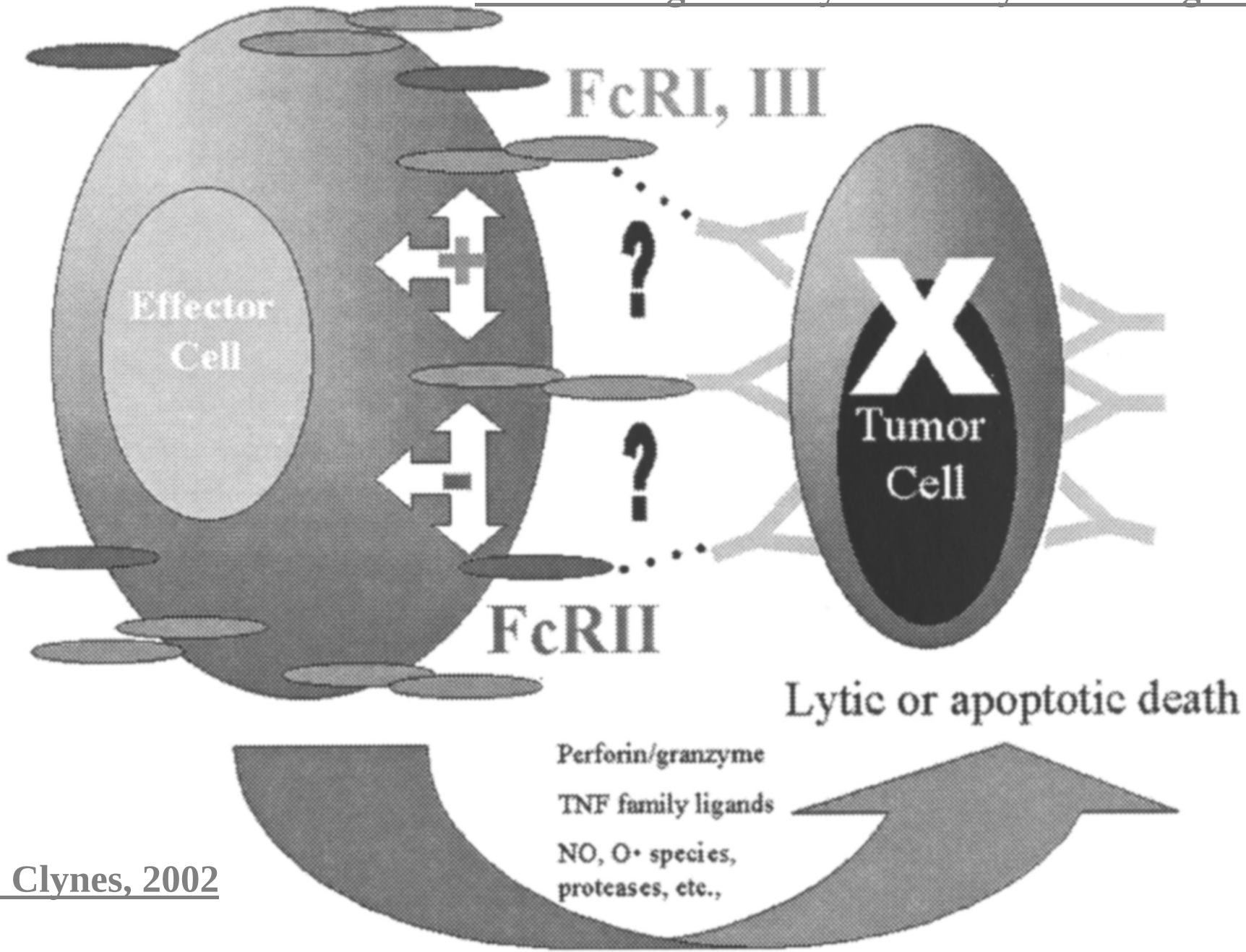
*Expression of complement regulatory proteins on CD20 expressing multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) B-cell lines, and cell line sensitivity to rituximab-mediated complement lysis*[a]

| Cell line | Type | CD59 | CD55 | CD46 | CD20 | Viability (%) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Rituxan | Rituxan/complement |
| ARH-77 | MM | ++ | ++ | +++ | ++ | 90.6 | 85.3 |
| DHL10 | NHL | ++ | ++ | ++ | ++ | 96.0 | 69.3 |
| NAWALMA | NHL | ++ | ++ | ++ | ++ | 98.7 | 30.7 |
| IM9 | MM | ++ | +++ | +++ | +++ | 88.0 | 28.7 |
| DHL4 | NHL | ± | ++ | ++ | ++ | 100.0 | 0.0 |
| HS SULTAN | MM | 0 | +++ | ++ | +++ | 84.0 | 0.0 |
| MM-AS | MM | 0 | + | +++ | ++ | 82.7 | 0.0 |
| MM-SV | MM | 0 | + | ++ | ++ | 96.0 | 0.0 |

[a] Myeloma and NHL B-cell lines were evaluated by single-color flow cytometry for expression of complement regulatory protein expression (CD46, CD55, and CD59) and CD20. Intensity of staining is denoted as follows: 0, no expression; ±, dim; +, moderate; ++, bright; and +++, very bright. Viabilities were assessed by trypan blue staining and represent means of triplicate samples.

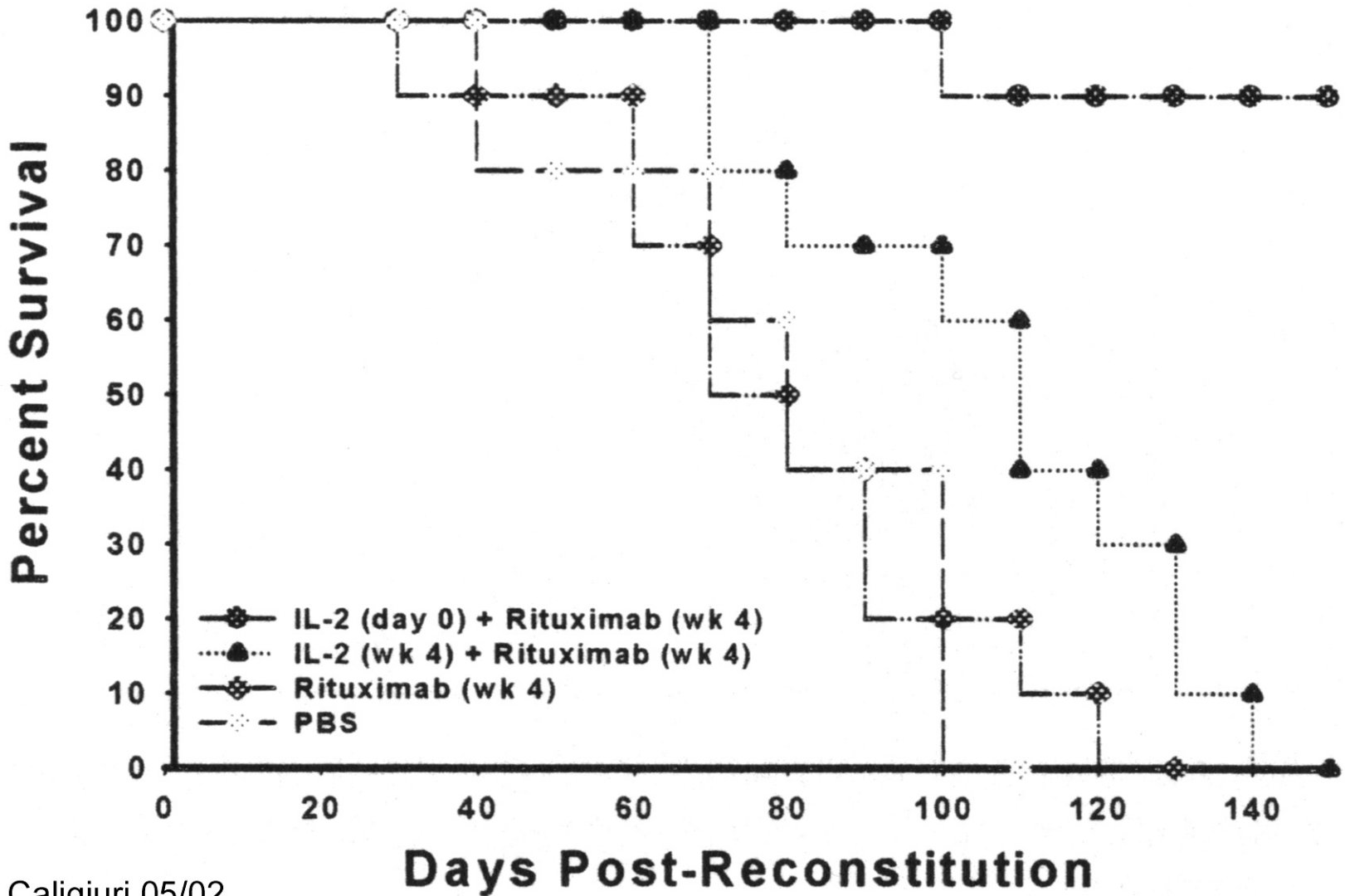
SP Treon et al. J. Immunother. 24:263, 2001

