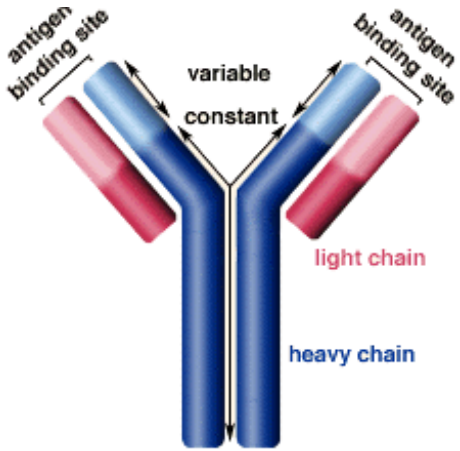
Carbone Cancer Center
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

UW Health
American Family Children's Hospital

Activation of Innate and Adaptive Immunity as an "*In Situ* Vaccine"



SITC

National Harbor, MD

11/11/17

Paul M. Sondel, MD, PhD

UW Depts. of Pediatrics, Human Oncology and Genetics

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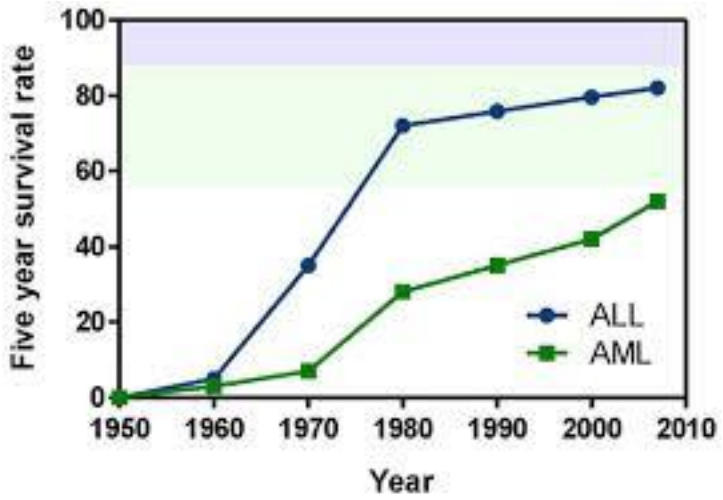
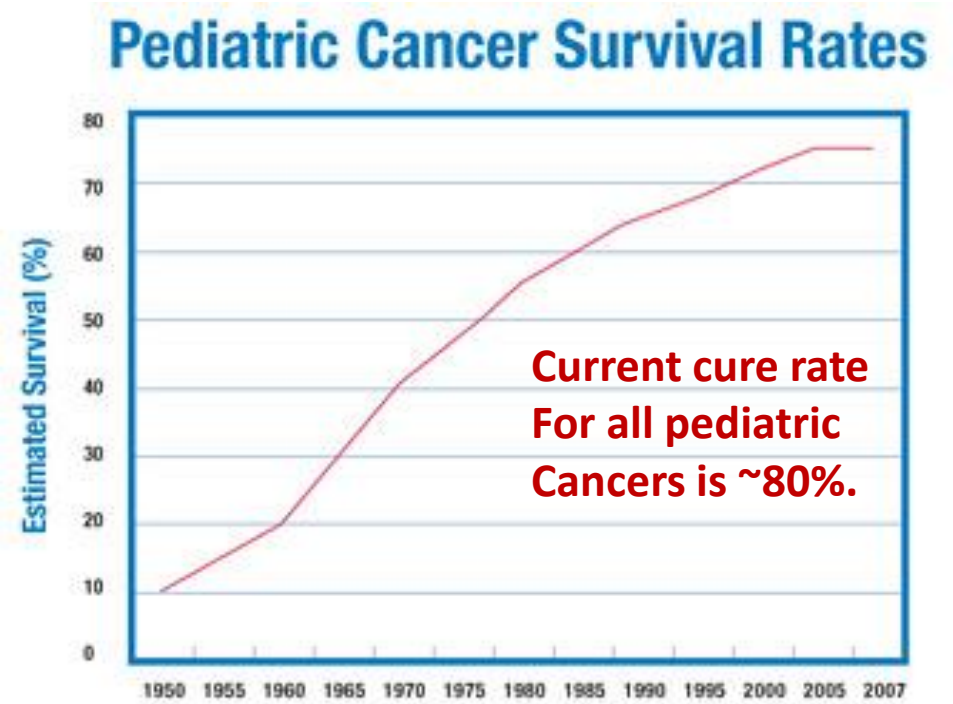
Disclosure:

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

65+ years of progress in childhood cancer treatment: Surgery, Radiation, Chemotherapy and *Collaboration* (Farber, Frei, Freireich, Pinkel, Hammond, CCG,POG, COG, etc.)



1949: Dr. Sidney Farber:
First prospective use of chemotherapy (amethopterin), hoping to kill the cancer, but not the child.



With this dramatic progress over 65+ years against childhood cancer

and

46 years since the USA declared "War on Cancer" in 1971

HOW ARE WE DOING?

Leading causes of Death in The USA (cases per year)

	All Ages-Male 1,328,241	All Ages-Female 1,298,177	1-19 Male 12,128	1-19-Female 6538
1	Heart 325,077	Heart 289,271	Accidents, unintentional 4409*	Accidents, unintentional 2023*
2	**Cancer 311,077**	**Cancer 280,403**	Suicide 1681**	**Cancer 757**
3	Accidents (unintentional) 85,448	Chronic lung 77,645	Homicide 1563**	Suicide 581**
4	Chronic Lung 69,456	CVA 77,632	**Cancer 1028**	Homicide 477**

***Most due to auto accidents**

****Childhood deaths due to firearms, 25X higher than other developed countries. Fowler K.A. et al. J. Peds, 2017**

Siegel et al
CA: A Cancer Journal for Clinicians
JAN 2017

But in 2017, why do so many still die of cancer?

- **Distant/advanced disease is seldom curable in adults, and difficult to cure in children**
- **Relapse is usually resistant to our "best" treatments.**

- **HOWEVER, we are finally seeing, and participating in, an expanding and promising clinical initiative, after 50-100 years of development: *Cancer Immunotherapy.***

- ***In the pediatric setting, the transfer of immune cells was the first effective immunotherapy, in the form of….***

BONE-MARROW TRANSPLANTATION IN A PATIENT WITH THE WISCOTT-ALDRICH SYNDROME

Fritz H. Bach
M.D. Harvard
ASSISTANT PROFESSOR OF MEDICAL GENETICS AND MEDICINE

RICHARD J. ALBERTINI
M.D. Wisconsin
POST-DOCTORAL FELLOW IN MEDICAL GENETICS

PATRICIA JOO
M.D. Wisconsin
ASSISTANT CLINICAL PROFESSOR OF PEDIATRICS

JAMES L. ANDERSON
M.D. Wisconsin
CHIEF RESIDENT IN PEDIATRICS

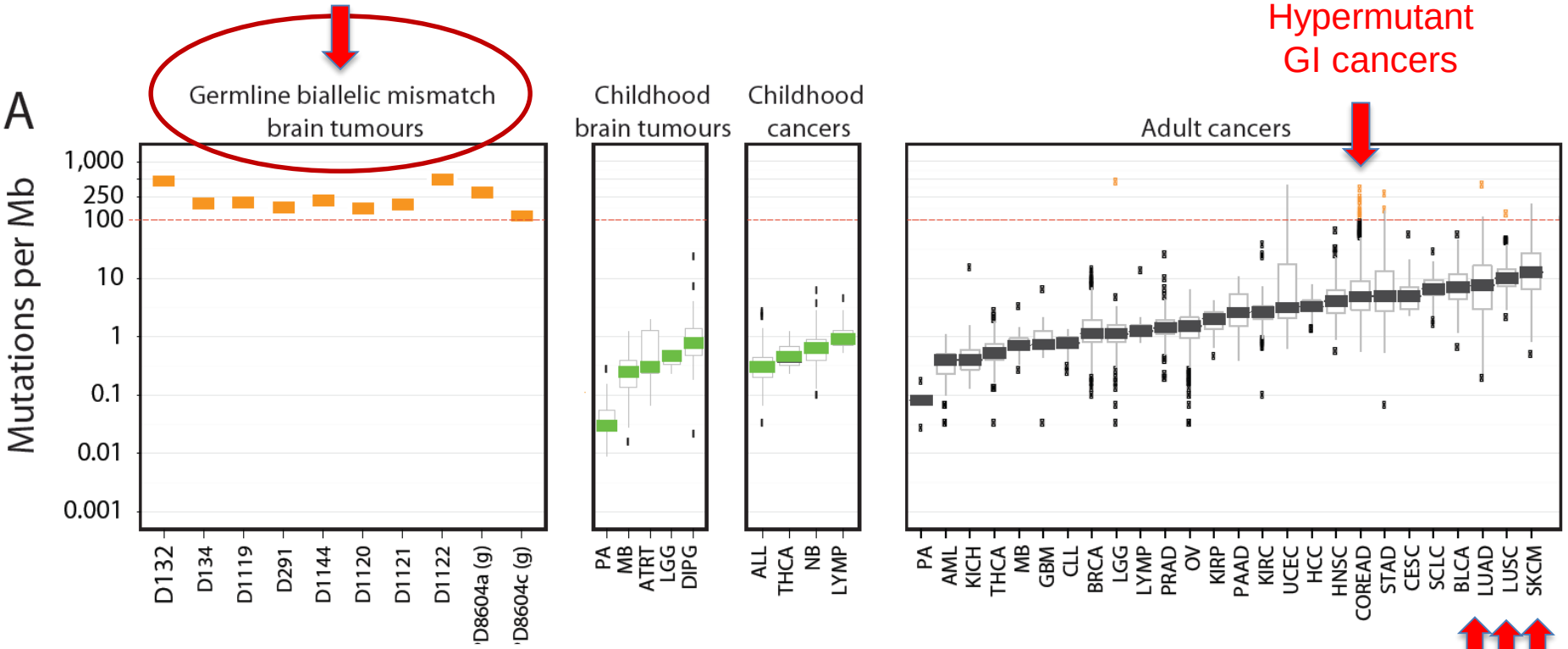
MORTIMER M. BORTIN
M.D. Marquette
ASSOCIATE CLINICAL PROFESSOR OF MEDICINE

From the Department of Medical Genetics, Medicine, and Pediatrics, University of Wisconsin, Madison, Wisconsin, 53706, the May and Sigmund Winter Research Laboratory, Mount Sinai Hospital, and the Department of Medicine, Marquette Medical School, Milwaukee, Wisconsin

First successful allogeneic BMTs, done in summer 1968 in Madison and Minneapolis (Robert Good et al); using method developed by F.H. Bach:
Now ~ 30,000 BMTs done yearly.

Cancer Immunotherapy 2017: Where are we now?

Mutation burden across 7000 cancers



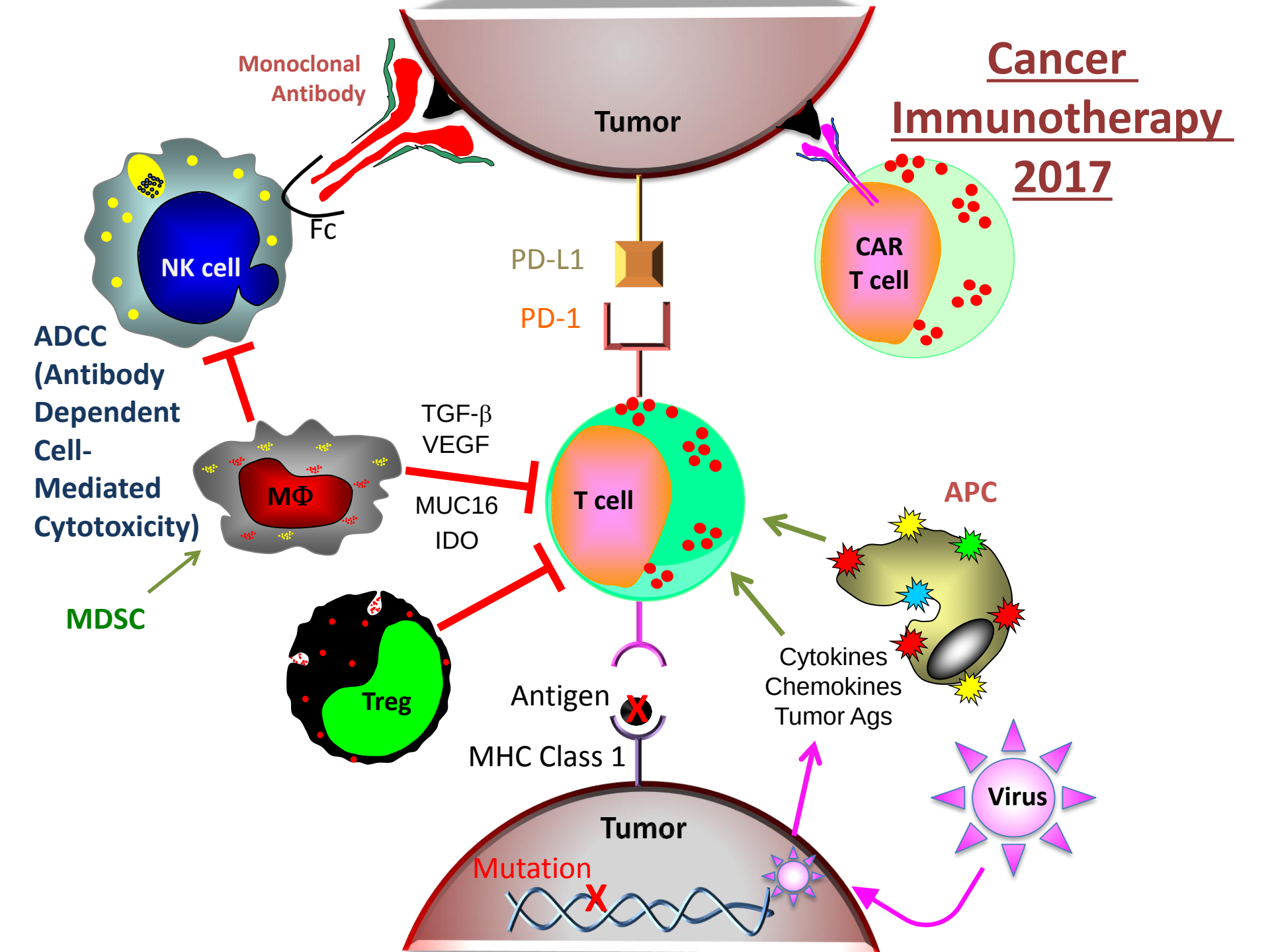
Checkpoint Inhibition (Allison, Honjo, and others), more effective in"hot"tumors:

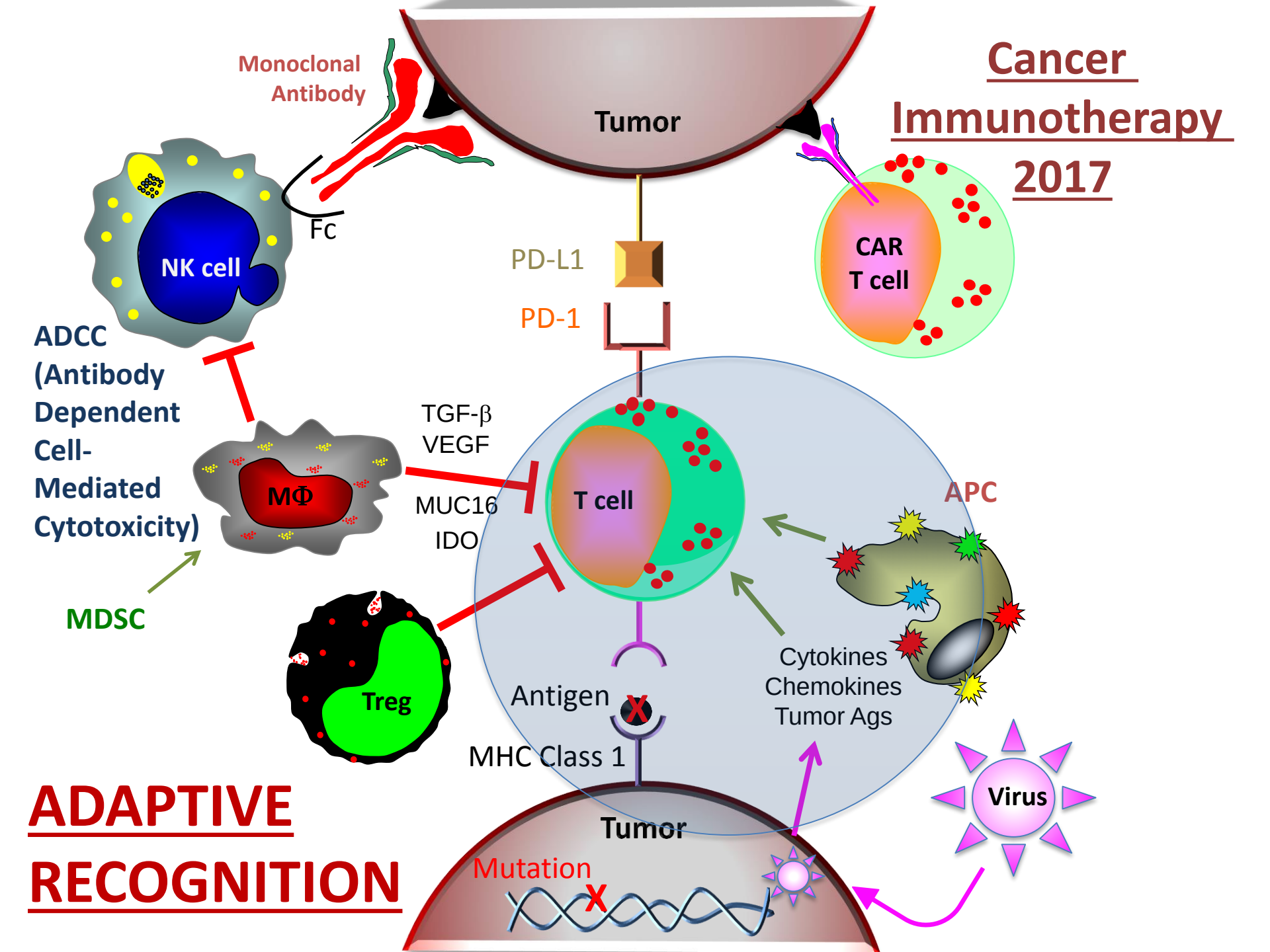
- **More neoantigens**
- **More immune infiltration**

WHAT CAN WE DO TO GET THE IMMUNE SYSTEM INTERESTED IN DESTROYING "COLD" TUMORS?

