University of Wisconsin
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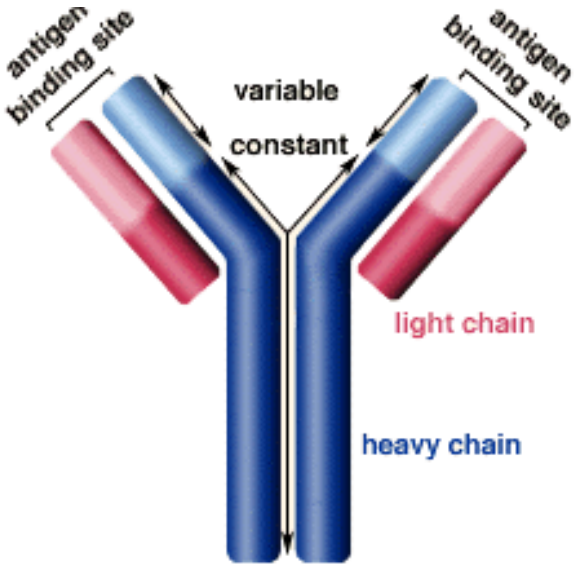
Children's Hospital

Clinical Antibody Dependent Cell-Mediated Cytotoxicity (ADCC): Targeting Neuroblastoma (and Lymphoma) with Monoclonal Antibodies

SITC



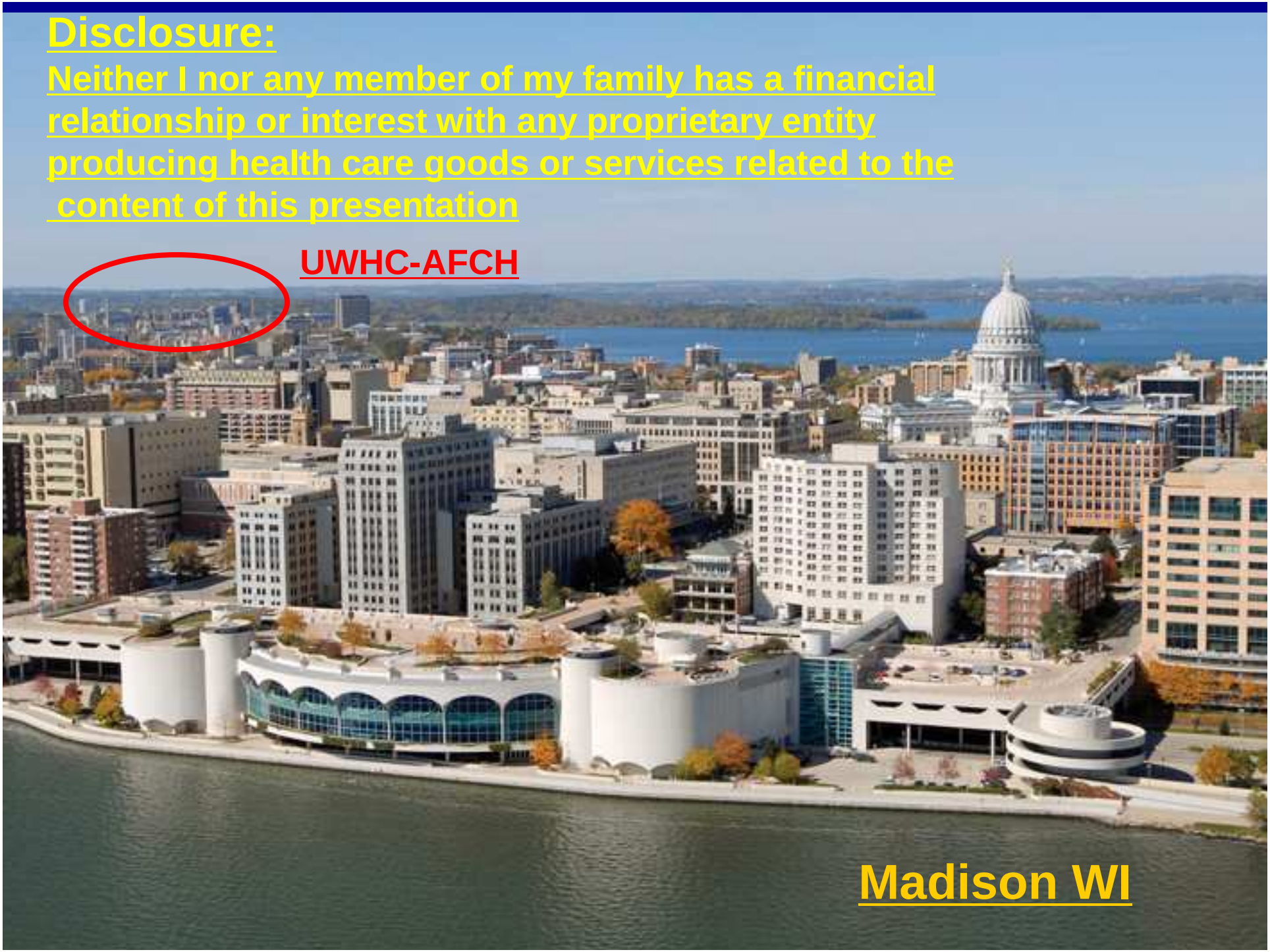
November 8, 2013

National Harbor MD

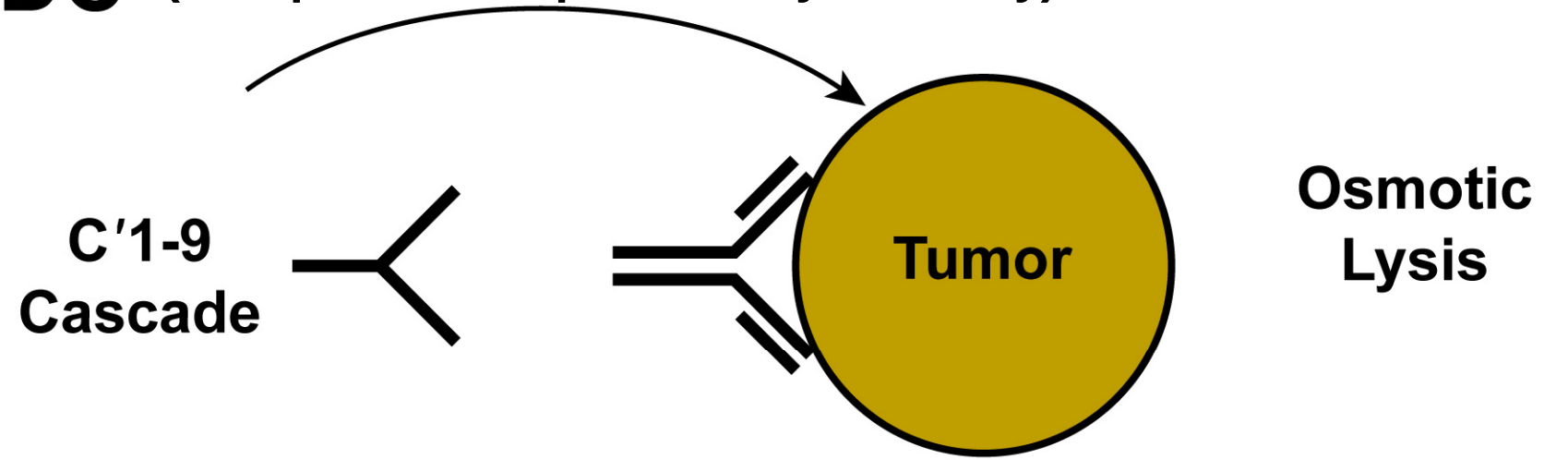
Paul M. Sondel MD PhD
UW-Madison

Disclosure:

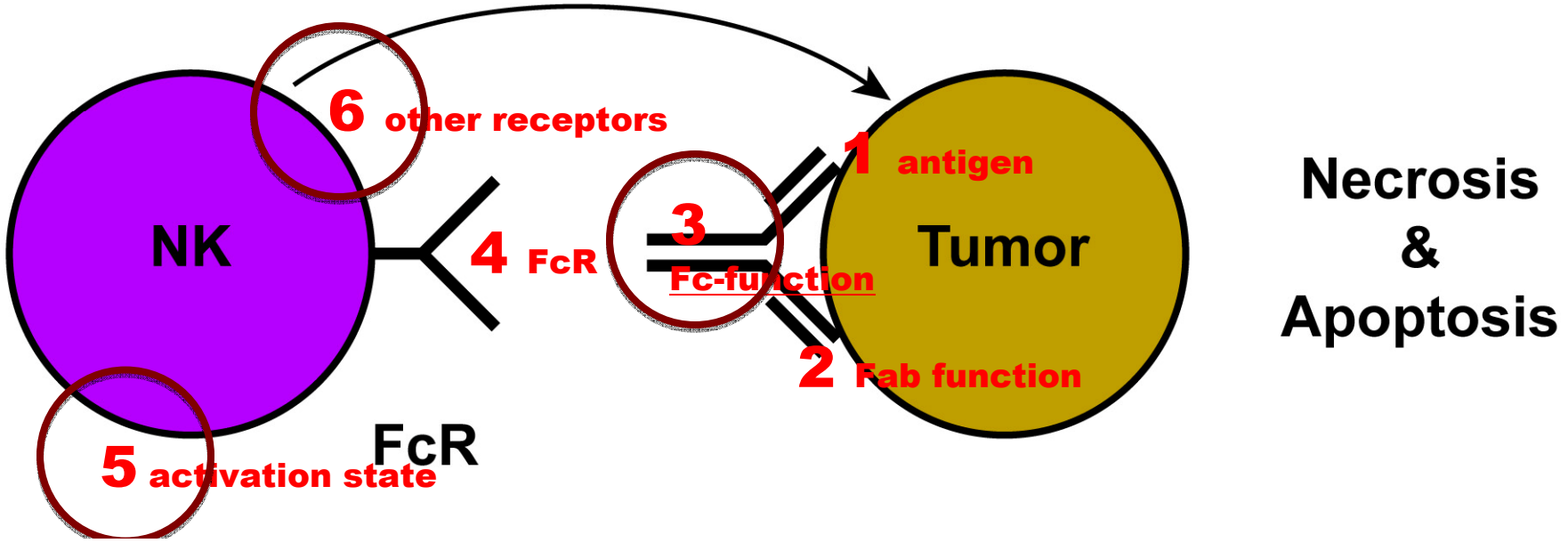
Neither I nor any member of my family has a financial relationship or interest with any proprietary entity producing health care goods or services related to the content of this presentation



