University of Wisconsin
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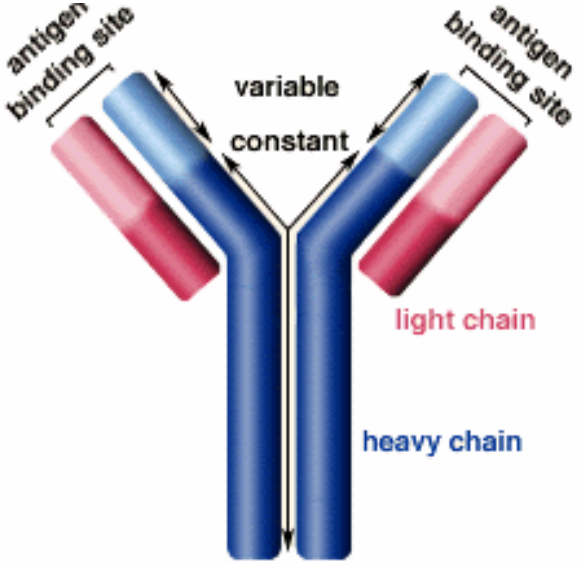
Children's Hospital

Antibody Therapy:
Biology, Immunocytokines, and Hematologic Malignancy

iSBTc Primer
Boston,
November 1, 2007



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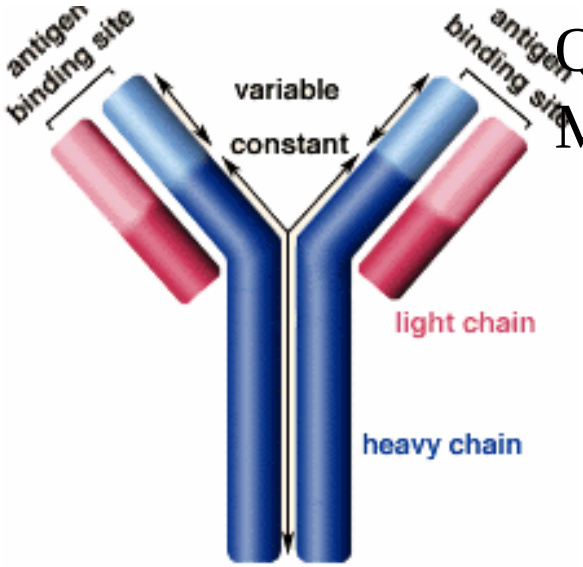
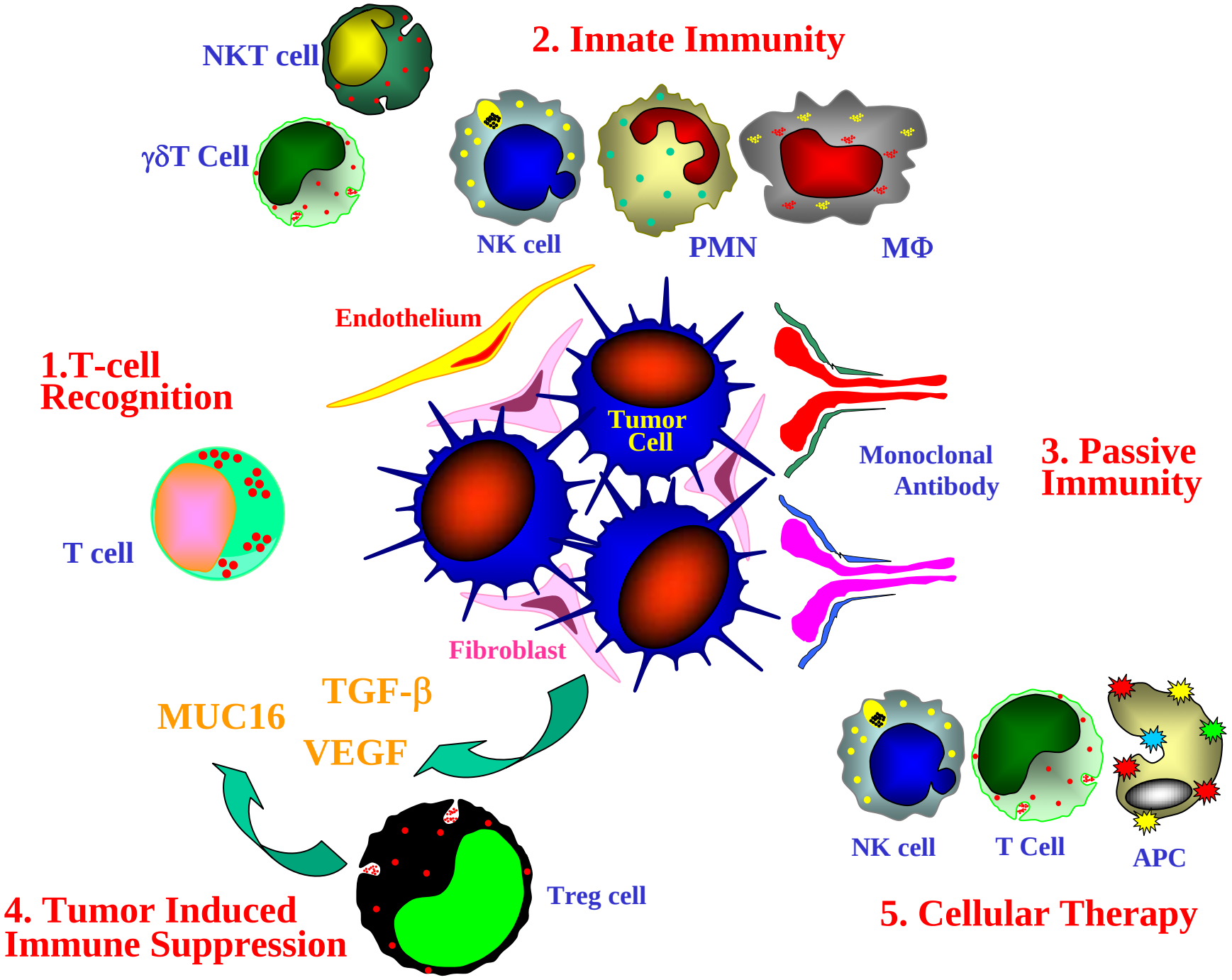
DISCLOSURE STATEMENT

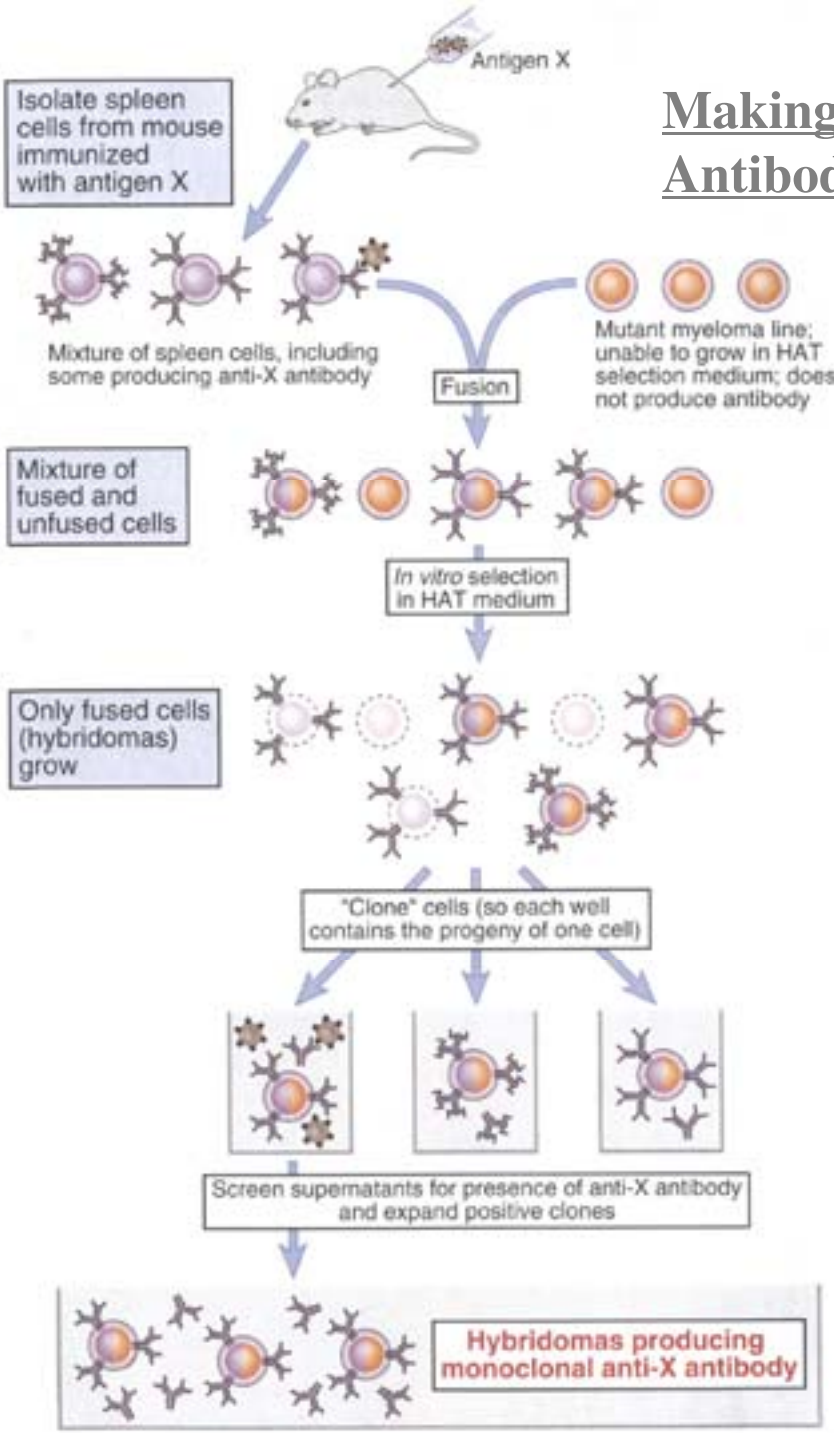
P. Sondel has disclosed the information listed below. Any real or apparent conflict of interest related to the content of the presentation has been resolved.

Organization	Affiliation
EMD-Pharmaceuticals	Scientific Advisor
Quintesence	Scientific Advisor
Medimmune	Scientific Advisor




NKT cell
γδT Cell
2. Innate Immunity
NK cell
PMN
MΦ
Endothelium
1.T-cell Recognition
Tumor Cell
Monoclonal Antibody
3. Passive Immunity
T cell
Fibroblast
TGF-β
MUC16
VEGF
NK cell
T Cell
APC
Treg cell
4. Tumor Induced Immune Suppression
5. Cellular Therapy

Making Monoclonal Antibody (mAb)



Abbas and Lichtman:2003

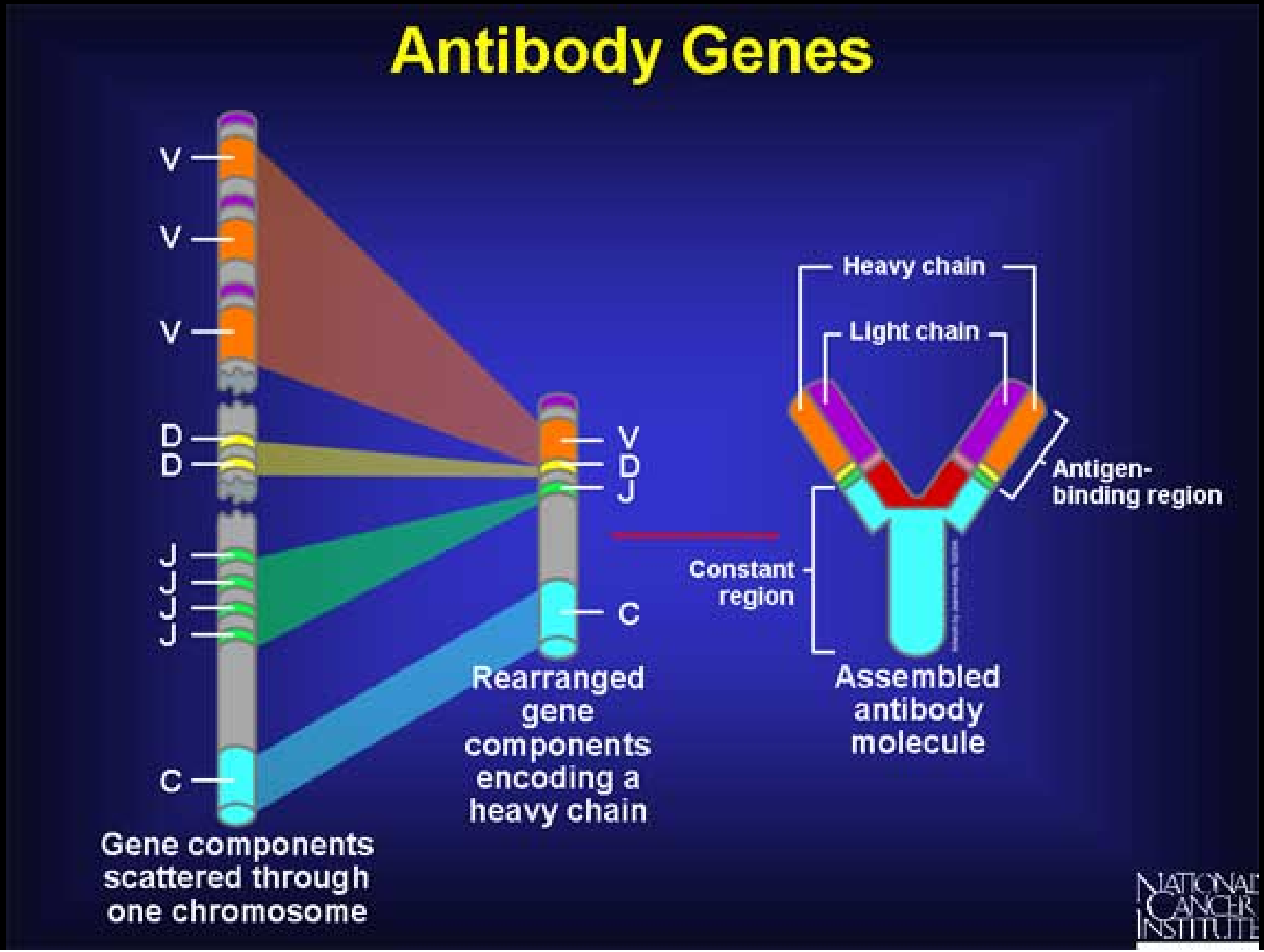
Underlying principle of mAb therapy

SELECTIVE recognition of tumor cells, but not most normal cells by therapeutic mAb