# ADCC is regulated by Inhibitory:Activating FcR




R. Clynes, 2002

# In Vivo IL-2/Rituximab Trial




M.A.Caligiuri 05/02

# Efficacy of FcR influences in vivo Rituxan Effects

**AA #158 of FcRIII**

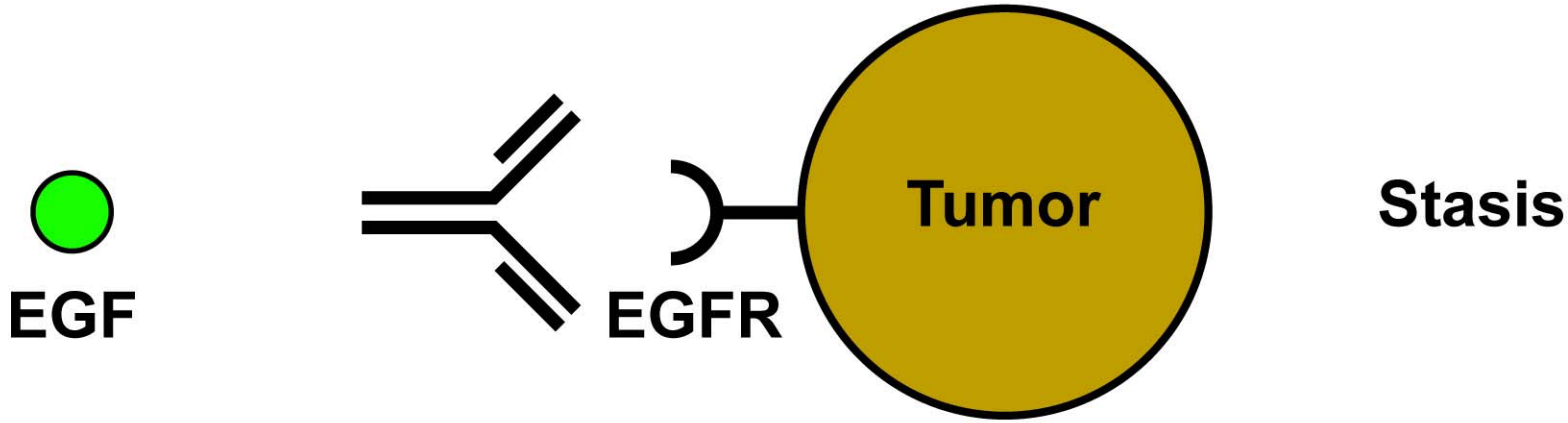
**V → Higher Affinity for $I_sG$**
**F → Lower Affinity**

| Phenotype | *In Vitro* ADCC | Reponse Rate *In Vivo* to Rituxan |
|---|---|---|
| V/V | ++++ | ++++ |
| V/F | +++ | +++ |
| F/F | + | + |