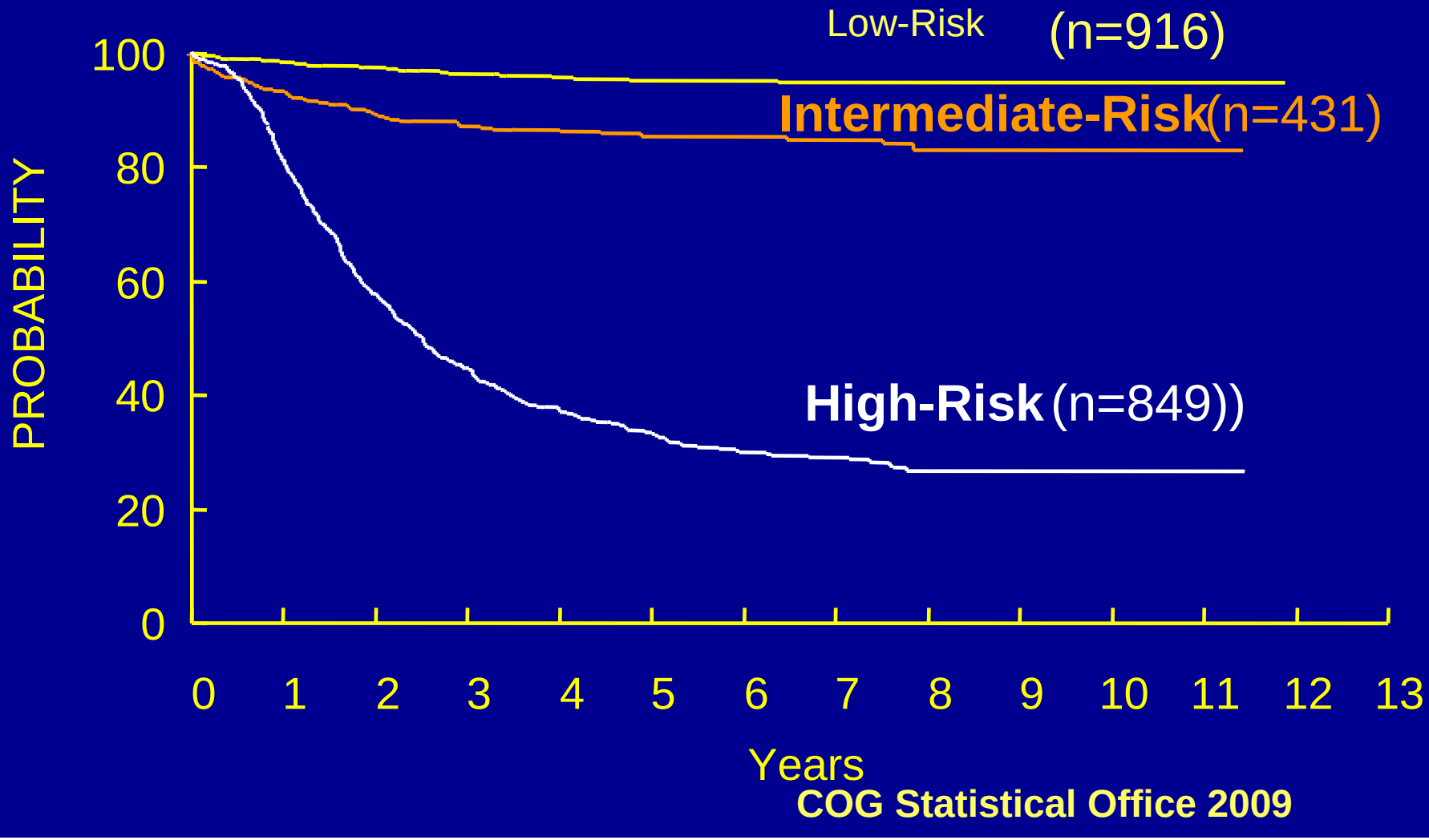
CDC (Complement Dependent Cytotoxicity)



ADCC (Antibody Dependent Cell-mediated Cytotoxicity)



Neuroblastoma, a major challenge: Survival According to Risk Group



Mujoo K, Spiro RC, Reisfeld RA. J Biol Chem. 1986

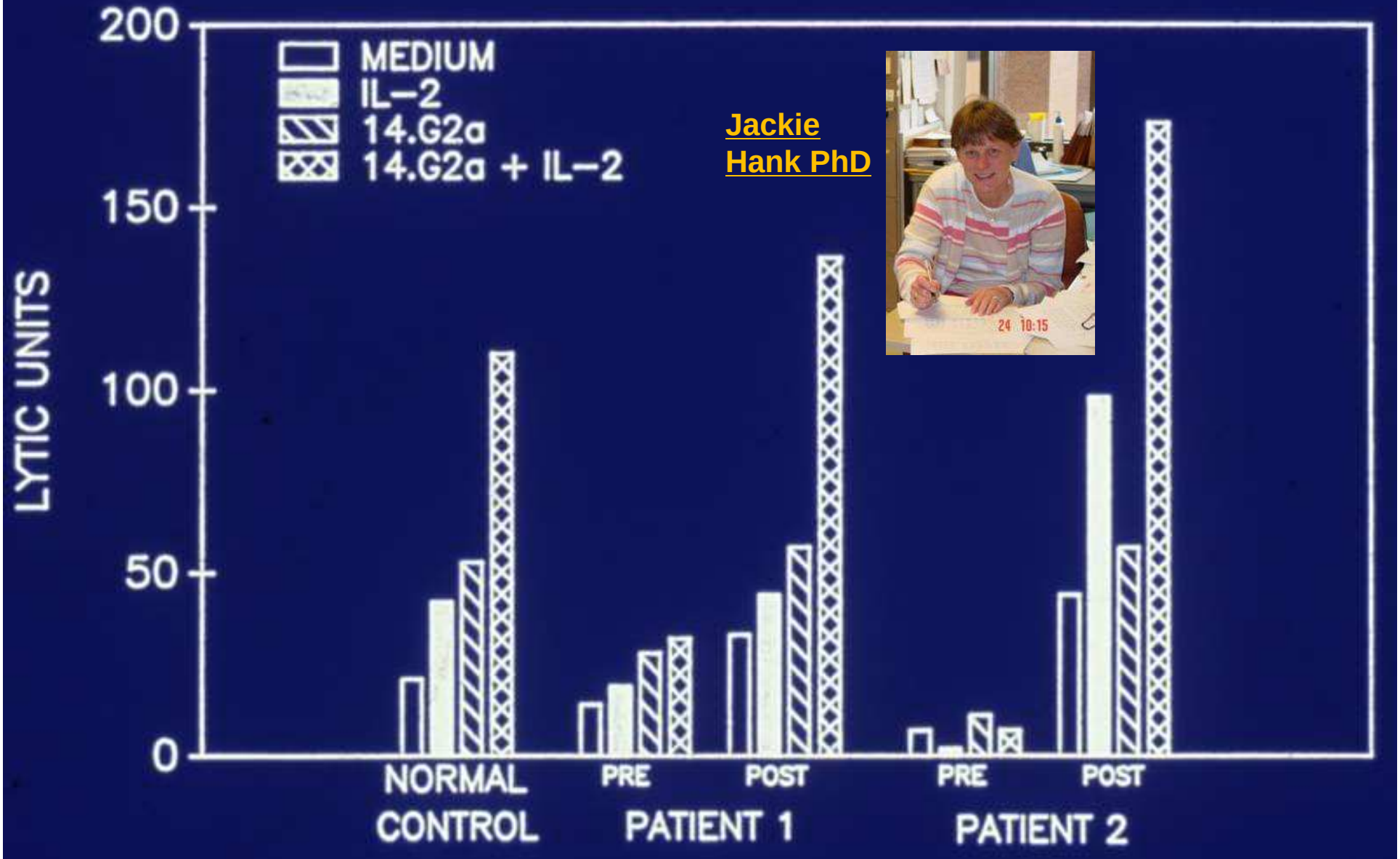
Comparable GD2 target To 3F8 mAb developed By N-K Cheung and Collaborators at MSKCC

Tissue Reactivity of mAb 14.18

Tumor Tissues	Reactivity*
Neuroblastoma	+++
Melanoma	+++
Glioblastoma	+++
Small Cell Lung Carcinoma	++
Osteosarcoma	++
Ewing's Sarcoma	±
Lung Adenocarcinoma	-
Stomach Carcinoma	-
Squamous Lung Carcinoma	-
Breast Carcinoma	-
Colon Carcinoma	-
Normal Tissues	
Lung	-
Colon	-
Liver	-
Kidney	-
Spleen	-
Pancreas	-
Thyroid	-
Cerebellum	++

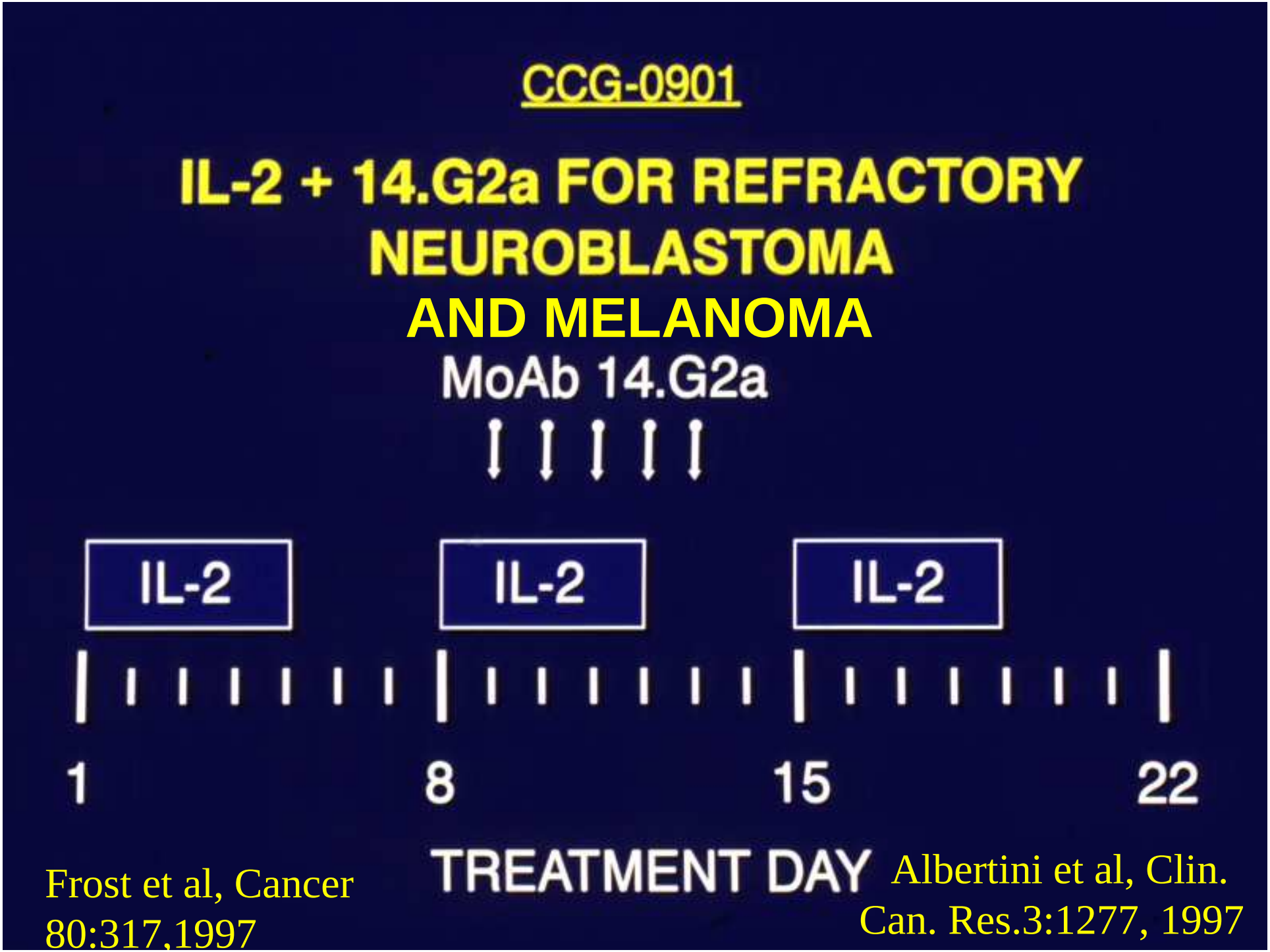
* +++, strongly positive; ++, positive; +, weak/positive; -, negative.