Some cancers responsive to Immune Checkpoint Inhibition (ICI)

Shlien, Campbell et al. 2015 *Nature Genet.*
Bouffet et al. 2016 JCO


Cancer Immunotherapy 2017
Monoclonal Antibody
Tumor
Fc
NK cell
PD-L1
PD-1
CAR T cell
ADCC (Antibody Dependent Cell-Mediated Cytotoxicity)
MΦ
TGF-β
VEGF
MUC16
IDO
T cell
APC
MDSC
Treg
Antigen
MHC Class 1
Cytokines
Chemokines
Tumor Ags
Virus
Tumor
Mutation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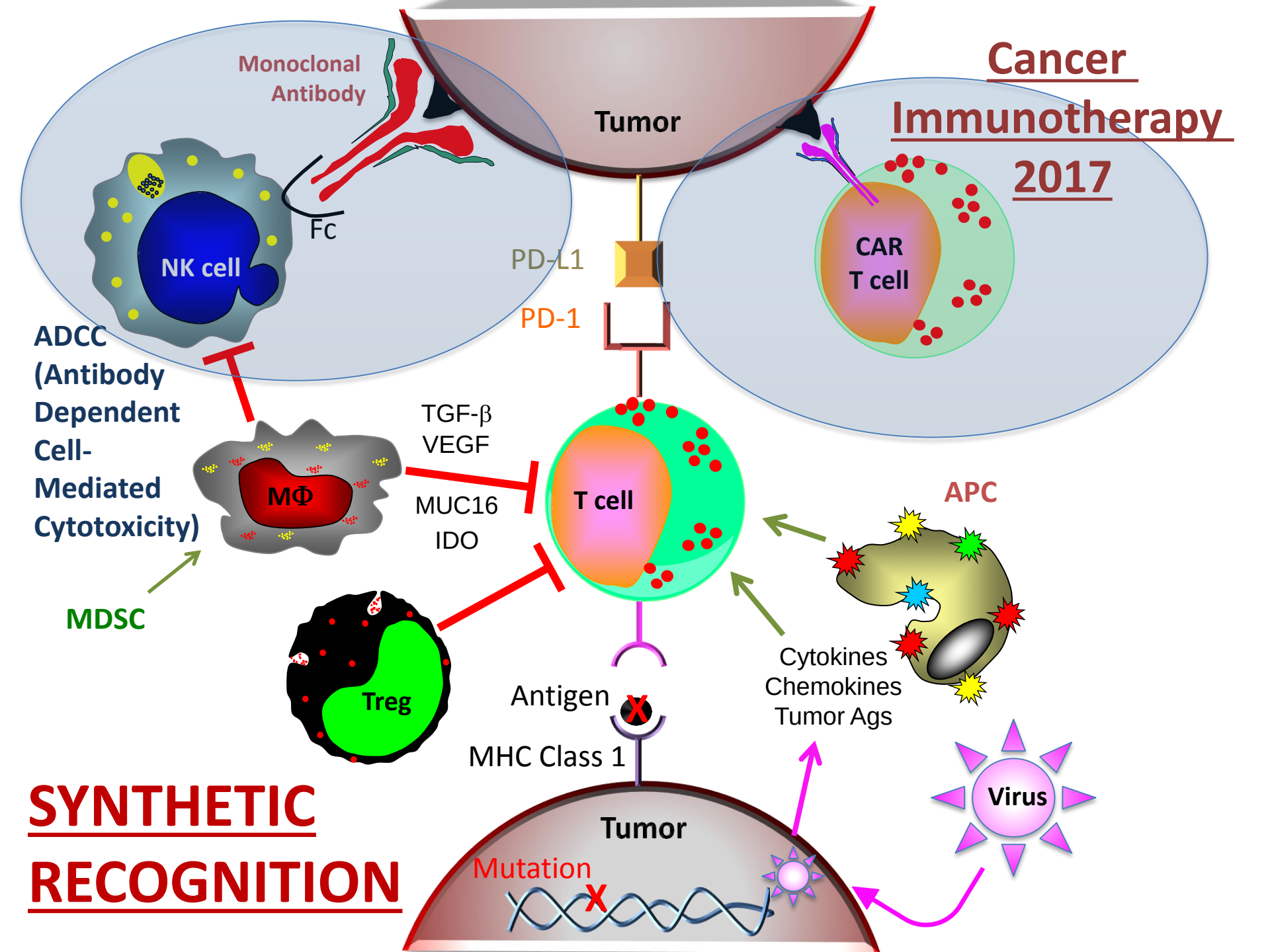
ADAPTIVE RECOGNITION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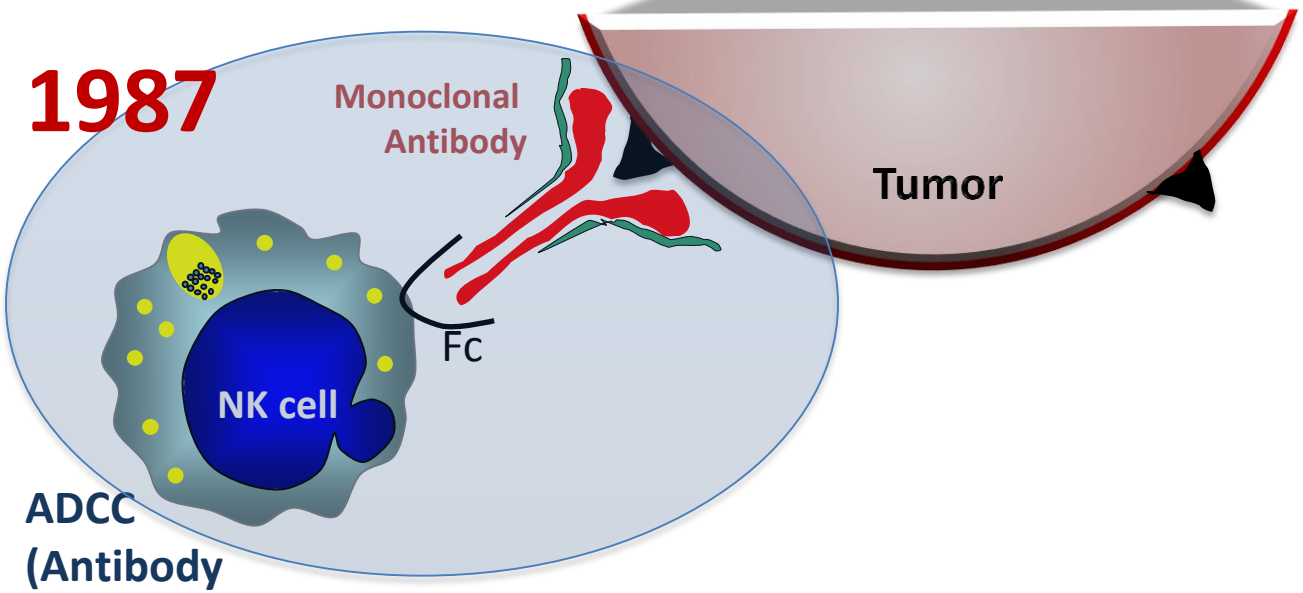
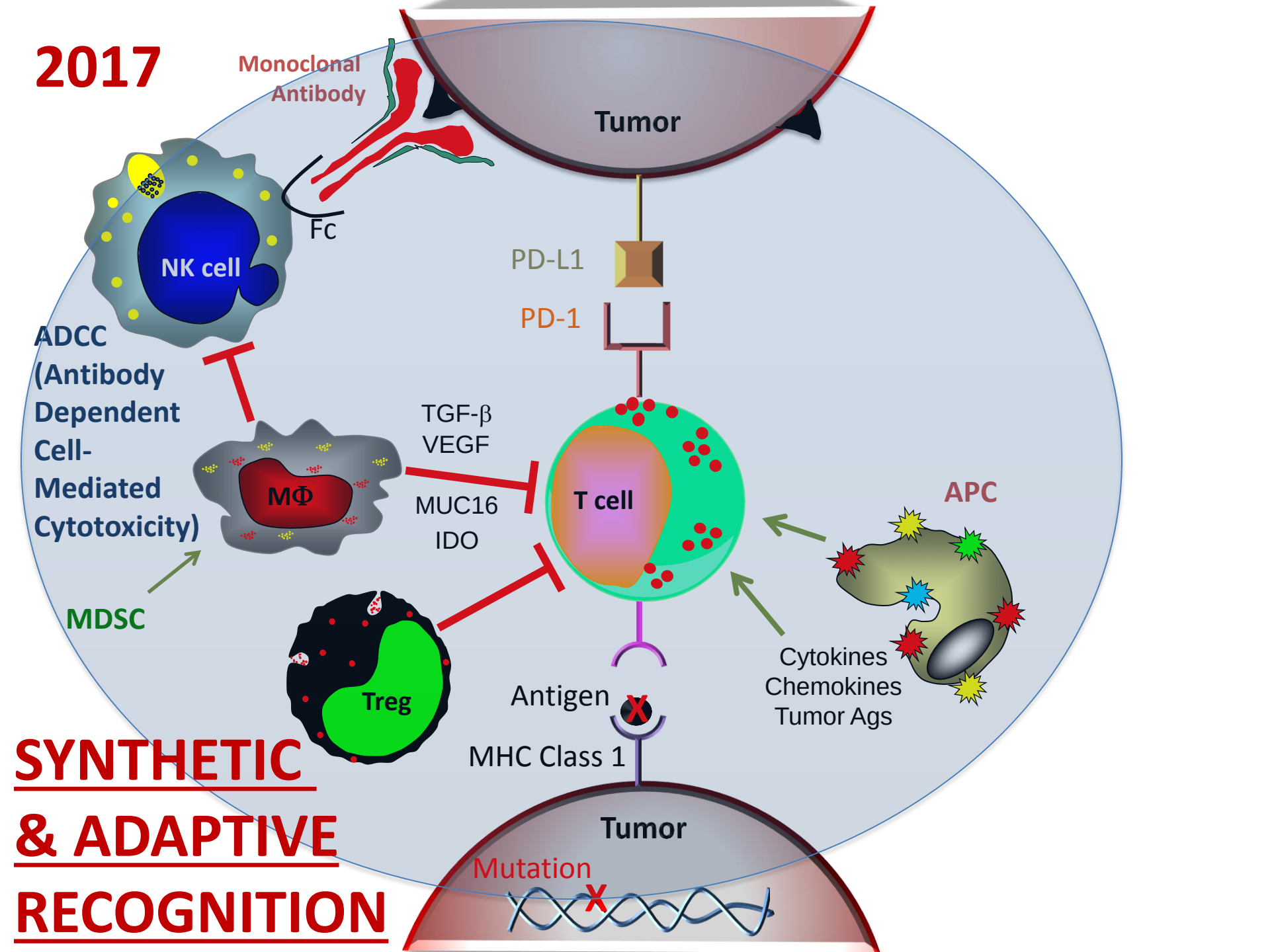
SYNTHETIC RECOGNITION



ADCC (Antibody Dependent Cell-Mediated Cytotoxicity)

Ralph Reisfeld PhD

SYNTHETIC RECOGNITION


2017
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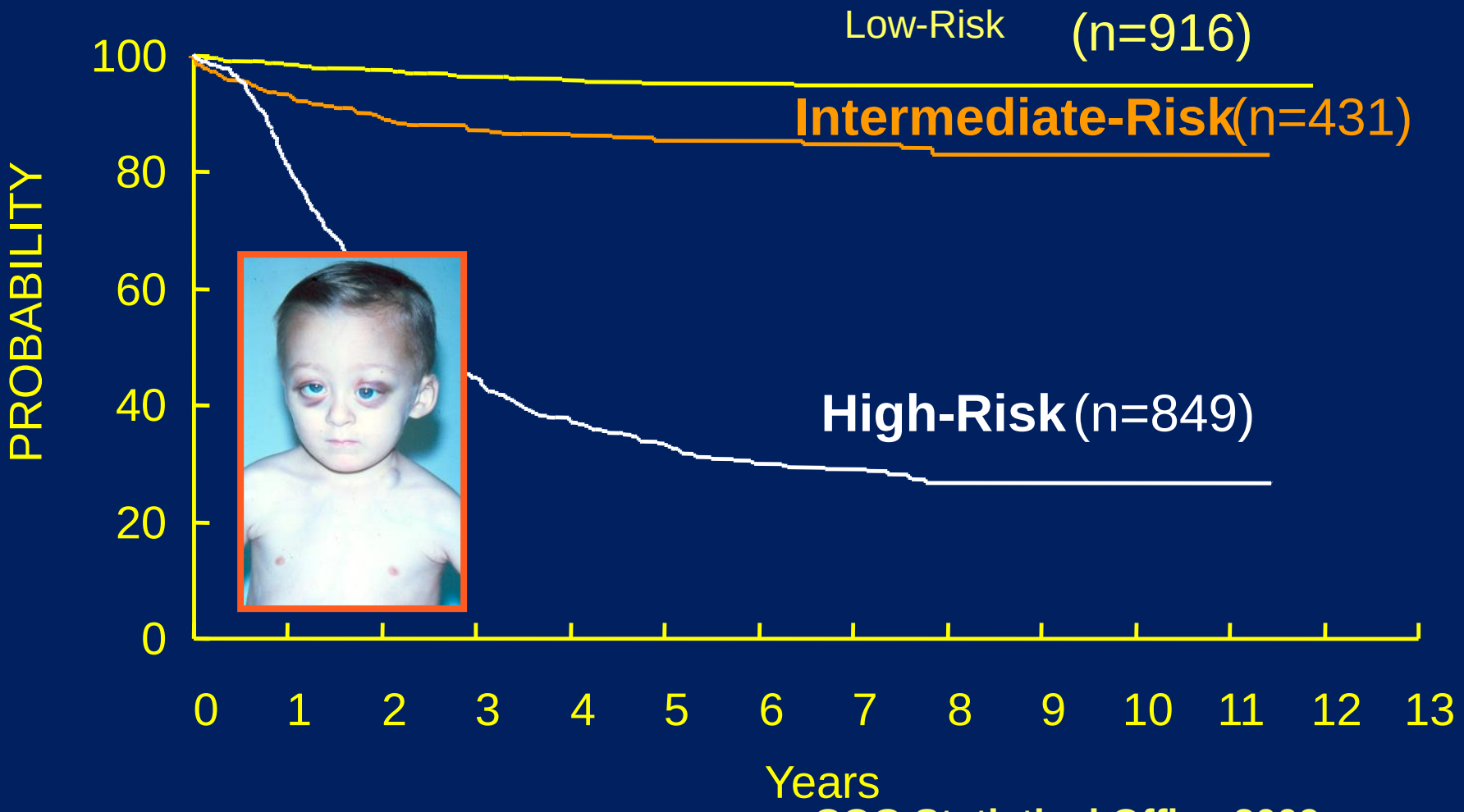
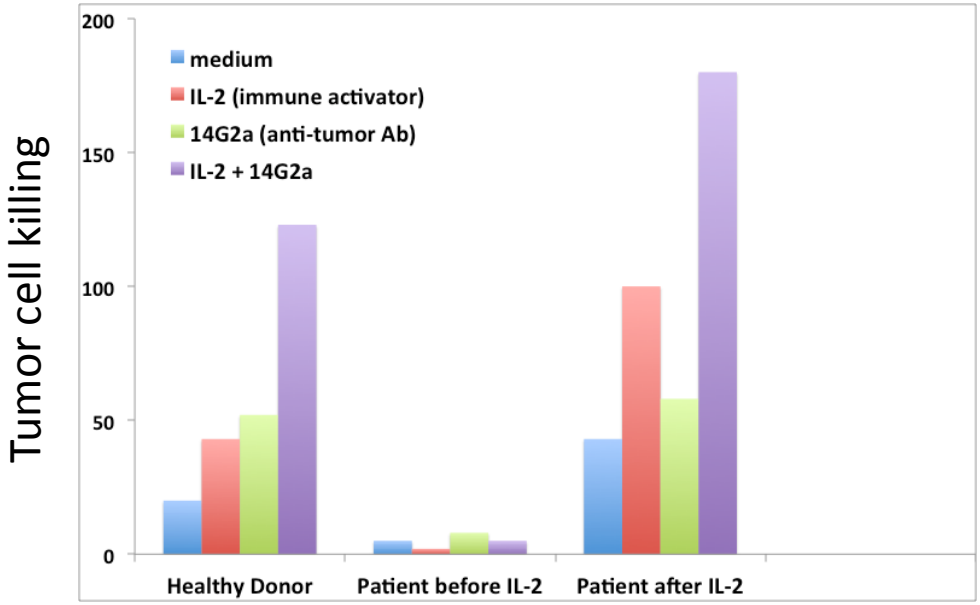
Cytokines
Chemokines
Tumor Ags
Antigen
MHC Class 1
Tumor
Mutation
SYNTHETIC & ADAPTIVE RECOGNITION

Neuroblastoma, a major challenge:
Can a "tumor-reactive" antibody help?



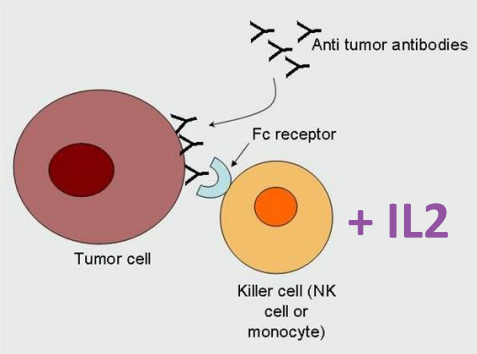
IL2 activates NK cells to kill neuroblastoma cells coated with an anti-GD2 mAb



Jackie
Hank PhD

Hank JA, Robinson RR, Surfus J, Mueller BM, Reisfeld RA, Cheung NK, Sondel PM. , Cancer Res. 50:5234, 1990

1. NK cells from healthy donors kill best with IL2 AND anti-GD2 mAb
2. NK cells from cancer patients receiving **IL2,** kill best with anti-tumor antibody AND IL2



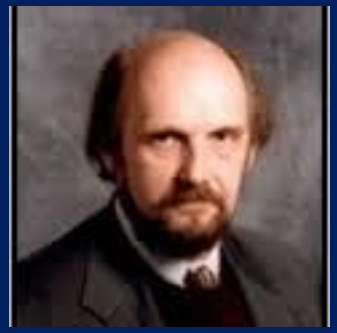
IL2 augments anti-GD2 mAb mediated NK ADCC

Ralph Reisfeld PhD

How to move this into effective clinical treatment? ***(Phase I/II studies at UWCCC and COG)***

Ernie Borden MD
UWCCC ImmRx Leader 1978-90
2nd SITC President (1986-88)

Steve Gillies PhD
Creator of ch14.18,
hu14.18-IL2 and
many other agents



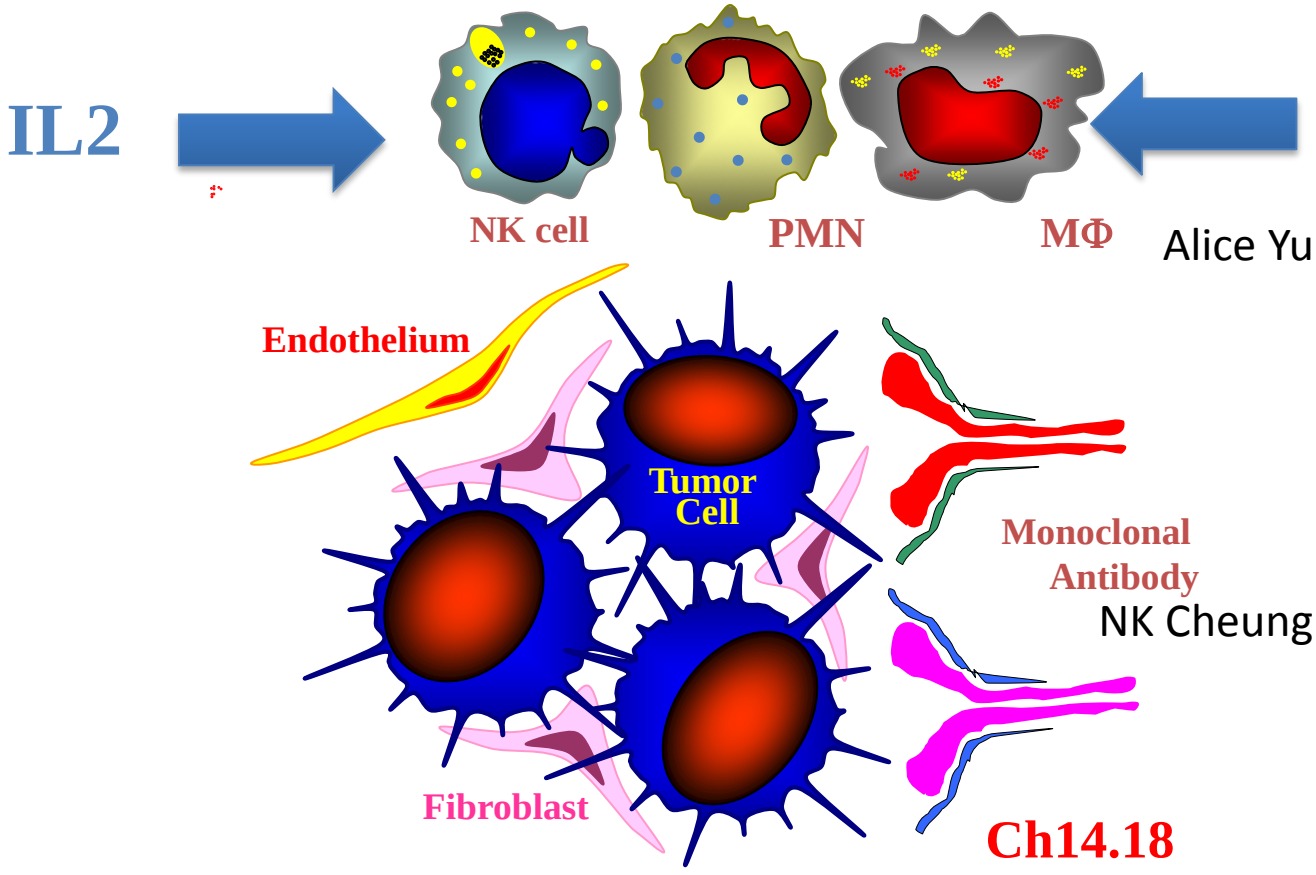
Phase I-II 14.G2a* or ch14.18* + IL2 studies:
PK, Tox., MTD, Biology but little **measurable** antitumor effect