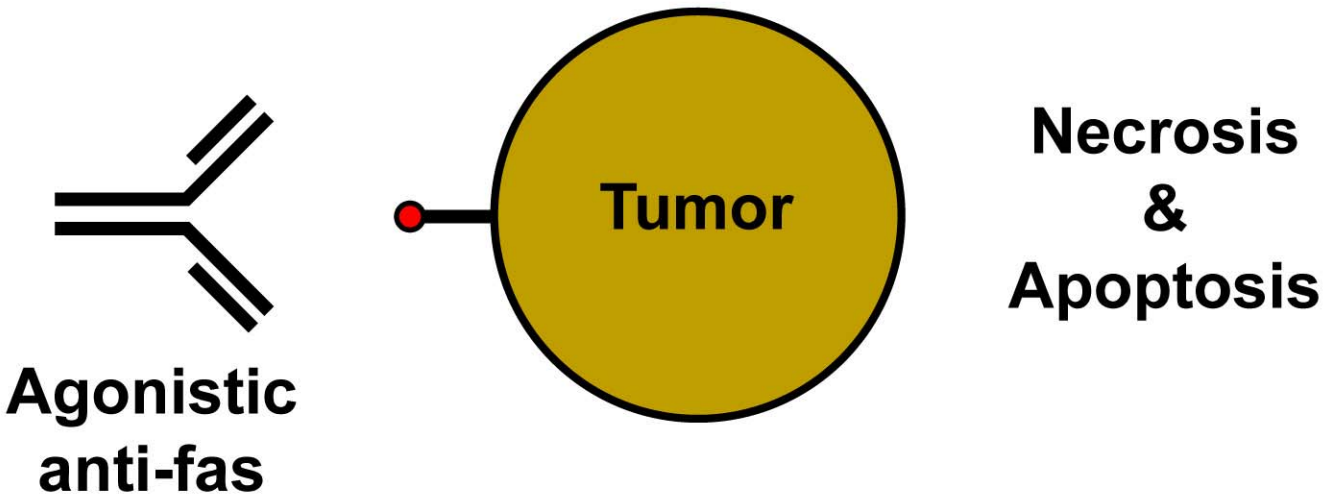
**p < .05**

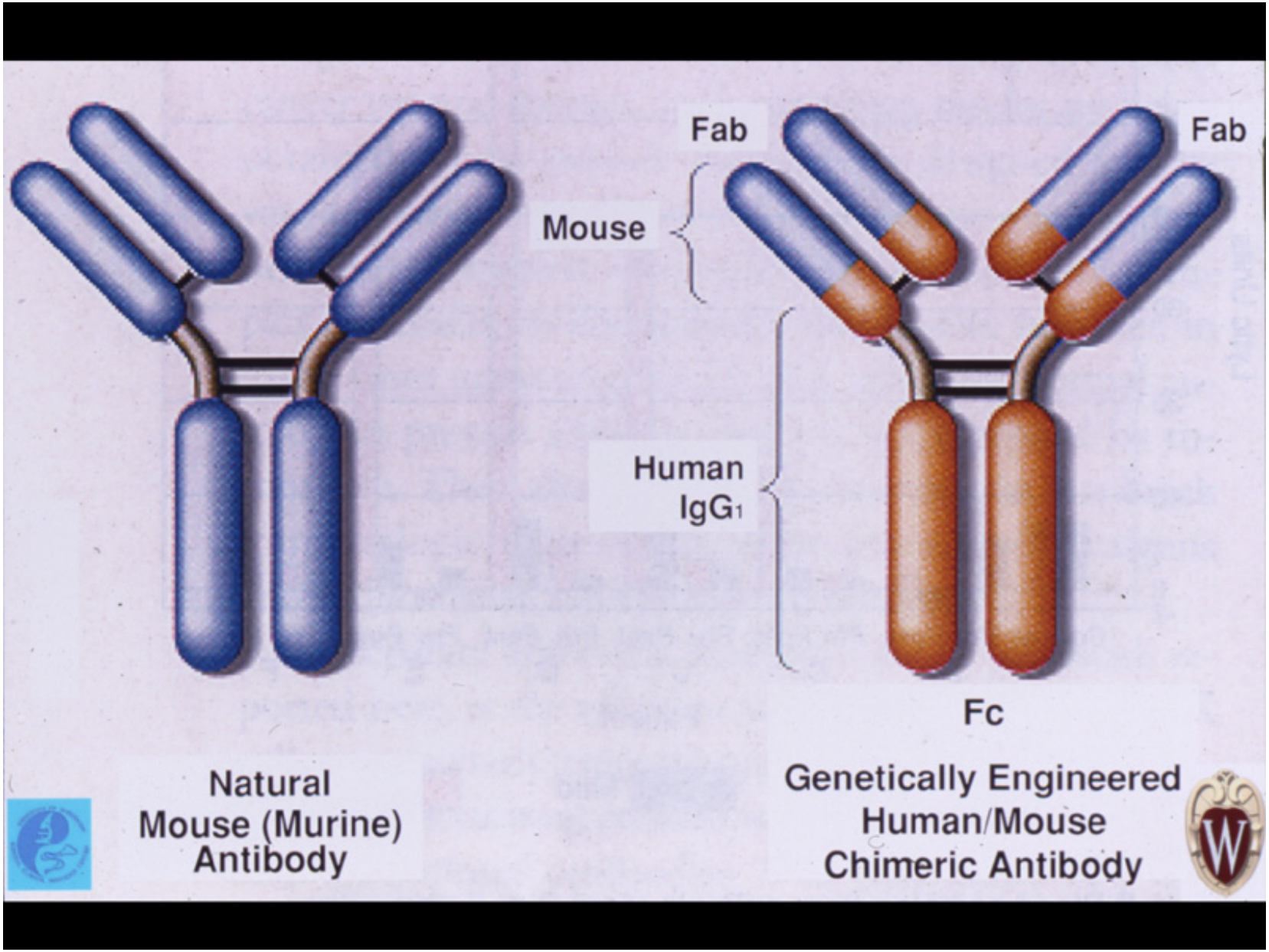
Cartron et al. Blood 99:754, 2002

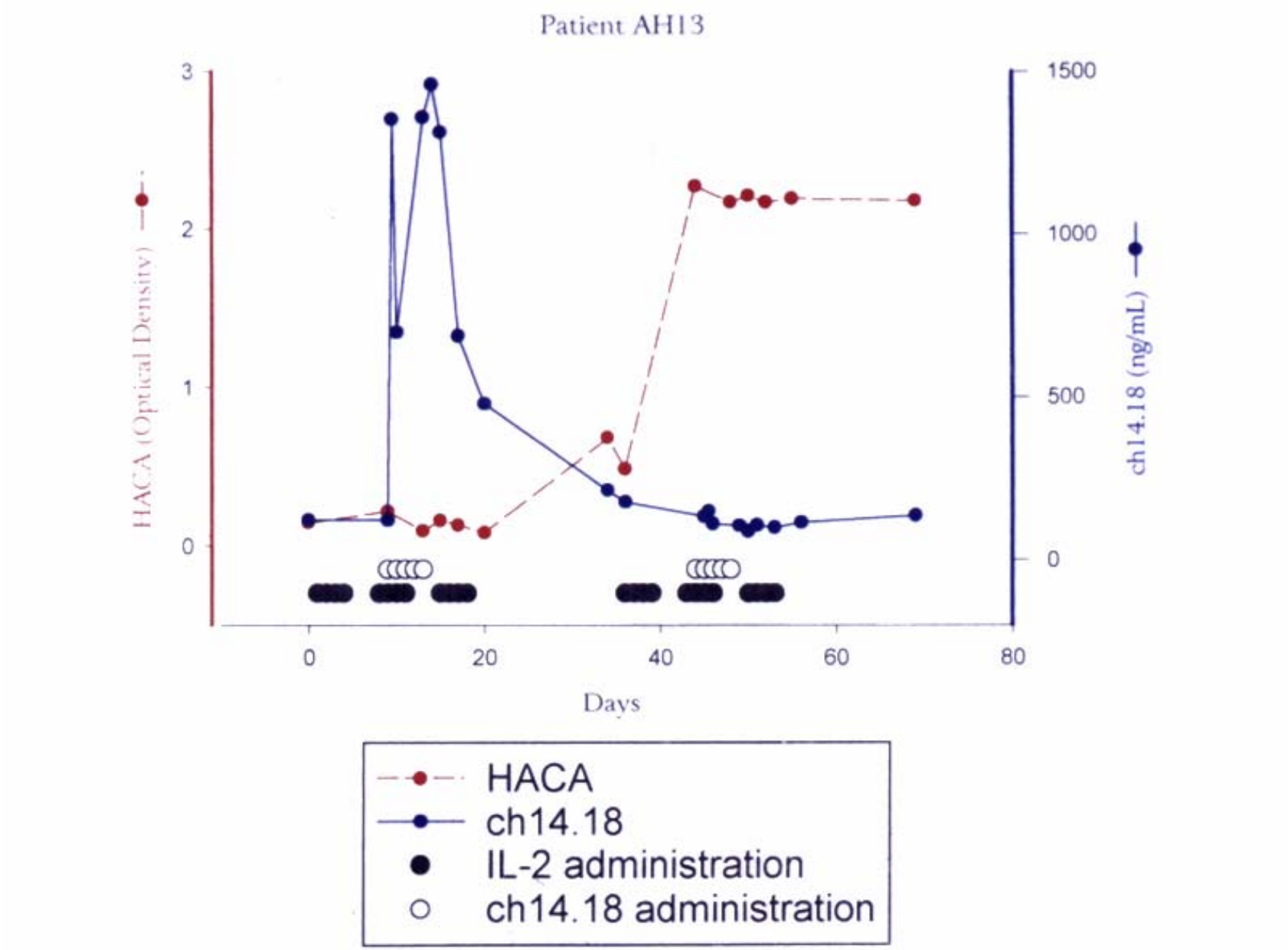
# Receptor Blockade




# Signal Activation

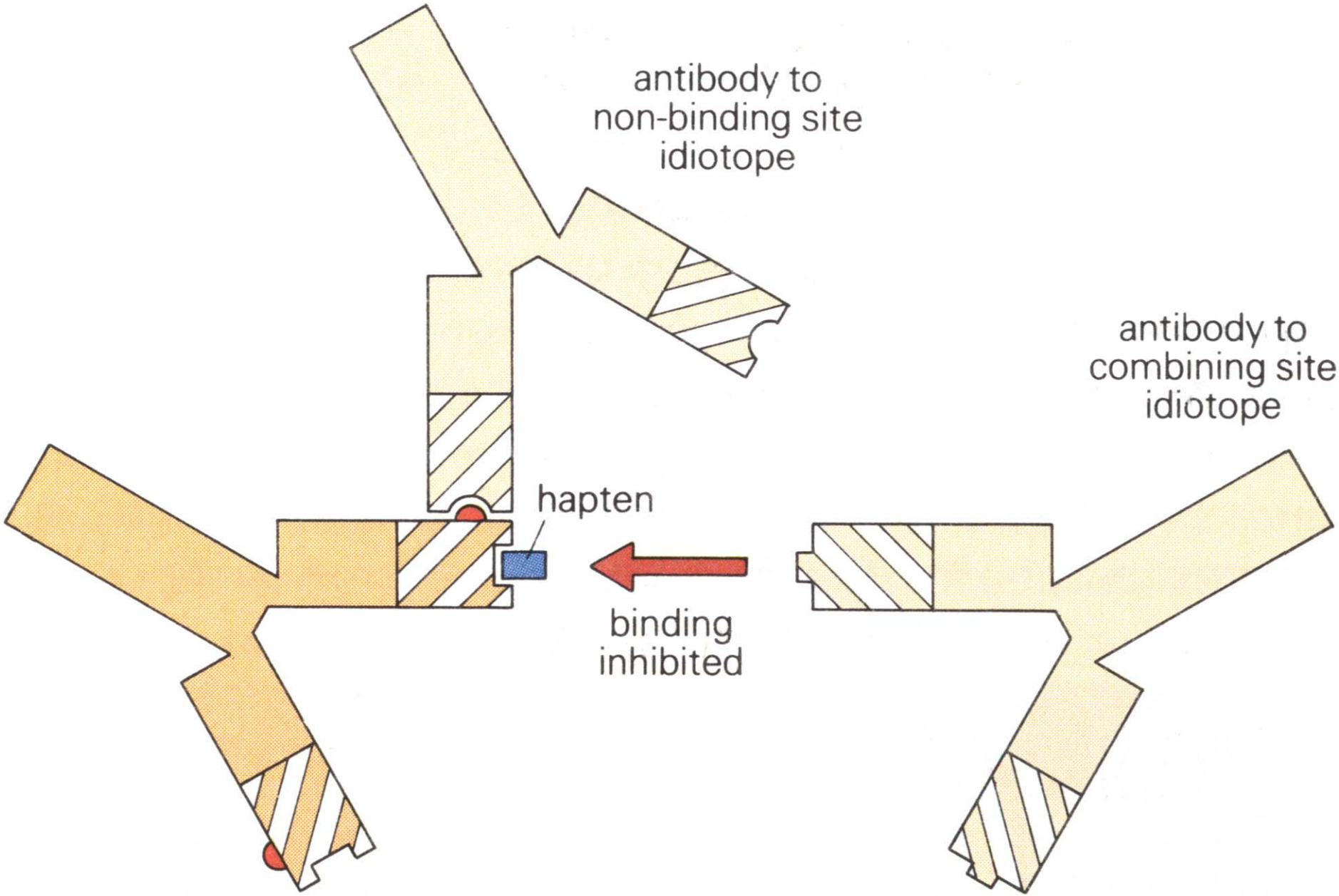



Fab
Fab
Mouse
Human
IgG1
Fc
Natural
Mouse (Murine)
Antibody
Genetically Engineered
Human/Mouse
Chimeric Antibody




Albertini et al 1998

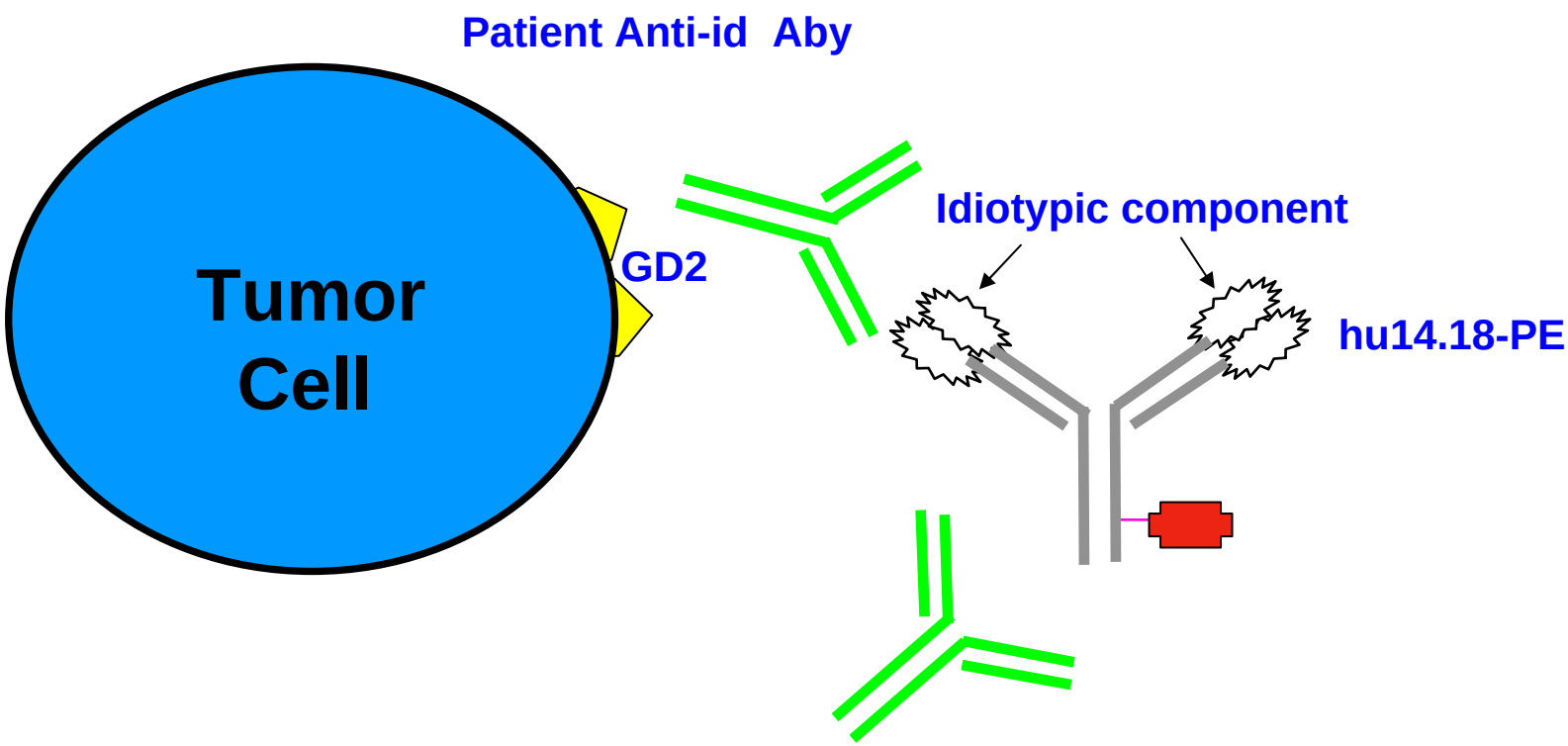
# Some anti-id antibodies can inhibit antigen binding