IL2 Facilitated ADCC of LAN5 Neuroblastoma (NBL)



Jackie
Hank PhD

Hank et al , Cancer Res. 50:5234, 1990



Frost et al, Cancer 80:317,1997

Albertini et al, Clin. Can. Res.3:1277, 1997

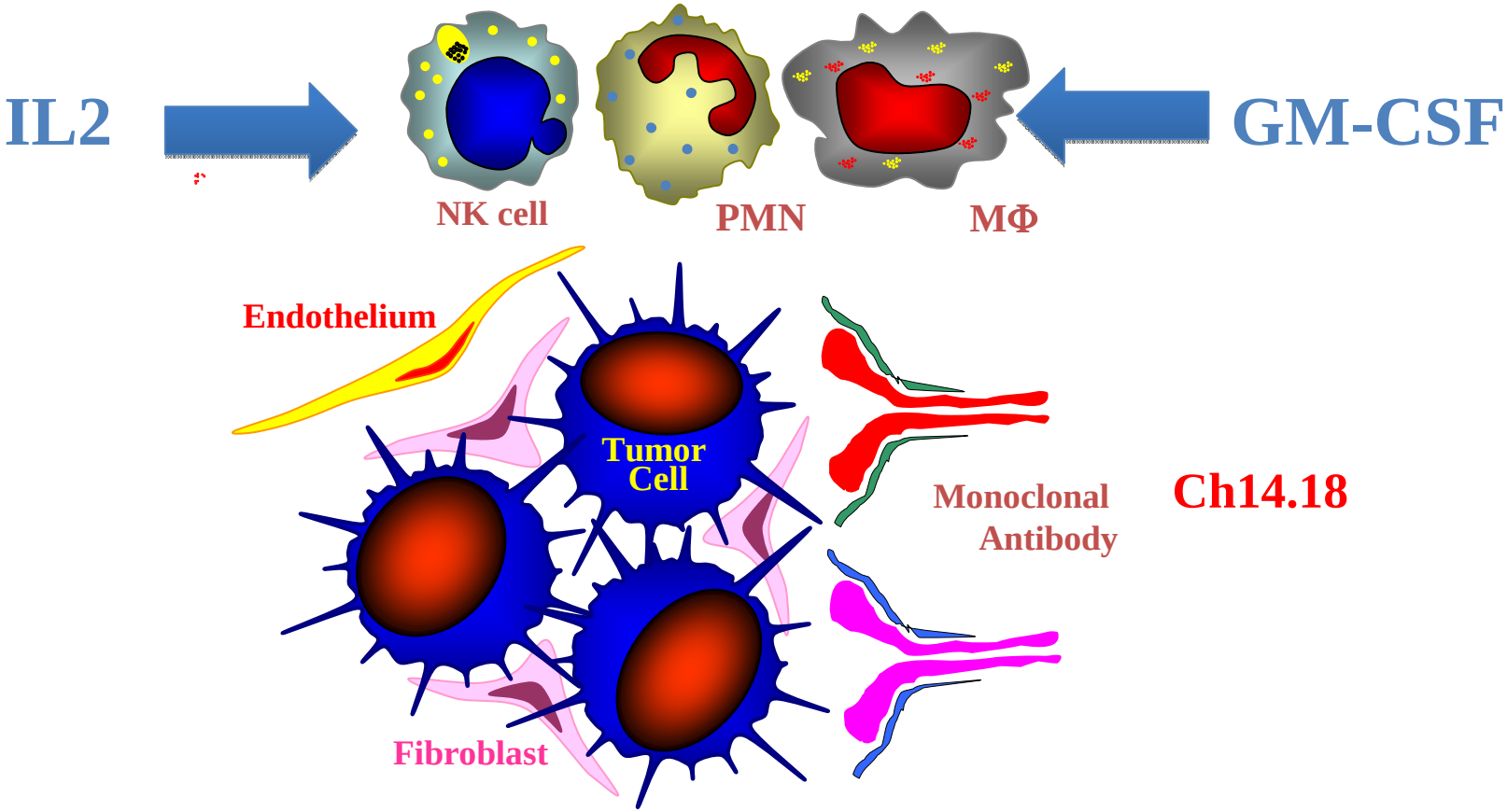
Published 14.G2a* and ch14.18* phase I studies: PK, Tox., MTD, Biologic effects, but little **measurable** antitumor effect

- Melanoma -UWCCC
  M.Albertini Chair

  - 14.G2a + IL2
  - Ch14.18 + IL2
  - Influence of IL2 on HACA
  - ch14.18 + R24 +IL2

- Neuroblastoma-COG
  - 14.G2a + IL2
  - Ch14.18 + GM-CSF after ASCT
  - Ch14.18 + GM-CSF + IL2 after ASCT
  - *14.G2a and ch14.18 available via NCI: groundwork by Drs. Reisfeld, Yu and others

COG's approach to Innate Immunity and ADCC for NBL (ANBL0032)



1. Activate Multiple Pathways of ADCC (ie: stimulate and engage several different populations of ADCC effector Cells: GM-CSF A. Yu, NK Cheung)

2. Administer Immunotherapy in Minimal Residual Disease

[ie: patients in remission, at risk of relapse, *to circumvent poor penetration, Tregs, myeloid derived supressor cells (MDSCs)]*

CCG-

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 J. Clin. Oncol. 18:4077, 2000
  - and Gilman et al, J. Clin. Oncol. 27:85-91, 2009)

Overall survival ~75% at 2 years

Schema: C.O.G. NBL Study ANBL0032

(2003) - A. Yu Chair

Accrual of 386 randomized patients needed

High Risk Newly Diagnosed NBL

Induction ChemoRx

Study stopped at 61% Accrual Feb. 2009

Ablation + Stem Cell Rescue (Surgery and XRT)

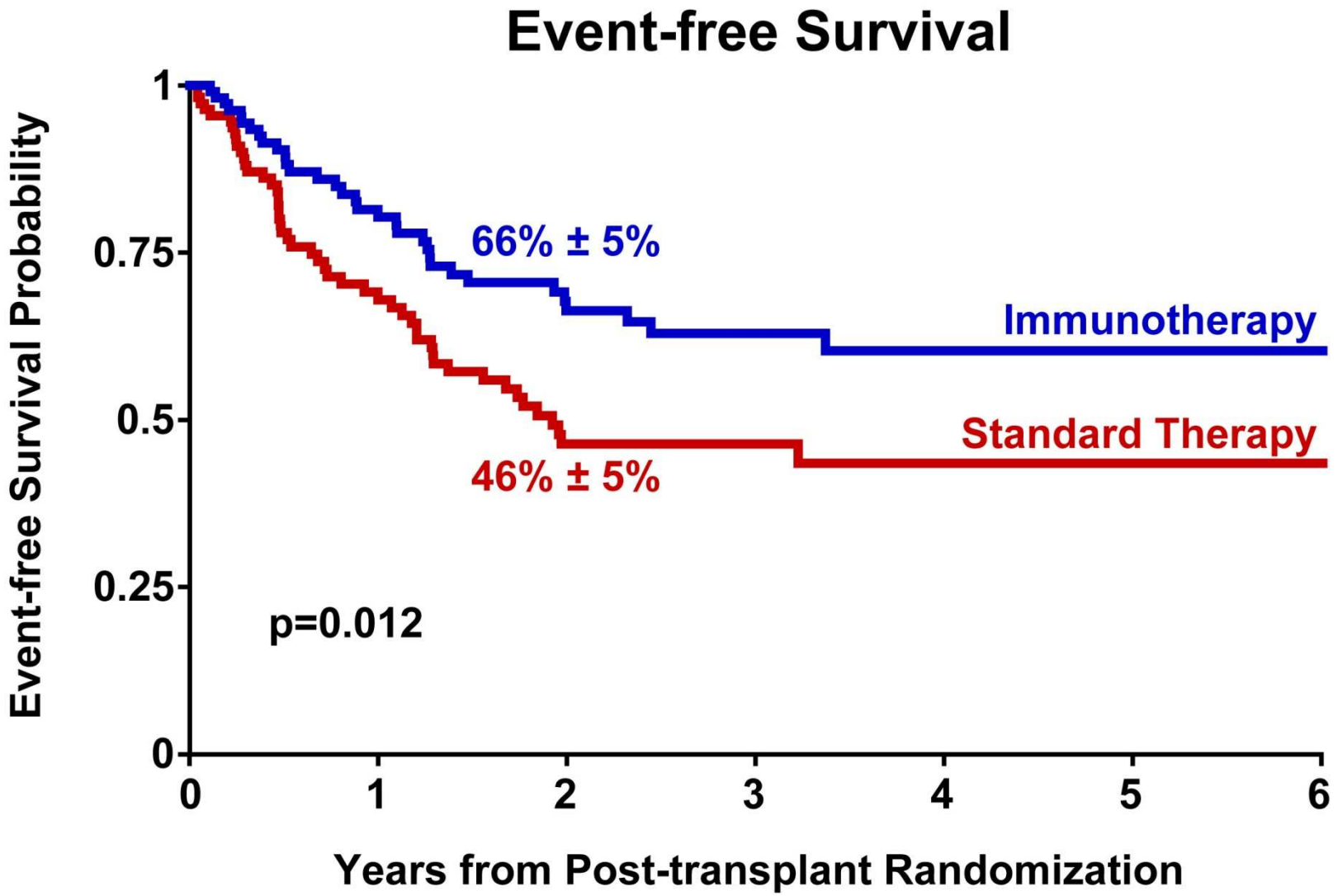
Randomize

Observe

ch14.18 + GM-CSF + IL2

Cis-Retinoic Acid (CRA)

Event free survival for 226 children randomized to ImmRx vs CRA



Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman S, Chen H, Smith M, Anderson B, Villablanca J, Matthay KK, Shimada H, Grupp SA, Seeger R, Reynolds CP, Buxton A, Reisfeld RA, Gillies SD, Cohn SL, Maris JM, Sondel PM. New Eng. J. Med. 335: 1324, 9/30/10

Implications of this result for neuroblastoma clinicians:

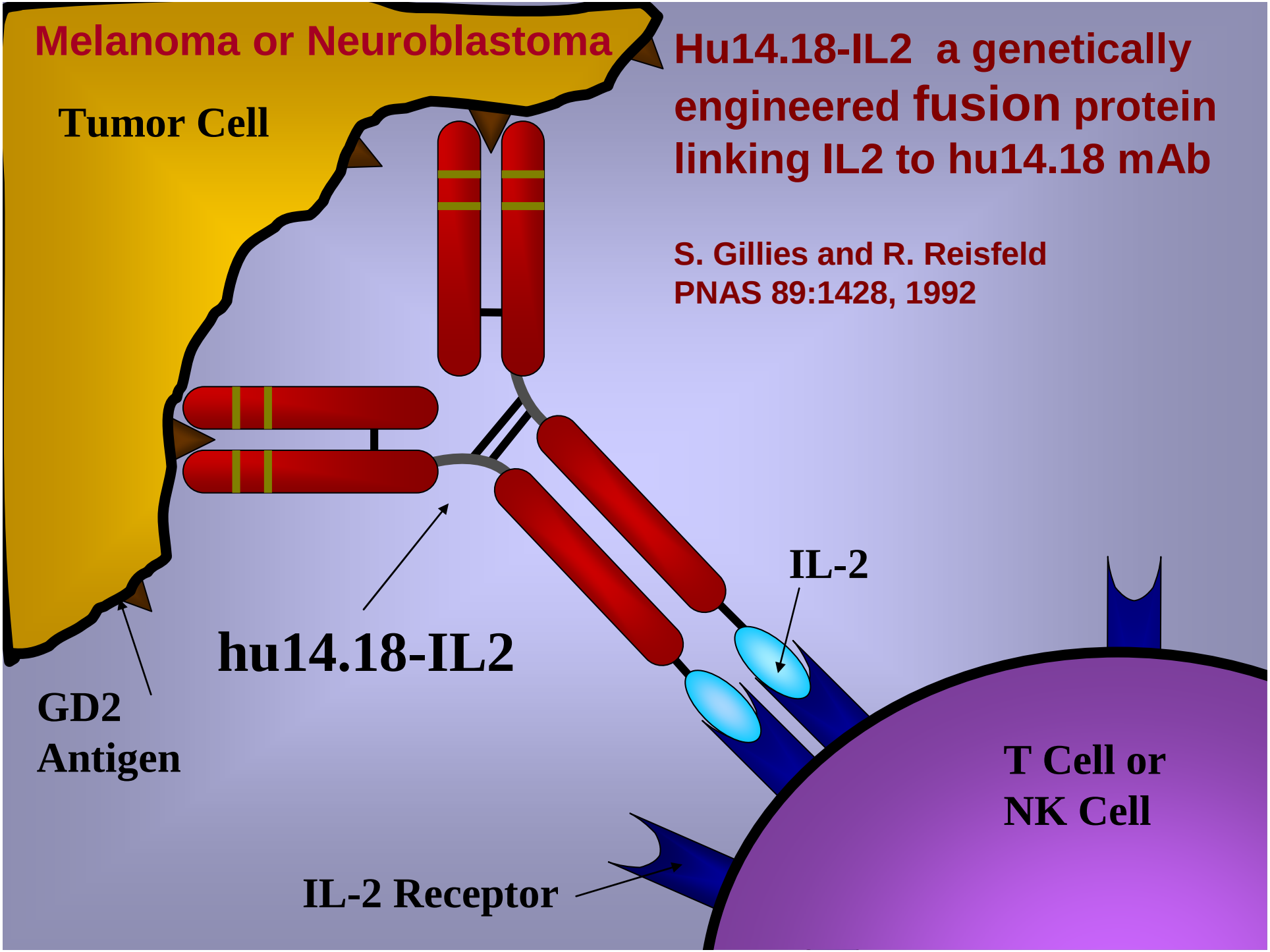
Simon et al (J.C.O 22:3549, 2004) 334 pts treated after consolidation, 166 got ch14.18 (no cytokines). Multivariate analyses showed **no benefit in OS or EFS***

"Because of these results, the MAB ch14.18 treatment is not continued in the current German NBL trial".

Why did the COG trial show the ch14.18 + cytokine regimen provides clear benefit for OS and EFS?