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

From Genes to Antibodies

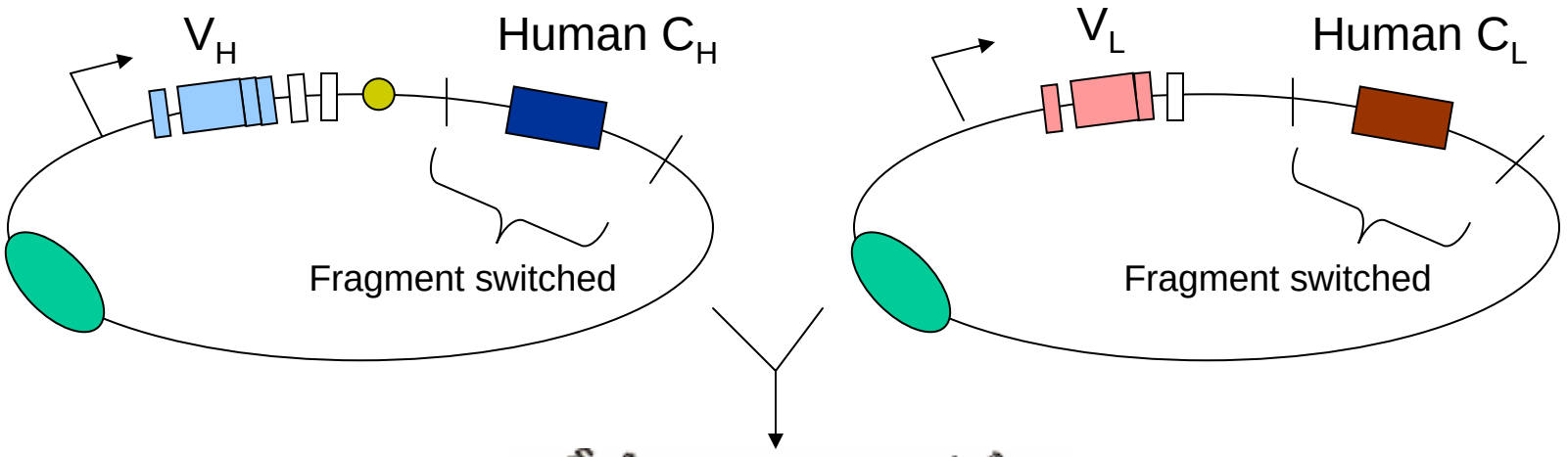


From S. Gillies

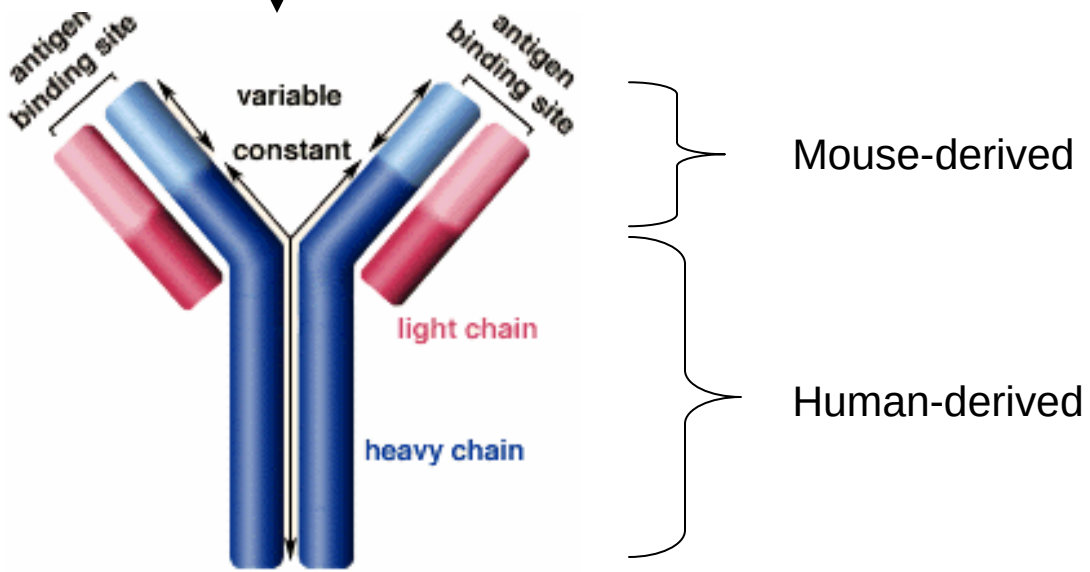
Antibody Engineering

- First step - development of monoclonal antibodies
  - Fusion of antibody-producing B cell with myeloma
  - Results in immortalized monospecific Ab-producing cell line
- Second step - ability to clone and re-express Abs
  - Initially done with cloned, rearranged genes from hybridomas
  - Parallel work with isolated Fab fragments in bacteria
- Third step - re-engineering for desired properties
  - Reducing immunogenicity of mouse antibodies
  - Tailoring size and half-life for specific need
  - Adding or removing functions
- Engineered diversity – phage display approach

Chimeric Mouse-human antibodies

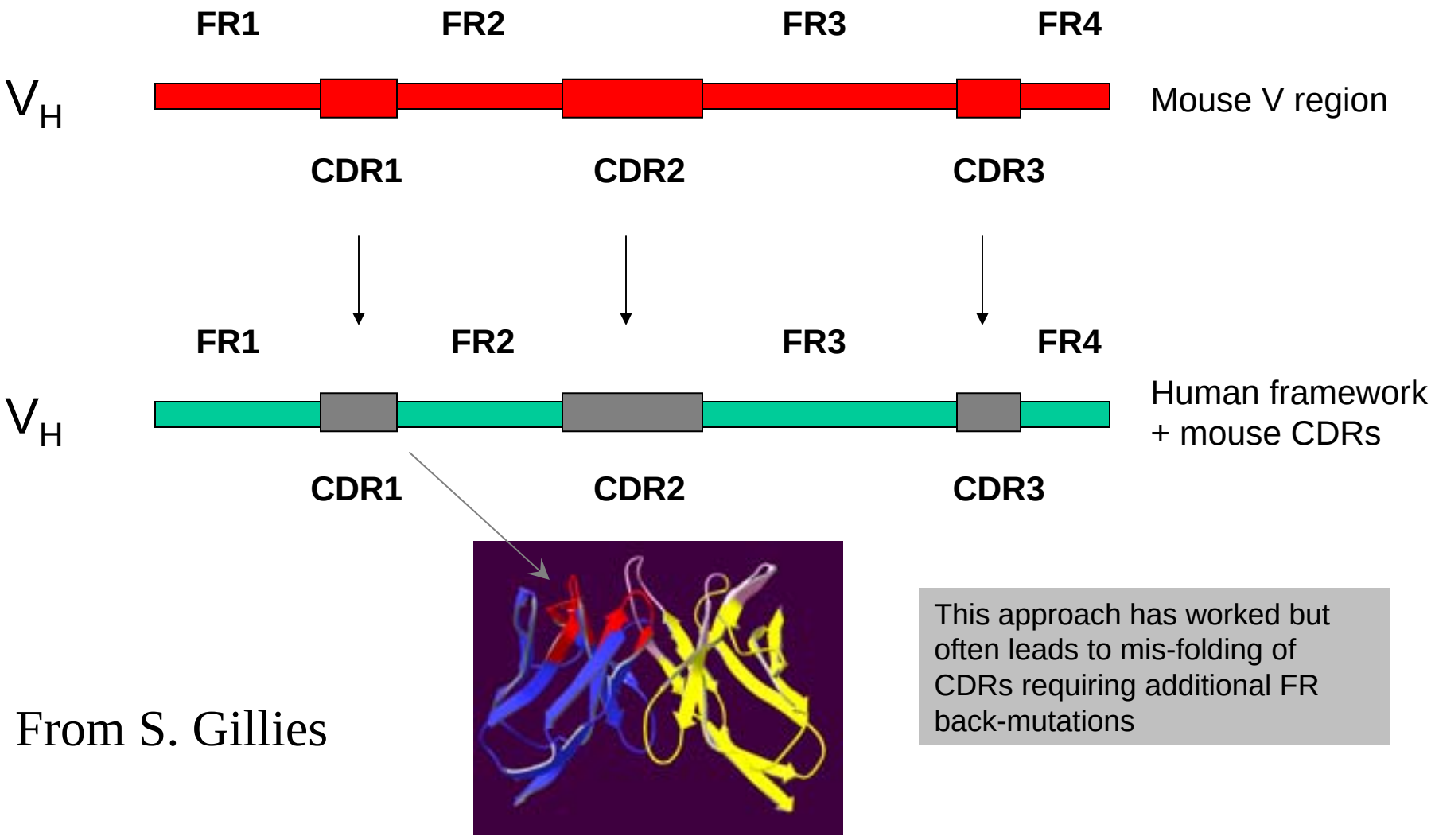


e.g. Erbitux



From S. Gillies

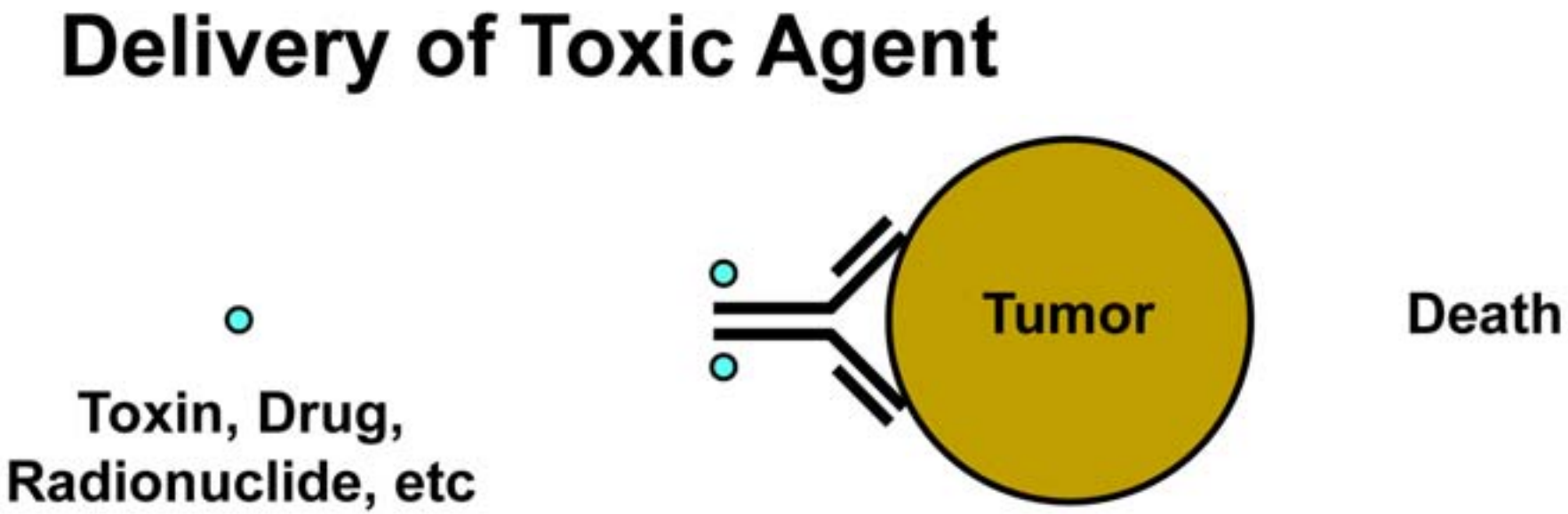
V Region “humanization”



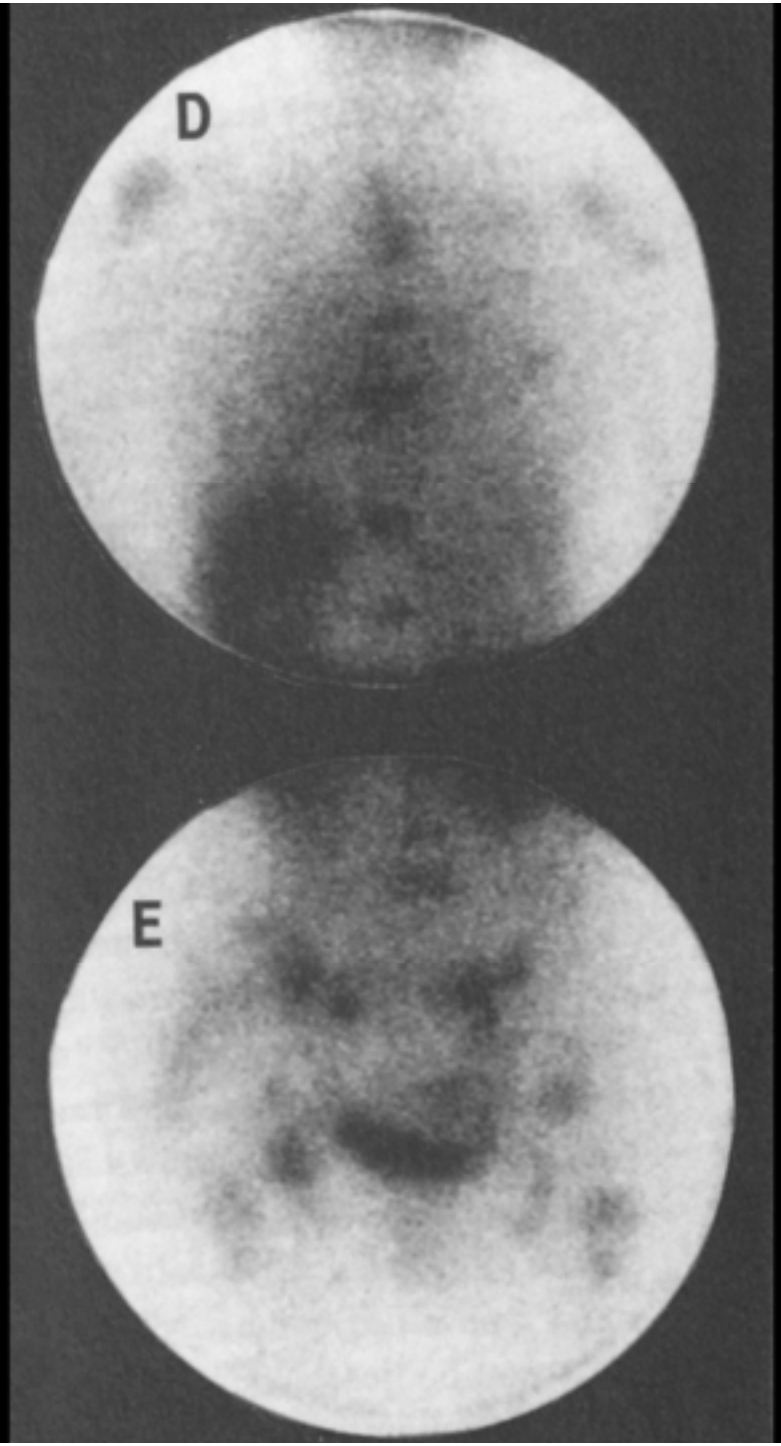
From S. Gillies

This approach has worked but often leads to mis-folding of CDRs requiring additional FR back-mutations