Roitt et al. 1985

# Patient Anti-id Antibody Inhibits Binding of hu14.18 to Tumor cells

## ( Flow Cytometry Assay)

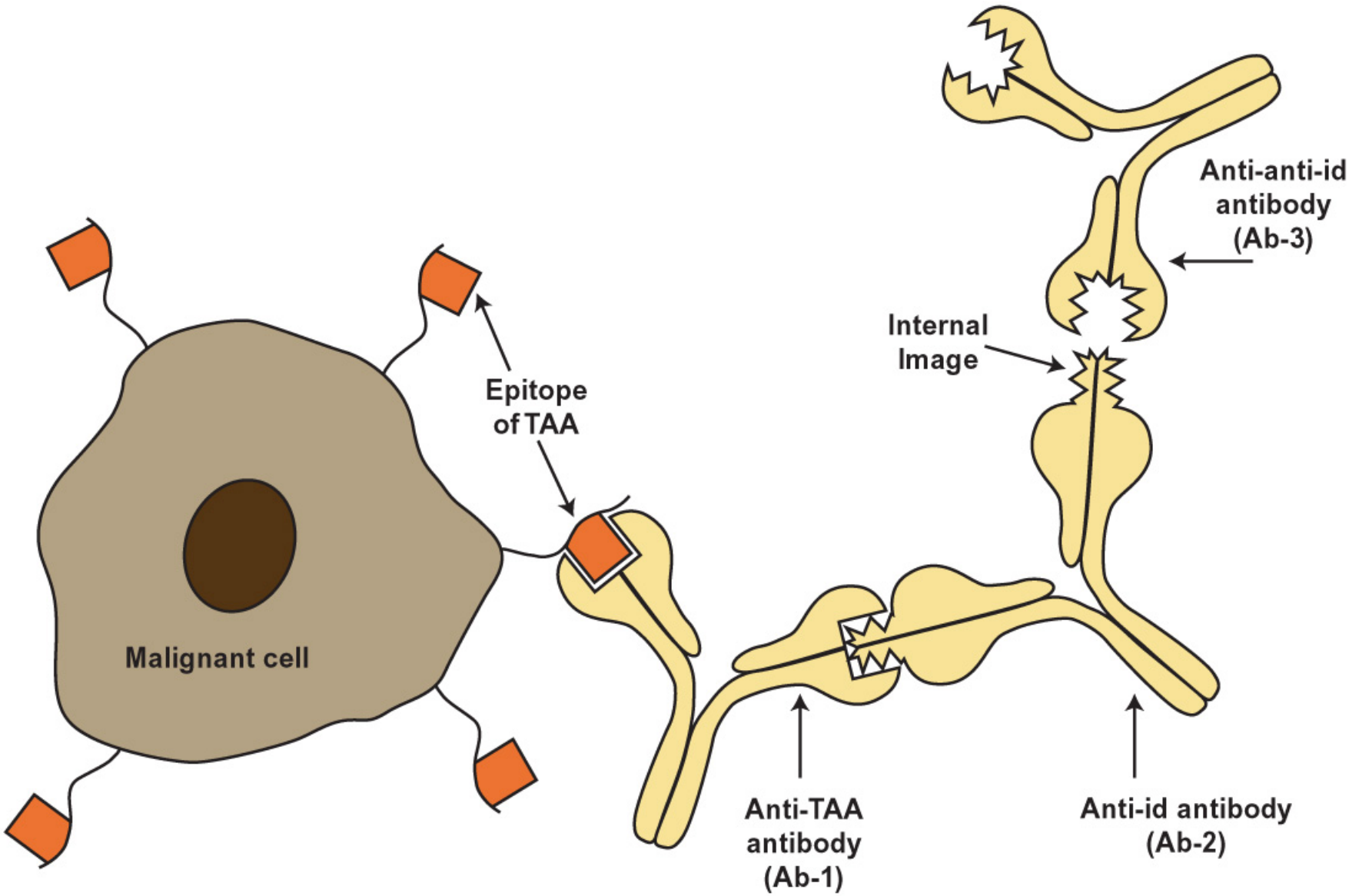


# Inhibition of hu14.18 mAb binding to GD2 on cells or in ELISA by post Rx pt. sera

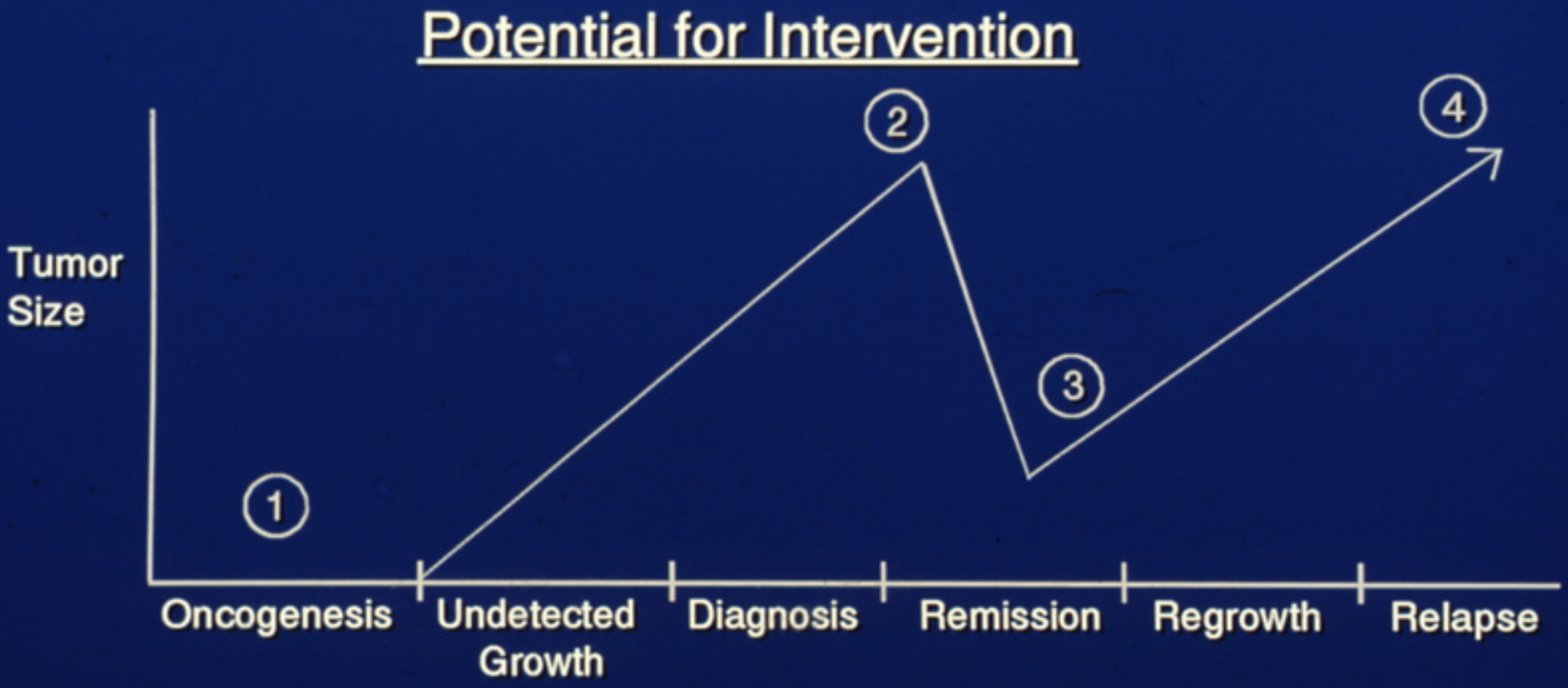
| | Patient Pre | Patient D15 |
|---|---|---|
| ELISA Inhibtion | 0% | 99% |
| Flow MFI | 357 | 16 |
| Flow inhibition | 0% | 96% |