- Melanoma -UWCCC
  M.Albertini Chair

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

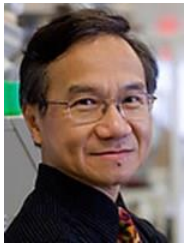
- Neuroblastoma-COG
  - 14.G2a + IL2
  - Ch14.18 + GM-CSF after ASCT
  - Ch14.18 + GM-CSF + IL2 after ASCT
  - *14.G2a and ch14.18 available via NCI: groundwork by Drs. Reisfeld, Gillies, Yu and others
  - Frost et al .Cancer 80:317, ‘97
  - Albertini et al, Clin.Can.Res. 3:1227, ’97
  - Albertini et al,J. Immther. 4:278, ’96
  - Choi et al, Canc. Imm. Imm. 7:761, ‘06

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



Alice Yu MD PhD et al;

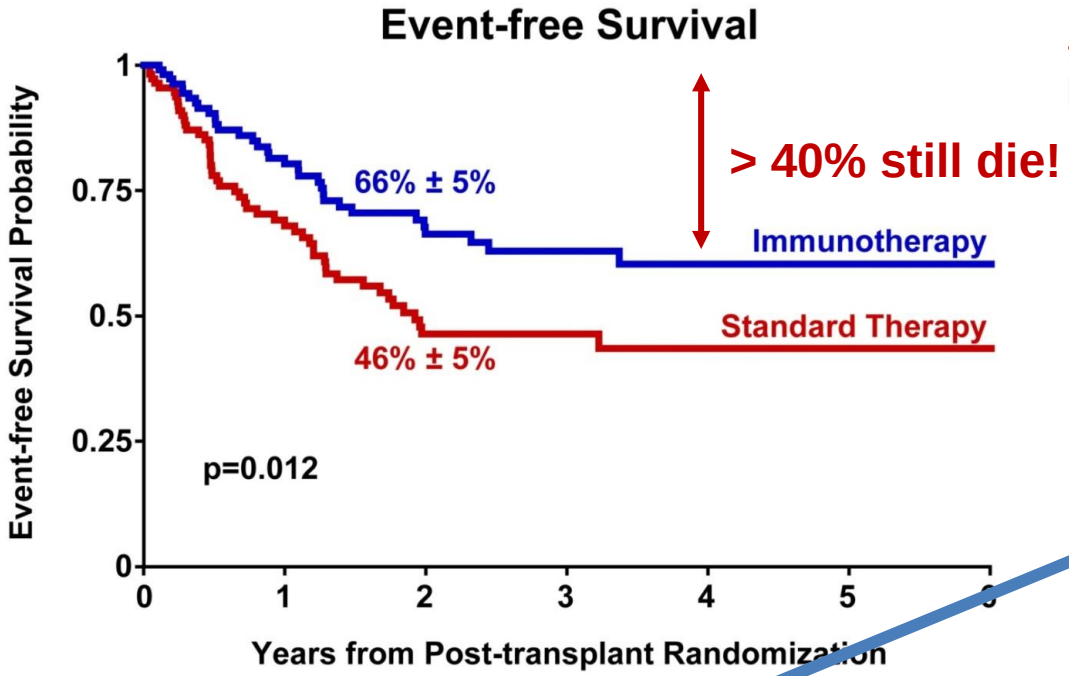
NK Cheung MD PhD et al



1. Activate Multiple Pathways of ADCC (ie: stimulate and engage several different populations of ADCC innate effector Cells)
2. Administer Immunotherapy in Minimal Residual Disease

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

Cancer-Free Survival for 226 Children with Neuroblastoma

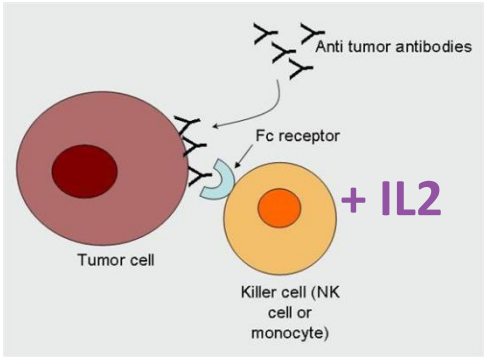


The NEW ENGLAND JOURNAL of MEDICINE

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

JA Hank PhD

Alice Yu MD PhD



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

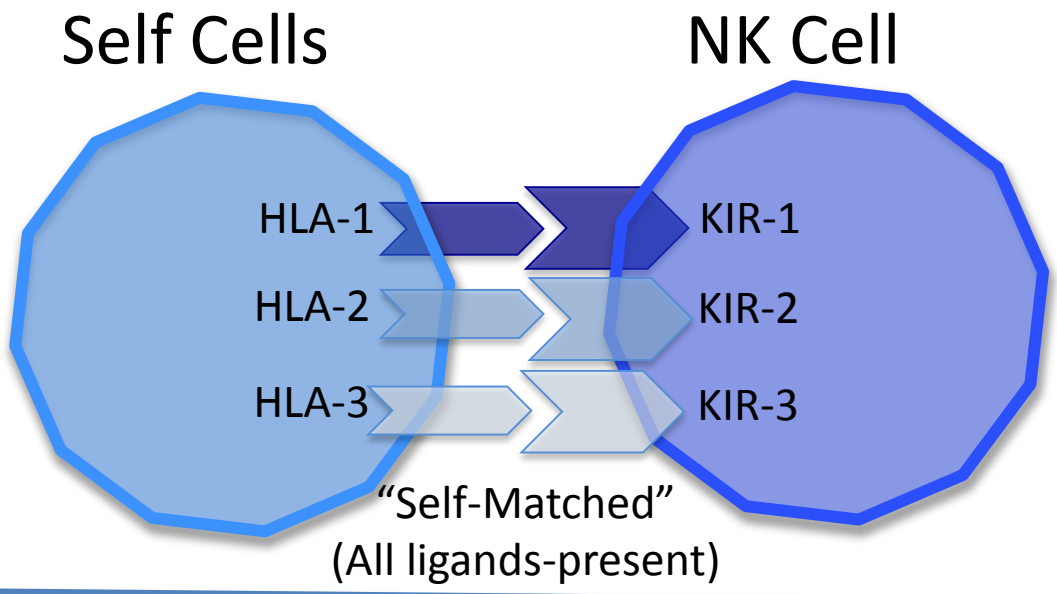
FDA approves as standard of care in 2015: Dinutuximab

Clinical benefit for some, and only in Minimal Residual Disease Setting:

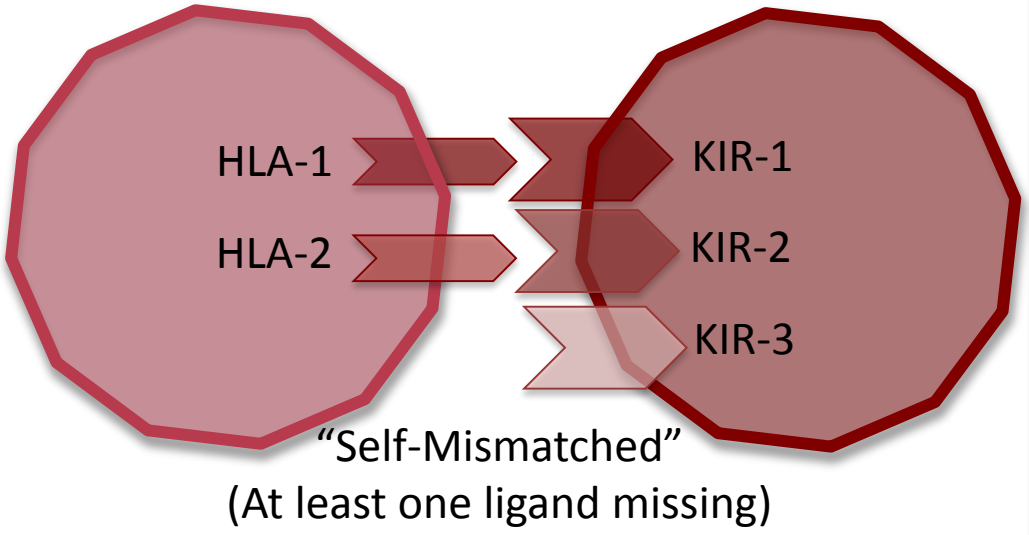
We must improve further and FASTER!

Basic in vitro observation, leads to ultimate clinical regimen of ch14.18 (dinutuximab) anti-GD2 mAb + IL2 + GM-CSF for high risk neuroblastoma in remission.

The Role of KIR/KIR-Ligand Interactions (Biomarker for ADCC?)

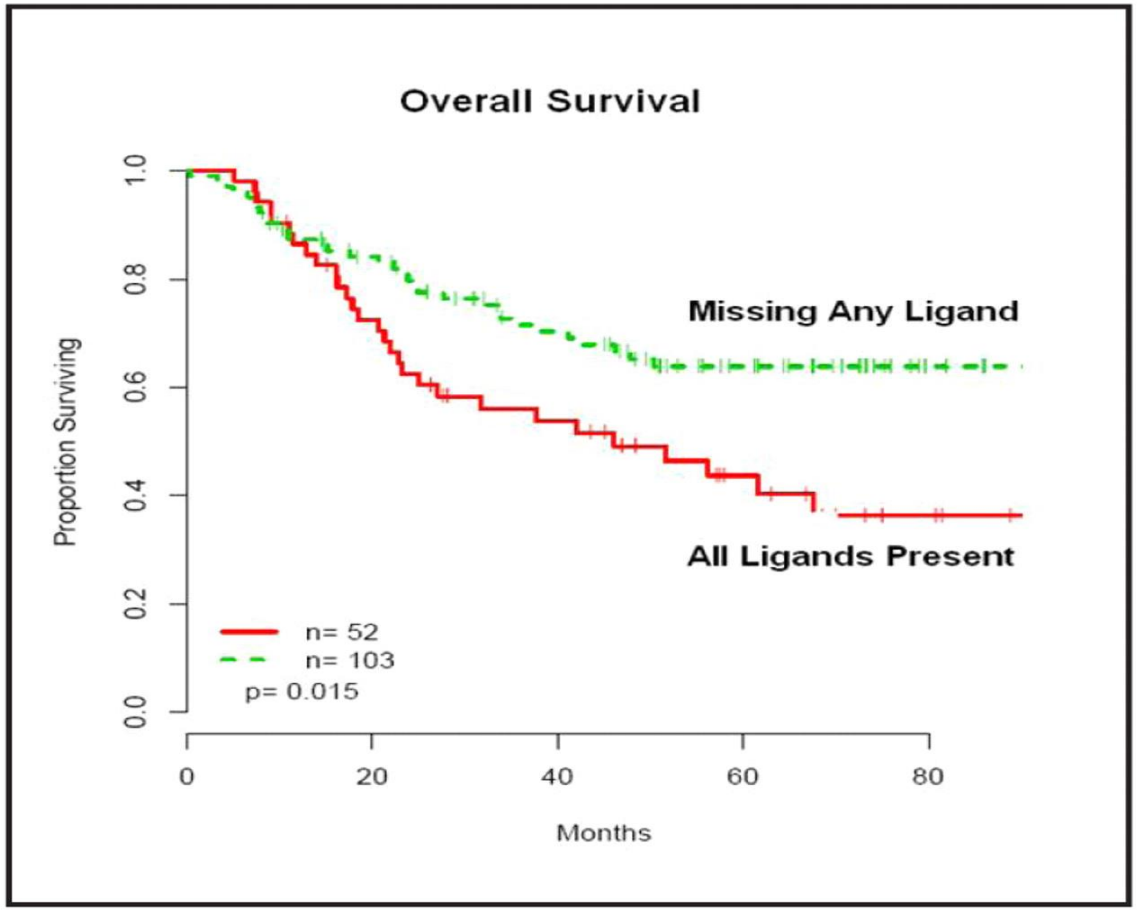


40% of population:
all iKIR present have a corresponding ligand, and thus are INHIBITED by HLA-expressing self



60% of population:
(at least 1 iKIR does not have a corresponding Ligand; thus SOME NK Cells are NOT INHIBITED By HLA-expressing self): IMPLIES BETTER ANTI-TUMOR NK FUNCTION!

KIR ligand mismatch helps ABMT



Less Inhibited NKs (KIR-mismatched, ~60% of pop.)

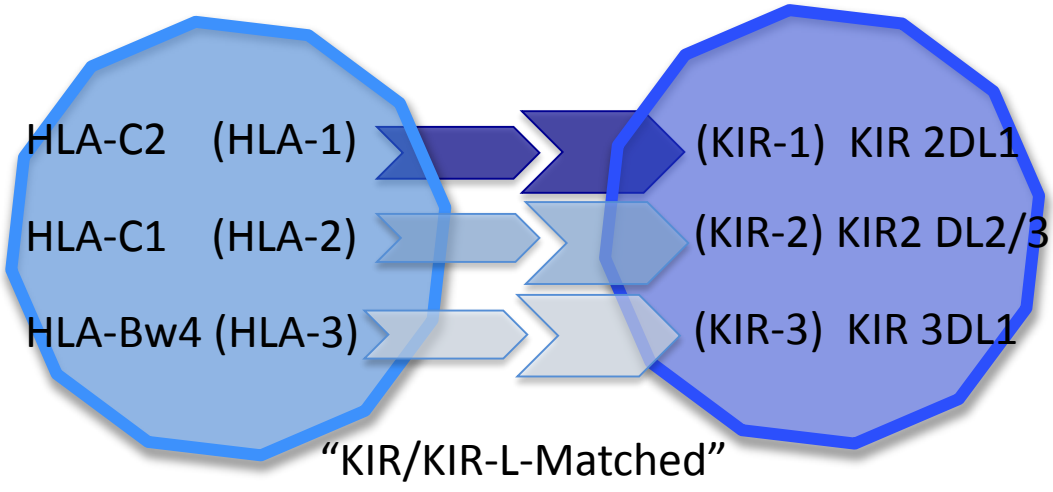
More Inhibited NKs (KIR-matched, ~40% of pop.)

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

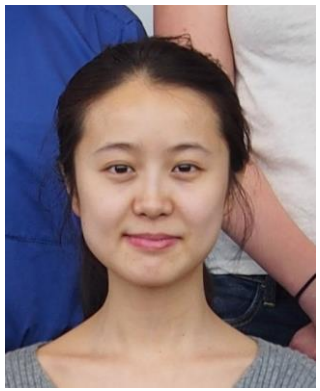
Autologous KIR-Ligand Repertoire (Chr.6)

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

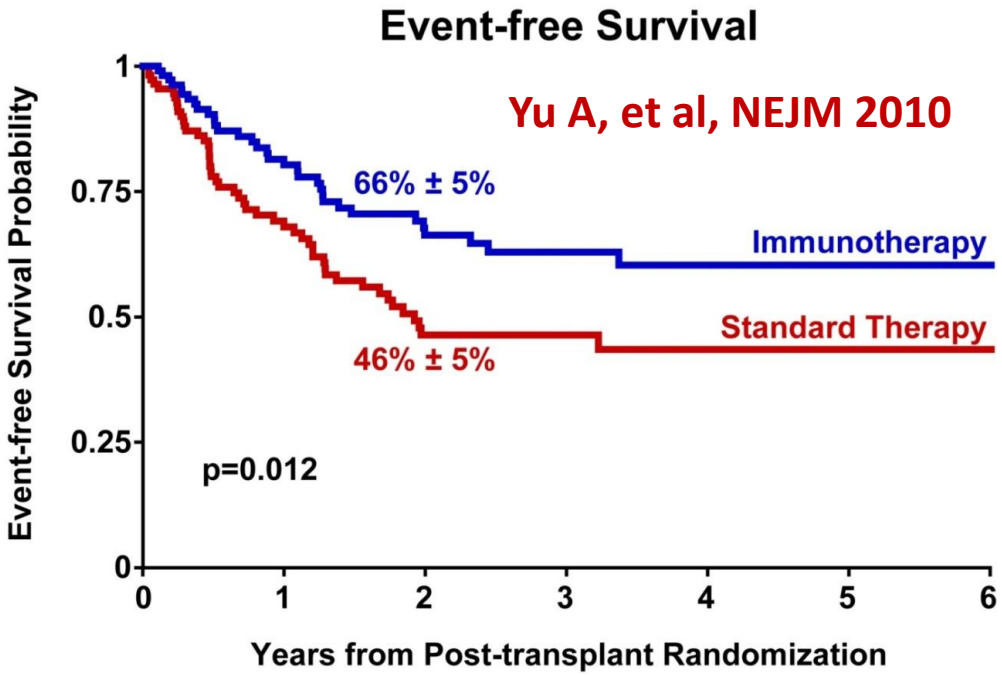


"KIR/KIR-L-Matched"

Inhibitory KIRs on NK cells and their Ligands: Biology and association with ImmRx outcome



Drs. Amy Erbe-Gurel and Wei Wang

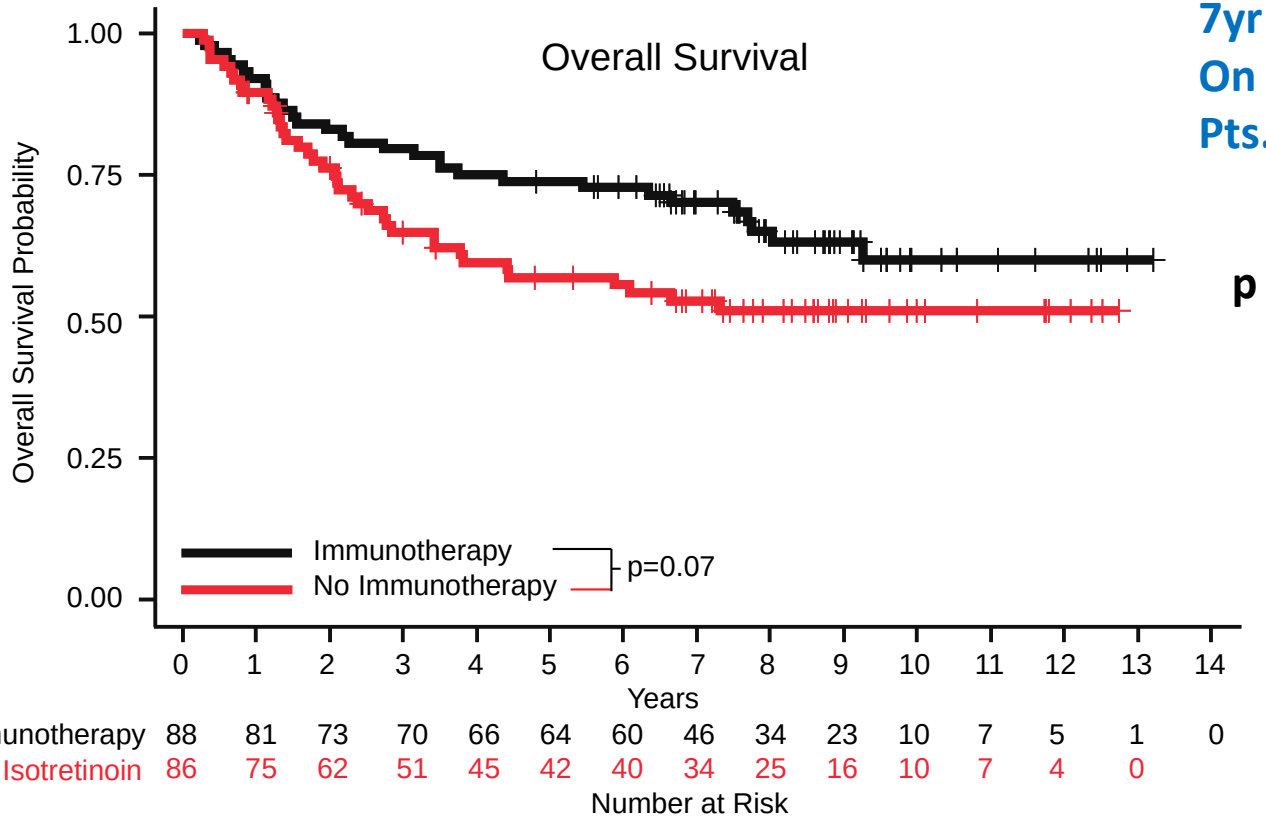


Evaluation of KIR and KIR-Ligand Genotype and associations with Outcome in this COG trial of Anti-GD2 + IL2 + GM-CSF:

Do some genotypes predict response to Immunotherapy?

Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

Overall Survival for 174 pts: based on **Immunotherapy** vs. **No Immunotherapy**.



Updated Clinical Data;
7yr Median Follow Up
On the 174 (of 226)
Pts. With DNA Available

p = 0.07

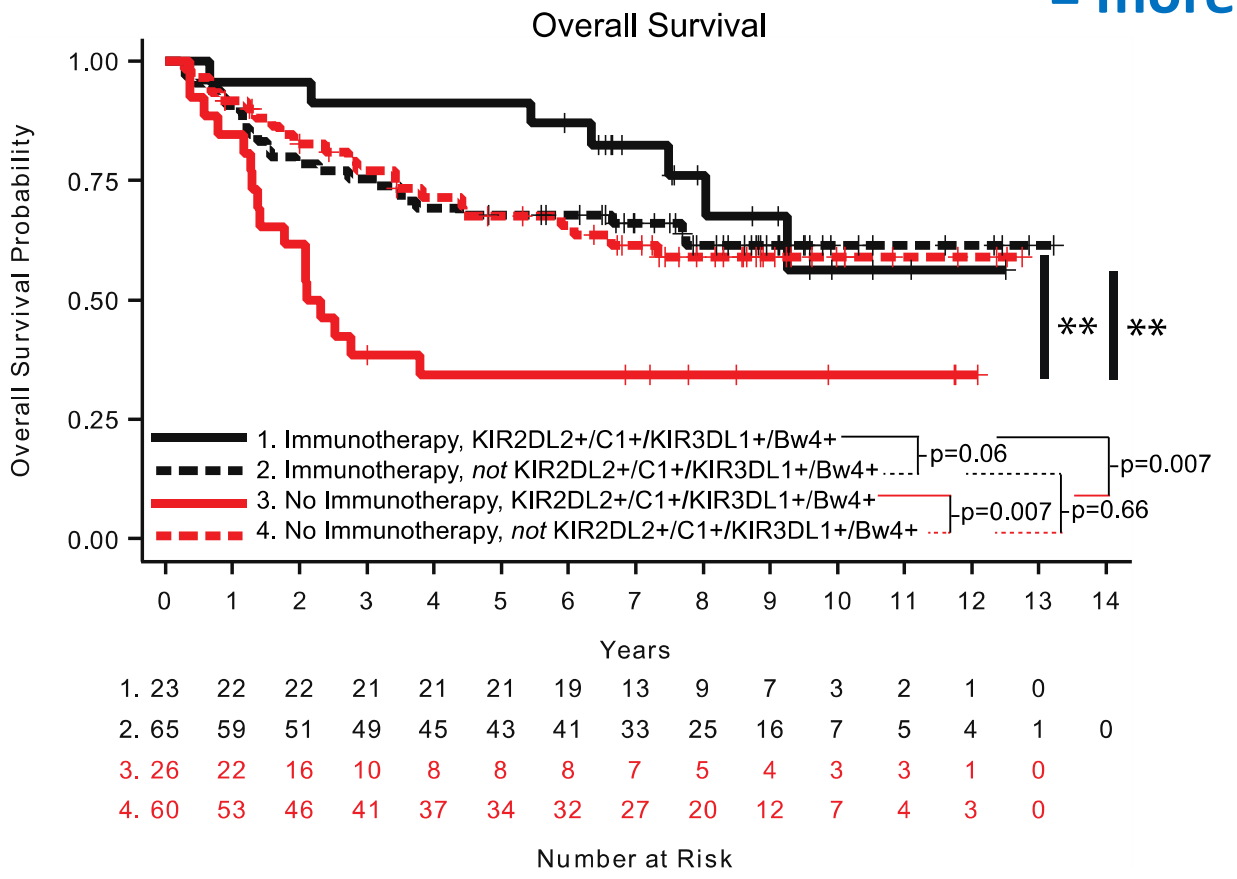
Erbe, Wang et al,
SITC Poster P26 2017;
Clin. Canc. Res. 2017

Overall Survival for 174 pts: based on

*KIR-2DL2+/C1+/KIR3DL1+/Bw4+:#Yes(——)or No (- - - -)

ImmRx vs. No ImmRx

 # = more inhibited



*Analyzed based on findings from labs of J Venstrom and H Lode and our findings from an ECOG NHL study

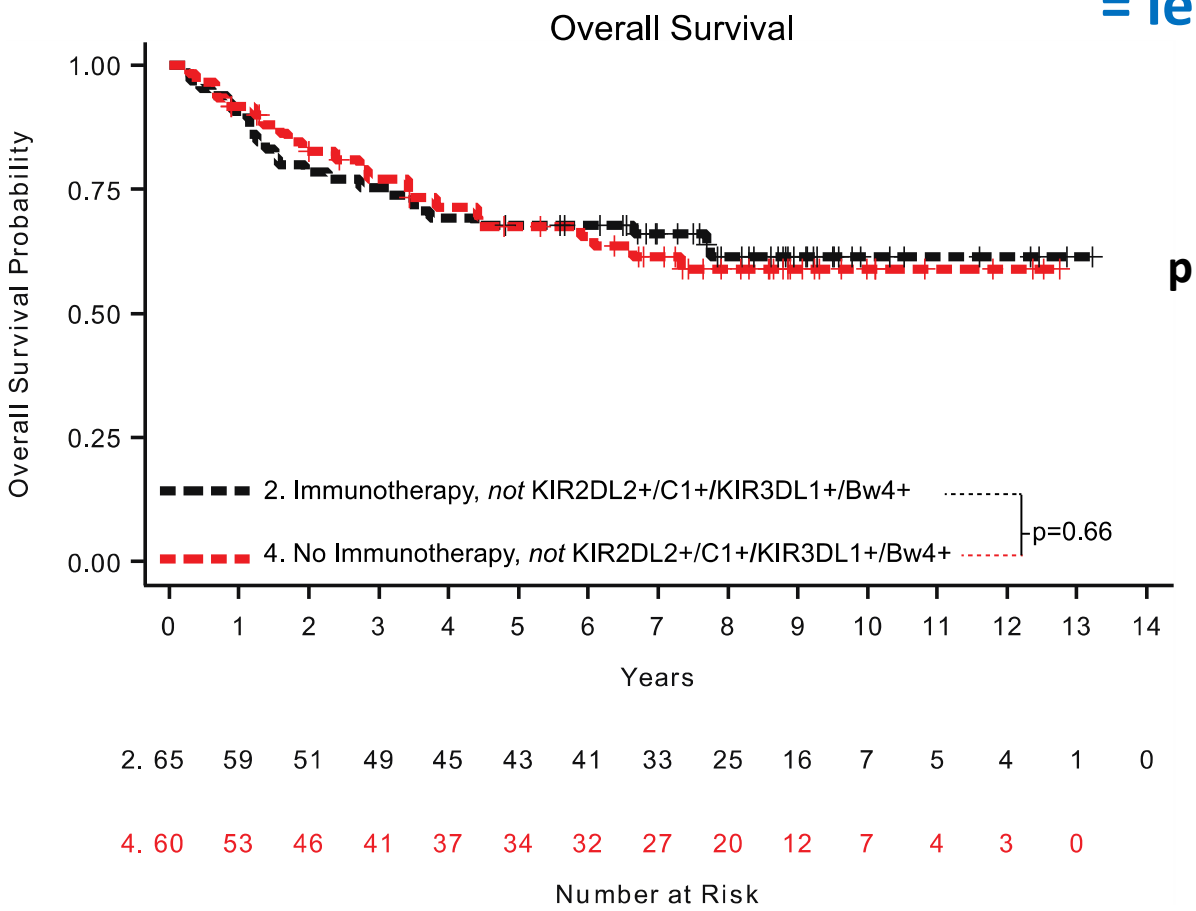
Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

Overall Survival for **125** of 174 pts (72%):

KIR-2DL2+/C1+/KIR3DL1+/Bw4+ : ##No (- - - -).

ImmRx vs. **No ImmRx**

= less inhibited



p = 0.66

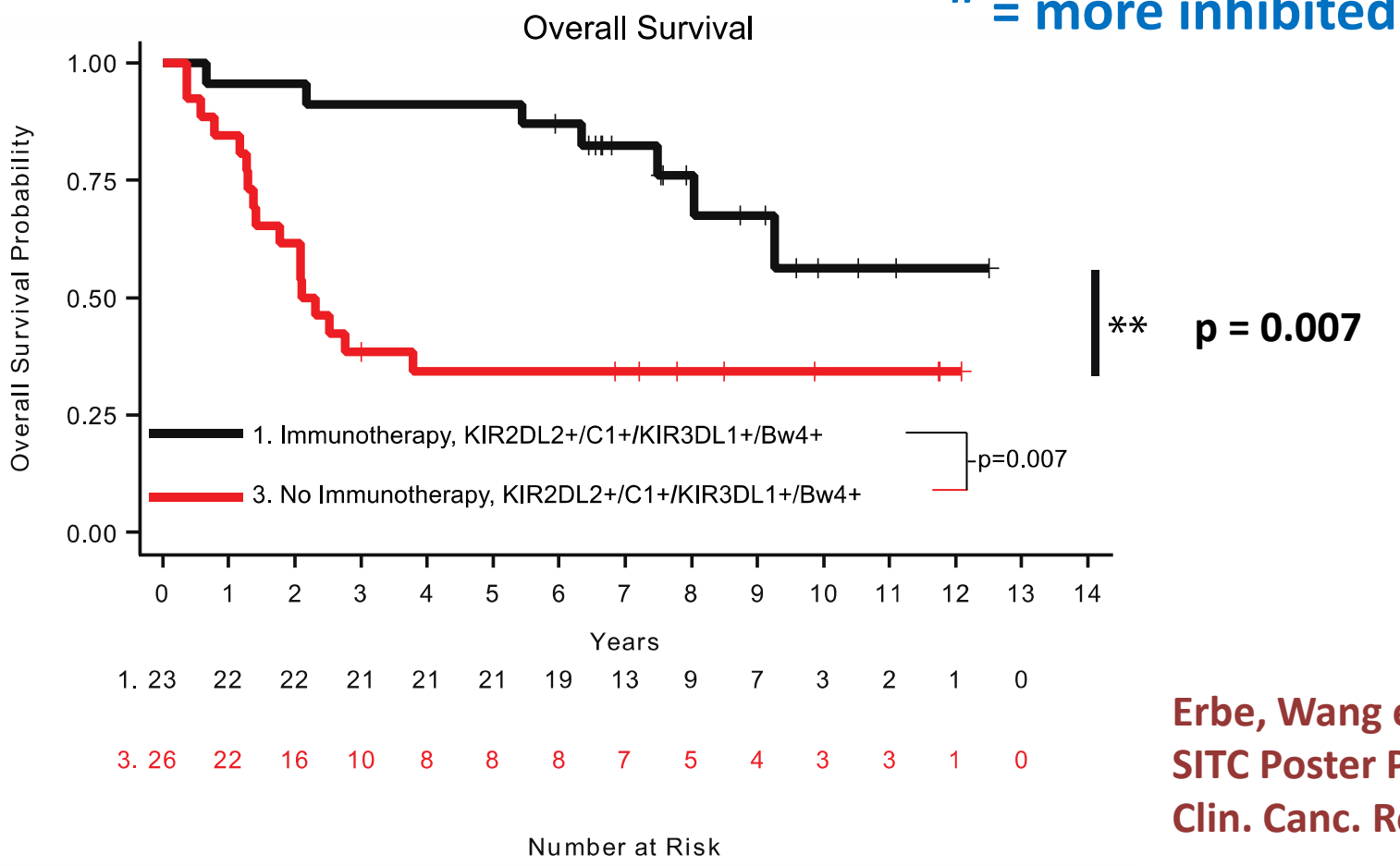
Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

Overall Survival for **49** of 174 pts (28%):

KIR-2DL2+/C1+/KIR3DL1+/Bw4+: #Yes (—— ——).

ImmRx vs. **No ImmRx**

 # = more inhibited



Erbe, Wang et al,
SITC Poster P26 2017;
Clin. Canc. Res. 2017

Summary of KIR Analyses for this Anti-GD2 + GM-CSF + IL2 regimen:

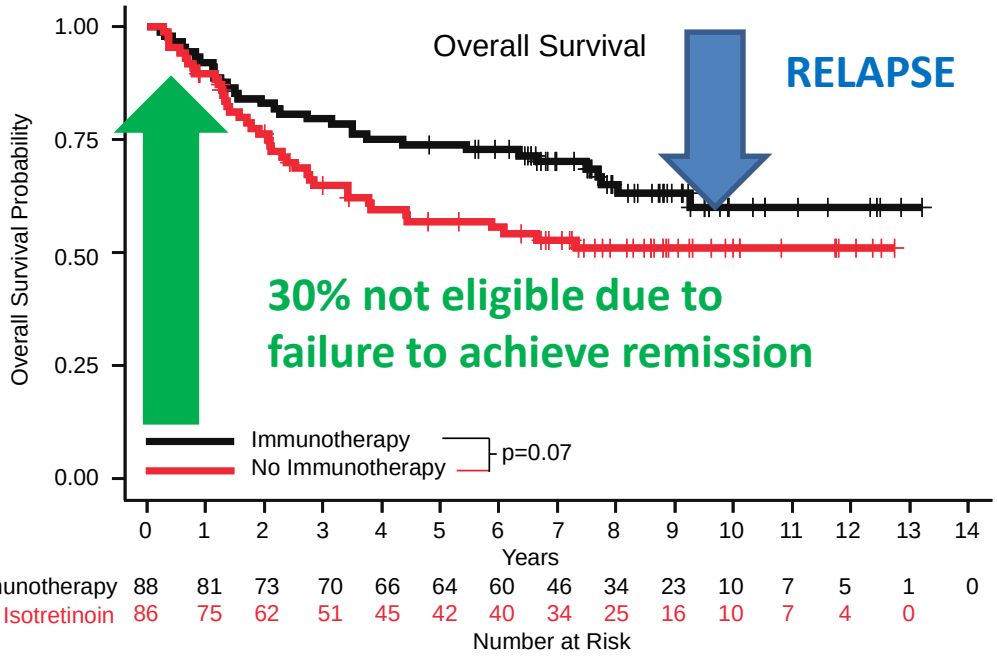
1. **KIR/KIR-L interactions modify ADCC in vitro* and clinical outcome** (NK cells involved)**
2. **This ImmRx may selectively help patients with some KIR/KIR-L genotypes:**
   2DL2+/C1+/KIR3DL1+/Bw4+ (49 of 174 = 28%);
   But the others (72%) don't seem to benefit
3. **IF VALIDATED, *could use KIR/KIR-L genotype as potential eligibility criteria.***

*Wang W, Erbe A. et al. Cancer Imm. Immunother. 65:1047, 2016

**Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

Challenges for Anti-GD2 based ImmRx for NBL:

>40% of patients in remission still die of disease and 30% don't achieve remission. What else can be done for Minimal Residual and Measureable Disease?



Ongoing Clinical and Preclinical Initiatives:

1. **Anti-GD2 mAb + chemotherapy***
2. **Anti-GD2 based CAR-T cells****
3. **Anti-GD2 based immunocytokine**
4. **Local delivery of anti-GD2-based Rx**

*Mody R, et al, Lancet Oncology, 2017

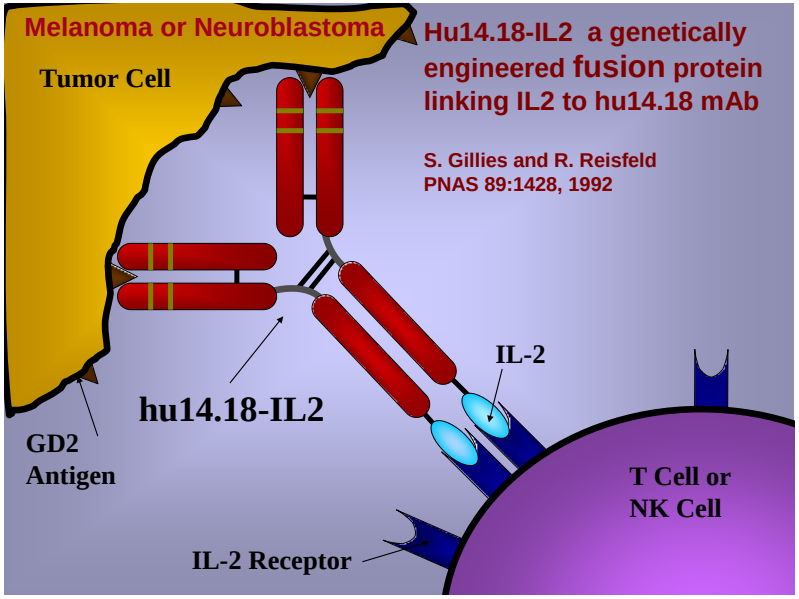
**Long A, et al, Nature Med. 2015

hu14.18-IL2 Immunocytokine (Anti-GD2/IL2 fusion protein)

1. Anti-GD2/IL2 fusion protein14.18-IL2
2. More effective than 14.18 + IL2; I.V.
3. NK cells involved (ADCC)
4. Efficacy in minimal disease setting**

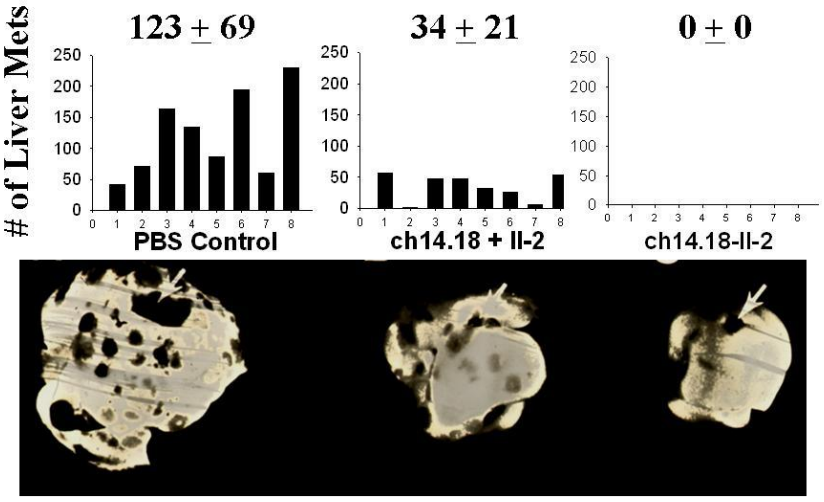
(mouse)*Neal ZC, et al Clin. Cancer Research 2004

(human)*Shusterman S. et al, J. Clin. Onc., 2010, and ASCO Abstract 2015.

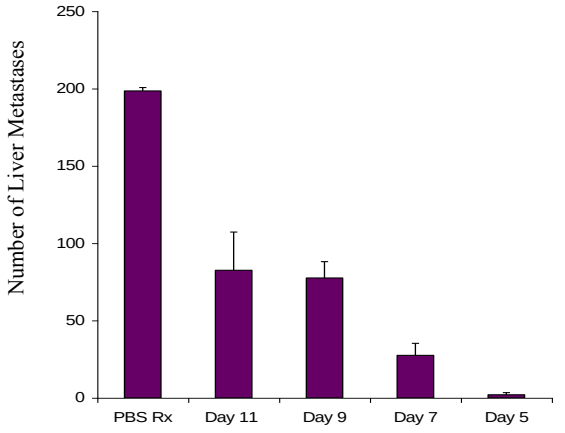


Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

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



Effective anti-GD2 Immunotherapy: Dependence on Minimal Tumor Status



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.

Neal ZC, et al Clin. Cancer Research 2004

An NK cell mediated response

Preclinical Conclusions for hu14.18-IL2 (IC)

More Effective:

1. **than 14.18 mAb + IL2 when given IV**
2. **in MRD setting**

WHY?

Gubbels J, et al: CII , 2011

Buhtoiarov IN, J. Leukocyte Bio. 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)
- **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, et al. J.Clin. Oncol. 28:4969, 2010)

Confirmatory Phase II trial from COG presented at ASCO-2015

(Shusterman et al, ASCO-2015; MS in preparation)

Response associated with KIR-ligand missing genotype:

(Delgado DC, et al. Cancer Res. 70:9554-61. 2010.)