Mechanisms of mAb mediated anti-tumor effects

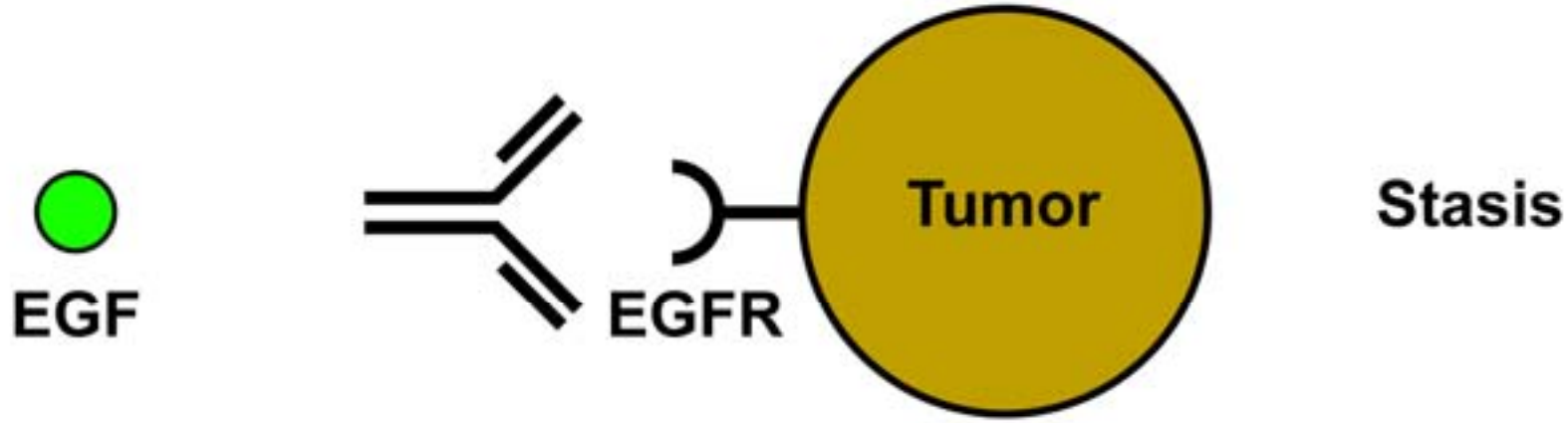


^{131}I-3F8 binding to melanoma

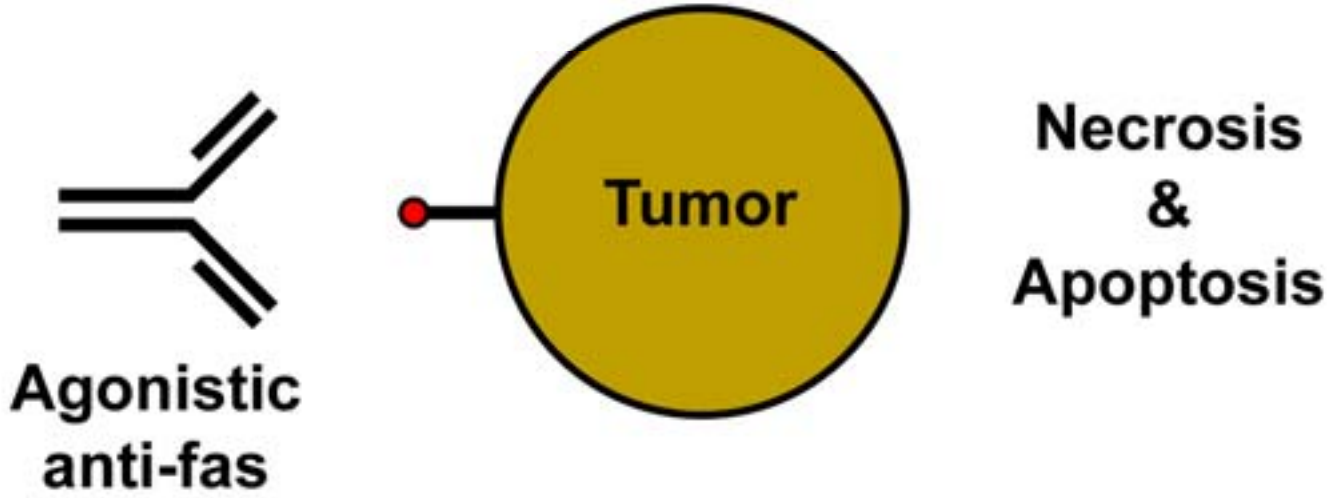


Cheung et al. Biol Ther. Of Cancer, 1995

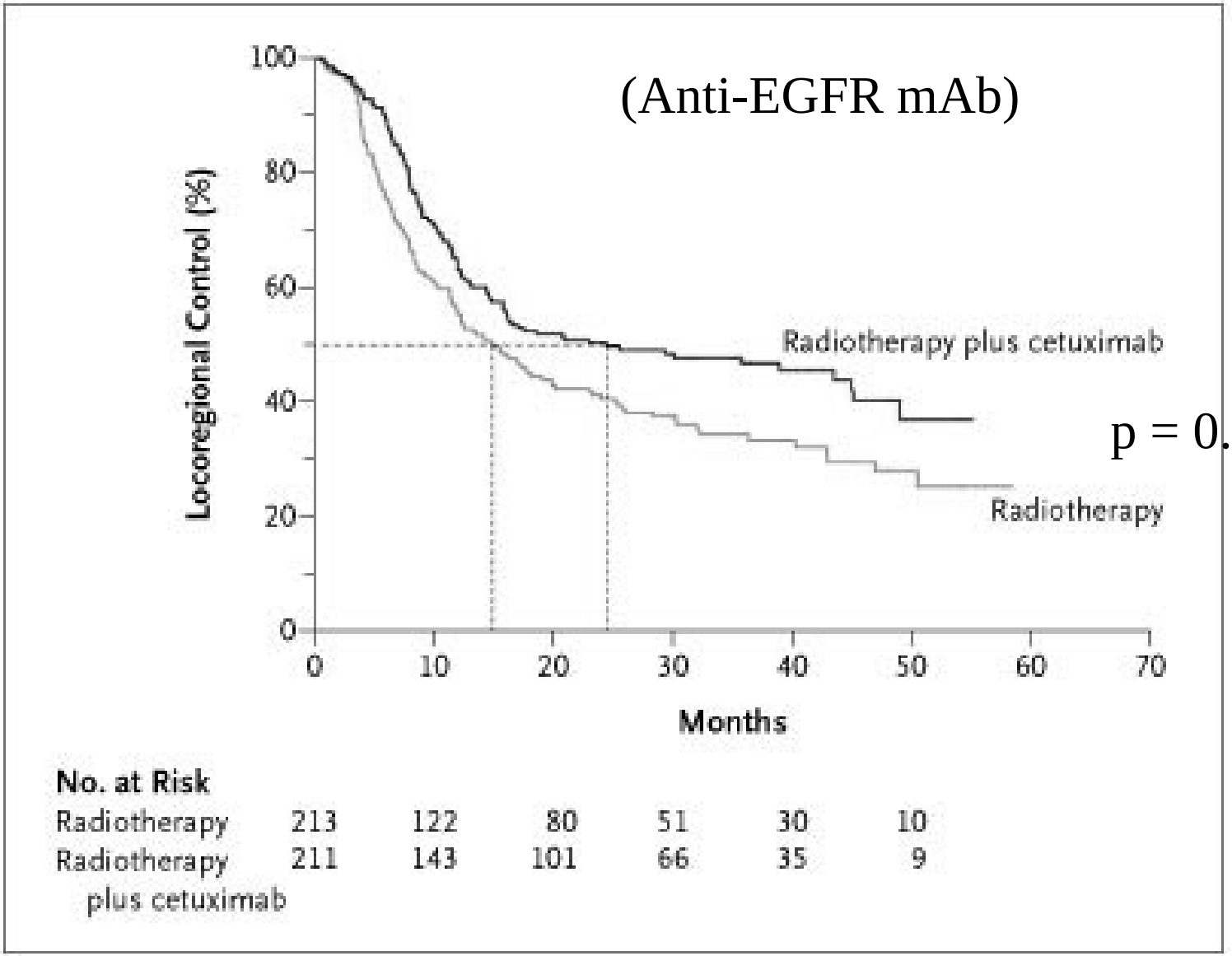
Receptor Blockade



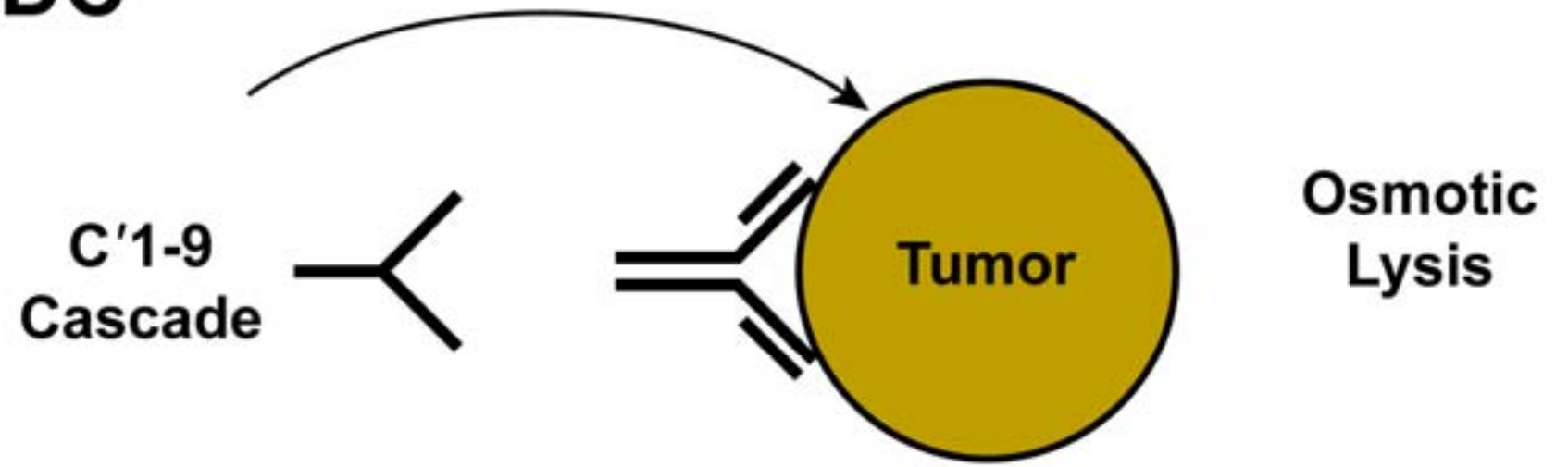
Signal Activation



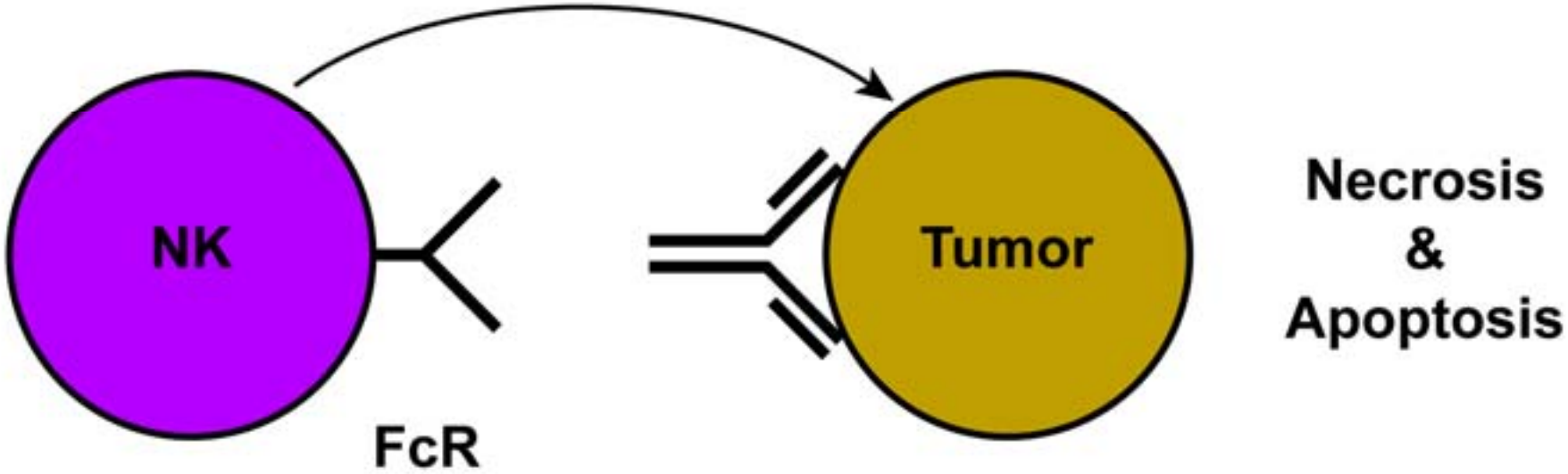
Bonner JA, Harari PM et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. NEJM, 354:634, 2006



CDC

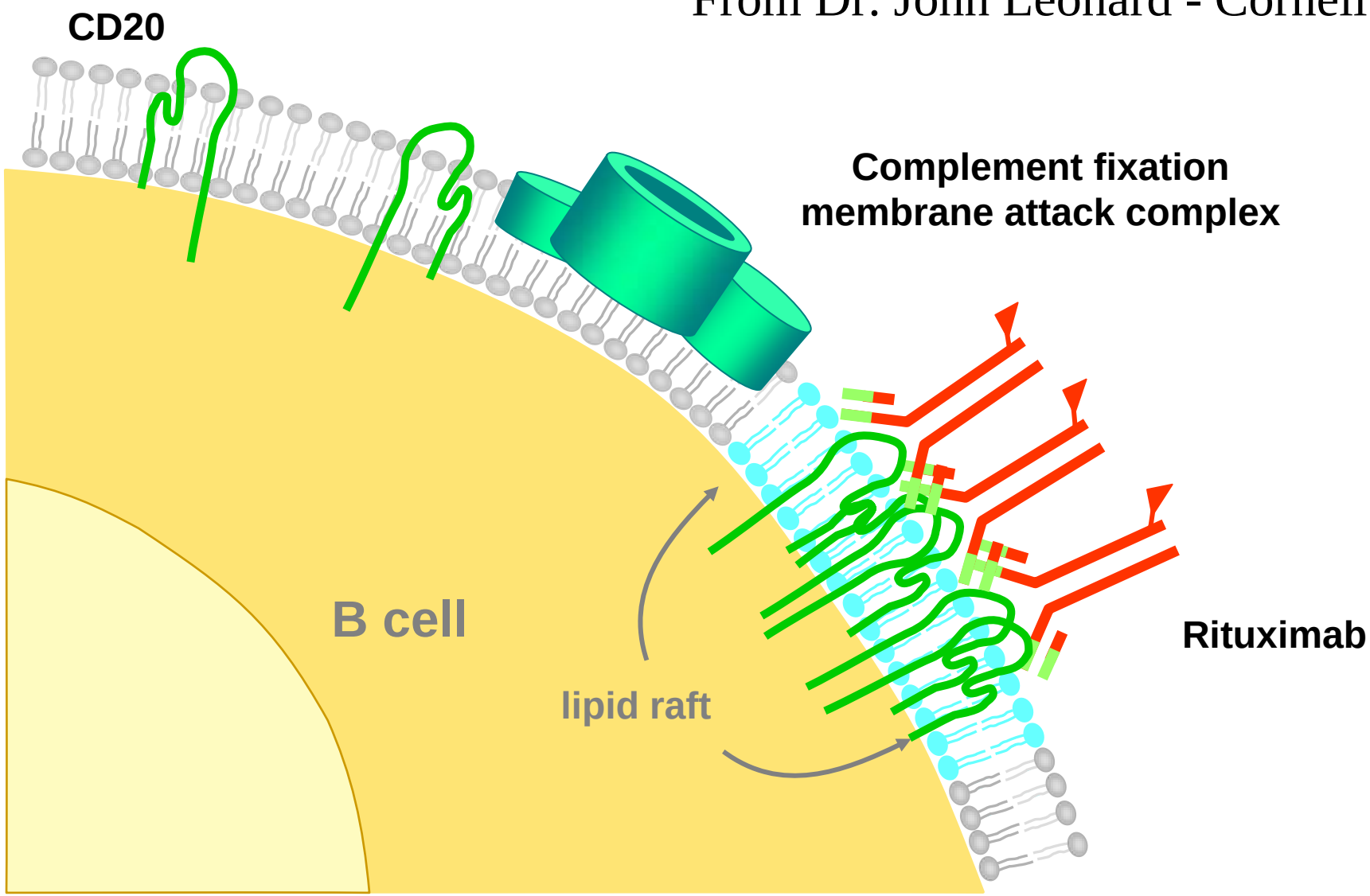

C′1-9
Cascade
Tumor
Osmotic
Lysis

ADCC

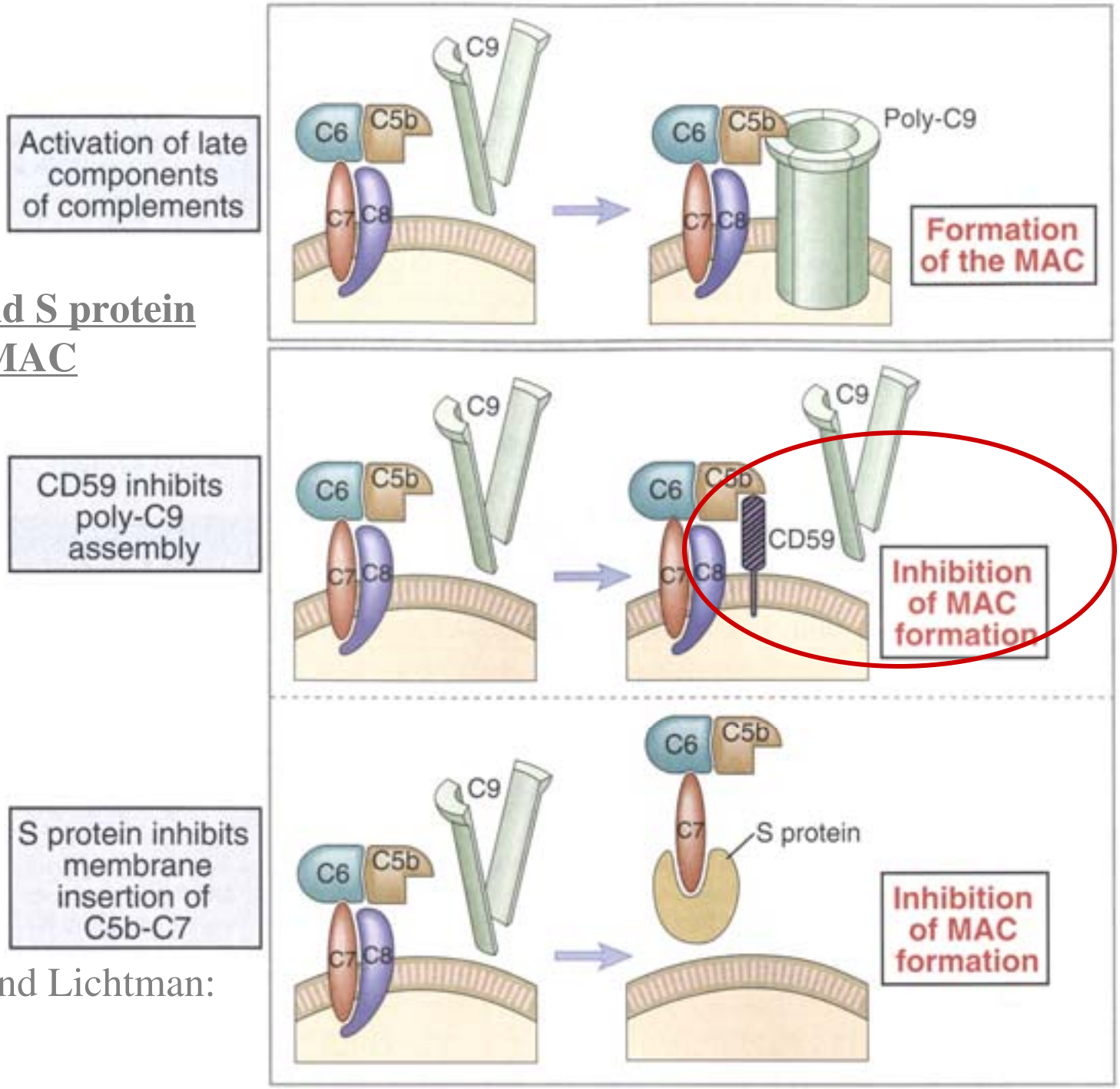

NK
FcR
Tumor
Necrosis
&
Apoptosis

Mechanisms of anti-CD20 therapy: CDC

From Dr. John Leonard - Cornell



CD59 and S protein Inhibit MAC



Abbas and Lichtman: 2003

Complement mediated destruction is inhibited in vitro by CD59 (blocks MAC)