Hank et al, unpublished

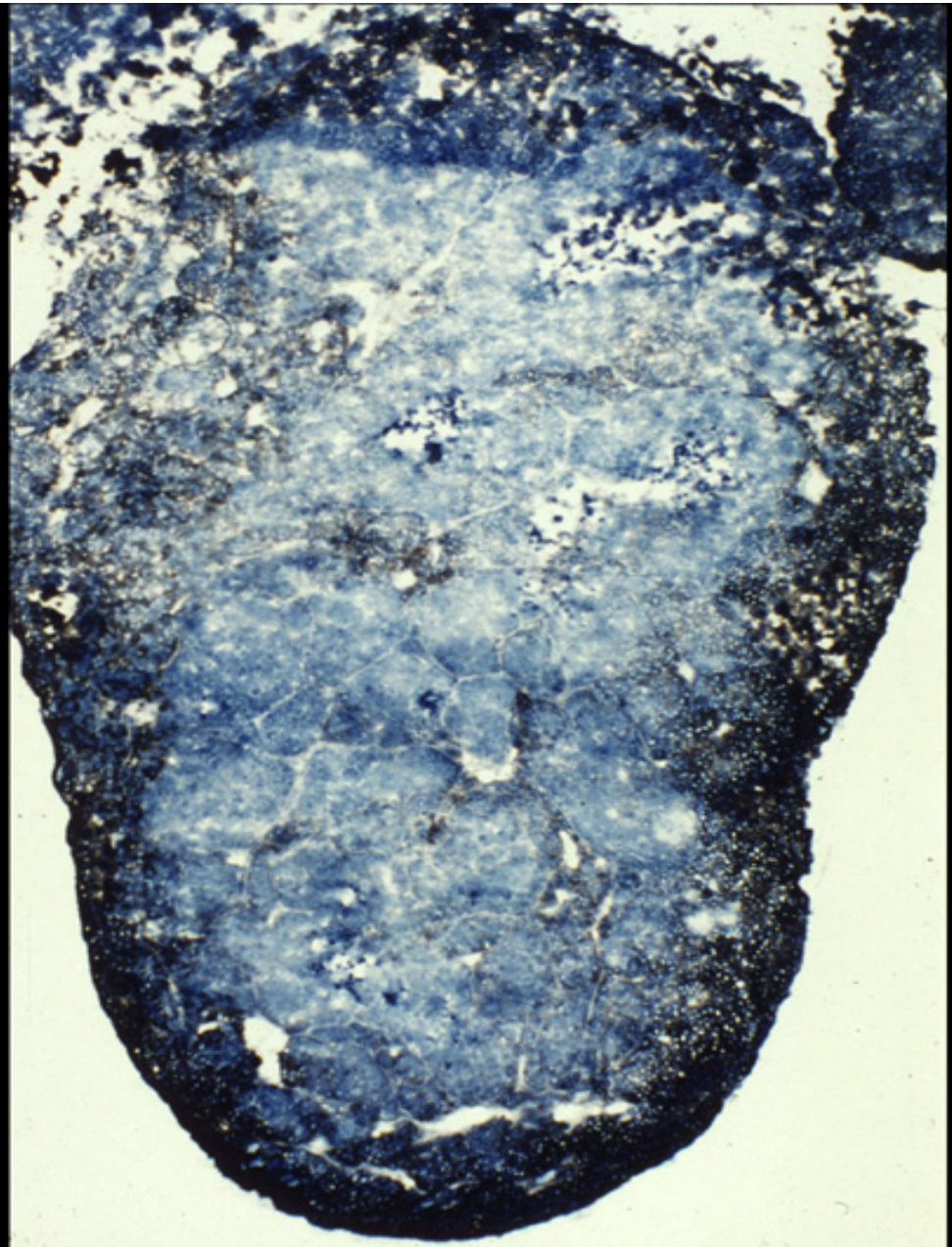
# Mimicry of a TAA determinant by anti-id antibodies



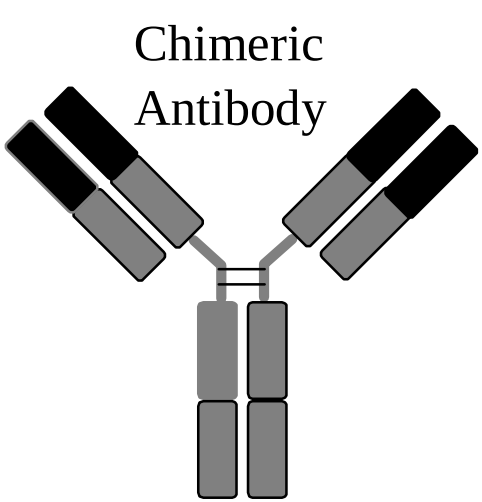
# IMMUNOTHERAPY?

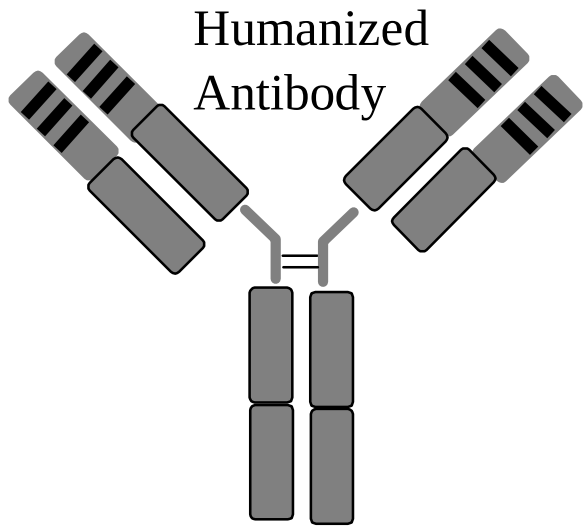


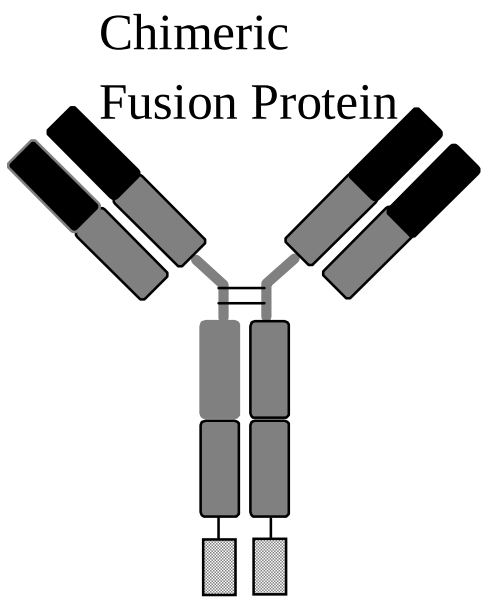
# Ch14.18 mAb does not penetrate well into measurable tumors

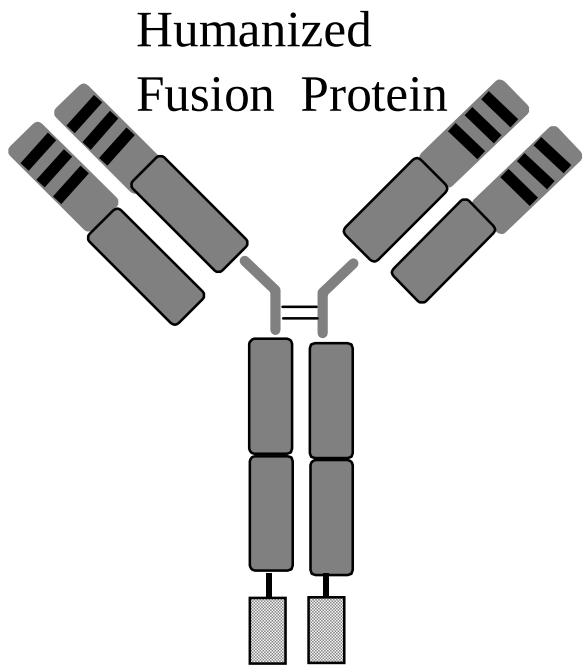


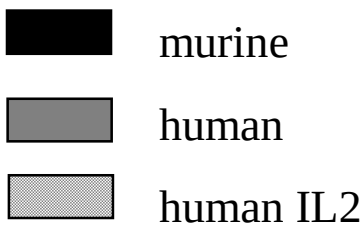
Kendra K et al.
J. Of Immunother.
22:423, 1999