How to increase the efficacy of IC against macroscopic solid tumors?

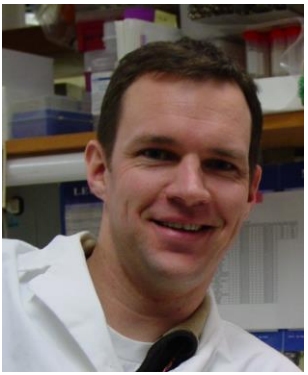
BETTER ENGAGE ADAPTIVE RESPONSE

1. Local delivery, intratumoral injection, provides better activity against *macroscopic tumors*
2. Combine IC with
   a. immunomodulatory radiotherapy
   b. checkpoint blockade

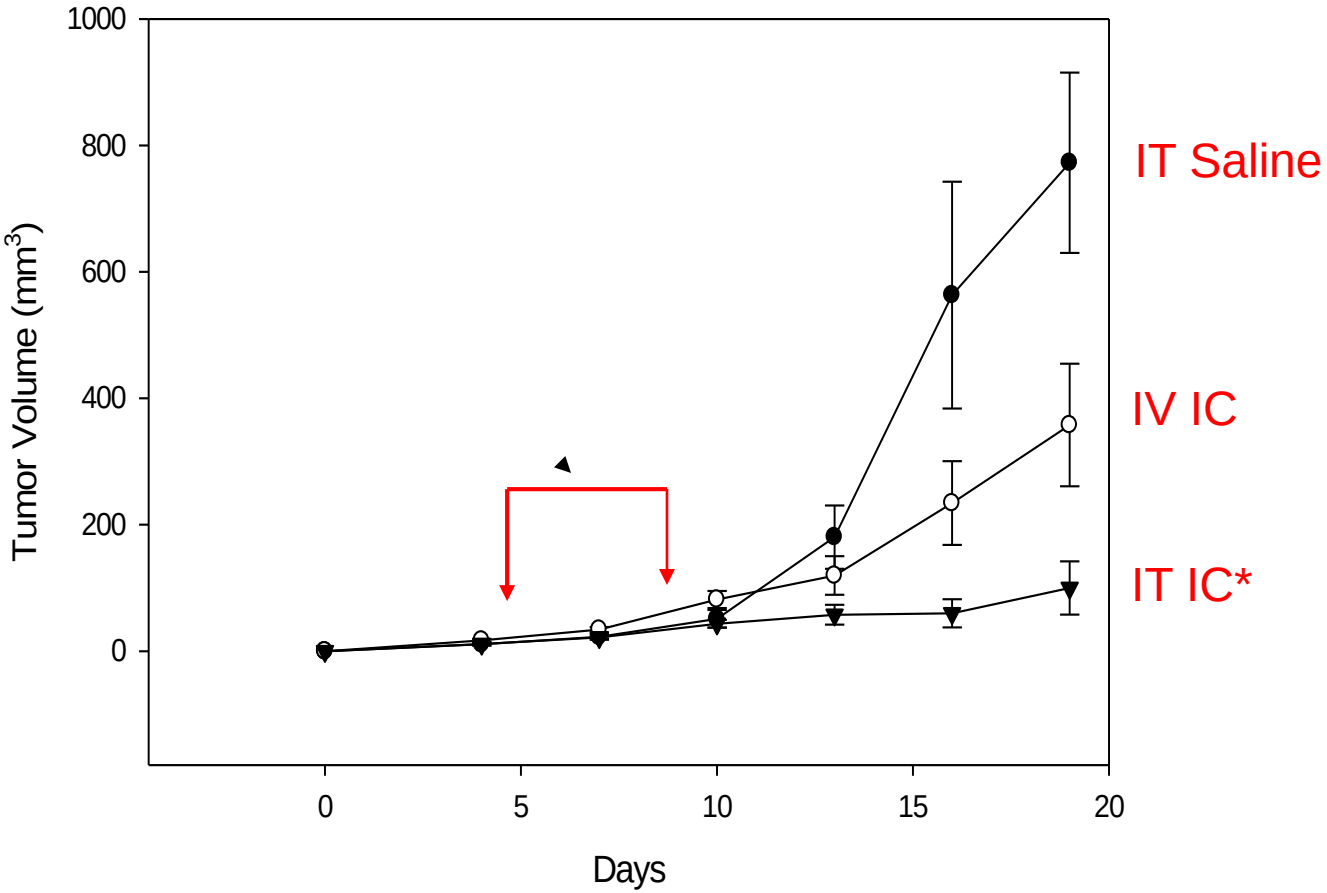
3. Goal: *Make the Tumor an *In Situ Vaccine*

* Marabelle A, Kohrt H, Caux C, Levy R. Clin. Cancer Res. 2014
* Moynihan KD, …… Wittrup KD, Irvine DJ. Nat. Med, 2016

IT IC is More Effective than IV IC in the Treatment of Macroscopic-Palpable (day 5) Murine Melanoma



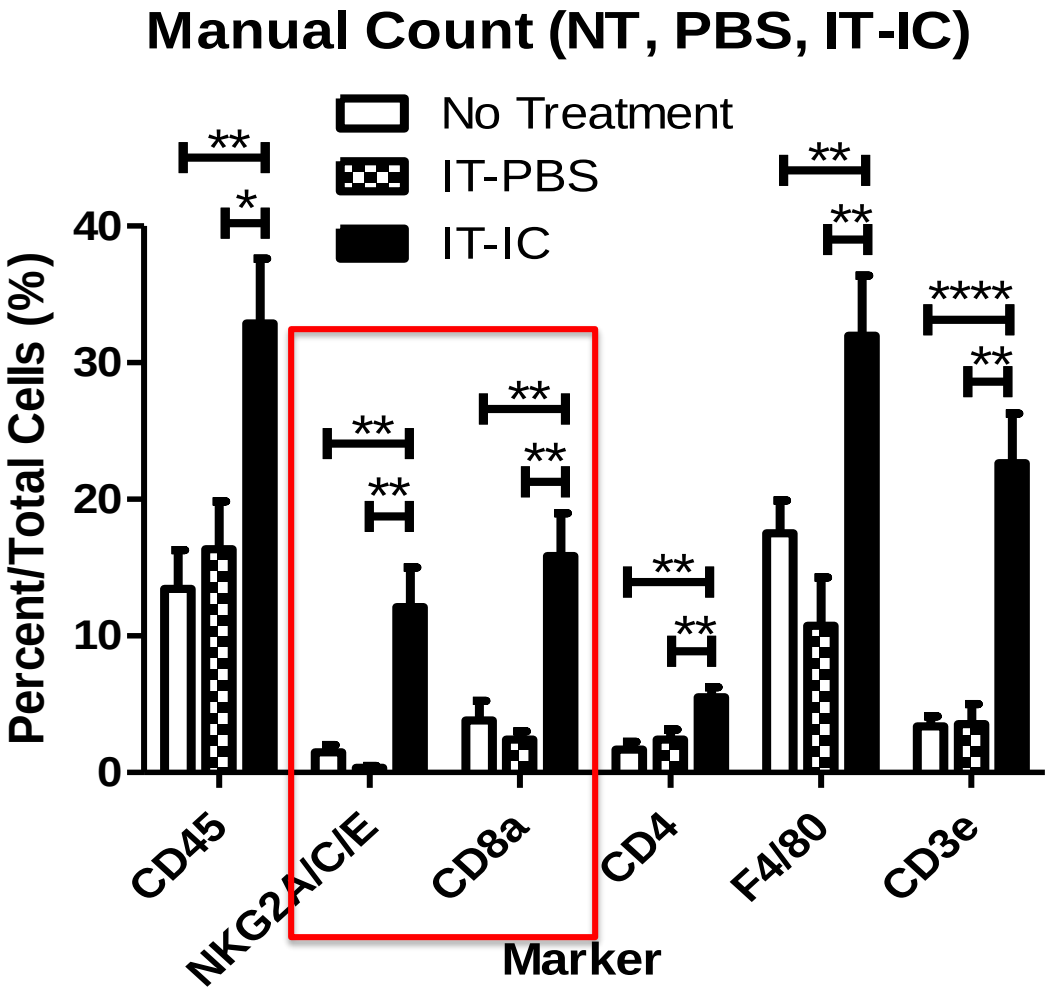
Drs. Eric Johnson and Alexander Rakhmilevich

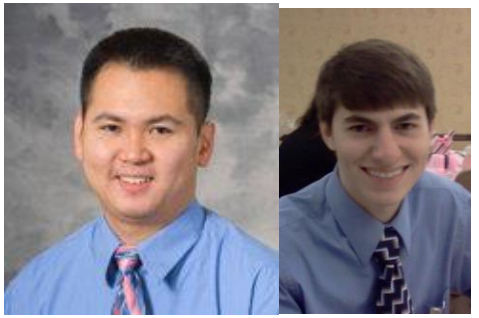


Conclusion: better response with IT than IV IC (5 daily doses).
(Johnson et al, Canc. Imm. Immunother. 57:1891, 2008)

*T-cells are required here and in murine neuroblastoma (not shown)
Yang RK et al. JI. 189:2656, 2012

Intratumoral hu14.18-IL2 is Distinguished by Many Increased Tumor (NXS2-NBL) Infiltrating Lymphocytes (TILs)



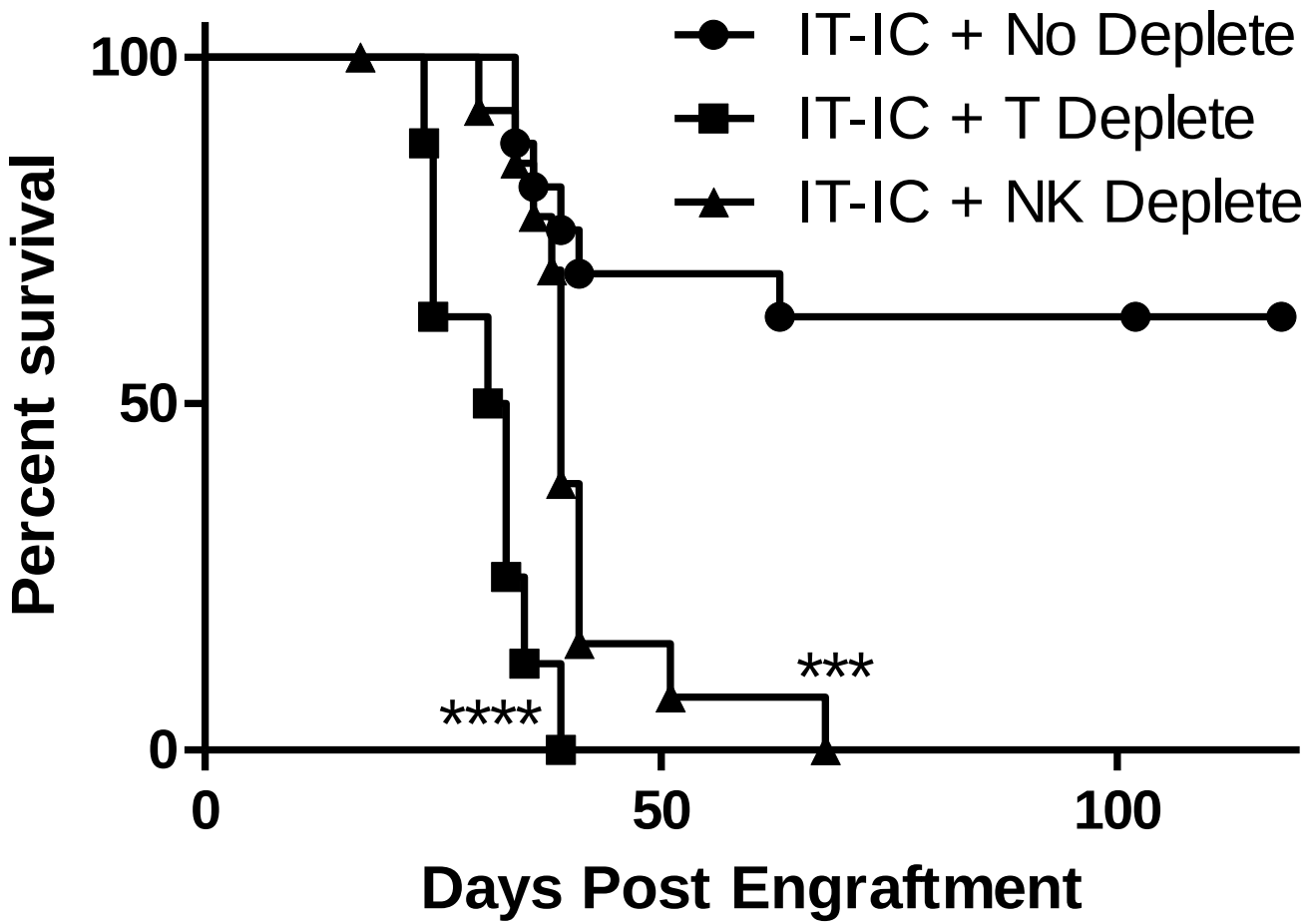


Richard Yang MD PhD,
Nick Kalogriopoulos
And
Erik Ranheim MD PhD

Yang RK et al. JI. 189:2656, 2012

Clinical translation – Yang et al SITC Poster P103 2017

T cell and NK cell Depletion Disrupts Full IT-IC Induced Anti-NXS2 NBL Effects

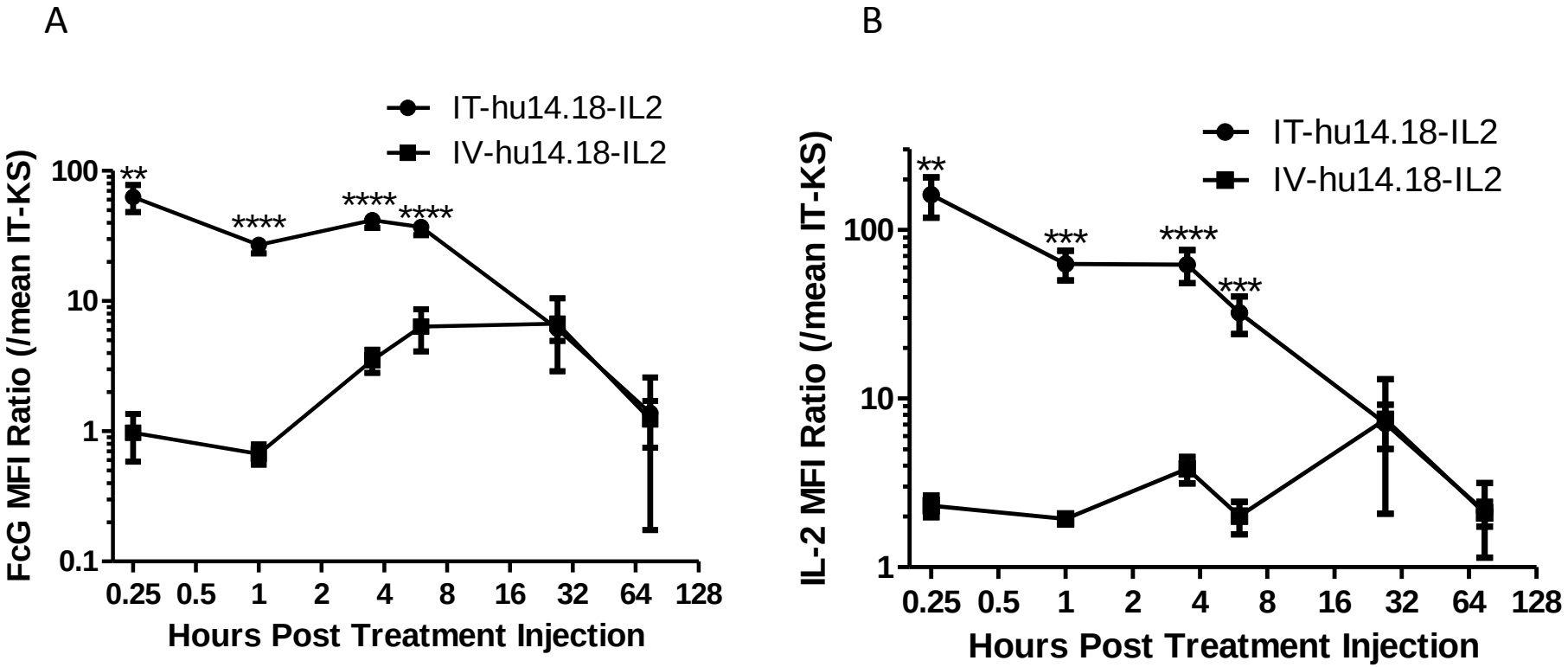


Yang RK et al. JI. 189:2656, 2012

T cells and NK cells Depletion Disrupts Full IT-IC Induced Anti-Tumor Effects

T cell or NK cell depleted, but IT-IC treated mice bearing NXS2 tumor are characterized by increased tumor growth and worse survival outcomes compared to non-depleted IT-IC treated mice bearing NXS2 tumor. (C) Kaplan-Meier survival curves of IT-IC treated subcutaneous NXS2, with and without NK and T cell depletion.

IT-IC Shows 100-fold Augmented IC Localization and Increased IC Retention Compared to IV-IC



Yang RK et al. JI. 189:2656, 2012

IT-IC Shows Augmented IC Localization and Increased IC Retention Compared to IV-IC
Tumor-bearing mice given hu14.18-IL2 IT or IV were sacrificed at varying times and their tumors disaggregated. (A) Flow cytometric measurements of levels of human IgG FcG antibody fragment on NXS2 tumor cells ex-vivo at various times post treatment. (B) Flow cytometric measurements of levels of human IL-2 on NXS2 tumor cells ex-vivo at various times post treatment. All values of MFI (mean fluorescent intensity) are normalized to an intratumoral non-specific control immunocytokine (IT-KS-IL-2).

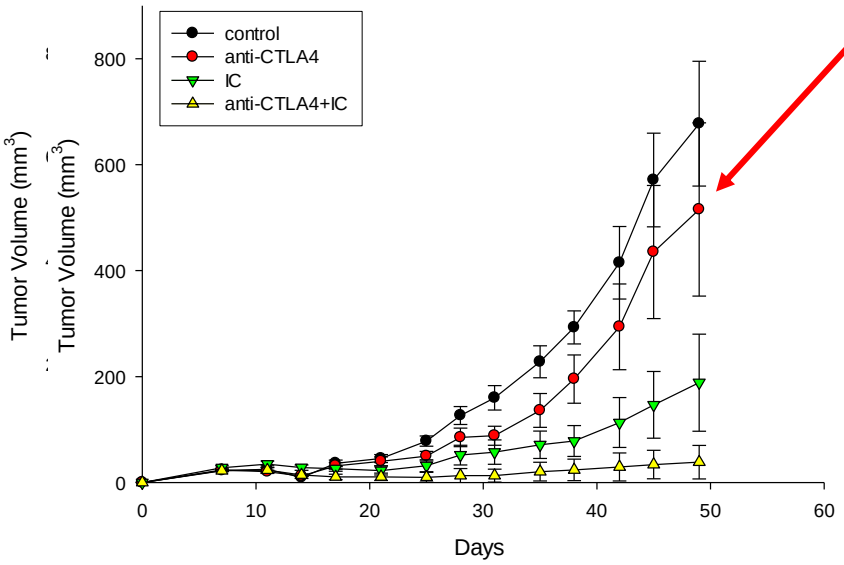
Since T cells are now involved, can we enhance the response with checkpoint blockade?

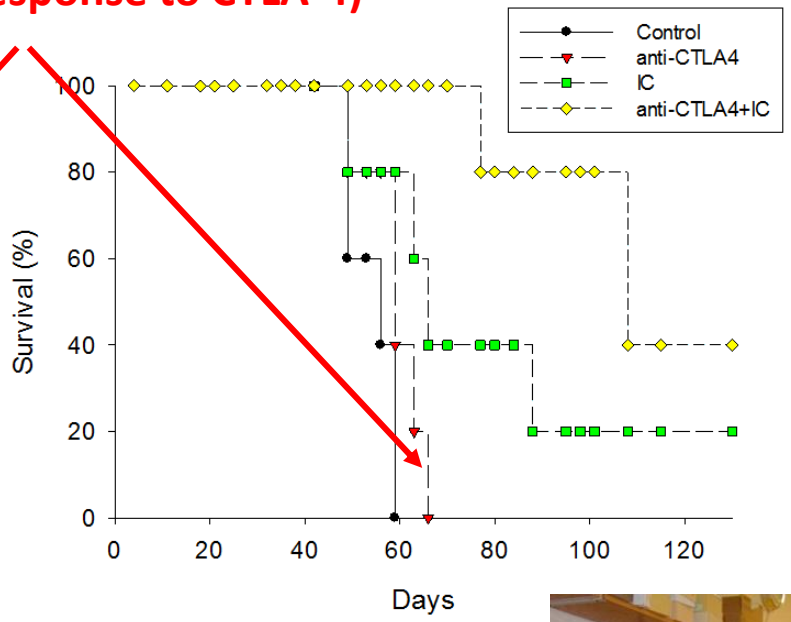
Can Checkpoint Blockade enhance this T-cell response?