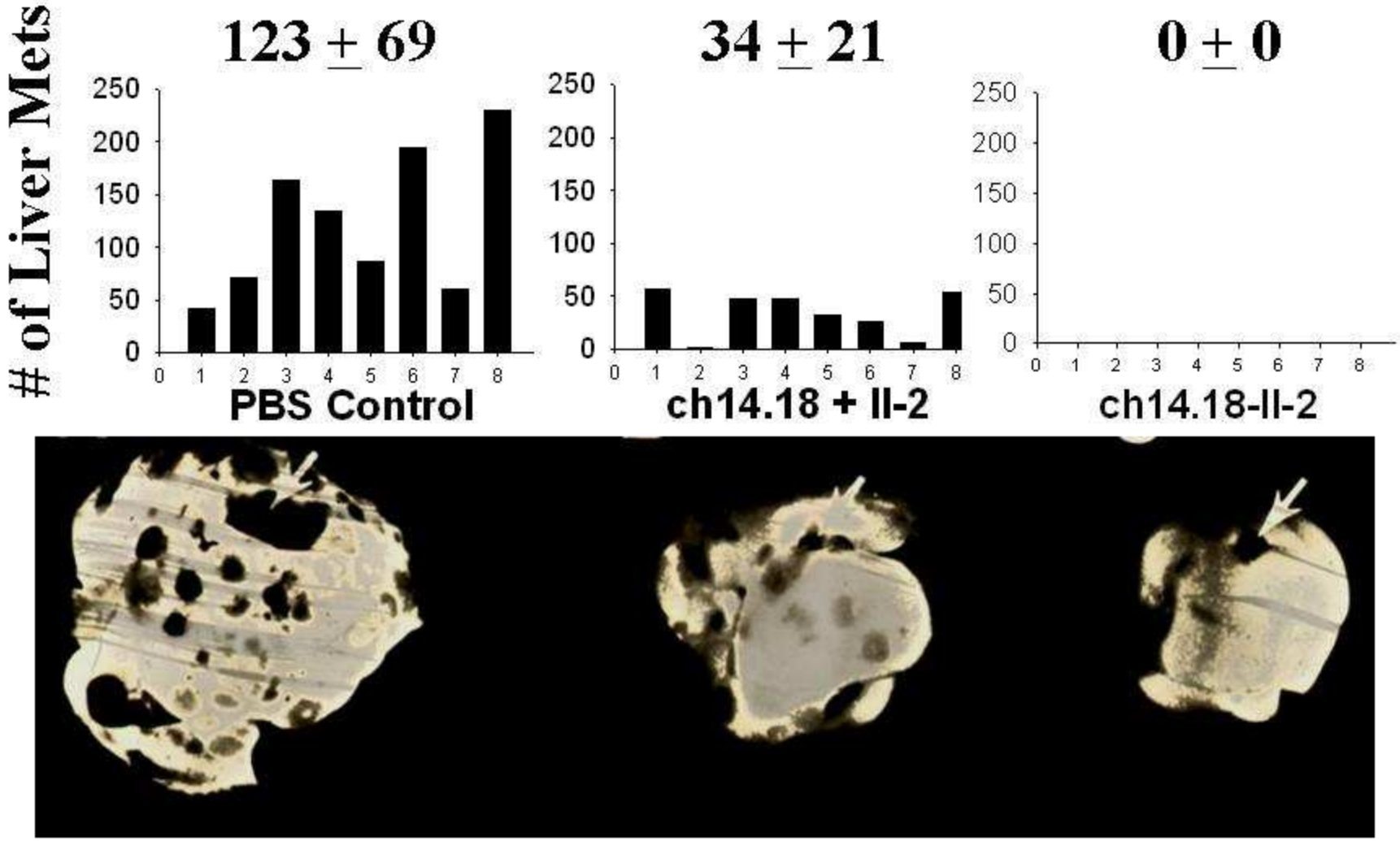
Might it be the addition of the IL2 + GM-CSF?

*2011 follow up shows OS benefit


Melanoma or Neuroblastoma
Tumor Cell
Hu14.18-IL2 a genetically engineered fusion protein linking IL2 to hu14.18 mAb
S. Gillies and R. Reisfeld
PNAS 89:1428, 1992
IL-2
hu14.18-IL2
GD2
Antigen
T Cell or
NK Cell
IL-2 Receptor

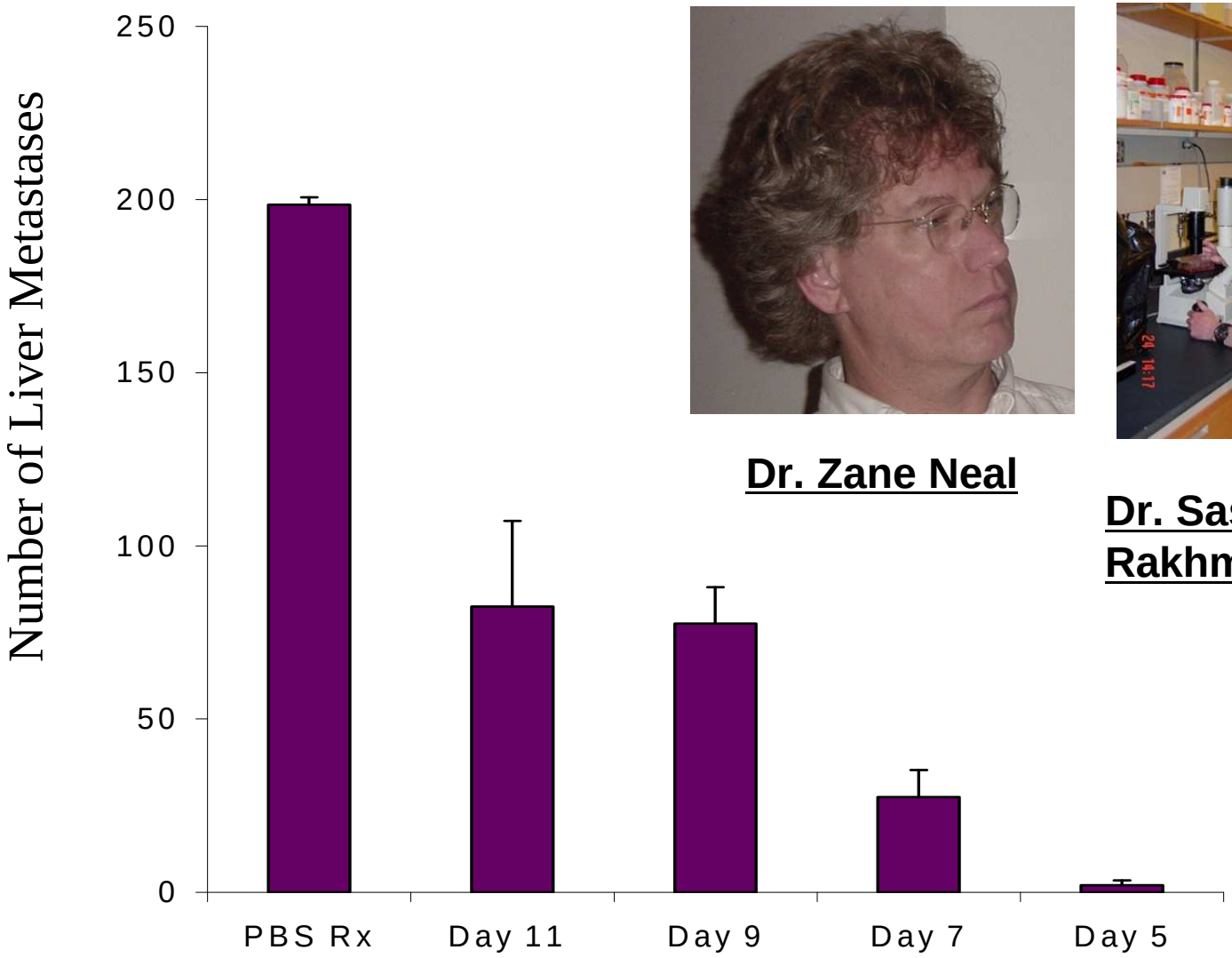
Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

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



Effective anti-GD2 Immunotherapy: Dependence on Minimal Tumor Status

Neal ZC,et al Clinical Cancer Research, 10:4839-4847, 2004



Dr. Zane Neal

Dr. Sasha Rakhmilevich

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.

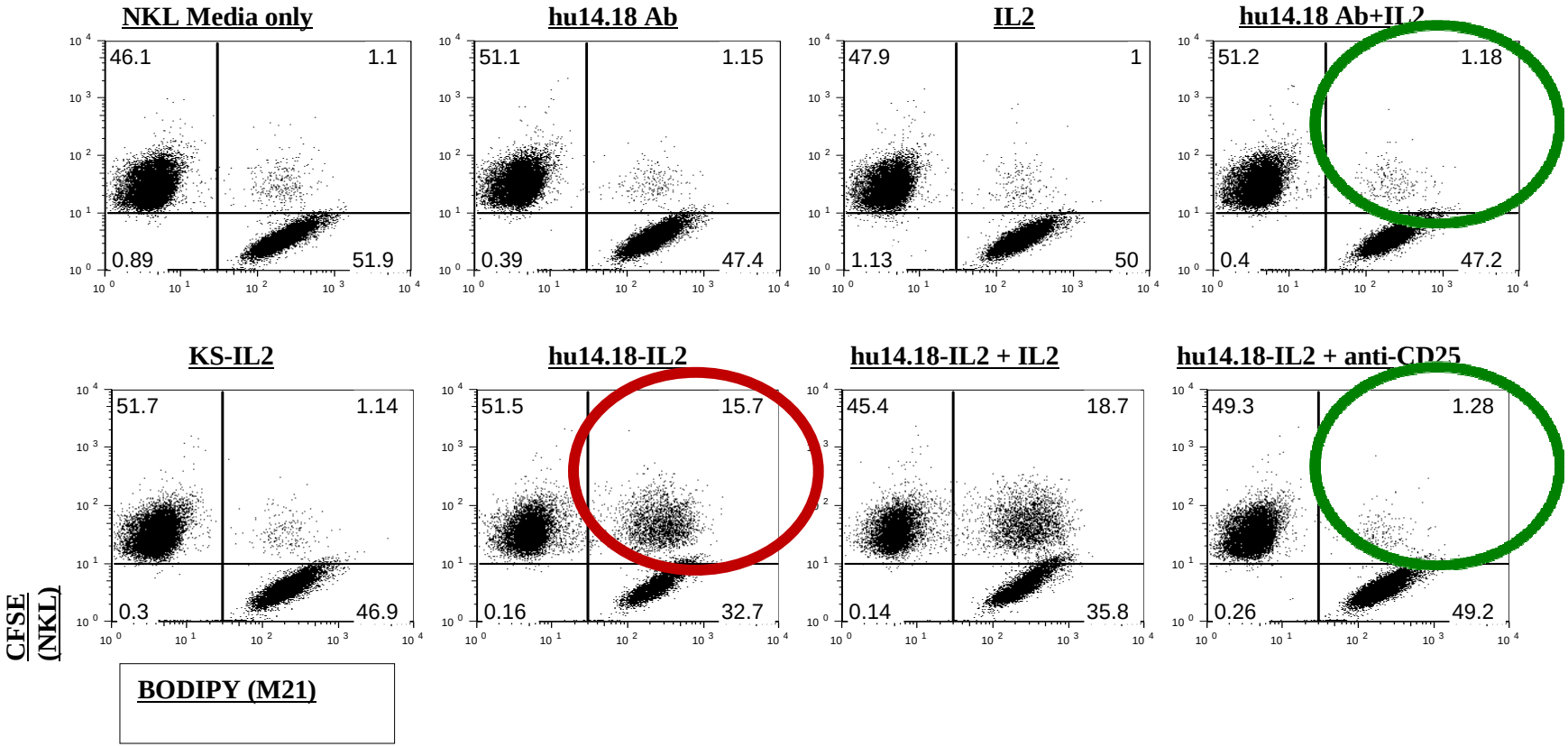
Preclinical Conclusions for hu14.18-IL2

1. NK cells and T cells can be involved in the response
2. Antibody Dependent Cellular Cytotoxicity (ADCC) is involved
3. Efficacy in MRD setting

4. **14.18-IL2 is more effective than 14.18 + IL2**

WHY?