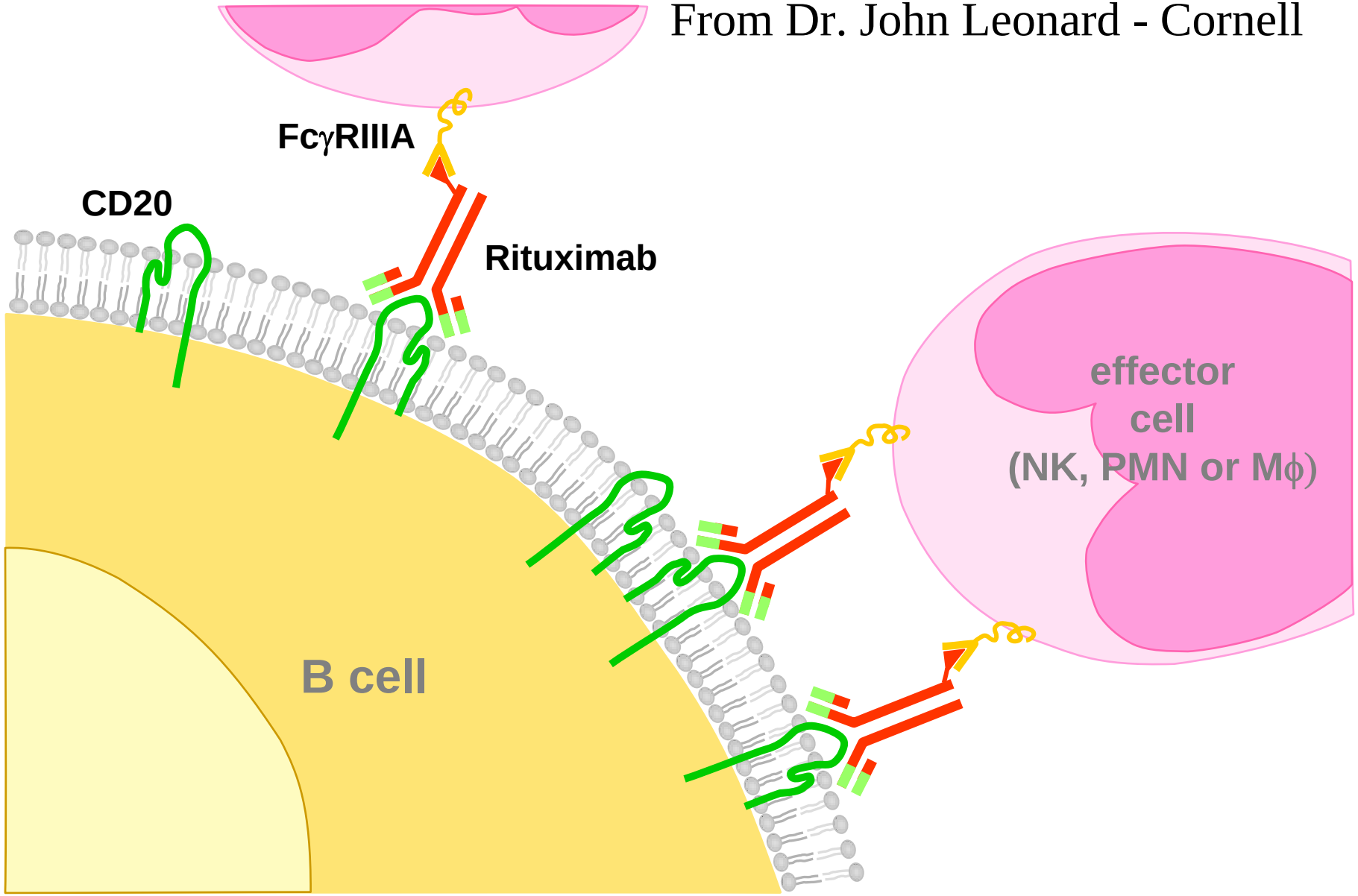
Tumor cell type	CD59	CD20	Viability	Viability
			Rituxan	Rituxan + C’
NHL	++++	++++	100%	90%
NHL	-	++++	100%	0%

BUT, there is no correlation in vivo with CD59 and Rituxan response!

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

Mechanisms of anti-CD20 therapy: ADCC

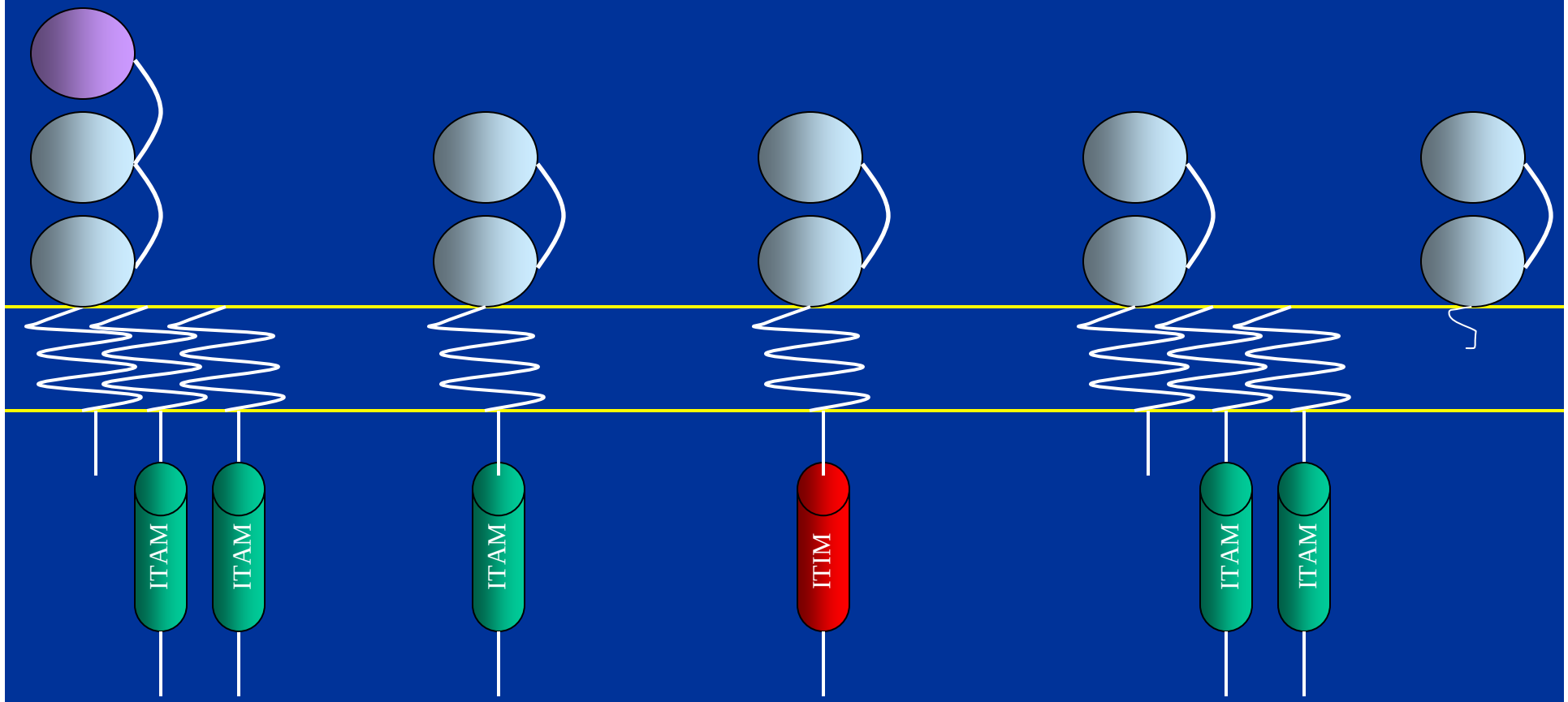
From Dr. John Leonard - Cornell



Human Fcγ Receptor Family

FcγRI FcγRIIA FcγRIIB FcγRIIIA FcγRIIIB

CD32 **CD16**



Dr. R. Clynes – Columbia U

2 Major Types of Activating FcR for IgG

- **FcγRIIA (CD32)**
- **Expressed on:**
  - **Macrophages**
  - **PMNs**
- **Functions:**
  - **Phagocytosis**
  - **ADCC**

- **FcγRIIIA (CD16)**
- **Expressed on:**
  - **NK Cells**
- **Functions:**
  - **ADCC**

Efficacy of FcR influences in vivo Rituxan Effects

AA #158 of FcRIII

V → Higher Affinity for huIgG
F → Lower Affinity

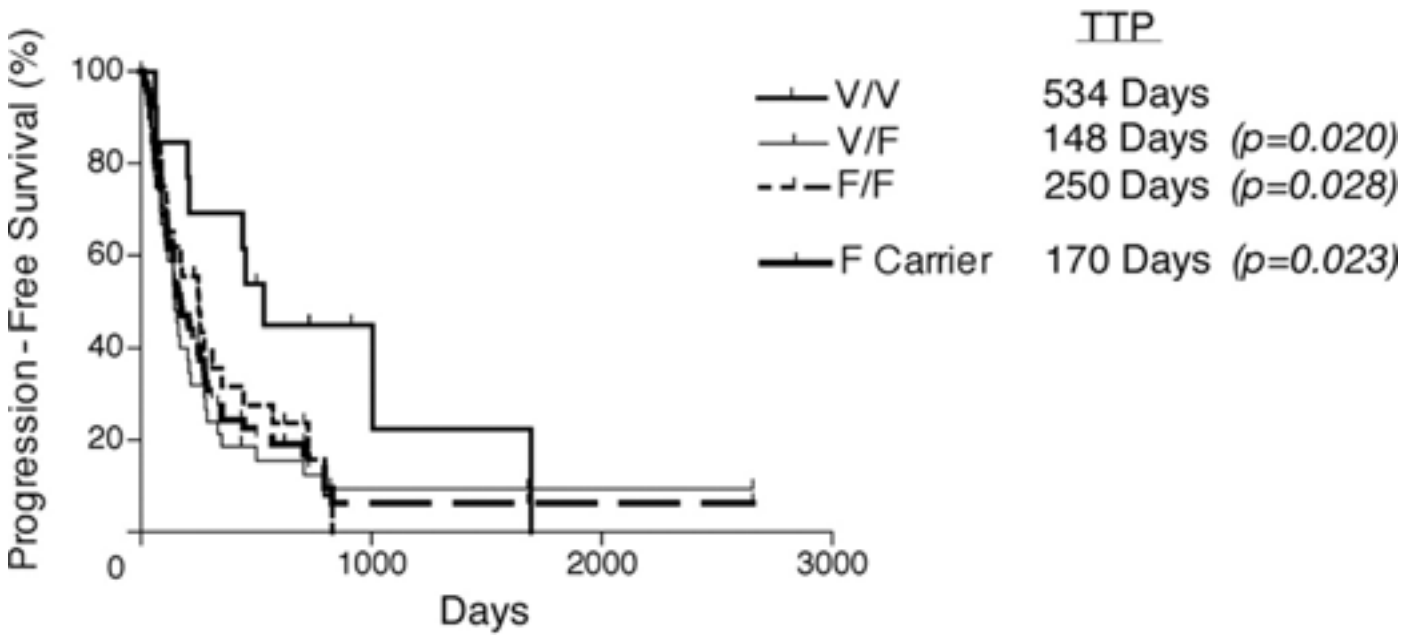
Phenotype	***In Vitro* ADCC**	**Reponse Rate *In Vivo* to Rituxan**
V/V	++++	++++
V/F	++	+
F/F	+	+

p < .05

Cartron et al. Blood 99:754, 2002

Importance of FcγRIIIA on NK cells in Rituxan Therapy

Fig. 2

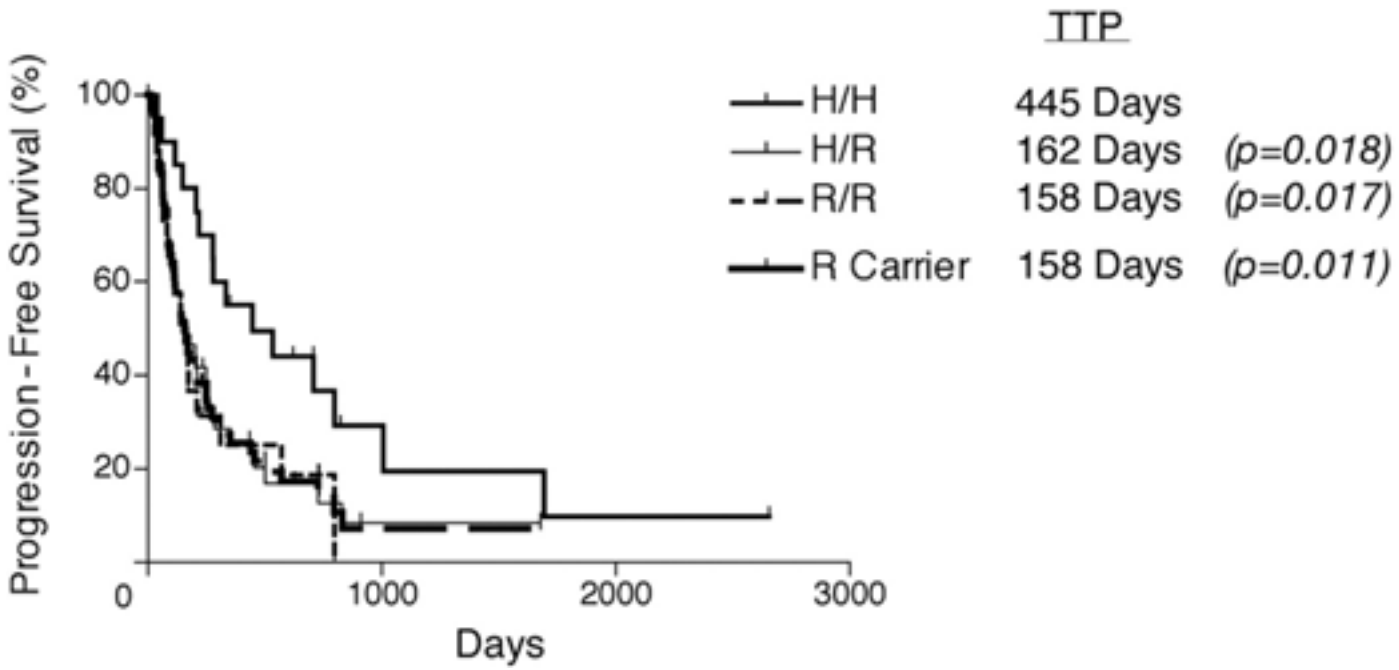


Weng, W.-K. et al. J Clin Oncol; 21:3940-3947 2003

Kaplan-Meier estimates of progression-free survival by immunoglobulin G fragment C receptor IIIa (Fc RIIIa) 158 valine (V)/phenylalanine (F) polymorphism.