[ALL SUBSEQUENT SLIDES WITH B78 (GD2+B16) MEL (weakly immunogenic)]

Effect of anti-CTLA-4 mAb and IT-IC are synergistic on d-7 B78 (<50mm^3)

B78 is a "cold" tumor
(no response to CTLA-4)



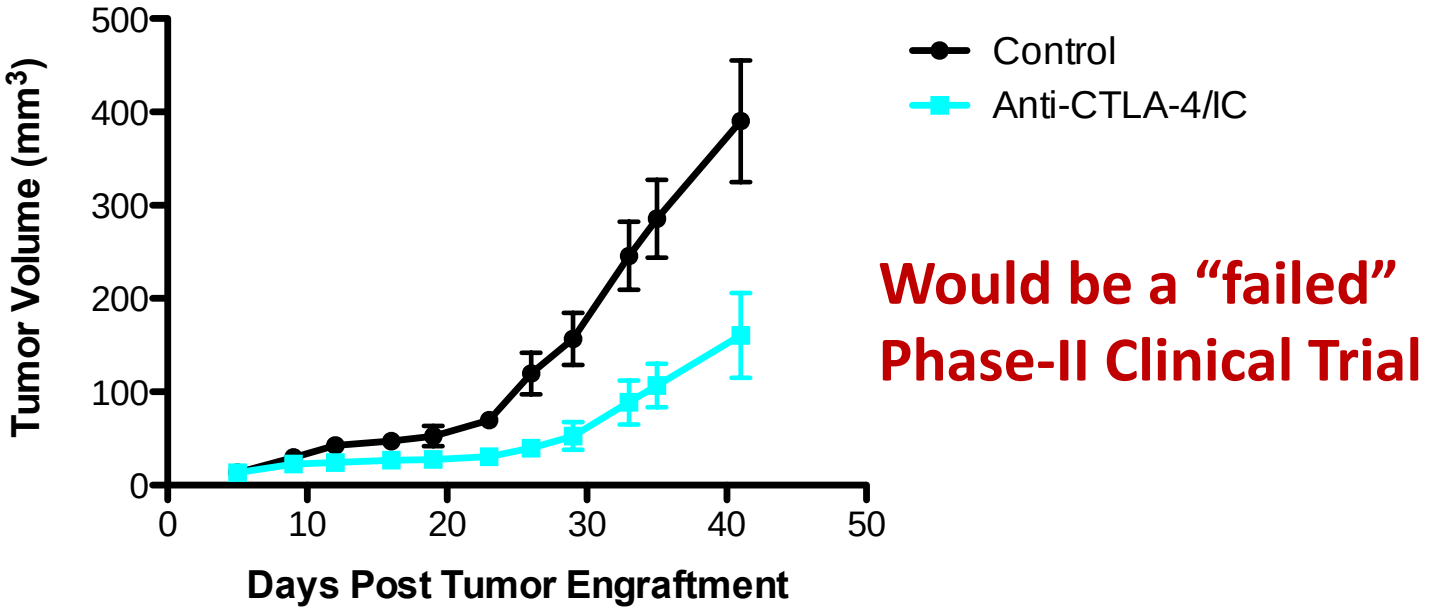


Day 0: B78 s.c. (2x10^6/mouse)
Day 7-11: 14.18-IL2 i.t. (5 mcg/mouse)
Day 7,9,11,14,16,18: anti-CTLA4 i.p. (200 mcg/mouse)

Rakhmilevich AL et al, JI, 2017.

Beneficial effect of IT-IC + anti-CTLA-4 is LESS EVIDENT on more advanced (d12) B78 tumors

Larger (d-12) tumors grow more slowly (but still grow)

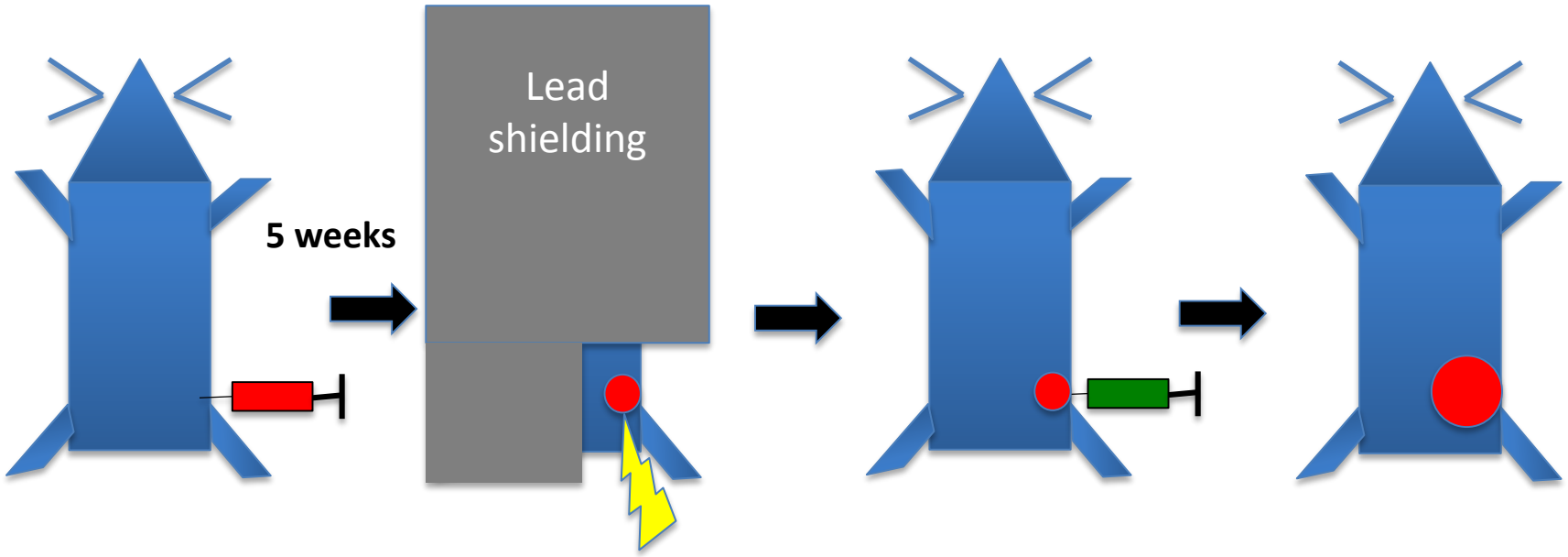


Would be a "failed" Phase-II Clinical Trial

Day 0: B78 s.c. ($2x10^6$/mouse)
IC IT (5 mcg), **d.12-16**; anti-CTLA-4 i.p., d. 12,14,16,19,26,33

Rakhmilevich A., et al; JI, 2017

Can augmented activity to **macroscopic disease (200mm^3)** be obtained by combination with immunomodulatory radiation therapy (RT)?



Tumor cells injected in syngeneic mice

DAY 1. Macroscopic tumors radiated to 12 Gy

DAY 6-10. Daily intra-tumor injections of IC

Tumor growth and animal survival monitored

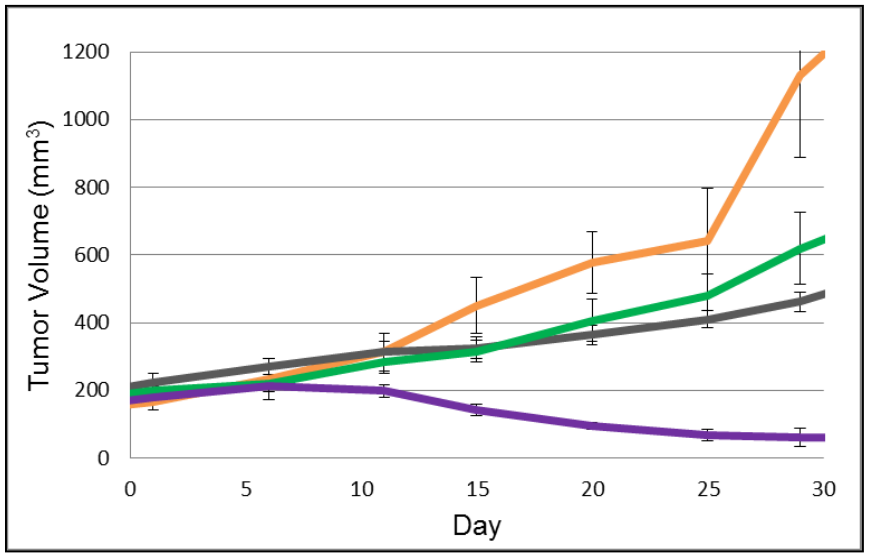
Tumor cells

B78 melanoma – poorly immunogenic B16 melanoma that expresses GD2

Morris Z et al Can. Res. 2016

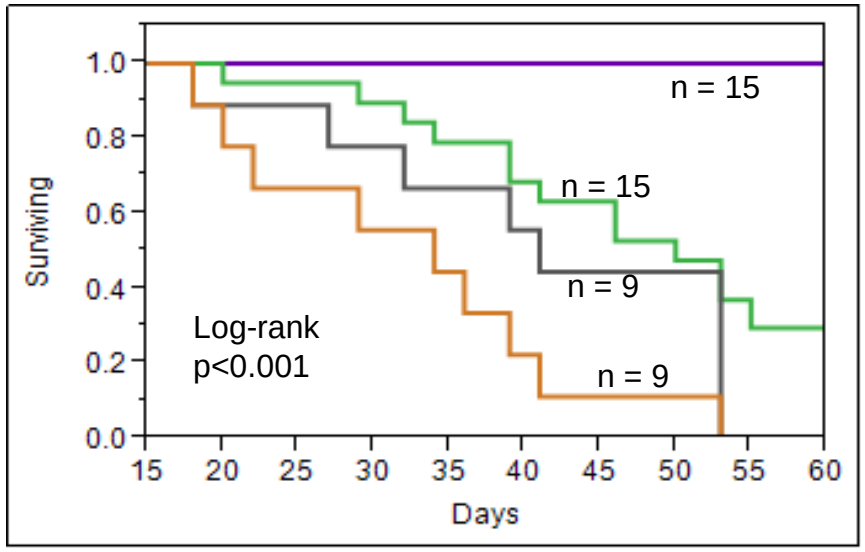
Zach Morris MD PhD

Radiation and IT hu14.18-IL2 results in cure of most ***5-week (200mm³)*** B78 tumors



Day 30 mean tumor volume (mm^3) +- SE
1161 +/- 233
619 +/-106
462 +/- 29
61 +/- 26**

12 Gy + hu14.18-IL2
12 Gy + IgG
hu14.18-IL2
IgG



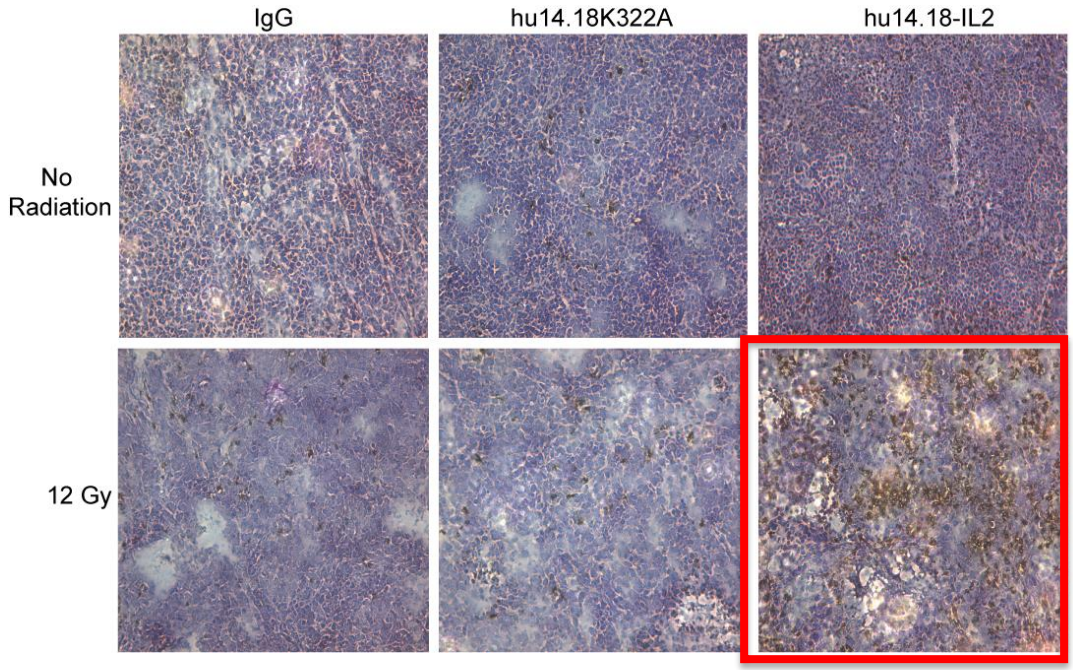
* $p < 0.05$

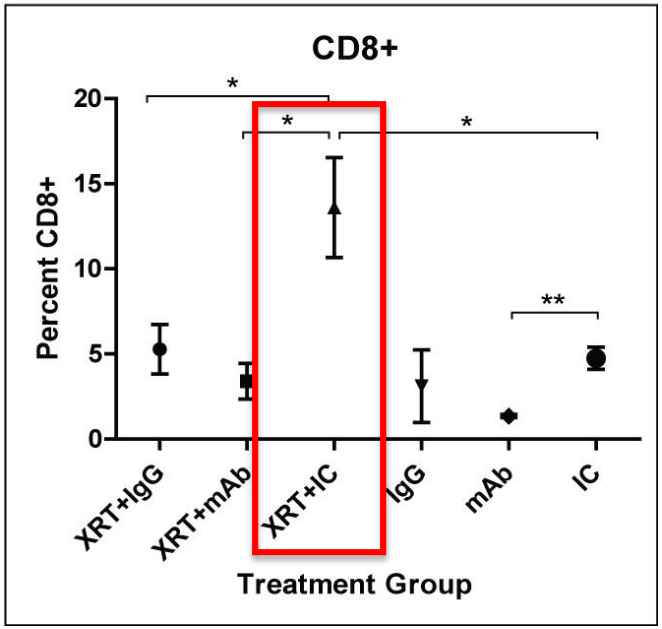
**** 73% (11/15)** of mice had durable complete tumor regression vs. none of the control mice

Morris Z. et al. Cancer Research 2016

RT + IT-IC makes B78 “hot”

(increases tumor infiltration by CD8+ T cells)





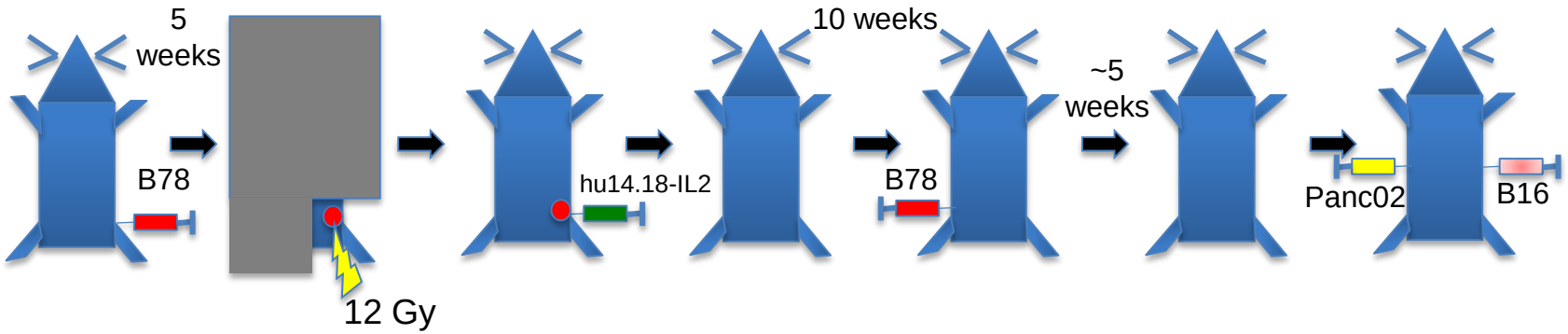
Day 12 post radiation
B78 melanoma tumors

* $p < 0.05$

Morris Z. et al. Can. Res. 76:3929, 2016

RT + IT-IC induces a tumor-specific T cell response with epitope spread

(In Situ Vaccine Effect, as introduced by R. Levy and colleagues)



Mechanisms for this In Situ Vaccine Effect[A] (not shown):

1. IC is needed (anti-GD2 alone with RT yields no vaccine effect)
2. T cells required (minimal effect if T-cells depleted)
3. FcR required (minimal effect in $FcR^{-/-}$ mice)[B]
4. GD2 on tumor is needed (weak effect on GD2- MEL)
5. Timing is key (give IT-IC 6d after RT)
6. Fas is upregulated by RT on tumor (no vaccine effect in $FasL^{-/-}$ mice)[C]
7. Tumor reactive antibody found in serum[D]
8. Protection from rechallenge is systemic (even in the brain)[E]

[A]Morris Z. et al. Cancer Research 76:3929, 2016

[B]*Suggests mAb/FcR-dependent antigen uptake/presentation rather than ADCC*

[C]Werner L. et al, Radiation and Oncology, 124; 418, 2017

[D]Heinze et al, Poster P37, SITC 2017

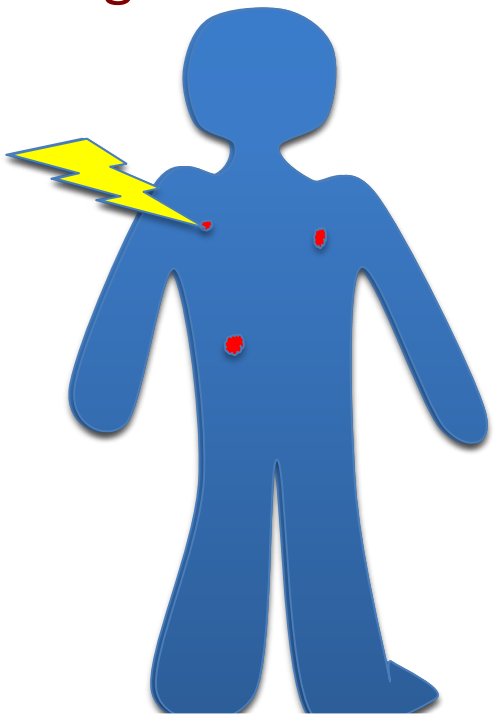
[E]Sriramaneni R. et al, Poster P142, SITC 2017

Cancer is seldom an isolated primary tumor. What about metastases?