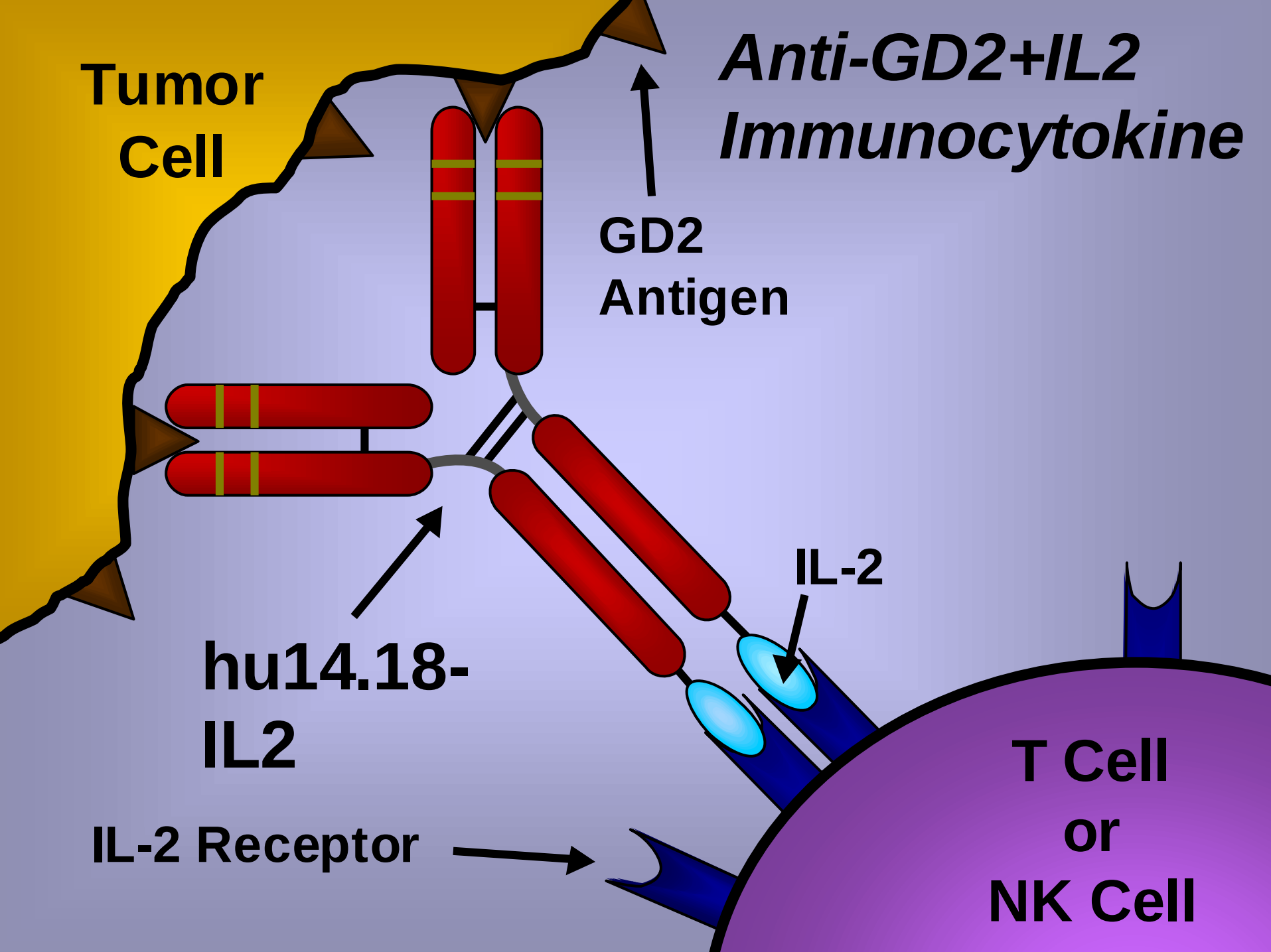

Chimeric
Antibody


Humanized
Antibody


Chimeric
Fusion Protein

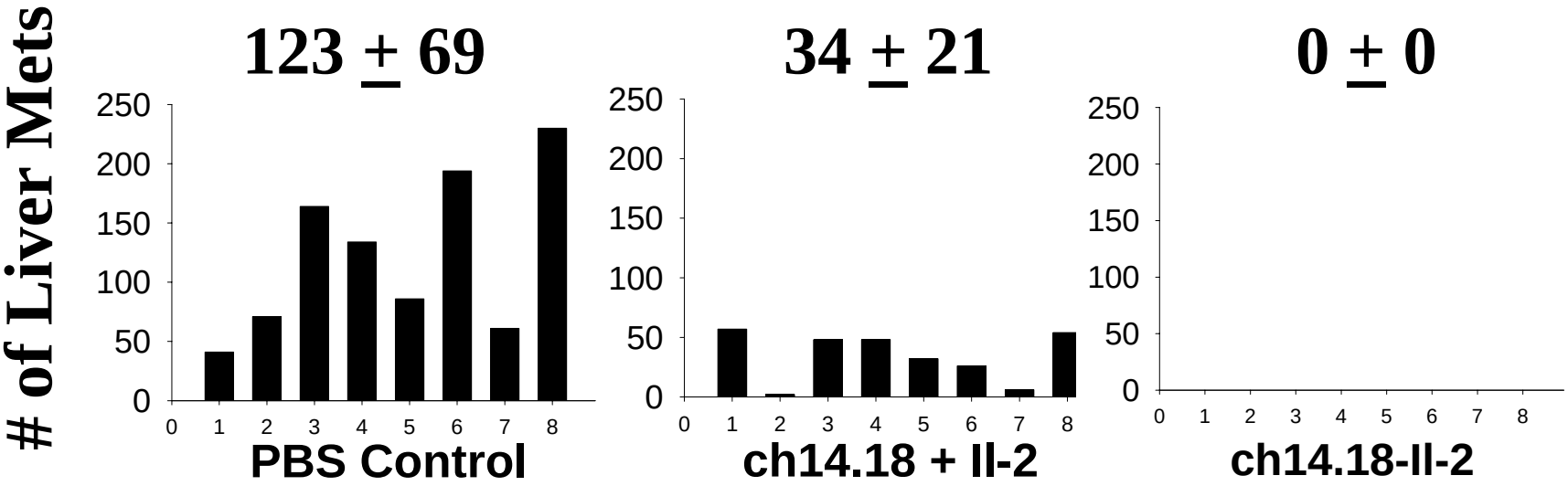

Humanized
Fusion Protein

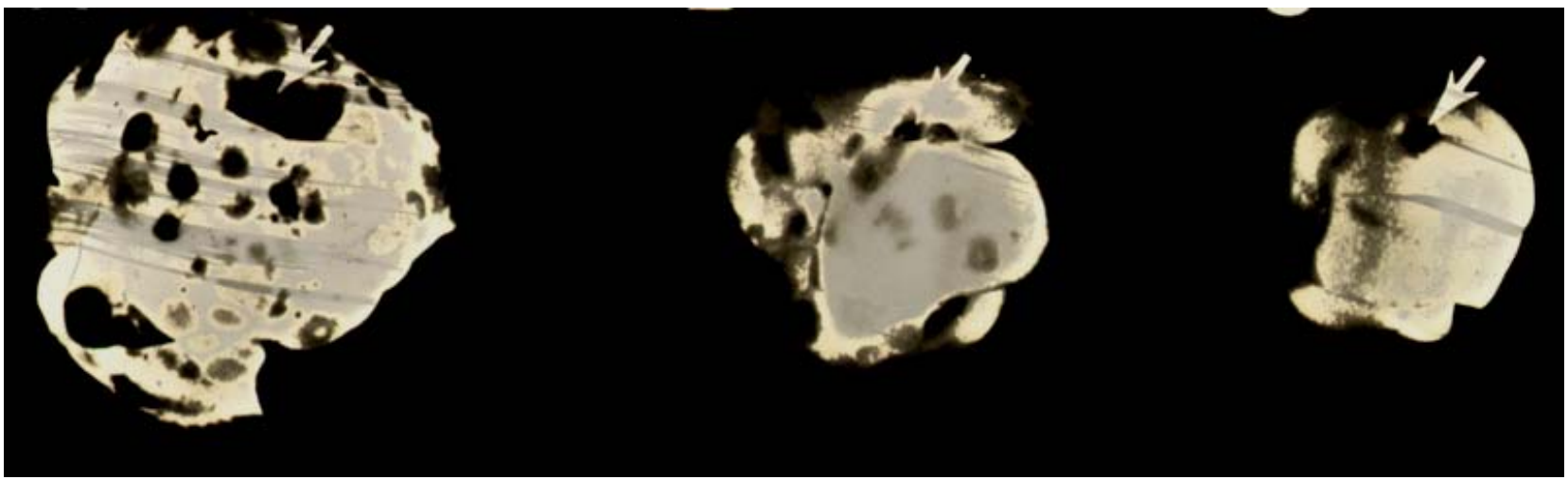

murine
human
human IL2


Tumor Cell
Anti-GD2+IL2 Immunocytokine
GD2 Antigen
IL-2
hu14.18-IL2
IL-2 Receptor
T Cell or NK Cell