Flow cytometric detection of IC- facilitated conjugates between NKL cells (FcR-negative / IL2R-pos) and M21 (GD2-pos) requires IC and IL2Rs

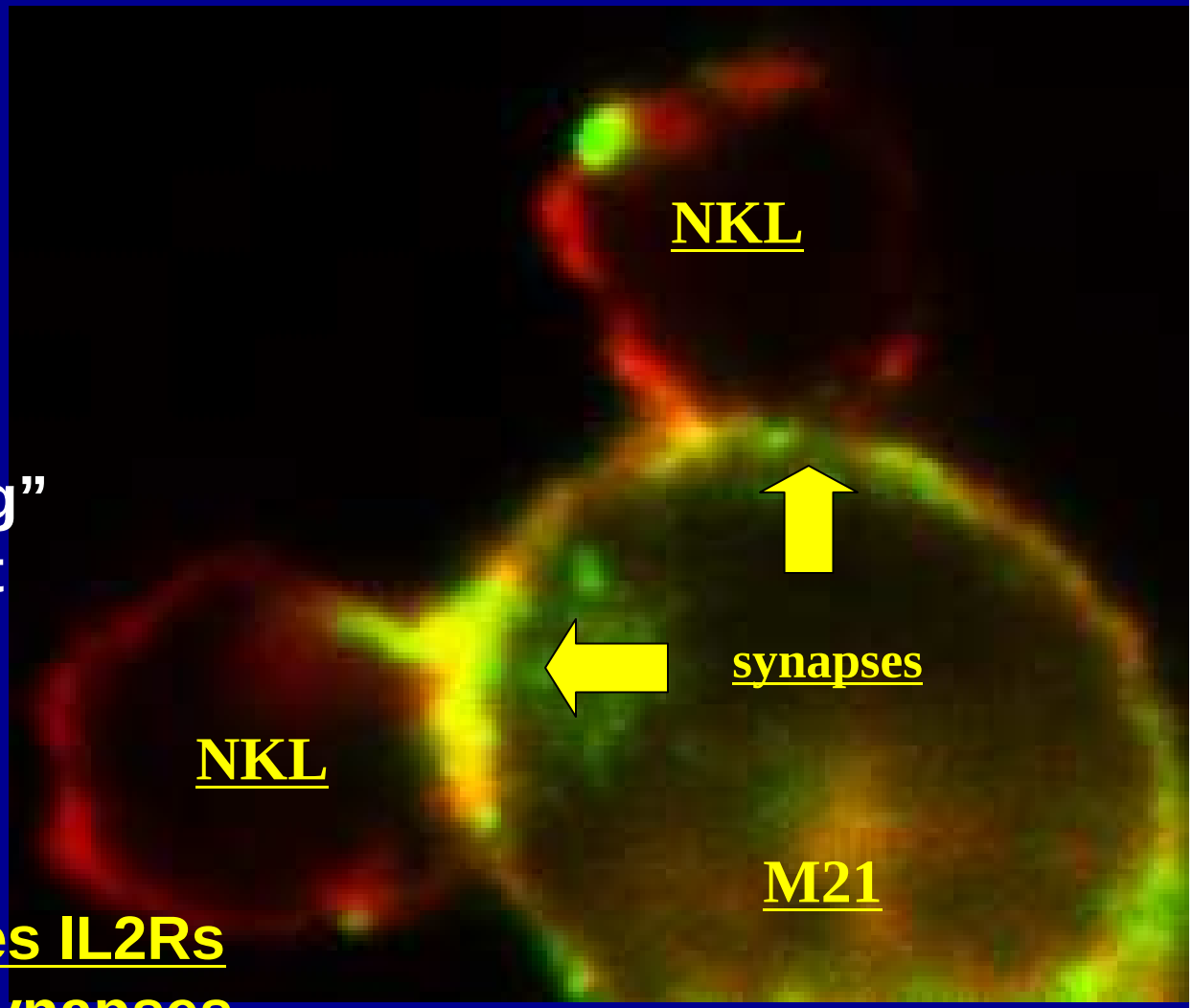


Buhtoiarov IN, Neal ZC, Jan J, Buhtoiarova TN, Hank JA, Yamane B, Rakhmilevich AL, Patankar MS, Gubbels JAA, Reisfeld RA, Gillies SD, Sondel PM. J. Leukocyte Bio. 2011

Hu14.18-IL2 (FITC) localizes at immune synapse of NKL-M21 conjugates

Form conjugates with Hu14.18-IL2-FITC + NKL + M21, and stain with actin.

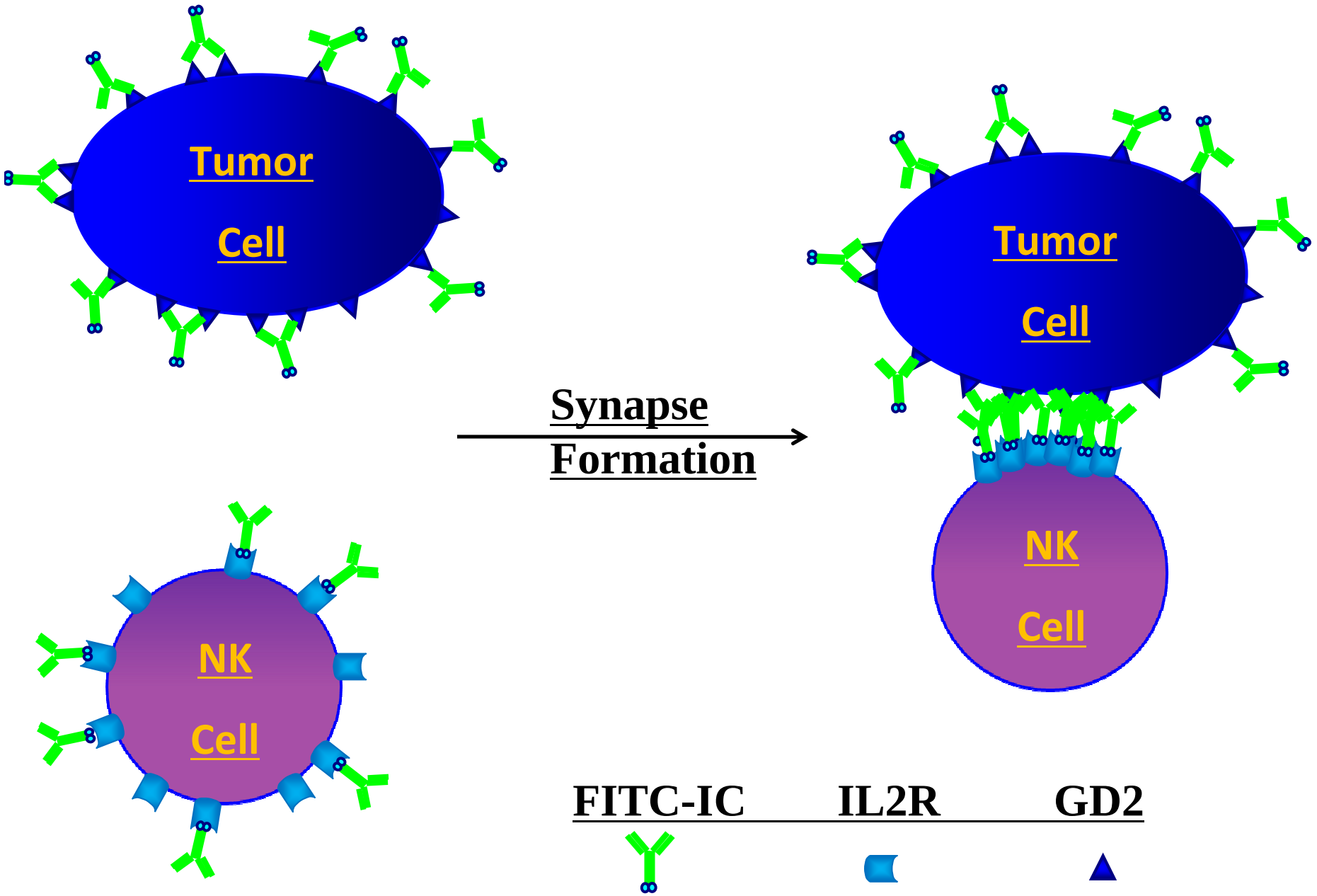
IC gives "ring staining" On M21 (via GD2), but localizes to synapse on NKL (CD25-pos., CD16-neg.)



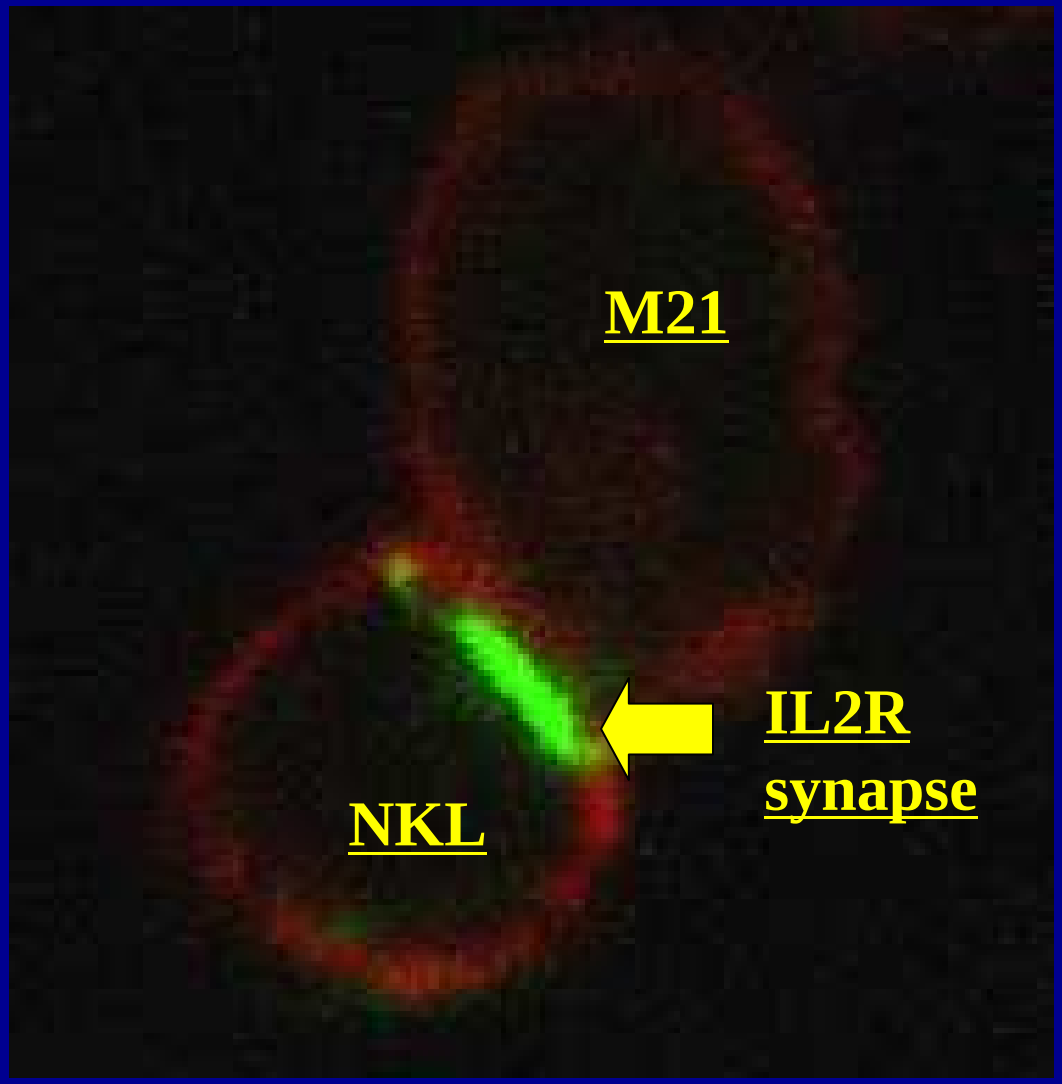
Cell-bound IL2 induces IL2Rs To cause activating synapses.

Gubbels et al: CII , 2011

FITC-IC Distribution



All IL2Rs on NKLs localize to immune synapse induced by hu14.18-IL2



Form conjugates with
NKL + M21 + HU14.18-IL2,
Then stain IL2Rs with
anti-CD25 mAb.