The abscopal response to radiation is thought to be immune-mediated

"The abscopal effect"

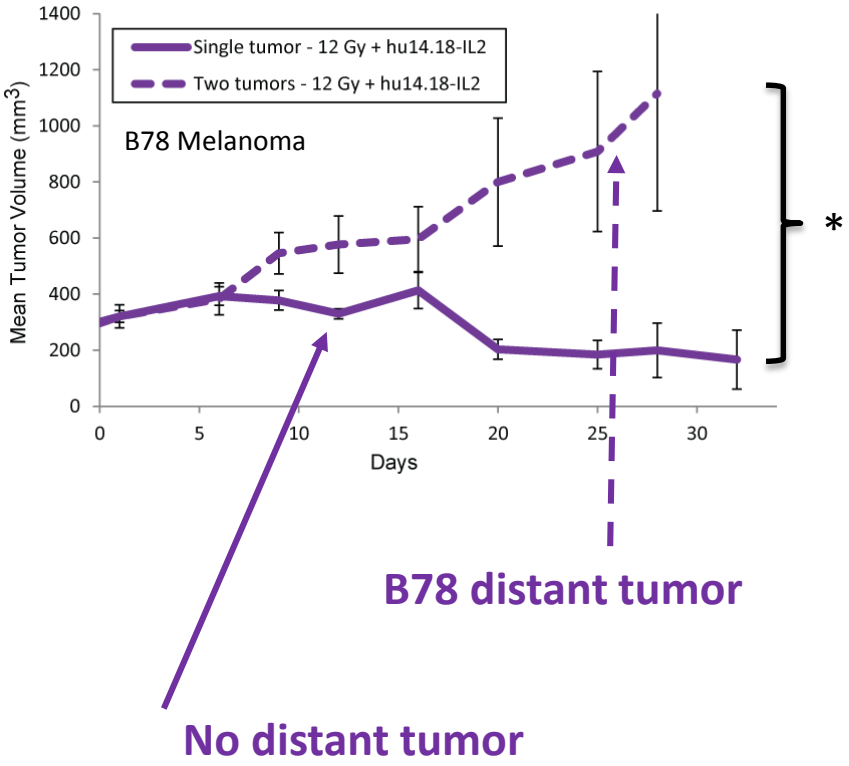
- *Mole, Br J Radiology 1953*

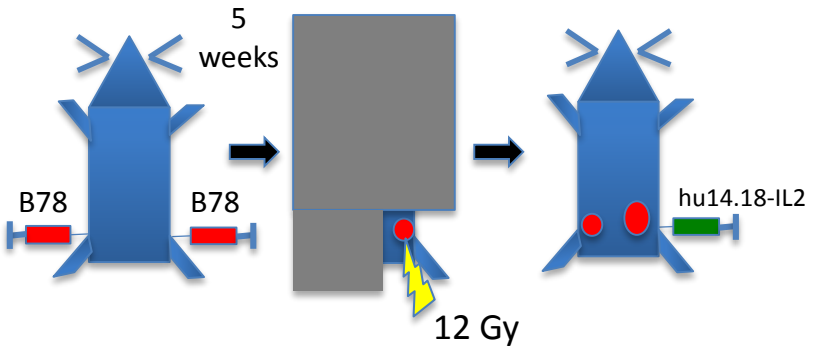


Morris Z et al. Abstract at AACR 2016, manuscript Submitted 2017

Concomitant Immune Tolerance of primary B78 tumor response to RT and IT-IC by a distant un-treated B78 tumor

Primary (treated) B78 tumor responses





Tumor-induced *Concomitant Immune Tolerance*

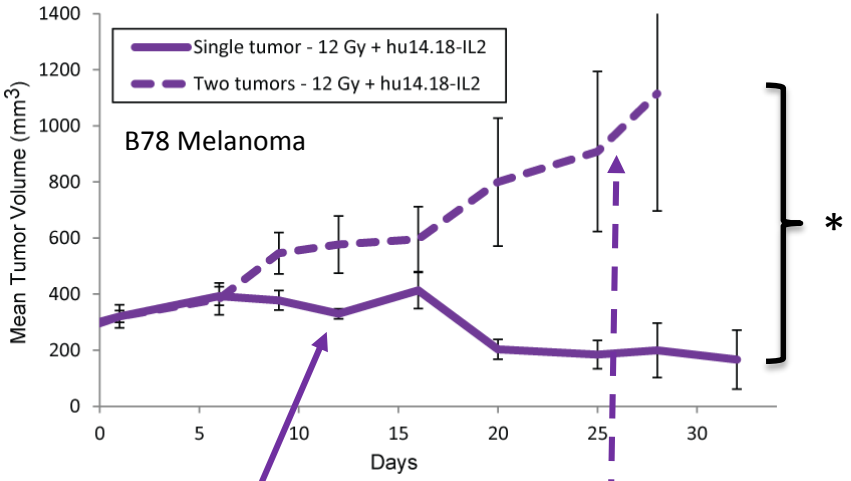
Morris Z et al. Abstract at AACR 2016, manuscript submitted

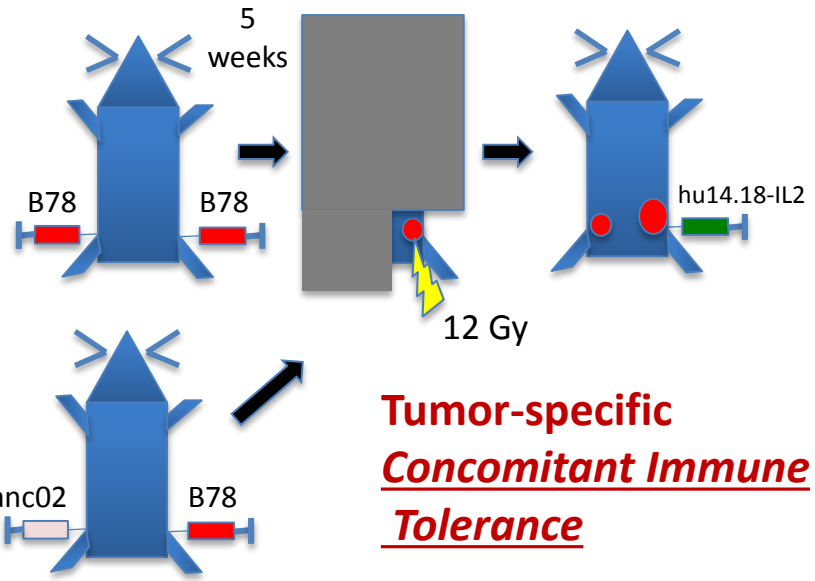
Radiation: 12 Gy x 1 - Day 1
hu14.18-IL2: 50 µg /mouse daily - Days 6-10

* $p < 0.001$

The B78 (but not Panc02) distant tumor suppresses In Situ Vaccination of the primary B78 tumor

Primary (treated) B78 tumor responses



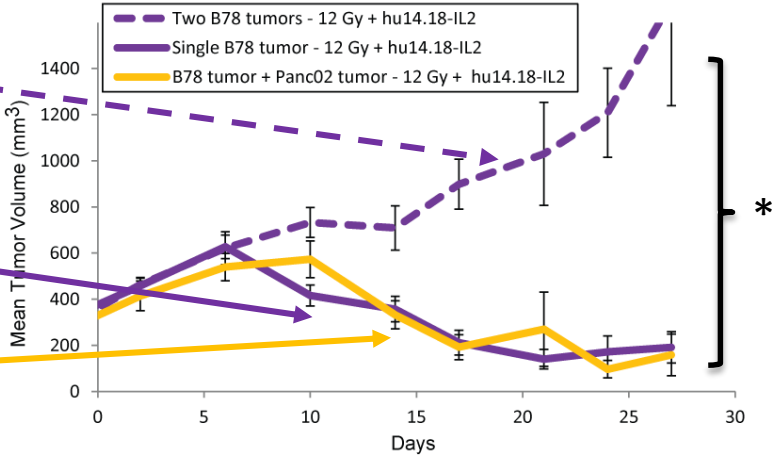


Tumor-specific *Concomitant Immune Tolerance*

B78 distant tumor

No distant tumor

Panc02 distant tumor



Radiation: 12 Gy x 1 - Day 1
hu14.18-IL2: 50 µg /mouse daily - Days 6-10

* $p < 0.001$

Morris Z et al. Abstract at AACR 2016, manuscript submitted

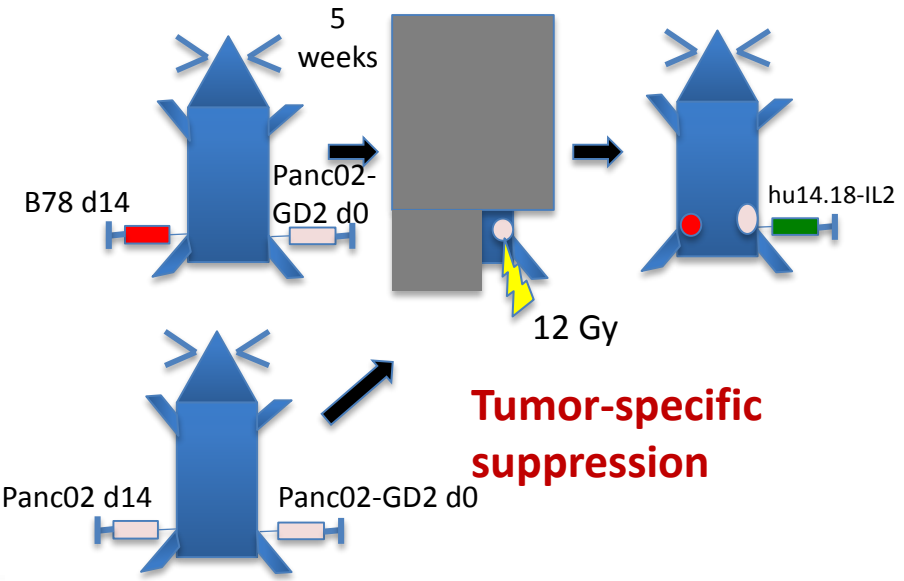
Concomitant Immune Tolerance shows reciprocal specificity.

Use Panc02-GD2 as the primary (d0) tumor.

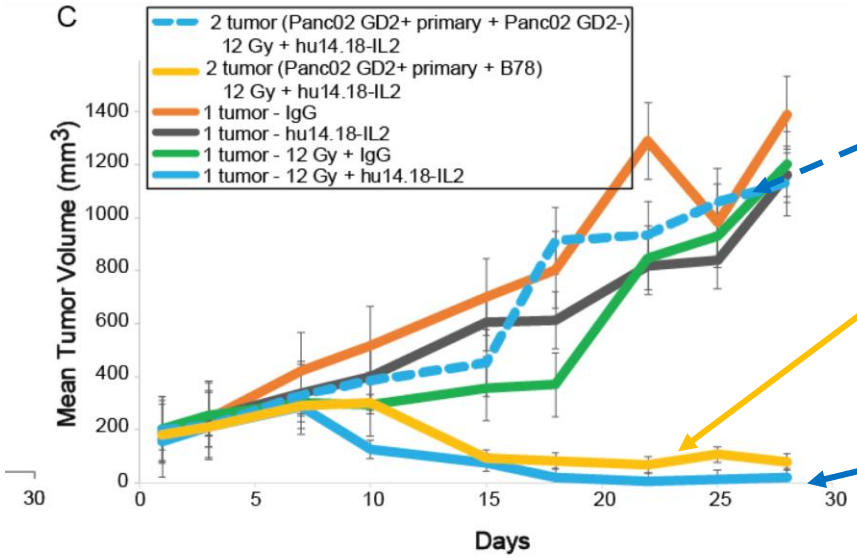
Immune destruction of the Primary Panc02-GD2 tumor is prevented in animals with a Panc02 2nd tumor

BUT occurs in animals with A B78 2nd tumor

Tumor Specific *Concomitant Immune Tolerance*



Tumor-specific suppression



Panc02 distant tumor

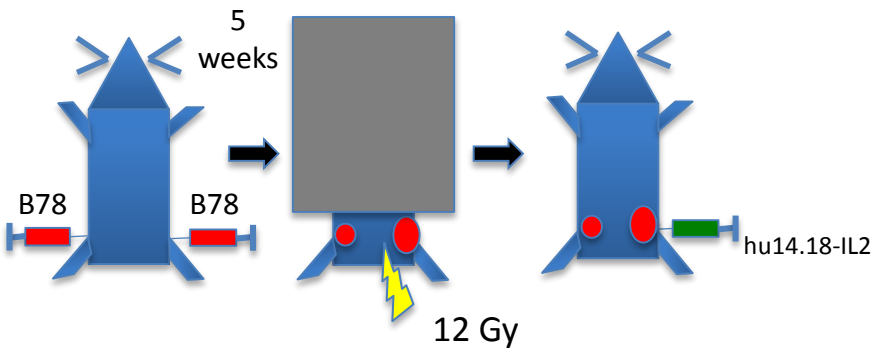
B78 distant tumor

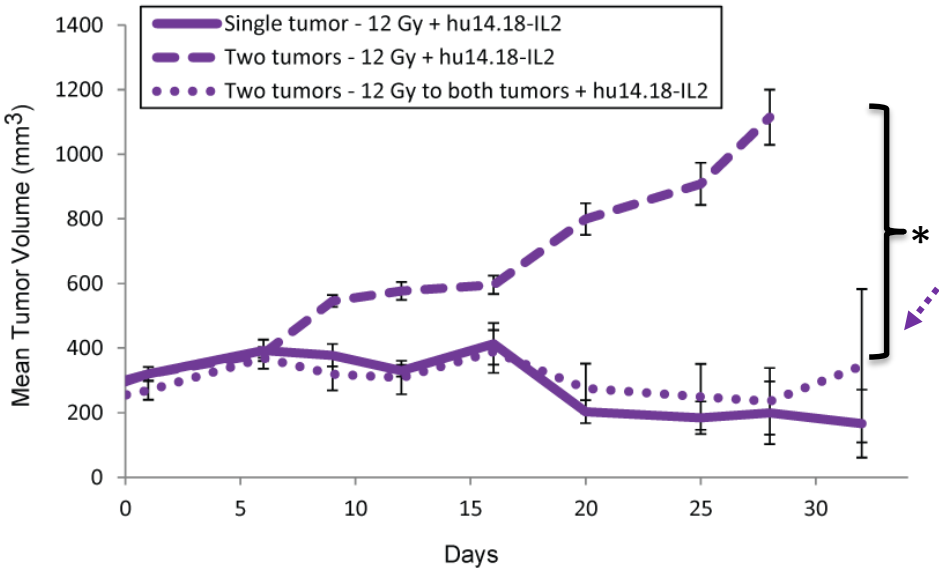
No distant tumor

Morris Z et al. Abstract AACR 2016, manuscript submitted

Can inhibition of primary tumor response to RT + IT-IC by 2nd tumor be overcome?

(How to overcome Concomitant Immune Tolerance?)





Yes; by RT to both tumors

* $p < 0.001$

Radiation: 12 Gy x 1 – Day 1
hu14.18-IL2: 50 µg /mouse IT - daily Days 6-10

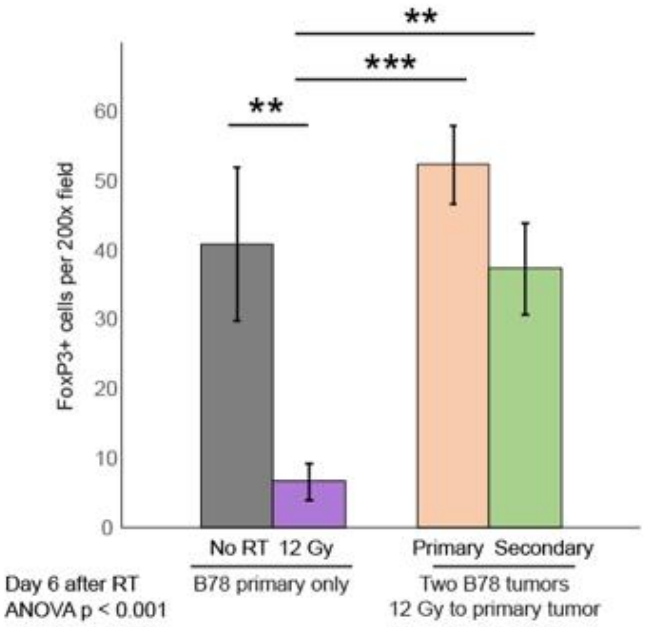
Primary tumor response

Morris Z et al. Abstract AACR 2016,
manuscript submitted

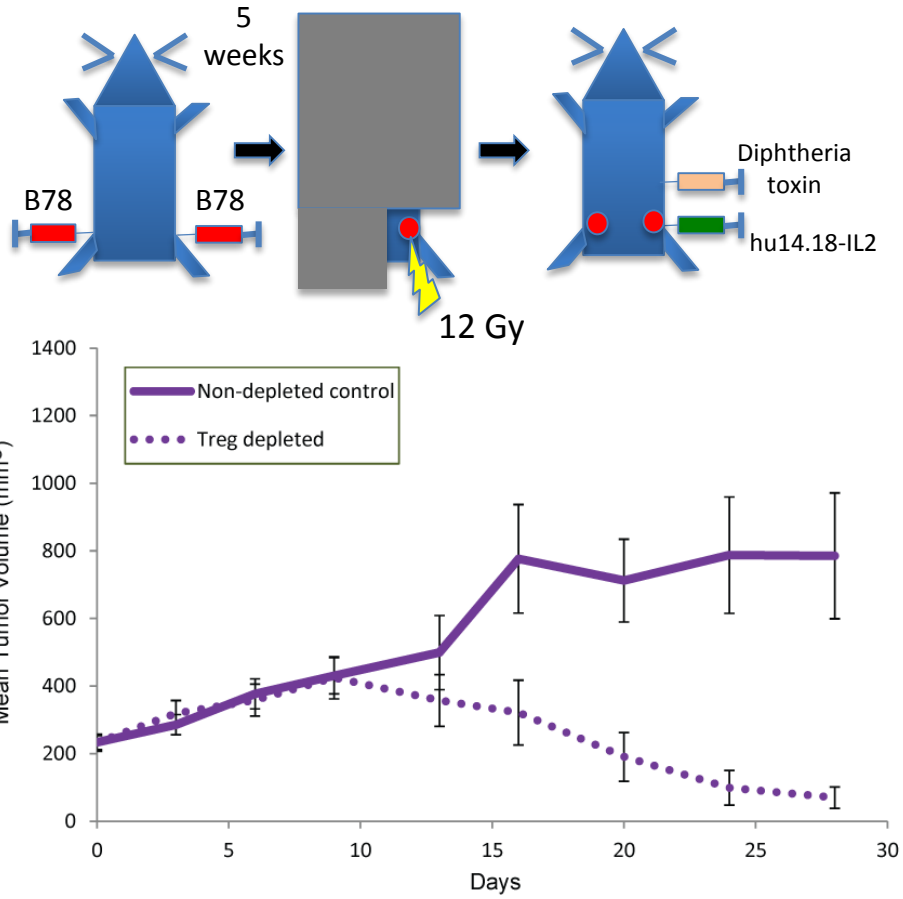
Tumor-specific, RT-sensitive immune tolerance: Role of Tregs

(Tregs cells from a distant B78 site suppress the In Situ Vaccine effect of RT + IT-IC)

Cross-talk with Treg distribution Between irradiated and non-irradiated tumor



C57BL/6-Tg(Foxp3-DTR/EGFP)23.2Spar/Mmjax
"DEREG mice"

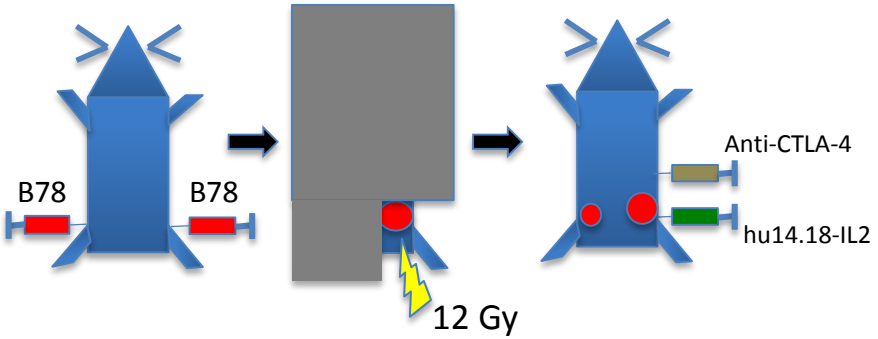


Morris Z et al. Abstract AACR 2016, manuscript submitted

B78 Melanoma
Radiation: 12 Gy x 1 – Day 1
hu14.18-IL2: 50 µg /mouse daily – Days 6-10
Diphtheria toxin: 1 µg IP – Day 1