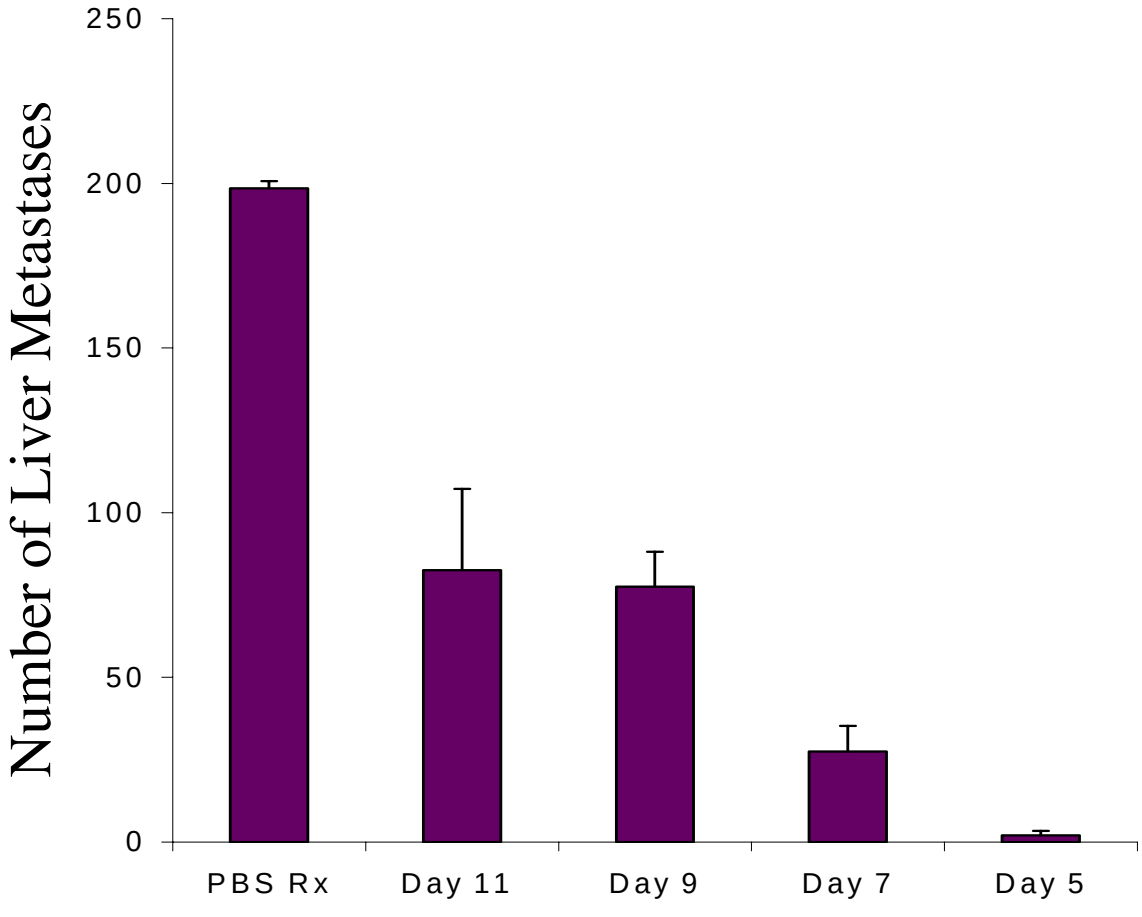
# Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

**Lode et al: *J. Natl. Cancer Inst.* 89:1586, 1997**

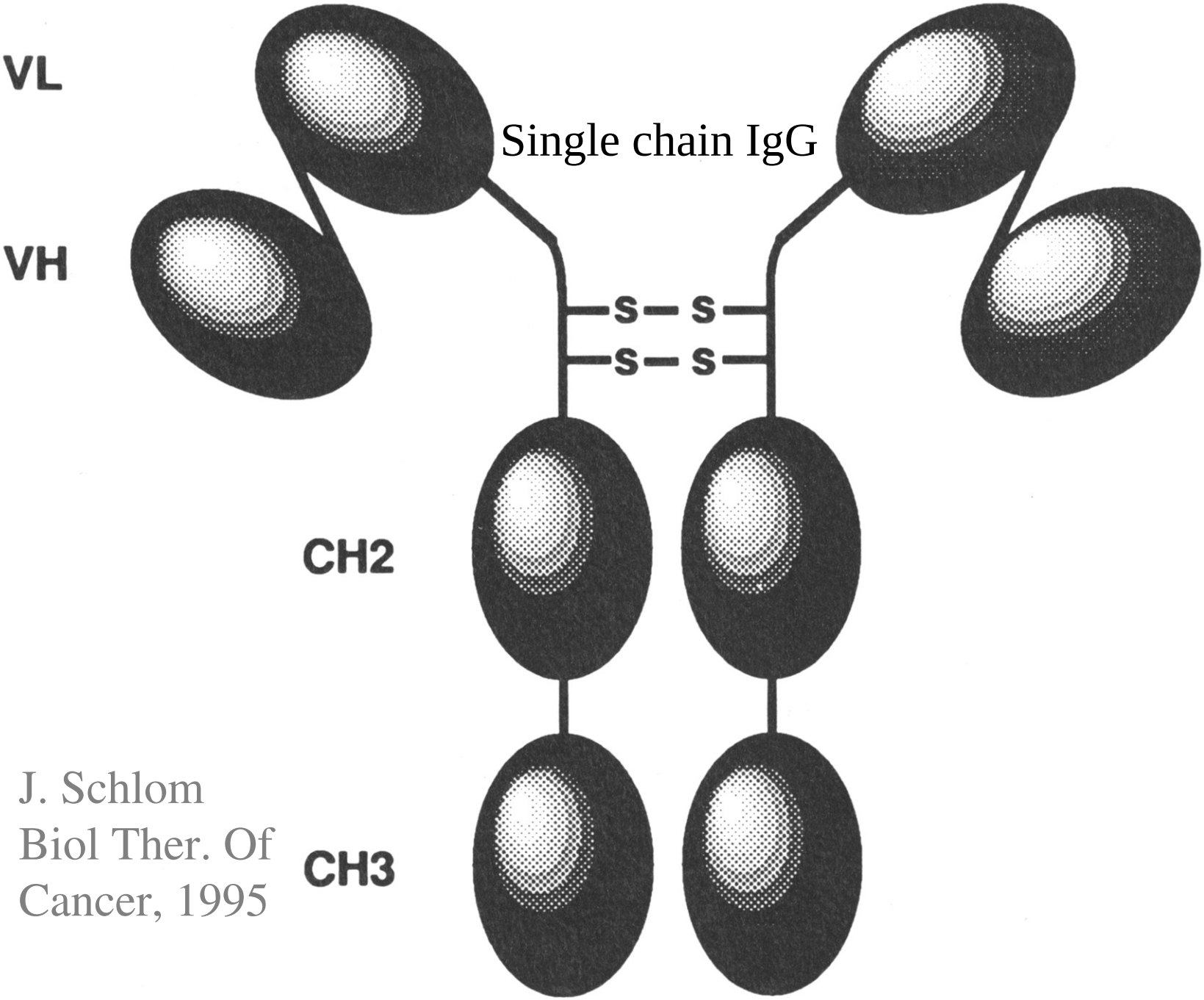


# Hu14.18-IL2 Efficacy: Dependence on Tumor Establishment

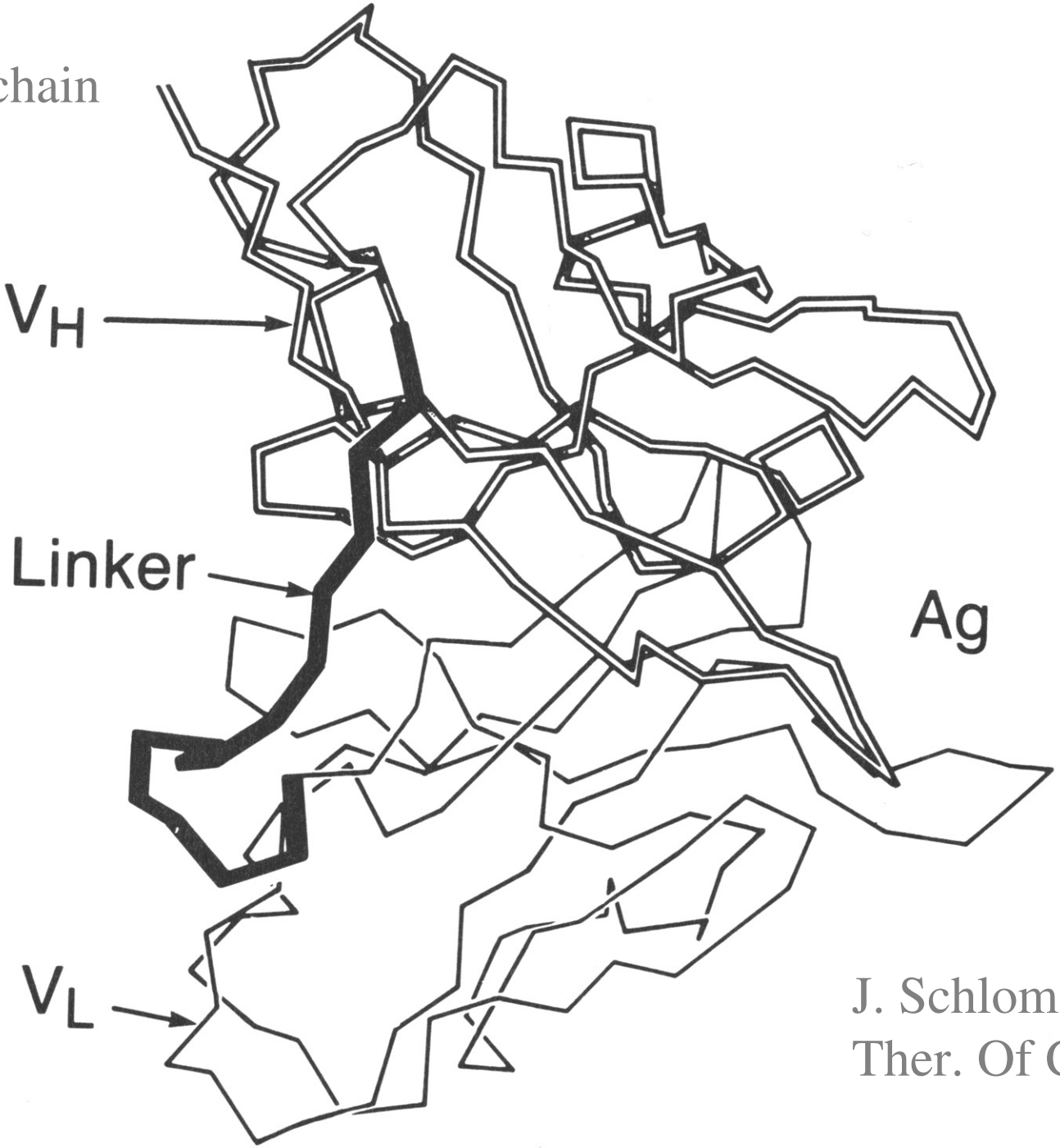



hu14.18-IL2 (10ug/d) for 5 days starting on day 5, 7, 9, or 11 following 5 X $10^5$ NXS2 cells injected on day 0, and harvested on day 28.