Proves that all IL2Rs
on NKL cells go to synapse

Suggests that hu14.18-IL2
mediates:
Conventional ADCC.
and
IL2R-facilitated ADCC

Gubbels, Buhtoiarov et al: CII, 2011

COG Phase II NBL Trial**- includes minimal residual disease (MRD) Stratum*

- Stratum 1: residual/refractory NBL measurable by standard radiographic criteria

- ***Stratum 2 : residual/refractory NBL not measurable by standard radiographic criteria, but evaluable by MIBG scanning or by bone marrow histology**

Shusterman S, London WB, Gillies SD, et al. Hank JA, Voss S, Seeger RC, Reynolds CP, Kimball J, Albertini MA, Wagner B, Gan J, Eickhoff J, DeSantes KD, Cohn SL, Hecht T, Gadbaw B, Reisfeld RA, Maris JM, Sondel PM. J.Clin. Oncol. 28:4969, 2010

Hu14.18-IL2 as a MRD agent

- **Stratum 1**: 0 of 13 patients respond
- **Stratum 2**: 5 of 24 patients with CR, (+ 2 with clear improvement)

- **5 of 24 responses (stratum 2) > 0 of 13 (stratum 1)**
  (p= 0.07)

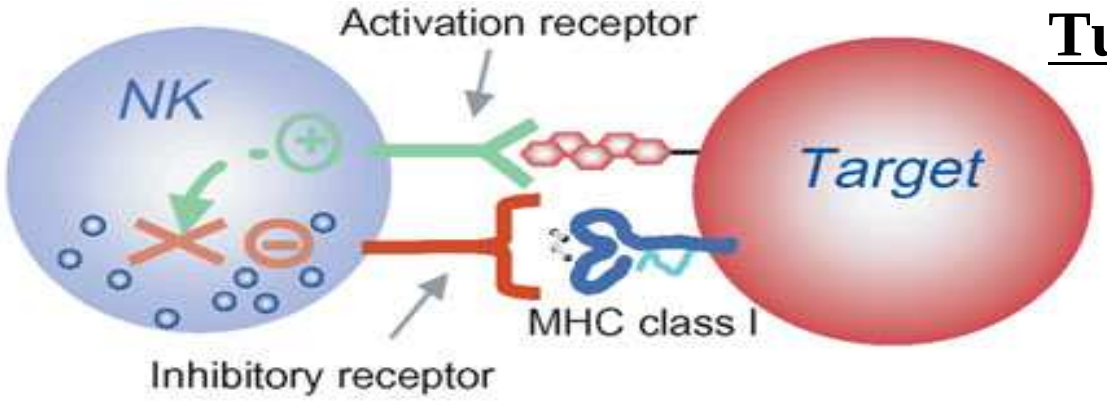
- **7 (improved) of 24 (stratum 2)> 0 of 13 (stratum1)**
  (p= 0.03) as hypothesized by preclinical data

IMPLICATION: Clinical studies confirm biology from preclinical studies **IF** the clinical study simulates the setting of the preclinical trial.

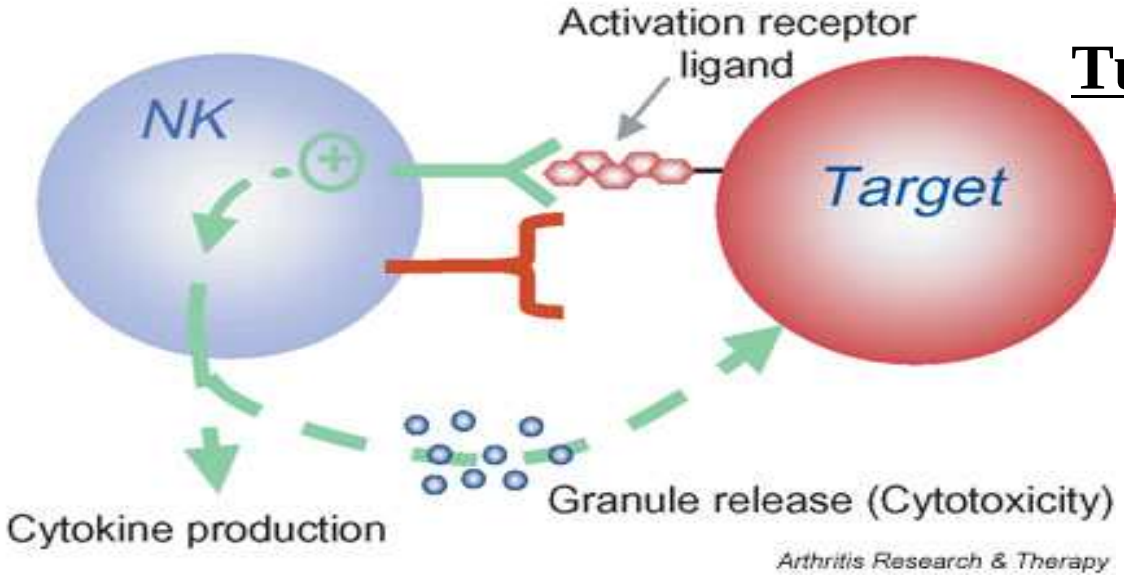
Shusterman S, London WB, Gillies SD, et al. Hank JA, Voss S, Seeger RC, Reynolds CP, Kimball J, Albertini MA, Wagner B, Gan J, Eickhoff J, DeSantes KD, Cohn SL, Hecht T, Gadbaw B, Reisfeld RA, Maris JM, Sondel PM. J.Clin. Oncol. 28:4969, 2010

"Missing Self Hypothesis" & KIR/KIR-L Mismatch*

KIR Match
= NK cell
Inhibition →
Tumor cell survival



KIR Mismatch (b)
(Missing self) →
= Tumor cell death

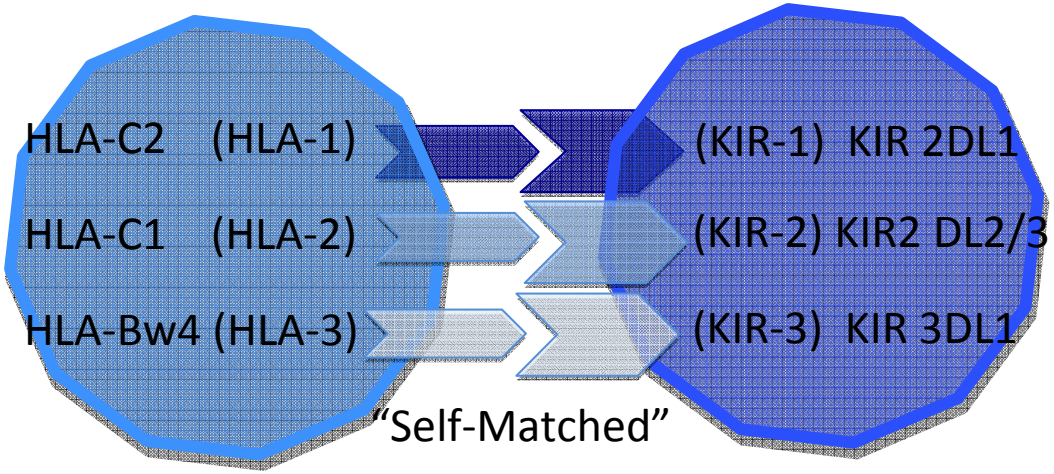


French and Yokoyama *Arthritis Res Ther* 2004 6:8-14

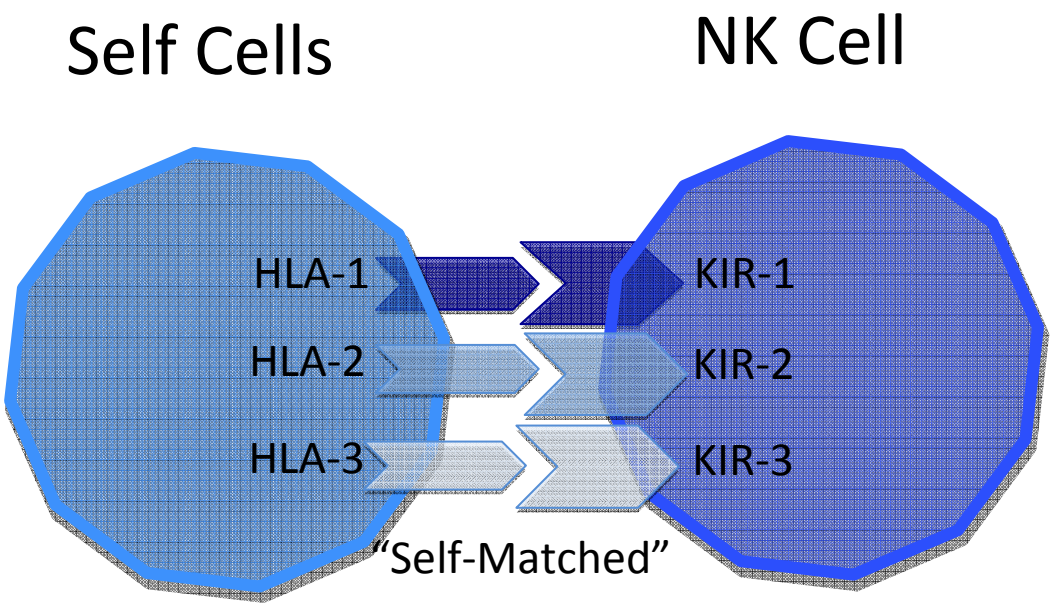
*** KIR/KIR-L mismatch can be allogeneic or autologous**

Autologous KIR-Ligand Repertoire (Chr.6)

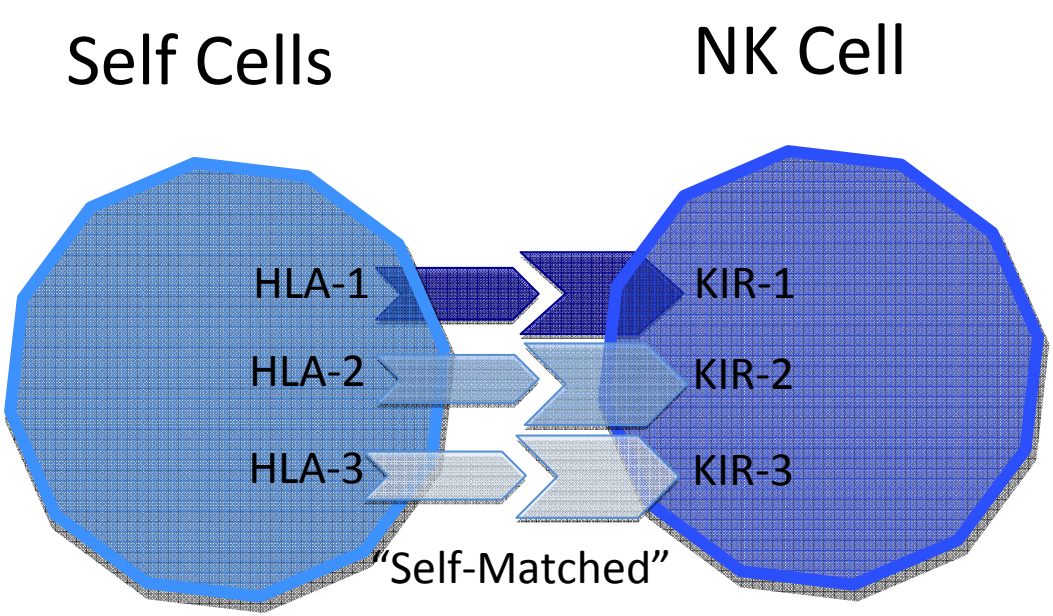
NK Cell **Inhibitory KIR** Repertoire (Chr.19)



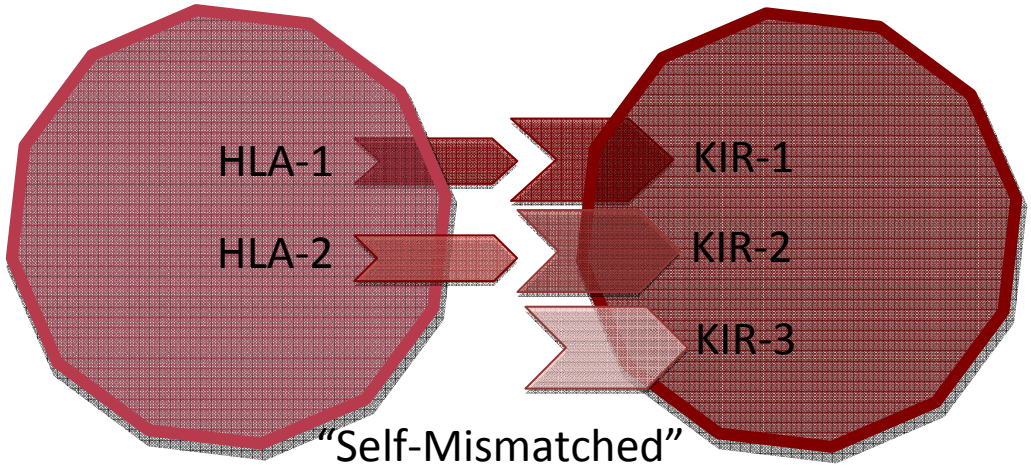
Inhibitory KIRs on Human NK cells and their primary Ligands