Importance of FcgRIIA on Mϕs and PMNs cells in Rituxan Therapy

Fig. 3



Weng, W.-K. et al. J Clin Oncol; 21:3940-3947 2003

Kaplan-Meier estimates of progression-free survival (PFS) by immunoglobulin G fragment C receptor IIa (Fc RIIa) 131 histidine (H)/arginine (R) polymorphism.

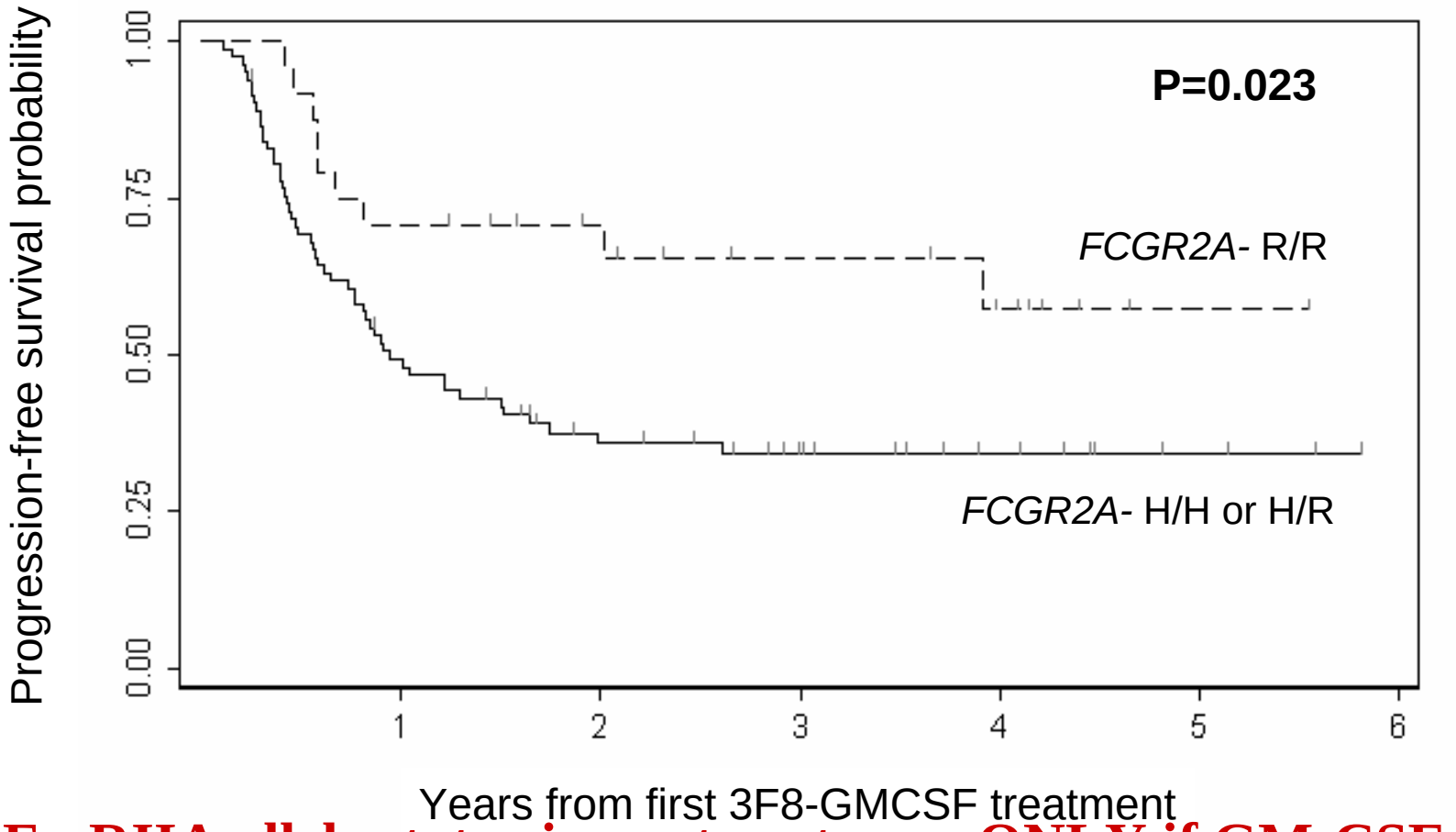
Activate cells with FcγRIIA (PMNs and Mϕs) with GM-CSF

- Treat with murine anti-GD2 mAb (3F8 or ch14.18) AND GM-CSF
- 3F8 is a murine IgG3 mAb:
- Murine IgG3 mAb binds better to the FcγRIIA-R131 than to the FcγRIIA-H131 allele
- **Does the FcγRIIA allele status impact on outcome?**

Cheung et al, JCO, 24:2885, 2006

MSKCC-IRB#9418, N=106, high-risk NB, no h/o prior relapse

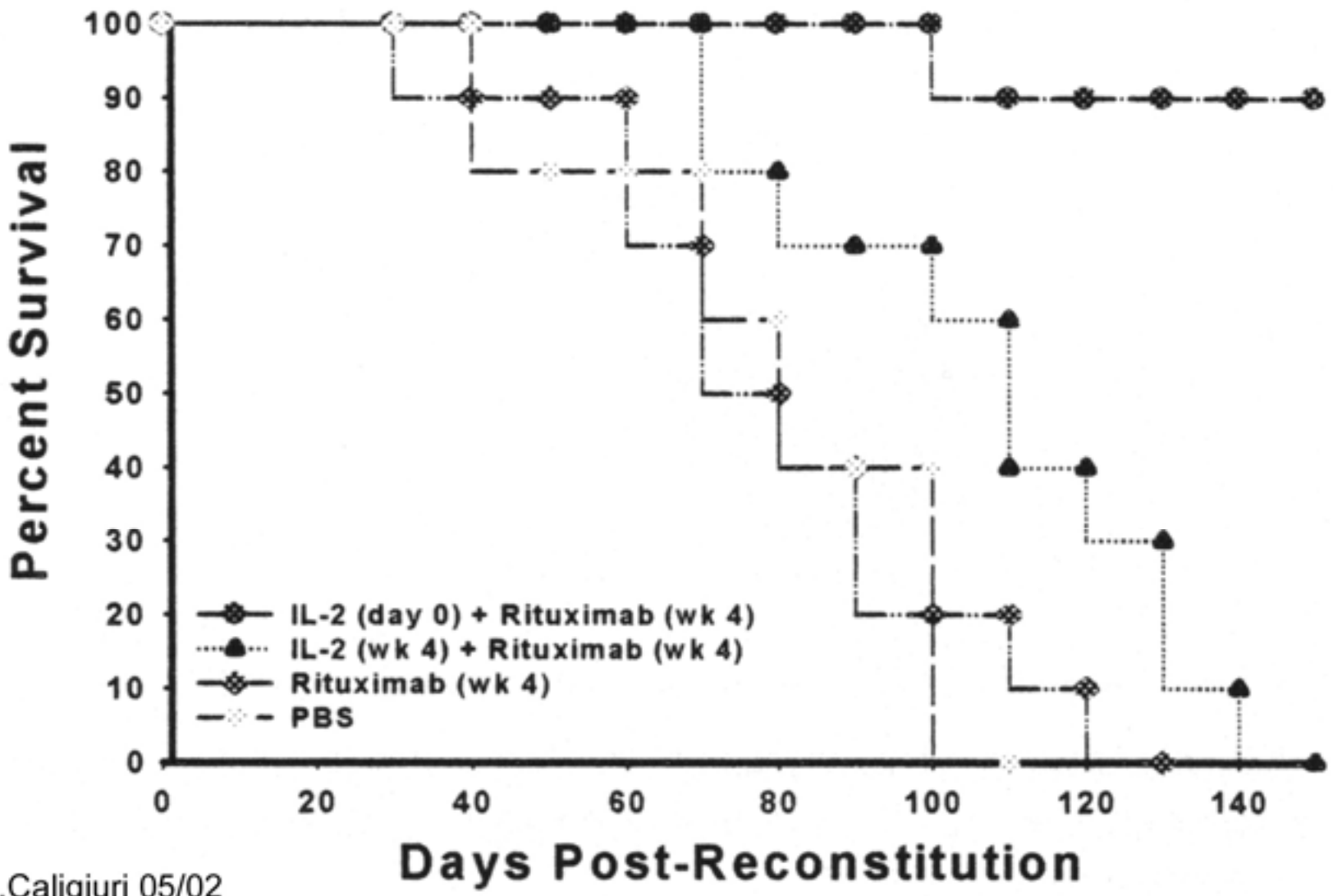
Cheung et al, JCO,24:2885, 2006



The FcγRIIA allele status impacts outcome ONLY if GM-CSF is given (based on historical controls receiving 3F8 without GM-CSF.

Activate NK cells to mediate ADCC

In Vivo IL-2/Rituximab Trial



M.A.Caligiuri 05/02

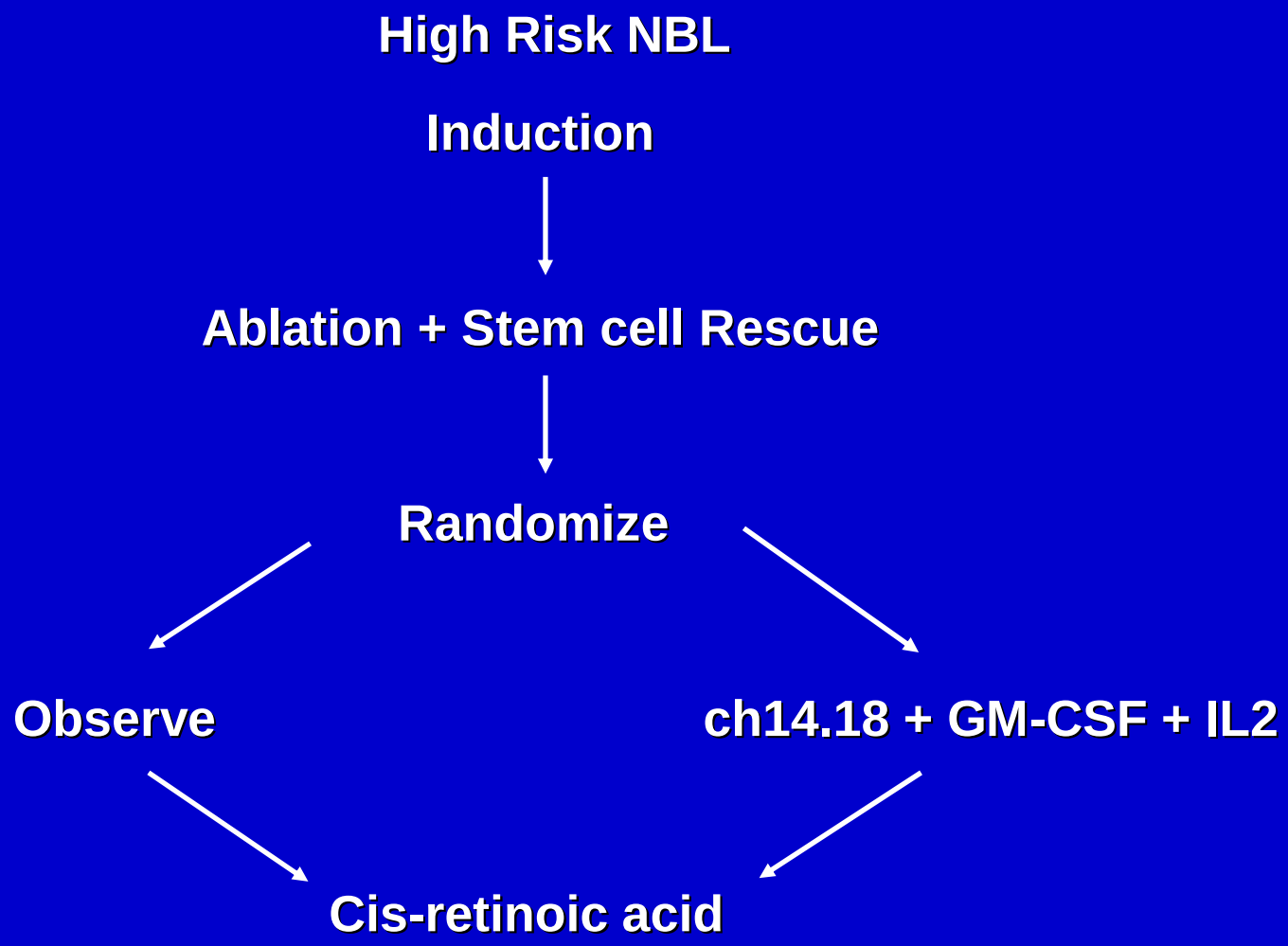
CCG-0935/0935A-

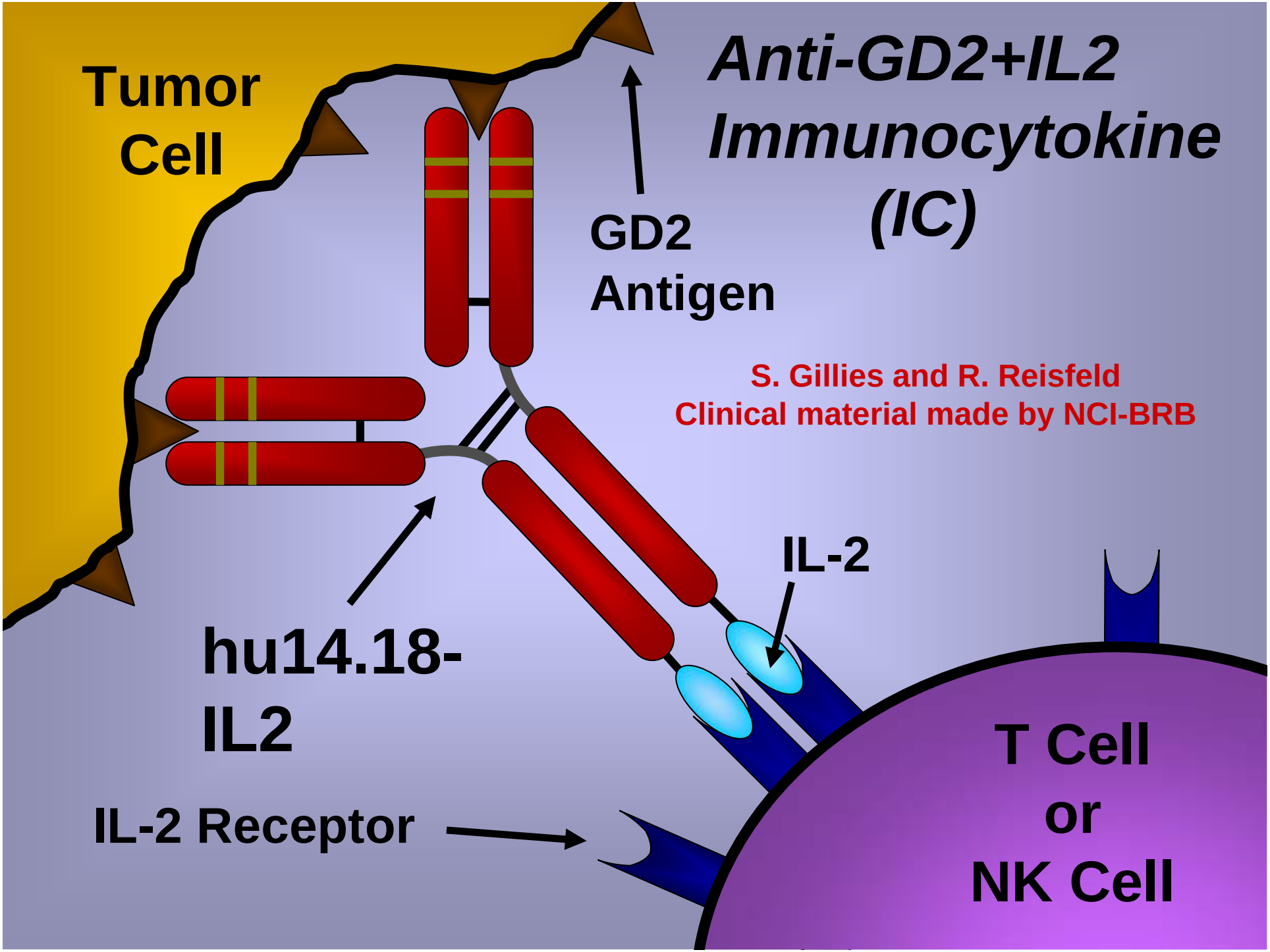
Pilot Phase-I study of ch14.18 + IL2 + GM-CSF following ABMT for NBL