J. Schlom
Biol Ther. Of
Cancer, 1995

Single chain
scFv




J. Schlom:Biologic
Ther. Of Cancer 95

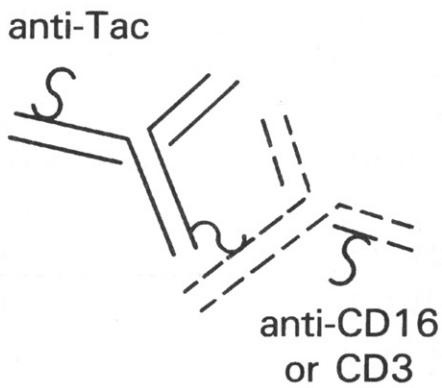
# Potential uses of scFv

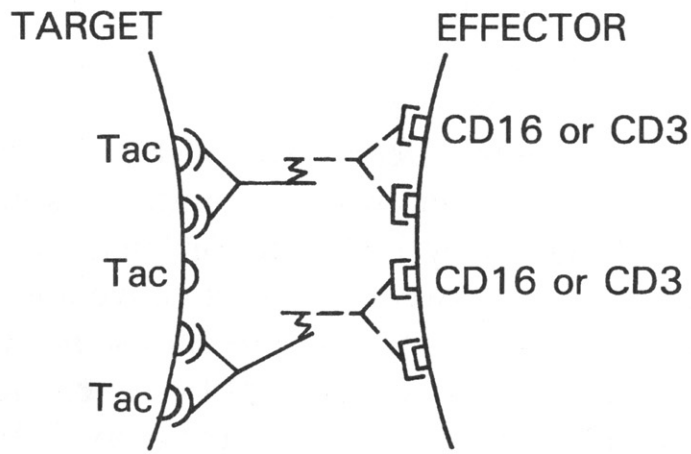
- Smaller molecule, penetrates better
- Link to toxins
- Link to TCR or FcR signaling components to provide mAb mediated specificity to T or NK cells (“T cell bodies”, or “artificial receptors”)

# Bifunctional mAb: Heteroconjugate vs. Quadroma

1) heteroconjugate

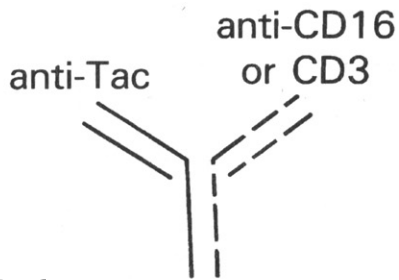
complete immunoglobulins,
chemically cross-linked,
multimeric form,
multivalent

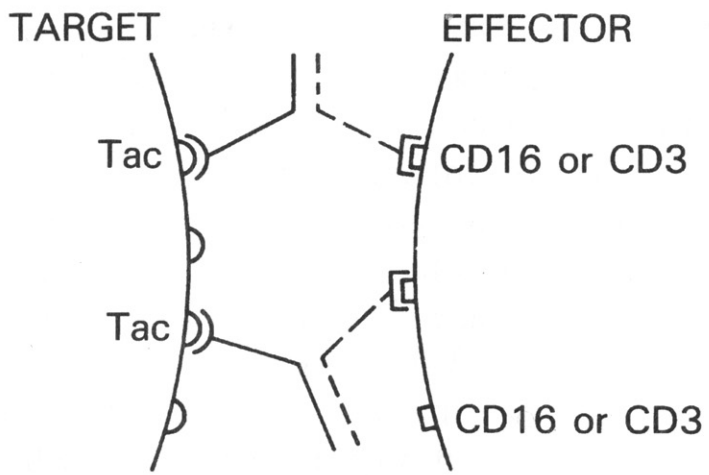



2) bispecific

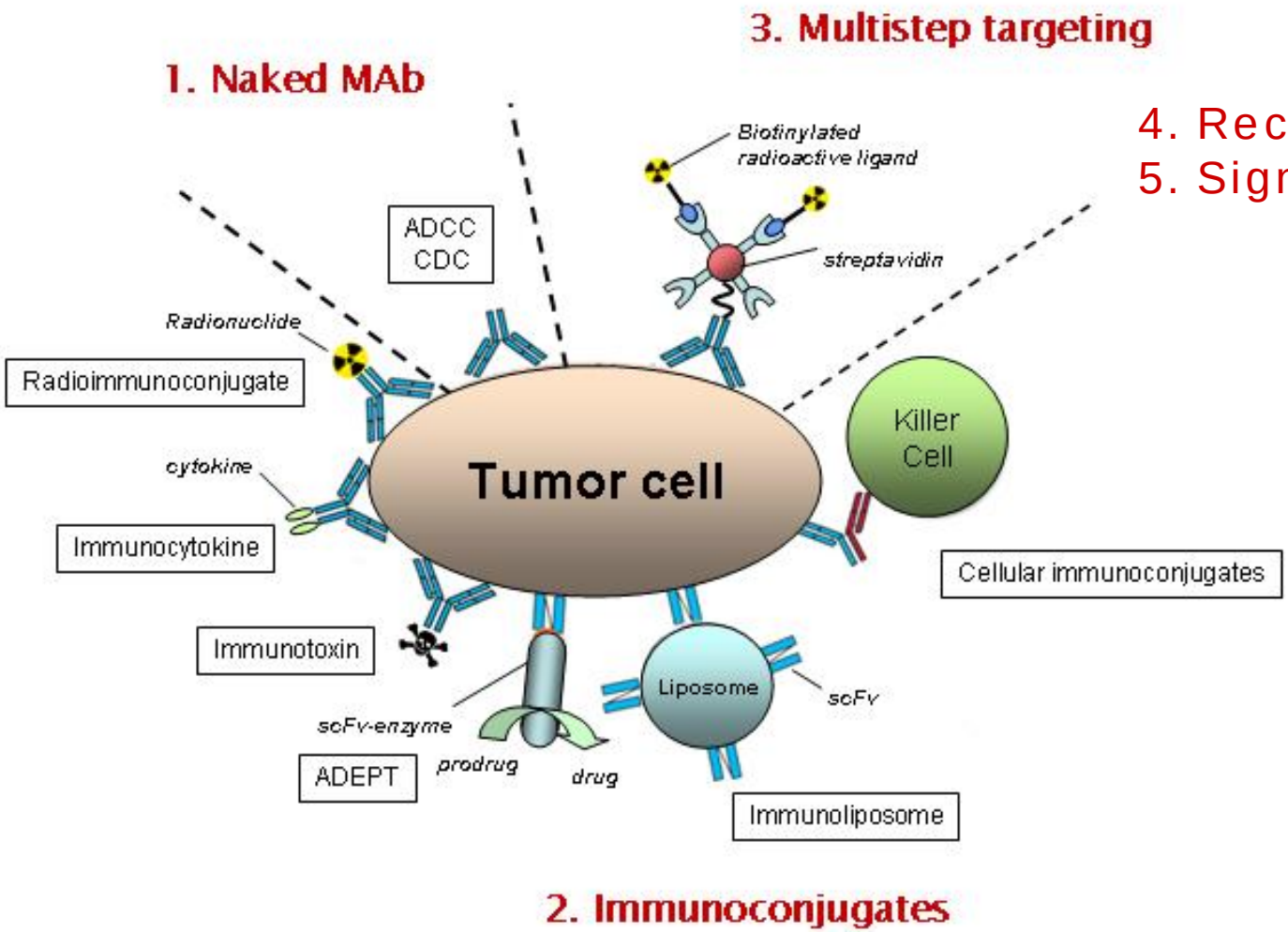
hemi globulins,
native disulfide linkage,
(hybrid hybridomas or
disulfide exchange)
monomeric form,
bivalent




R. P. Junghans et al, 1996

# Anti-tumor applications of mAb




4. Receptor blockade
5. Signal transduction

**Adapted from N-K Cheung 2003**

# Clinically Approved MoAb for Cancer-Rx-2004

| Generic | Brand | Target | Indication |
|---|---|---|---|
| Rituximab | Rituxan | CD20 | B cell NHL |
| Trastutumab | Herceptin | HER-2 | HER-2 Breast CA |
| Gemtuzumab | Mylotarg | CD33 | AML (mAb-toxin) |
| Alemtuzumab | Campath | CD52 | B-CLL, CTCL |
| Ibritumomab Tosifumomab | Zevalin Bexxar | CD2 | Refractory B NHL (Radiolabeled mAb) |
| Basiliximab/ Daclizumab | Anti-TAC | CD25 | Anti-Graft Rejection/ GVH |
| Bevacizumab | Avastin | VEGF | GI Malignancies |
| Edrecolomab | 17-1A | EpCam | GI Malignancies |

# Collaborators in UWCCC Immunocytokine Research-2004

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  - A Sternberg
- C.O.G and N.A.N.T.
  - Many Pediatric Oncologists
- Lexigen
  - S Gillies
- EMD
  - B Clements
- Scripps
  - R Reisfeld