For Simplicity, we'll refer to them
As KIR 1, 2 and 3
And
HLA 1, 2 and 3

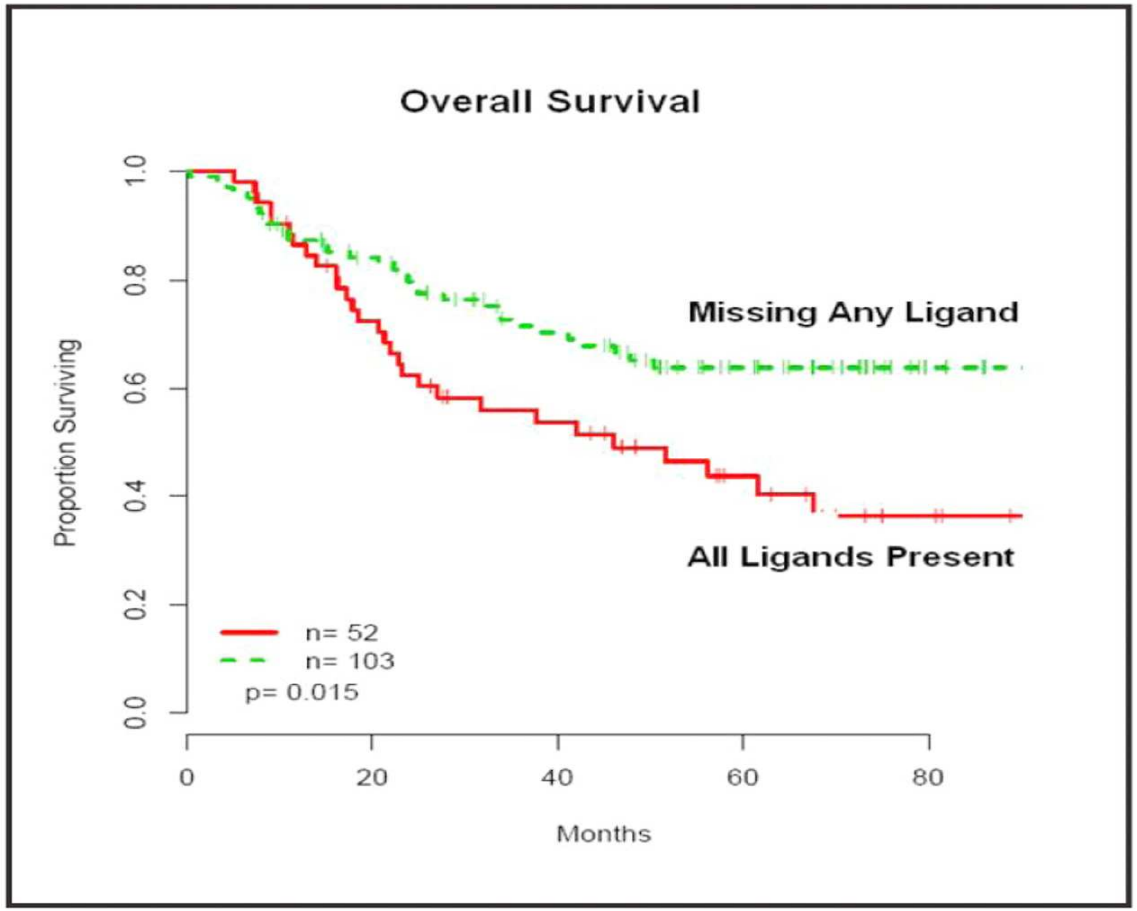


40% of population (all KIRS present have a corresponding ligand)



60% of population (at least 1 KIR does not have a corresponding ligand)

KIR ligand mismatch helps ABMT



KIR-mismatched
(~60% of pop.)
KIR-matched
(~40% of pop.)

HOW MIGHT THIS WORK?

155 neuroblastoma pts: those with KIR mismatch w/ 45% lower risk of death after ASCT
Venstrom et al, Clin. Can. Res 15:7330, 2009; similar to data from Leung et al, Br. J. Cancer, 97:539, 2007

Hypothesis: Autologous KIR/KIR-L mismatch will influence response to hu14.18-IL2.

Analysis of completed COG Phase II study:

Mismatch vs. Response/Improvement (Stratum 1 & 2)

	KIR-Mismatch	KIR-Match	Total
Response/ improvement	**7 (29%)**	**0 (0%)**	**7**
No Response/No improvement	17 (71%)	14 (100%)	31
Total	24 (63%)	14 (37%)	38

P= 0.03

1.Demonstrates an association between "mismatch" and clinical response

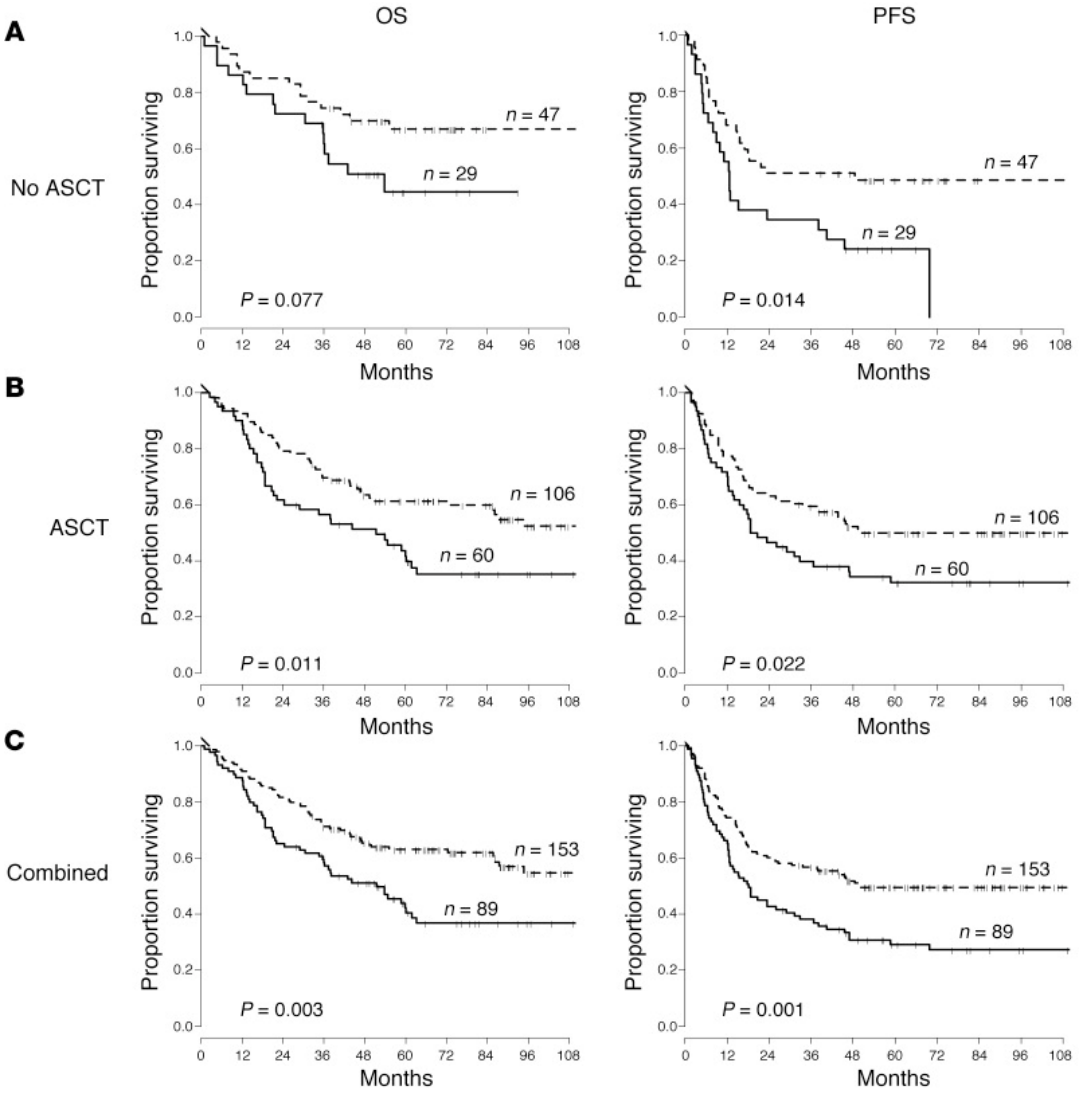
2. Consistent with in vivo role for NK cells in the anti-tumor response to hu14.18-IL2

3.Suggests NK receptors other than FcR and IL2R influence IC-induced in vivo ADCC

Is "mismatch" required for response to evaluable disease (this Phase II) or for MRD (Pts. In remission at high risk of relapse)?

Delgado DC, Hank JA, Kolesar J, Lorentzen D, Gan J, Seo S, Kim KM, Shusterman S, Gillies SD, Reisfeld RA, Yang R, Gadbaw B, DeSantes KD, London WB, Seeger RC, Maris J, and Sondel PM. Cancer Research, 70:9554, 2010

KIR/KIR-Ligand Mismatch Helps anti-GD2 mAb in Neuroblastoma



Tarek N. et al.
Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. J.C.I. 122:3260, 2012.

MSKCC

NEW, UNPRESENTED DATA:

Initial Analyses of contributions of KIR and KIR Ligand in the Response of Follicular Lymphoma to Rituximab (UWCCC analyses of data from an ECOG Study) [See Poster #44 here at SITC, presented by Wei Wang]

*Amy K. Erbe[1], *Wei Wang[1], *Bartosz Grzywacz[2], Erik A. Ranheim[2], Jacquelyn A. Hank[1], KyungMann Kim[3], Lakeesha Carmichael[3], Songwon Seo[3], Eneida A. Mendonca[3], Yiqang Song[3], Fangxin Hong[4], Randy D. Gascoyne[5], Elizabeth Paietta[6], Sandra J. Horning[7], **Brad Kahl[8],** Paul M. Sondel[1]

*Co-First Authors

209 patients with follicular lymphoma (FL) received 4 weeks Rituximab.