- Day 0 ABMT
- Day 35 Ch14.18 + GM-CSF
- Day 56 Ch14.18 + IL2
- Day 77 Ch14.18 + GM-CSF
- Day 98 Ch14.18 + IL2
- Day 119 Ch 14.18 + GM-CSF
  - Ozkaynak et al JCO 18:4077, 2000
  - Gilman et al, Submitted 2007

Schema: C.O.G. NBL Study ANBL0032

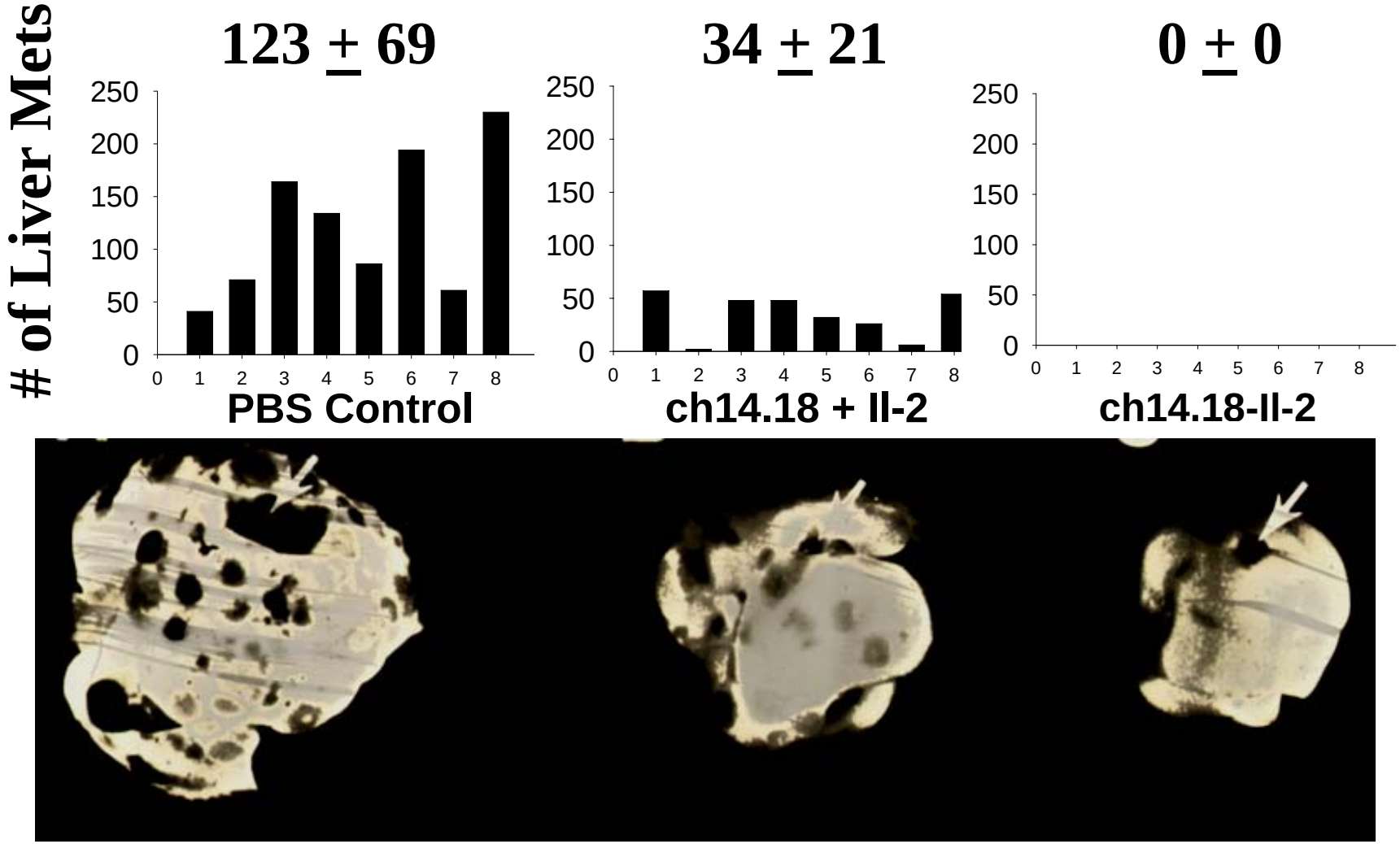
(2002)- A. Yu Chair



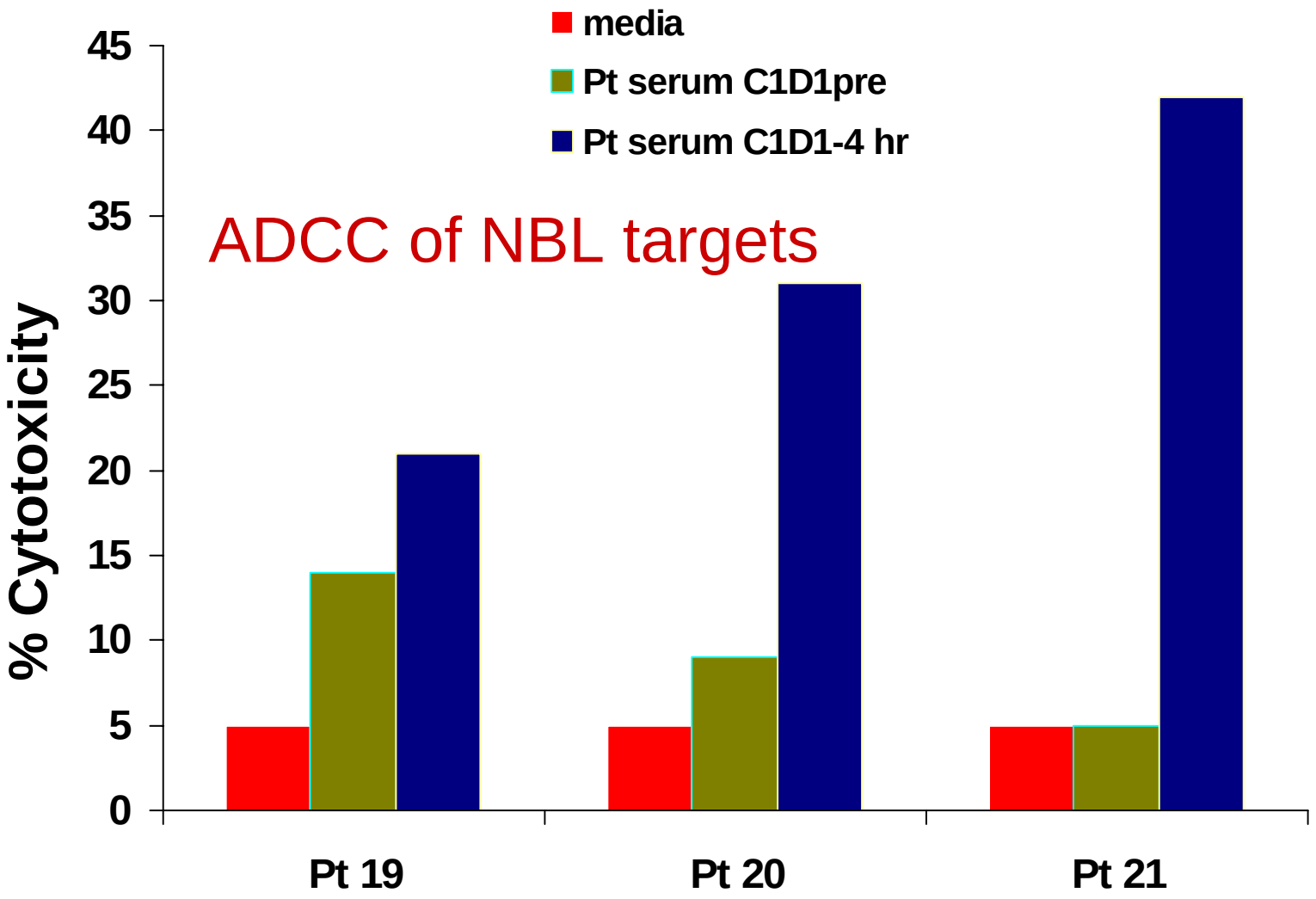

Tumor Cell
Anti-GD2+IL2 Immunocytokine (IC)
GD2 Antigen
S. Gillies and R. Reisfeld
Clinical material made by NCI-BRB
IL-2
hu14.18-IL2
IL-2 Receptor
T Cell or NK Cell

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

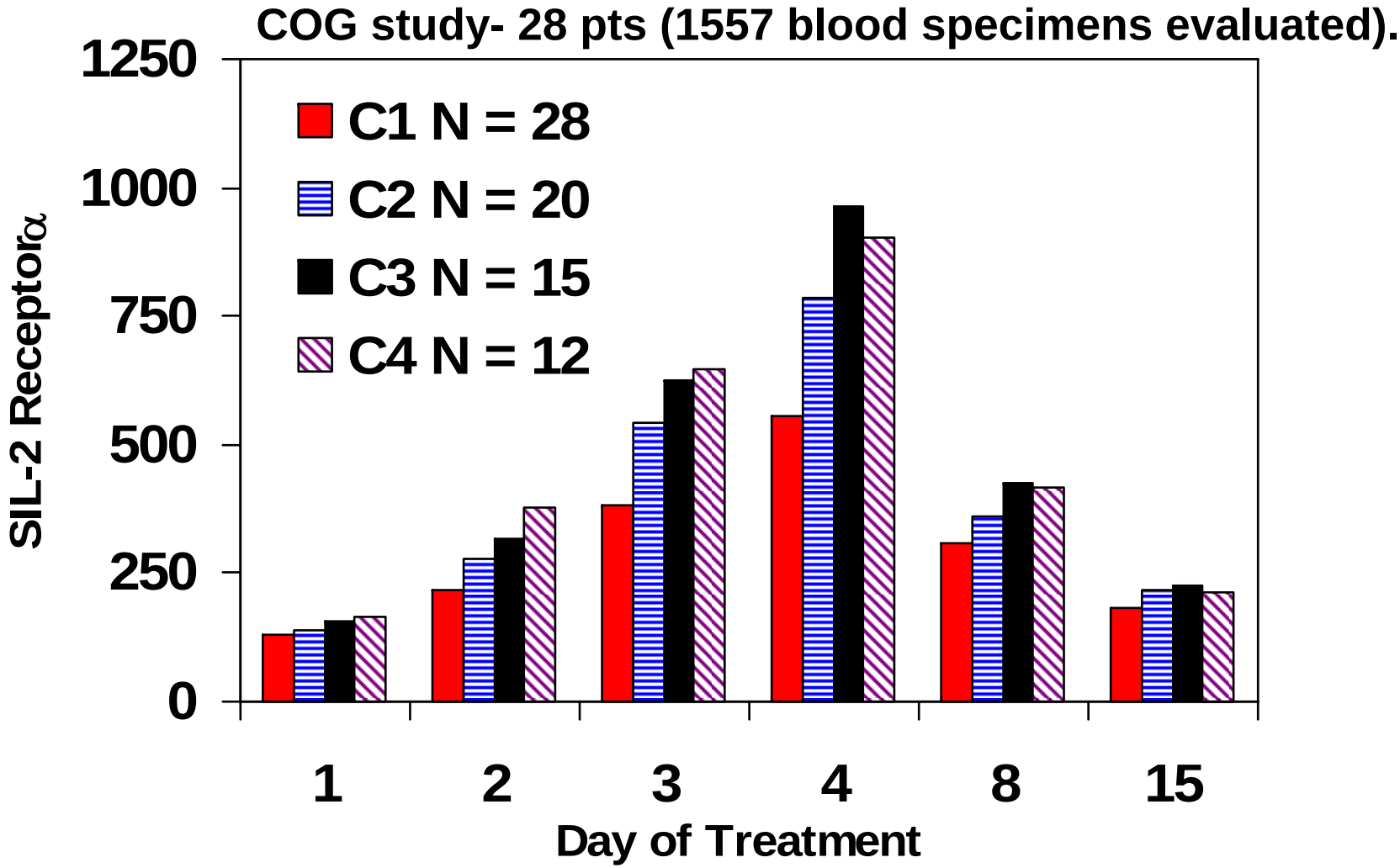
Lode et al: *J. Natl. Cancer Inst.* 89:1586, 1997



Assays with Patient Effectors (C1D8) And Serum Pre and Post hu14.18-IL2 Infusion (1144 Blood samples from 33pts)



King DM, Albertini MR, Schalch H, Hank JA, Surfus J, Mahvi D, Schiller JH, Warner T, Kim KM, Eickhoff J, Kendra K, Reisfeld R, Gillies SD, Sondel PM. J.Clin.Onc. 22:4463,2004



Soluble IL-2 receptor α level determined on days 1,2,3,4,8 and 15 of each course.

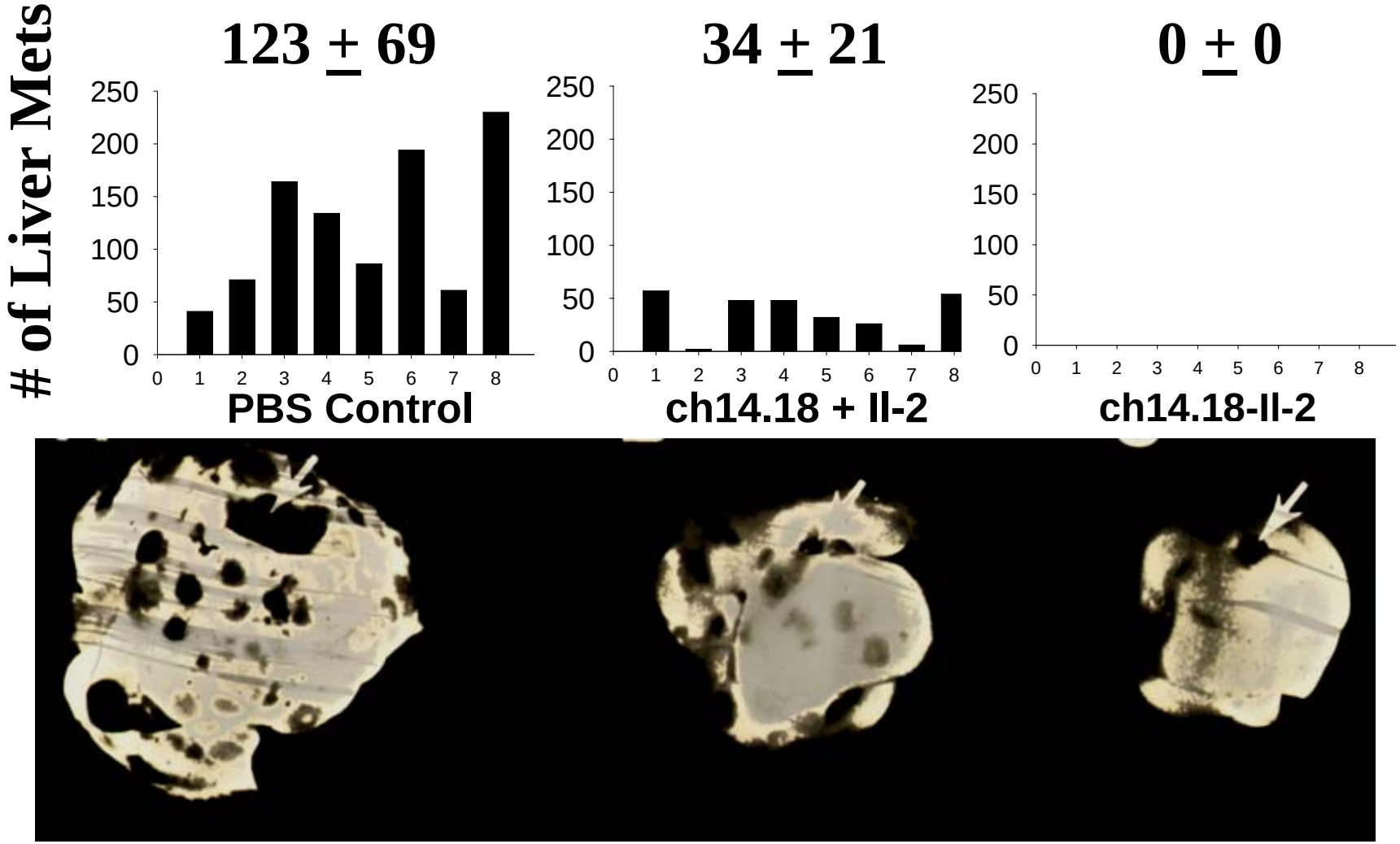
Osenga KL, Hank JA, Albertini MR, Gan J, Sternberg AG, Eickhoff J, Seeger RC, Matthay KK, Reynolds CP, Twist C, Krailo M, Adamson PC, Reisfeld RA, Gillies SD, Sondel PM. Clinical Cancer Research, 12:1750, 2006.