At week 13, 157 patients showed clinical response and were randomized to:

ArmA (no scheduled maintenance treatment)

ArmB (1 dose Rituximab every 13 weeks)

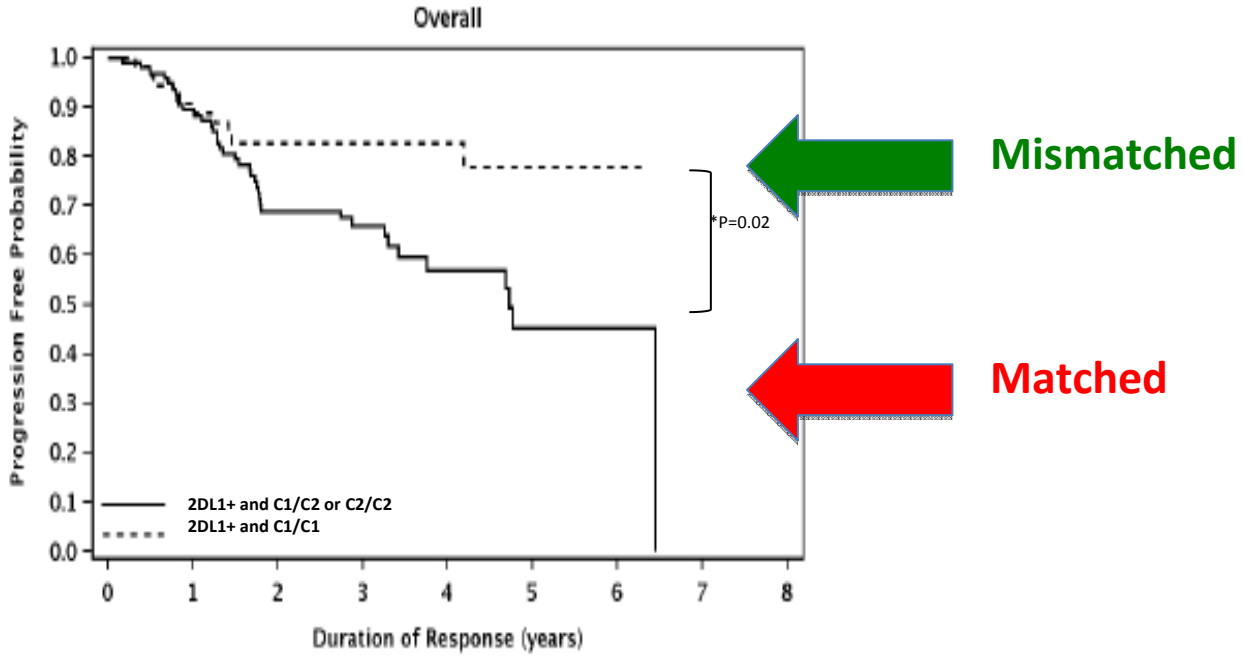
Result (ASH 2011): ArmB has longer time to progression but no benefit in overall survival

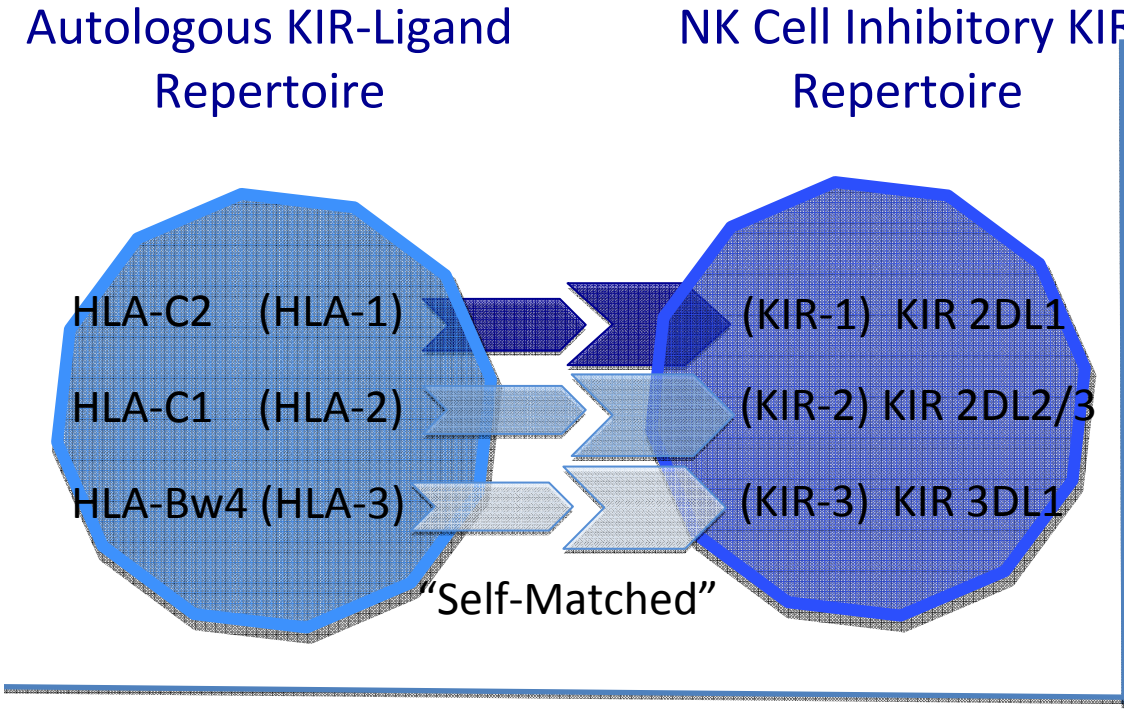
All patients had 15 KIR genes and corresponding KIR-Ligands genotyped by real time-PCR and PCR-SSP: **What is the influence of KIR/KIR-L?**

INHIBITORY KIR AND HLA STATUS (MATCHED VS. MISMATCHED)

- **Patients who are overall KIR/KIR-Ligand Mismatched showed no benefit.**
- **Patients mismatched at 2DL1** [namely positive for KIR 2DL1 and lacking its HLA-C2 ligand] **had a longer duration of response compared to those who are matched at 2DL1** [namely positive for 2DL1+ and its C2 ligand (C1/C2 or C2/C2)]**.**
- **Mismatching for KIR 2DL2/2DL3, or for KIR 3DL1 showed no benefit** (not shown)

FIGURE 1. KIR 2DL1 matched (N=55) vs. mismatched (N=98) status, duration of response.





Inhibitory KIRs on Human NK cells and their primary Ligands

Inhibitory and Activating KIR genes and their corresponding HLA ligand:

Inhibitory KIR Gene	**KIR Ligand**	***Activating KIR Gene**
KIR 2DL1	HLA-C2	KIR 2DS1
KIR 2DL2 AND/OR 2DL3	HLA-C1	# KIR 2DS2 and KIR 2DS3
KIR 3DL1	HLA-Bw4	##KIR 3DS1

Note:

*There is great homology in HLA binding domain between Inhibitory and Activating KIR genes

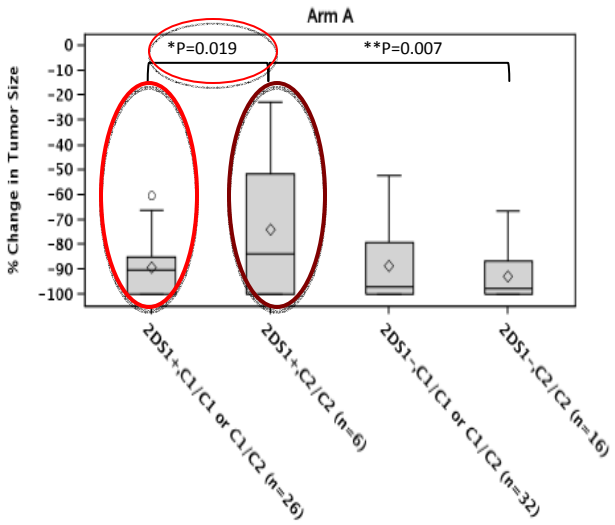
It is uncertain what the ligands may be for KIR2DS2 and KIR2DS3.

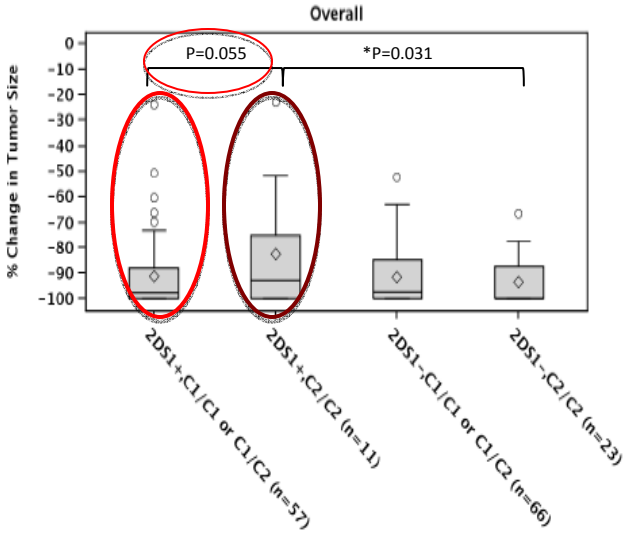
It remains uncertain whether KIR3DS1 actually recognizes HLA-Bw4 as its ligand.

Interaction of Activating KIR 2DS1 and its HLA-C2 ligand on Tumor Shrinkage

- **2DS1+ patients had less tumor shrinkage if they were C2/C2.**
- This wasn't just due to presence of C2/C2 as C2/C2 patients did better if they were negative for 2DS1 (2DS1-).
- These relationships were seen in Arm A and overall, but not in the Arm B (maintenance) group.

Interaction of KIR 2DS1 and HLA-C status, maximum tumor shrinkage.





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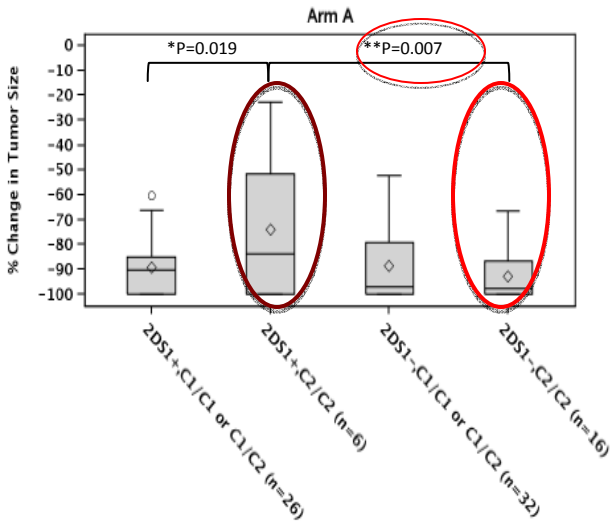
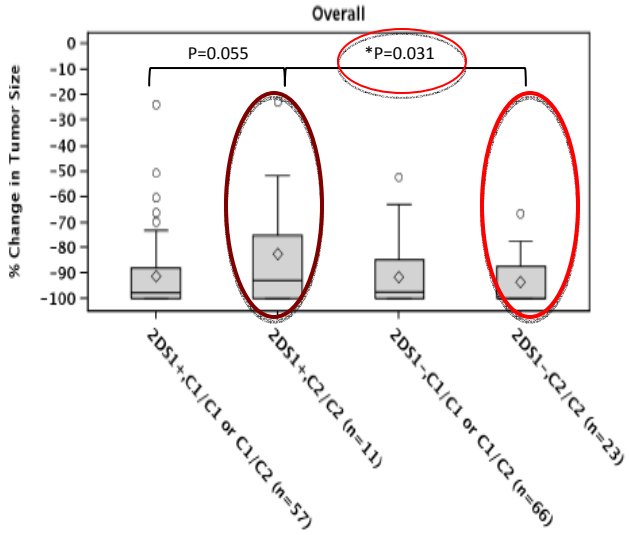
Interaction of KIR 2DS1 and HLA-C status, maximum tumor shrinkage.





Initial Conclusions from KIR analyses of ECOG Rituximab Study

Inhibitory KIRs and HLA Status

- **Patients mismatched at KIR 2DL1**(2DL1+, C1/C1) **had a longer duration of response vs. those matched at 2DL1** (2DL1+, C1/C2 or C2/C2)**.**
- Patients mismatched for KIR 2DL2/2DL3 or for KIR 3DL1 showed no advantage
- Patients that are overall KIR/KIR-Ligand mismatched showed no benefit, by any clinical outcome measure.

- This suggests that **in this clinical setting**, **not all inhibitory KIRs act identically.** Mismatching for certain inhibitory KIRs may have greater influence.

Initial Conclusions from KIR analyses of ECOG Rituximab Study

Activating KIR and HLA Status

- **In KIR 2DS1+ patients, the presence of the C2/C2 genotype correlated with less tumor shrinkage.**
- In KIR 3DS1+ patients, the presence of the HLA-Bw4 correlated with less tumor shrinkage (not shown) .

- **If** this worse outcome actually reflects deficient NK mediated ADCC *in vivo*, this clinical result would be consistent with *in vitro* results[1] that show that **the presence of an activating receptor and its ligand results in reduced responsiveness of NK cells.**

[1] **Pittari et al. J. Imm. 190, 4650, 2013**

Summary: In Vivo ADCC

- Ch14.18 mAb + IL2 + GM-CSF improves EFS for NBL
- Activating ADCC effectors may augment clinical ADCC in MRD
- Merits testing with other mAbs (Rituximab, Trastuzumab, Cetuximab), and other effector activators (IL15, anti-CD37)
- ICs are more potent preclinically than mAb+ IL2 via additional ADCC pathways (ie: polarizing role for IL2Rs)
- KIR/KIR-L associations suggest NK cells are involved and other effector receptors may influence ADCC
- Each disease, and each immunotherapy, may have its unique pattern for KIR/KIR-L effects
- Opportunities for “personalized” medicine, via genotyping and via application of additional agents (ie: anti-KIR mAb) based on genotyping

Collaborators in our Immunotherapy Research: 2013

- **UWCCC**
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  - S Gillies
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- **Research Support**
  - NCI
  - MACC Fund
  - SU2C- St. Baldrick's
  - Hyundai
  - Other agencies

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