Conclusions from Phase I Trials hu14.18-IL2

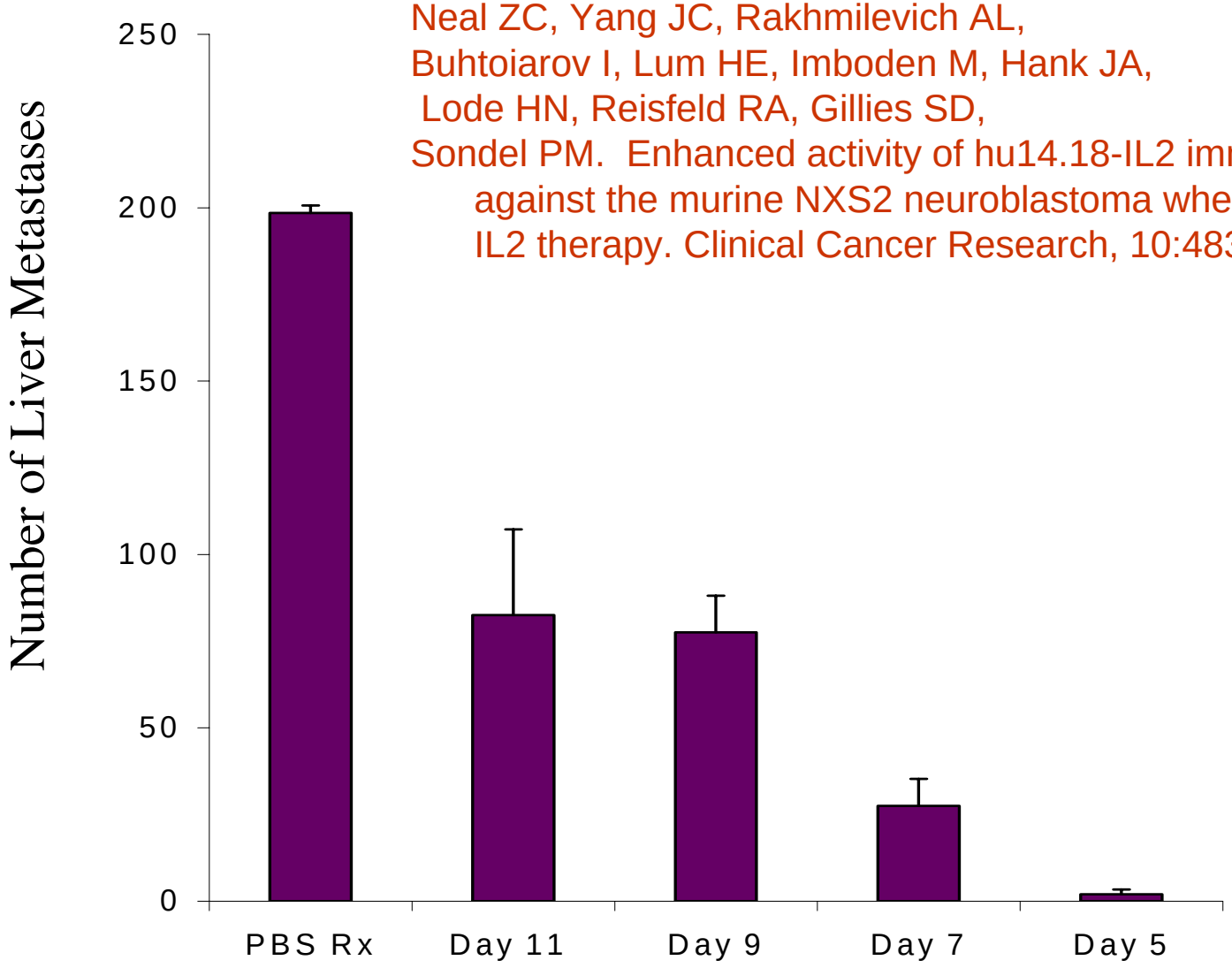
- **Dose, Schedule and MTD with acceptable toxicity found in pts. with NBL and MEL.**
- **MTD in children with NBL (heavily pretreated with chemo) is higher than MTD in adults, as expected. Similar PK.**
- **Hu14.18-IL2 induces immune activation in vivo (PBL, sIL2R, ADCC).**

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 Minimal Tumor Status



Neal ZC, Yang JC, Rakhmilevich AL, Buhtoiarov I, Lum HE, Imboden M, Hank JA, Lode HN, Reisfeld RA, Gillies SD, Sondel PM. Enhanced activity of hu14.18-IL2 immunocytokine against the murine NXS2 neuroblastoma when combined with IL2 therapy. Clinical Cancer Research, 10:4839-4847, 2004

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.

ANBL0322: A Phase II Trial of the HU14.18-IL2 Fusion Protein in Children with Refractory Neuroblastoma

Paul Sondel Chair

Suzy Shusterman Vice Chair

ANBL0322 Patient Accrual Goals

- Stratum 1 (n=20): residual/refractory NBL measurable by standard radiographic criteria
- *Stratum 2 (n=20): residual/refractory NBL not measurable by standard radiographic criteria, but evaluable by MIBG scanning or by bone marrow histology
- *Stratum 3 (n=20): residual/refractory neuroblastoma in clinical remission but with disease identified by BM ICC (>5 NBL cells per 1,000,000 cells)

*MRD Strata

Clinically Approved MoAb for Cancer (2007)

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
Cetuximab	Erbitux	EGFR	GI Malignancies

Potential role for mAbs in standard therapy – clinical goal

- Include a mAb-containing regimen (possibly combined with other therapy) in the standard care for patients with high-risk cancers (i.e. likely to relapse)
- Goal – to prevent recurrence

Antitumor applications of mAb

- Some are already working! (ie: FDA approved components of standard therapy)
- Continued development and efficacy appear likely.

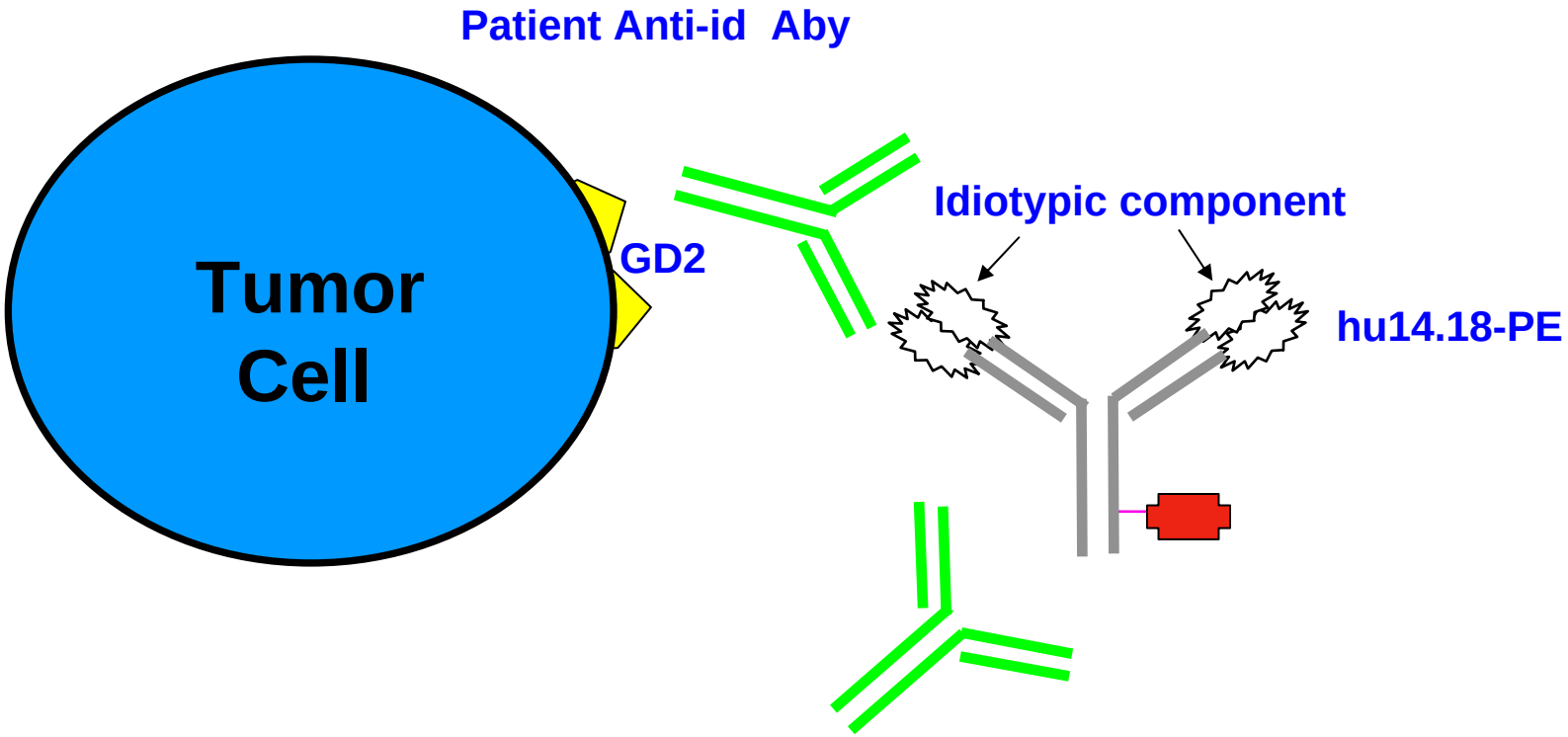
Important issues not covered

1. The IMMUNOGENICITY of mAbs in patients

- Does the patient's immune response (HAMA, HACA, anti-id) HELP or HINDER the anti-tumor effect?

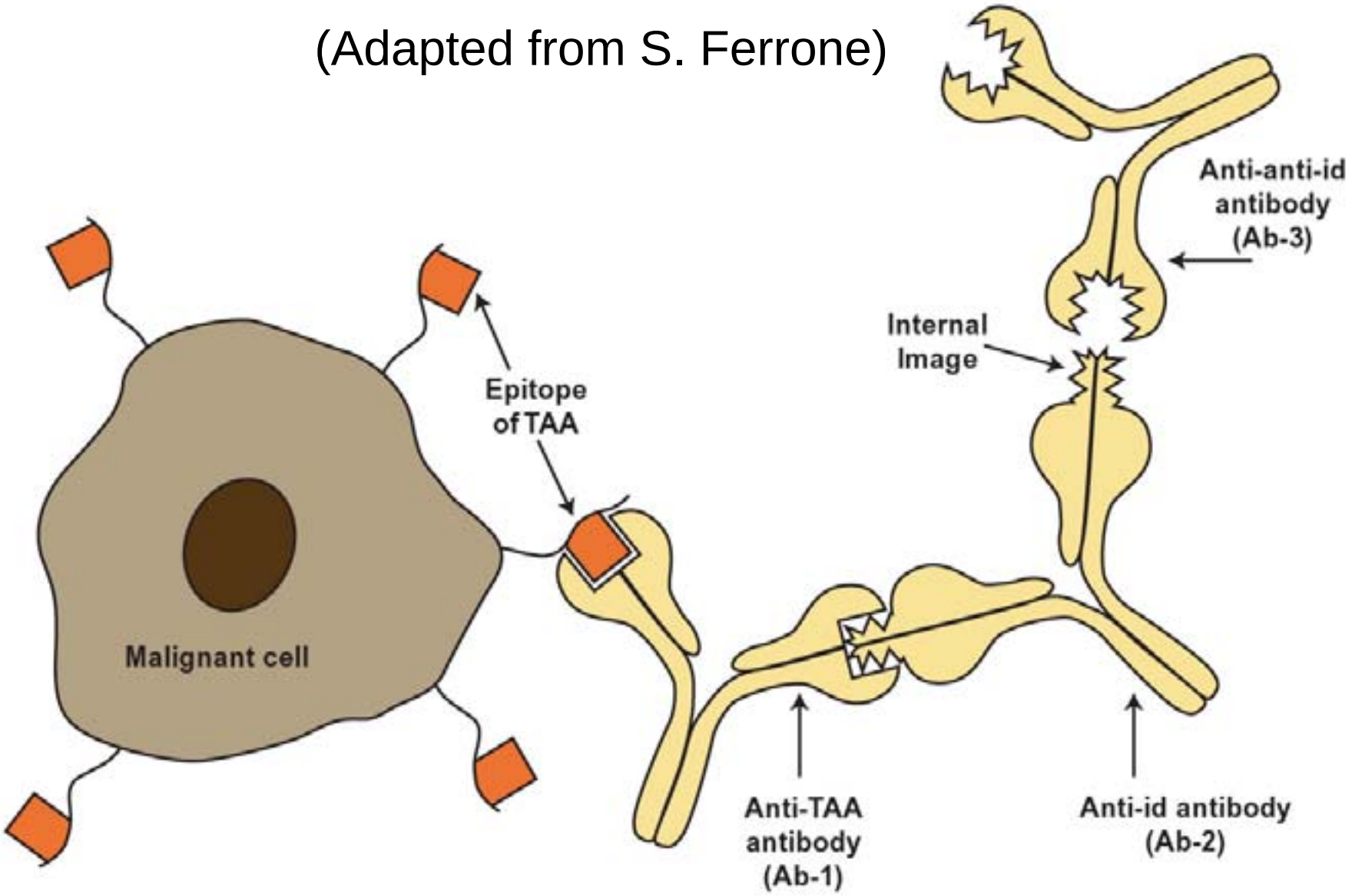
Patient Anti-id Antibody Inhibits Binding of hu14.18 to Tumor cells

(Flow Cytometry Assay)



Mimicry of a TAA determinant by anti-id antibodies

(Adapted from S. Ferrone)

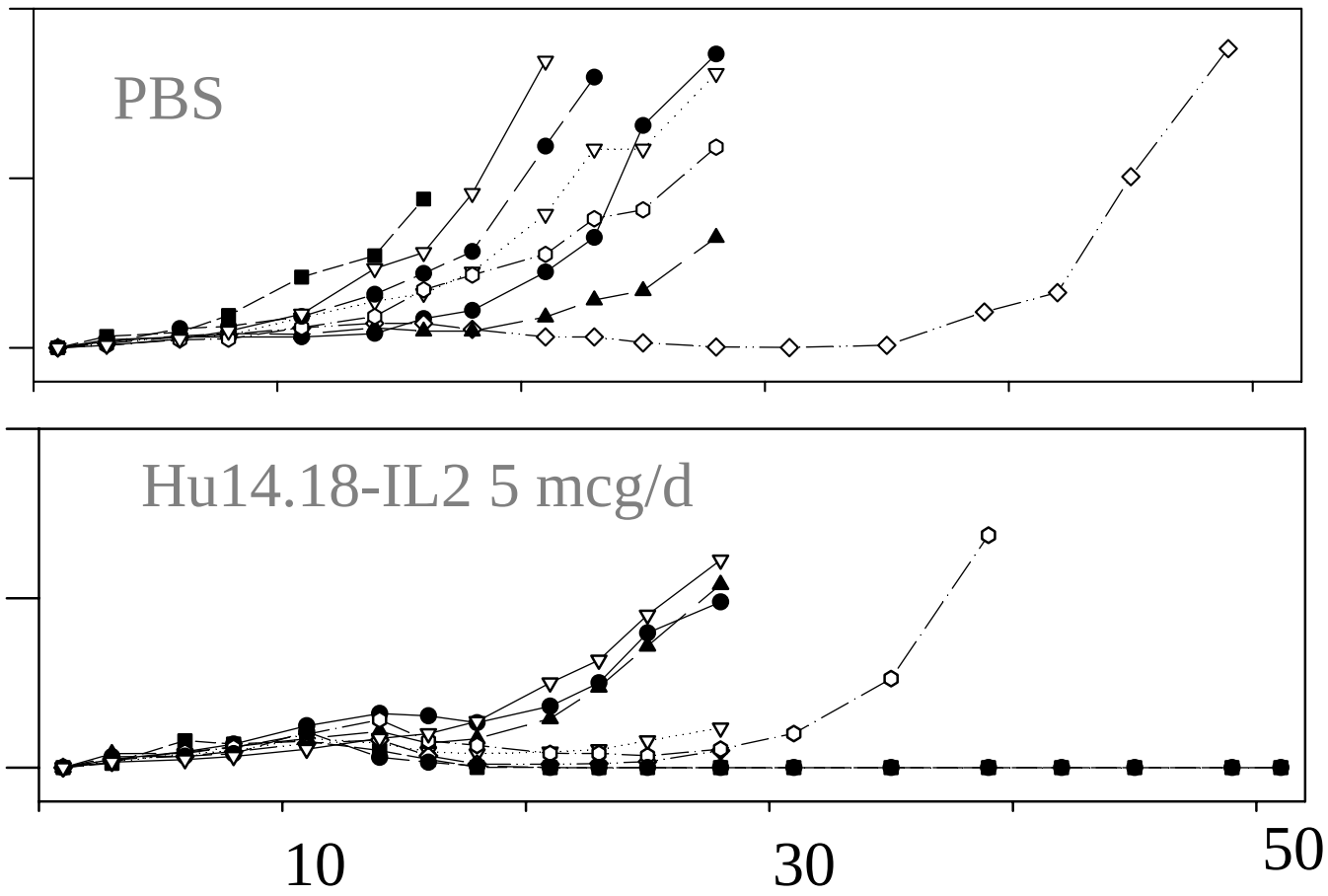


Important issues not covered

2. The ability of tumors to ESCAPE from immunotherapy (ie: antigen loss or MHC modulation)

- Equivalent to selecting cancer cells with multi-drug resistance

Suboptimal hu14.18-IL2 treatment causes transient antitumor effects in mice with palpable neuroblastomas

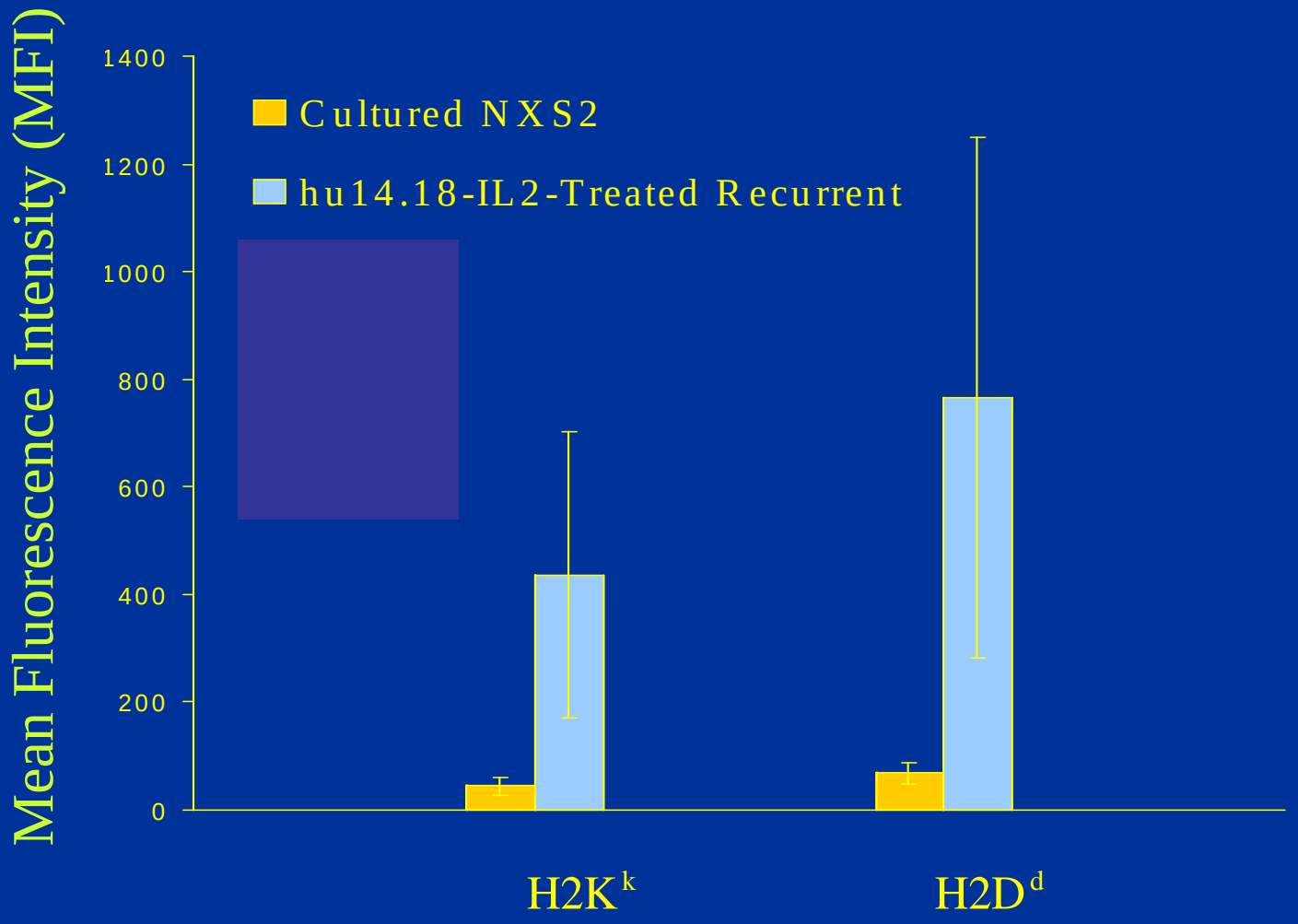


NXS2 Free Mice on d 50

0/8

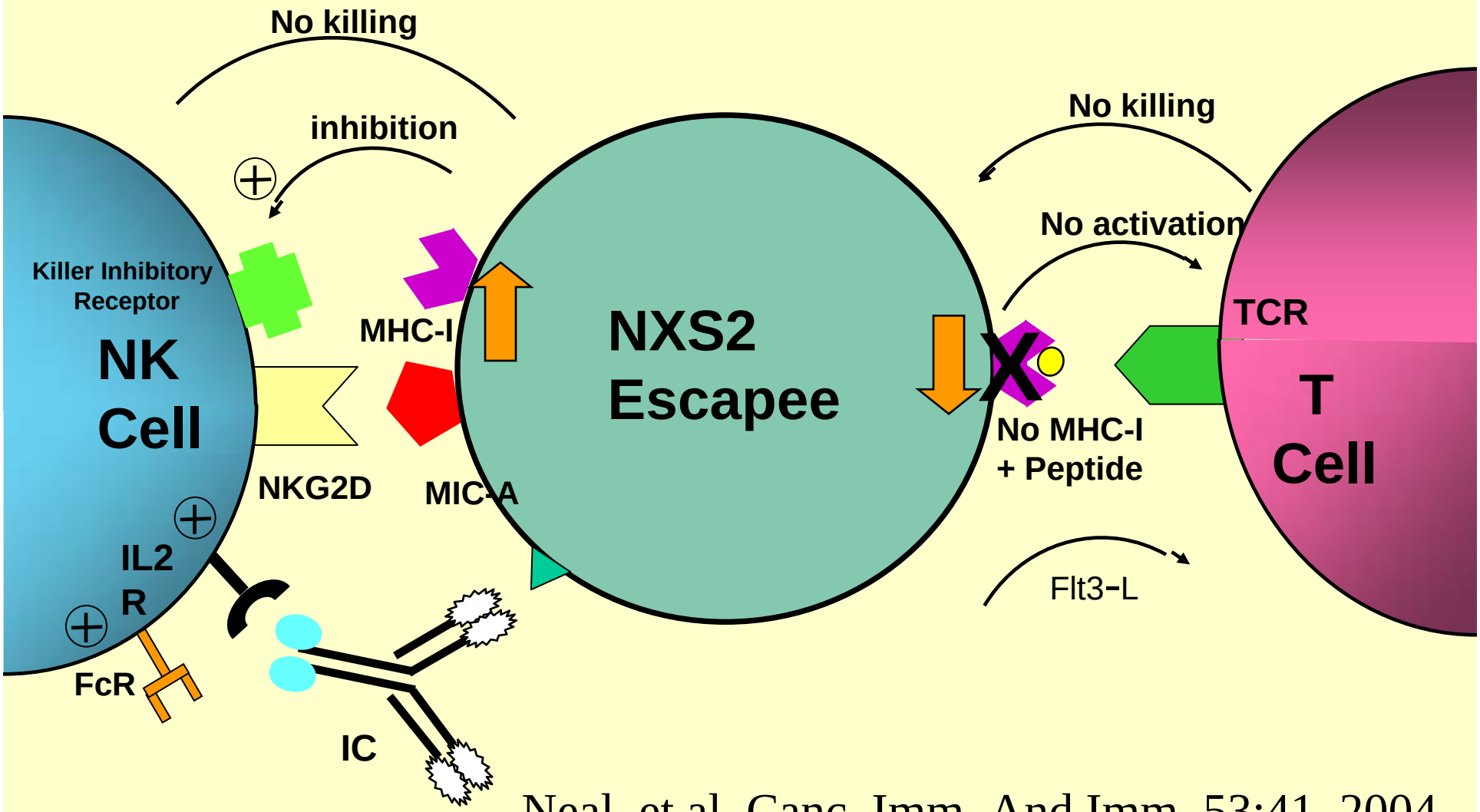
2/8

Neal et al. NXS2 NBL express increased MHC -I upon recurrence from NK dependent immunotherapy. Cancer Imm. and Imm. 53:41-52, 2004



Results: a > 9-fold increase in H2Kk expression and a > 10-fold increase in H2Dd expression

NXS2 Tumors modulate class I, down OR up, to escape T or NK mediated Immunotherapy



Neal et al. Canc. Imm. And Imm. 53:41, 2004

Collaborators in UWCCC Immunocytokine Research-2007

- UWCCC
  - J Hank
  - M Albertini
  - J Gan
  - A Rakhmilevich
  - I Buhtoiarov
  - H Lum
  - H Schalch
  - D Mahvi
  - KM Kim
  - J Eickhoff
  - A Sternberg
  - S Dean
  - R Cassaday
- C.O.G and N.A.N.T.
  - Many Pediatric Oncologists
- Lexigen-EMD
  - S Gillies and colleagues
- EMD-Merck
  - B Clements
  - Karl Joseph Kallen
  - Many others
- Scripps
  - R Reisfeld
- NCI-BRB
  - Toby Hecht and colleagues

UW-Pediatric Oncology Patient Reunion-Nov. 2003

PROOF THAT CANCER RESEARCH MAKES A DIFFERENCE!

Important issues not covered

3. The potential for combining mAb therapy with other immunotherapies to prevent ESCAPE from immunotherapy

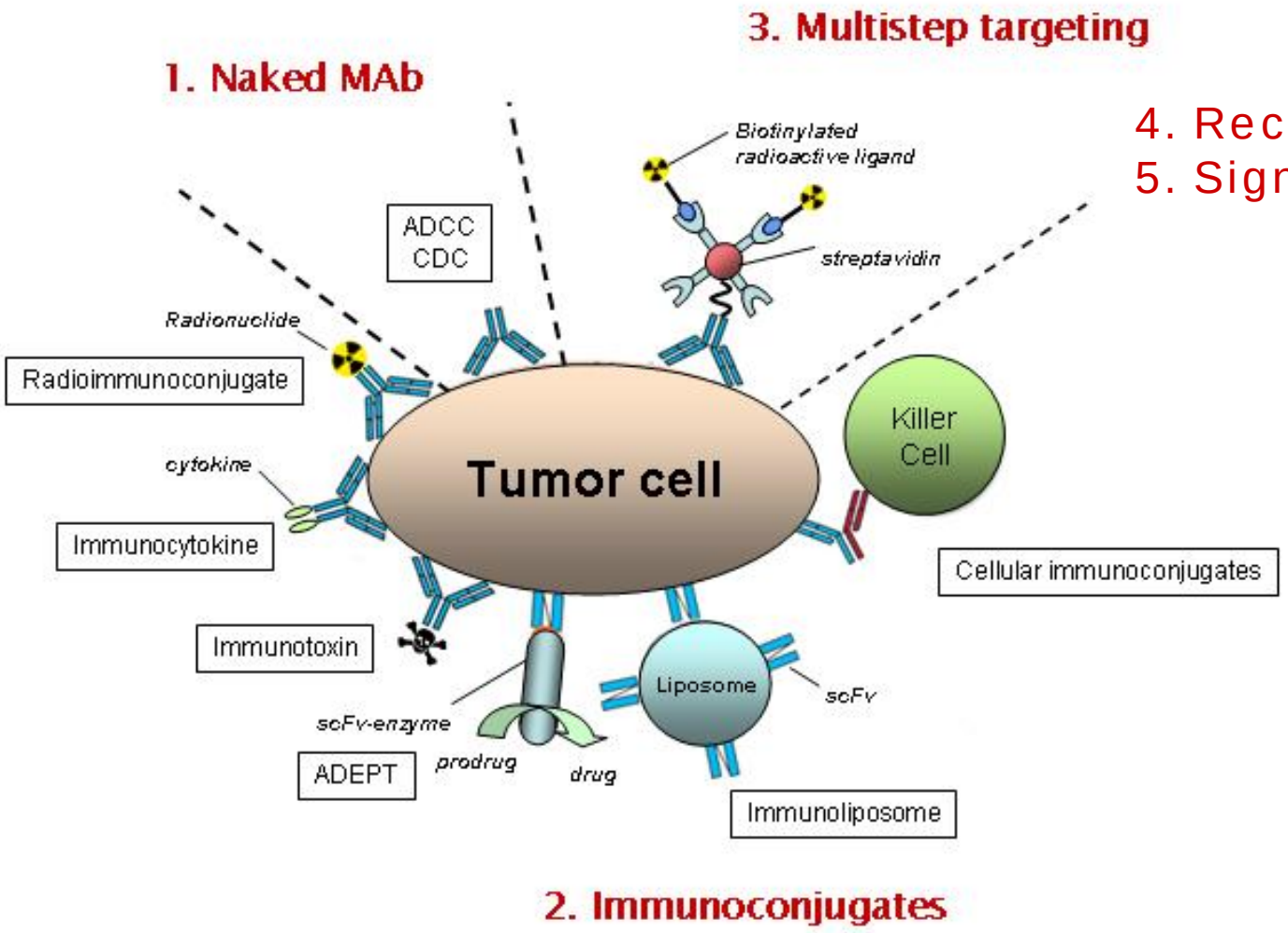
- Combine mAbs, or use mAbs together with cytokines or vaccines

Important issues not covered

4. The potential for combining mAb therapy with other treatments (ChemoRx, RadioRx)

- Timing may be key. Should mAb be given during chemo cycle or after immune recovery?

Anti-tumor applications of mAb



4. Receptor blockade
5. Signal transduction

Adapted from Cheung and Sondel 2005