Concomitant Immune Tolerance can be overcome by a Treg-depleting anti-CTLA4

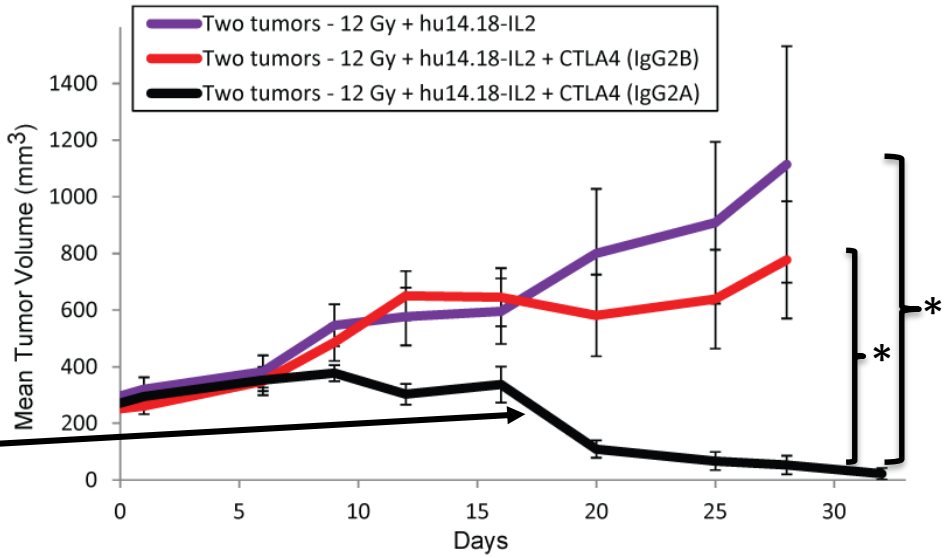
Alan Korman PhD, BMS



Treg depleting (IgG2a) anti-CTLA-4

Primary 200 mm^3 B78 tumor receiving RT + IT-IC

The IgG2a but not the IgG2b anti-CTLA4 depletes Tregs and enables the immunotherapeutic effect to the PRIMARY (injected) tumor



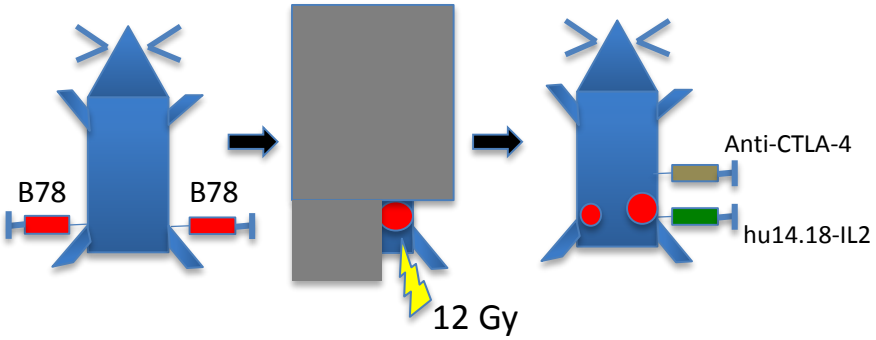
Radiation: 12 Gy x 1 – Day 1
hu14.18-IL2: 50 µg /mouse IT - daily Days 6-10
anti-CTLA-4: 200 µg /mouse IP – Days 3, 6, 9

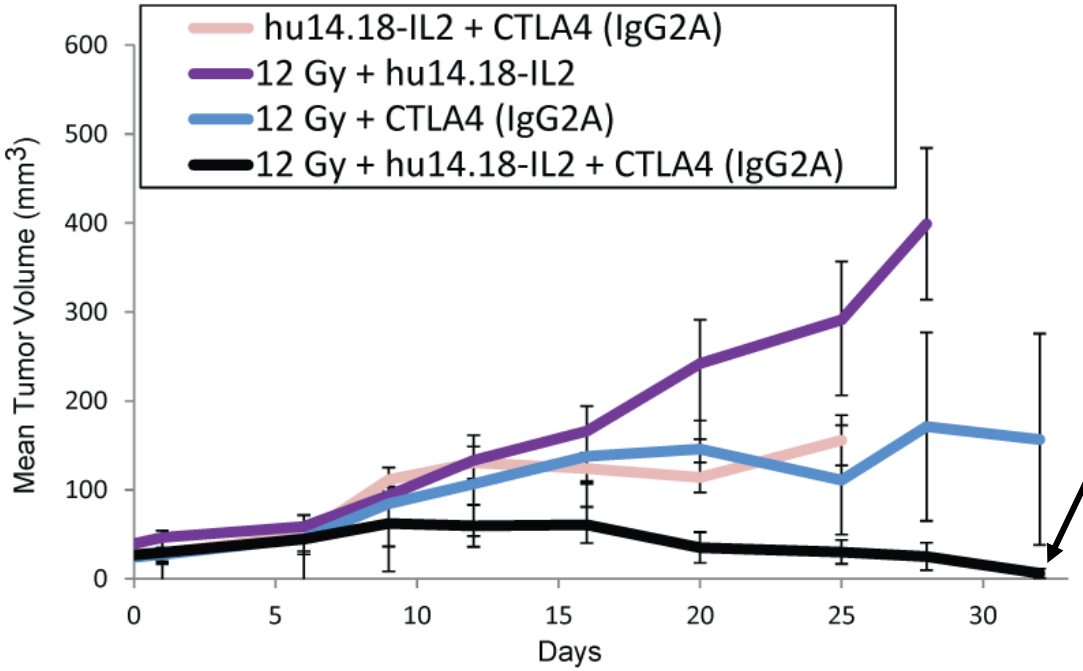
Morris Z et al. Abstract AACR 2016, manuscript submitted

* $p < 0.001$

Concomitant Immune Tolerance can be overcome by a Treg-depleting anti-CTLA4 mAb And effectively eliminate the non-irradiated non-injected tumor

Response of the 2nd (non-irradiated, non-injected) tumor



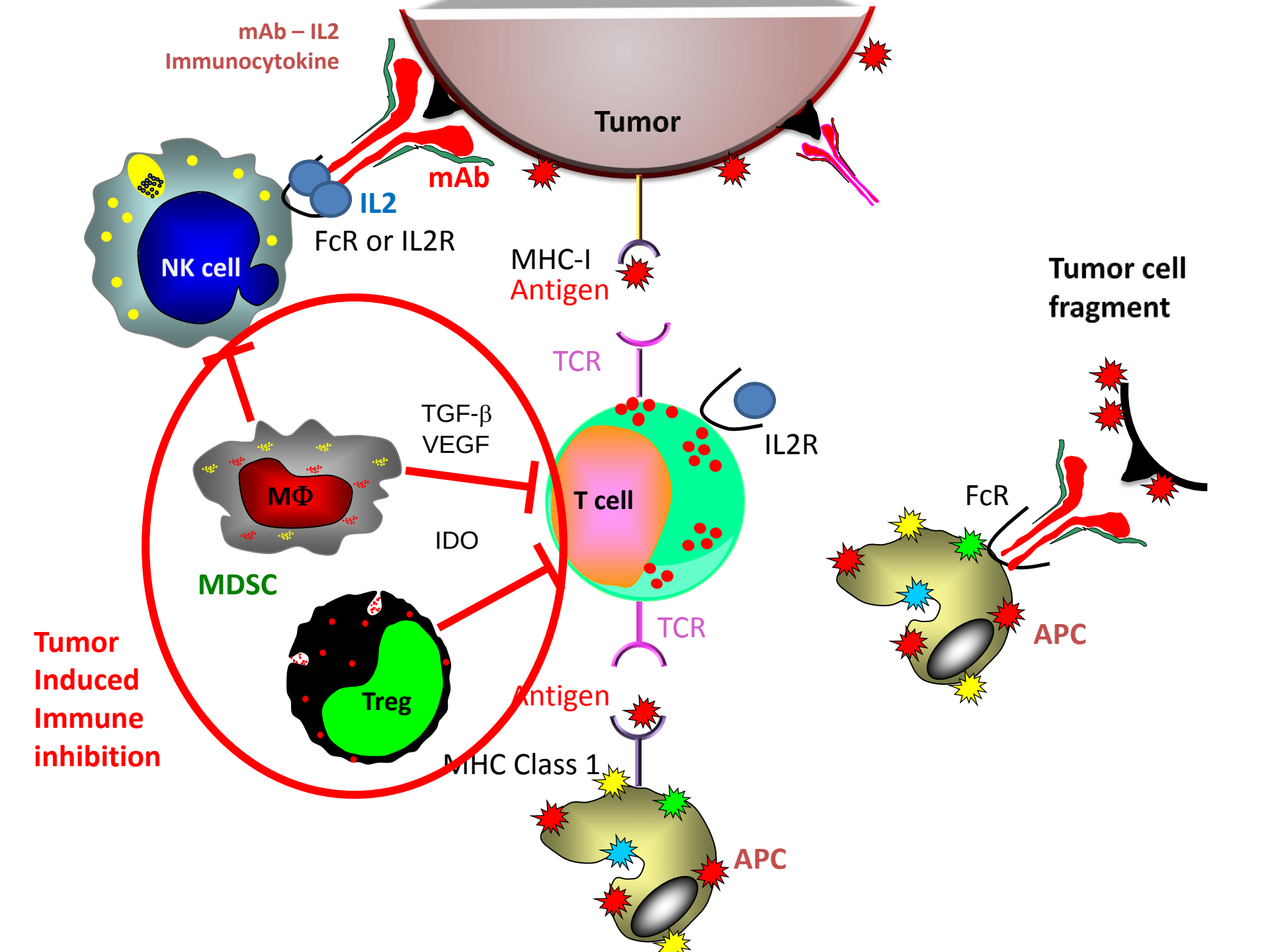


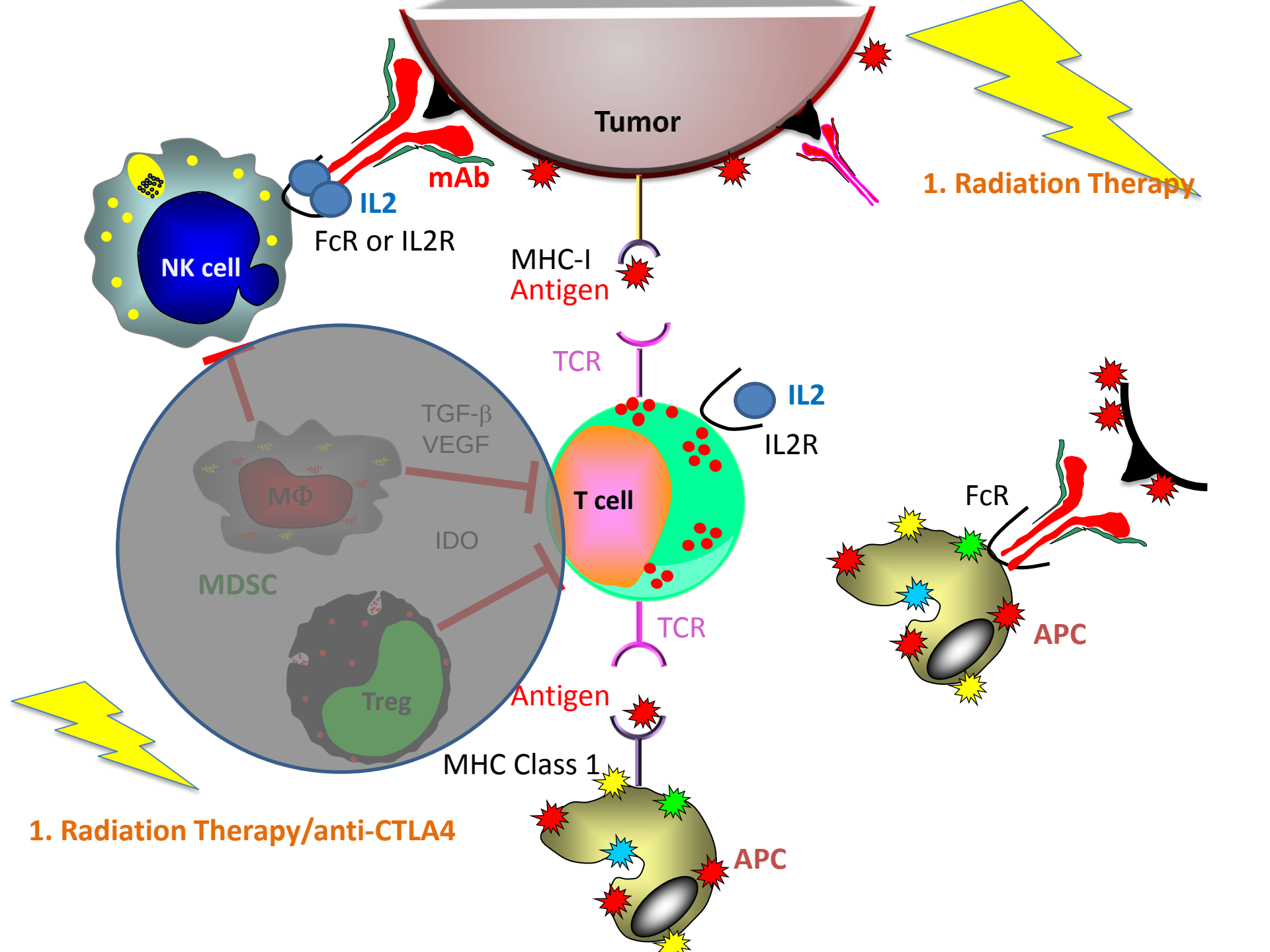
Alive at 60 days: RT + hu14.18-IL2 + CTLA4 = 75% (12/16)
systemic immune response

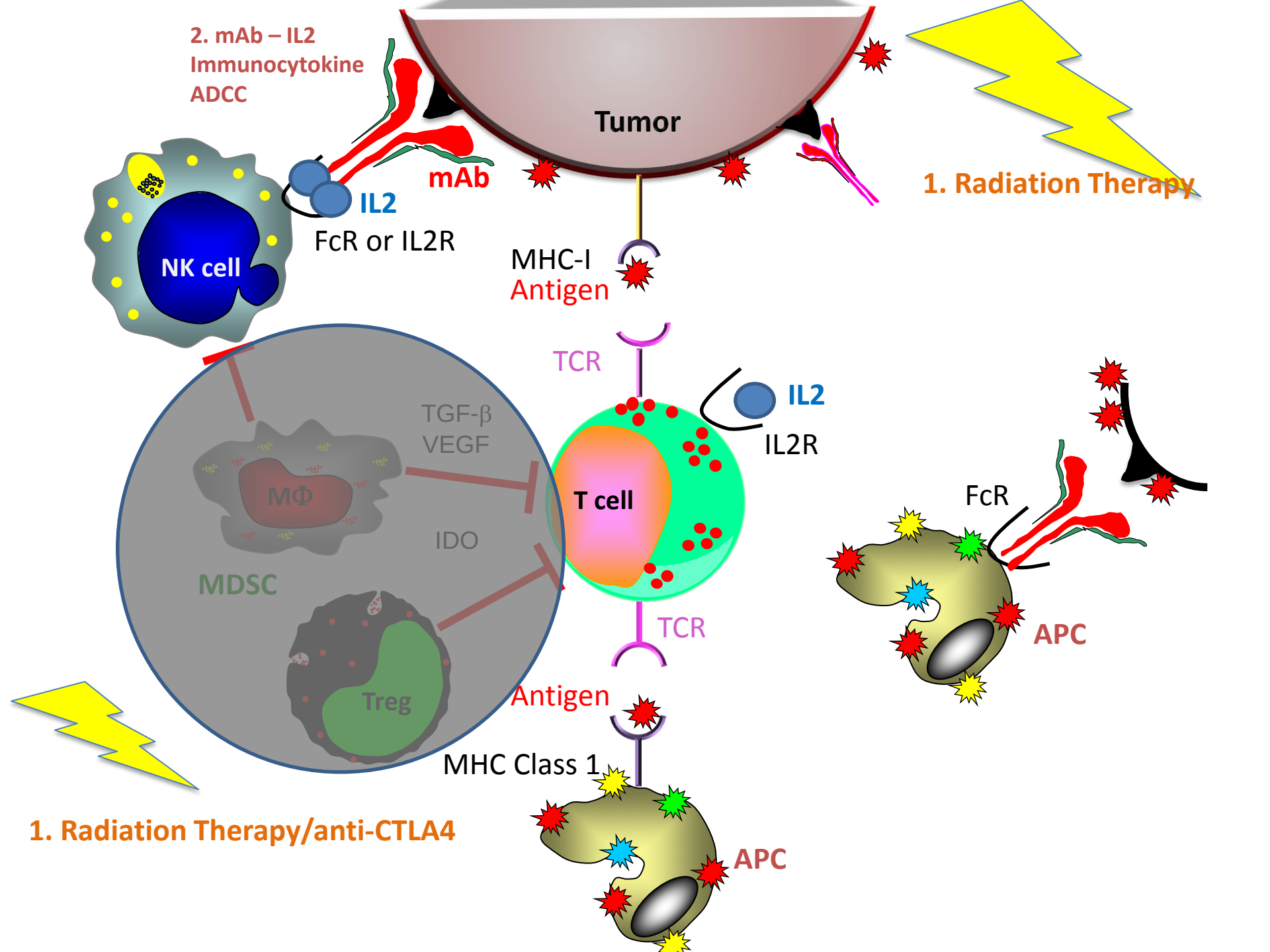
* $p < 0.001$

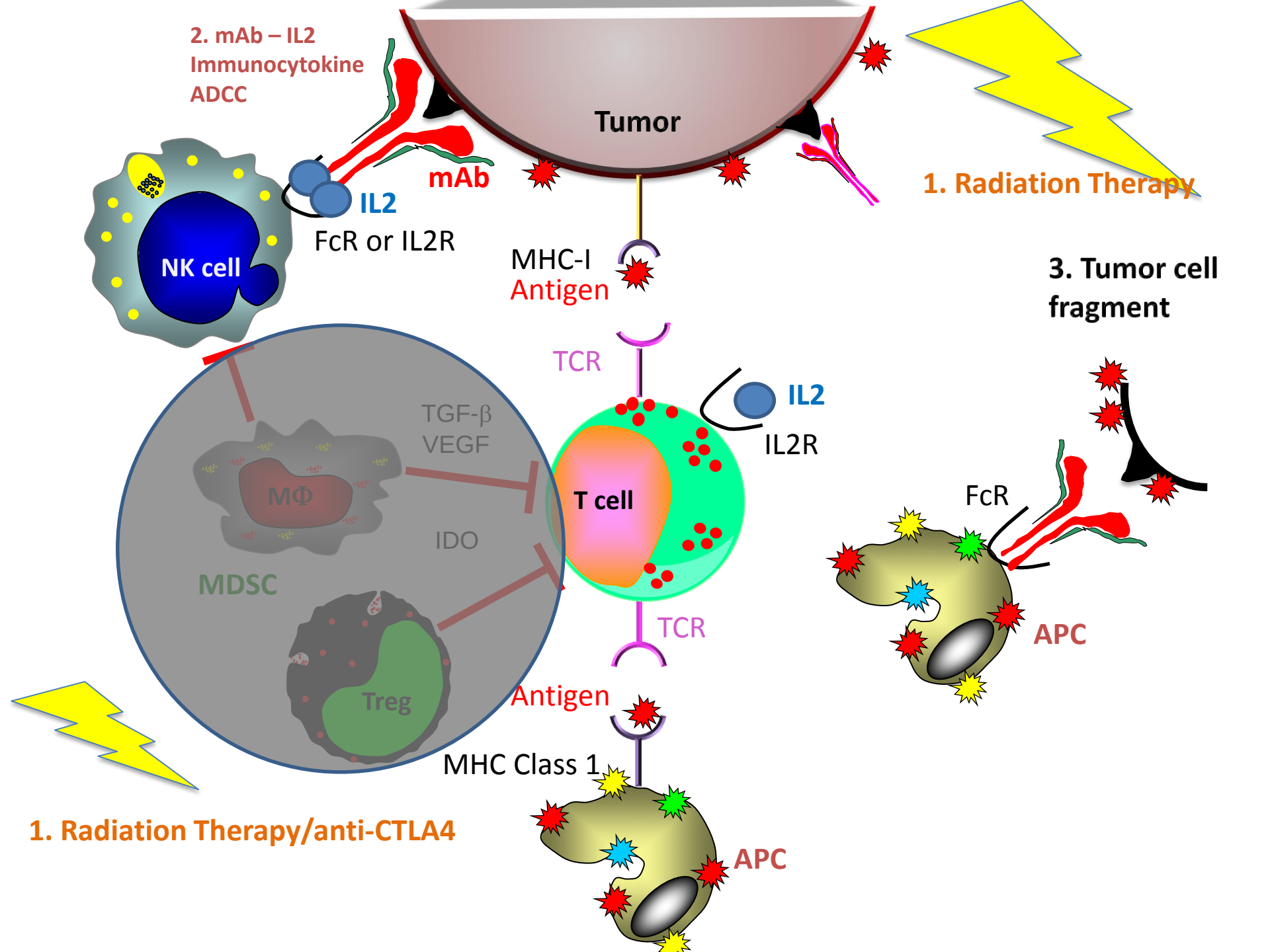
Morris Z et al. Abstract AACR 2016, manuscript submitted

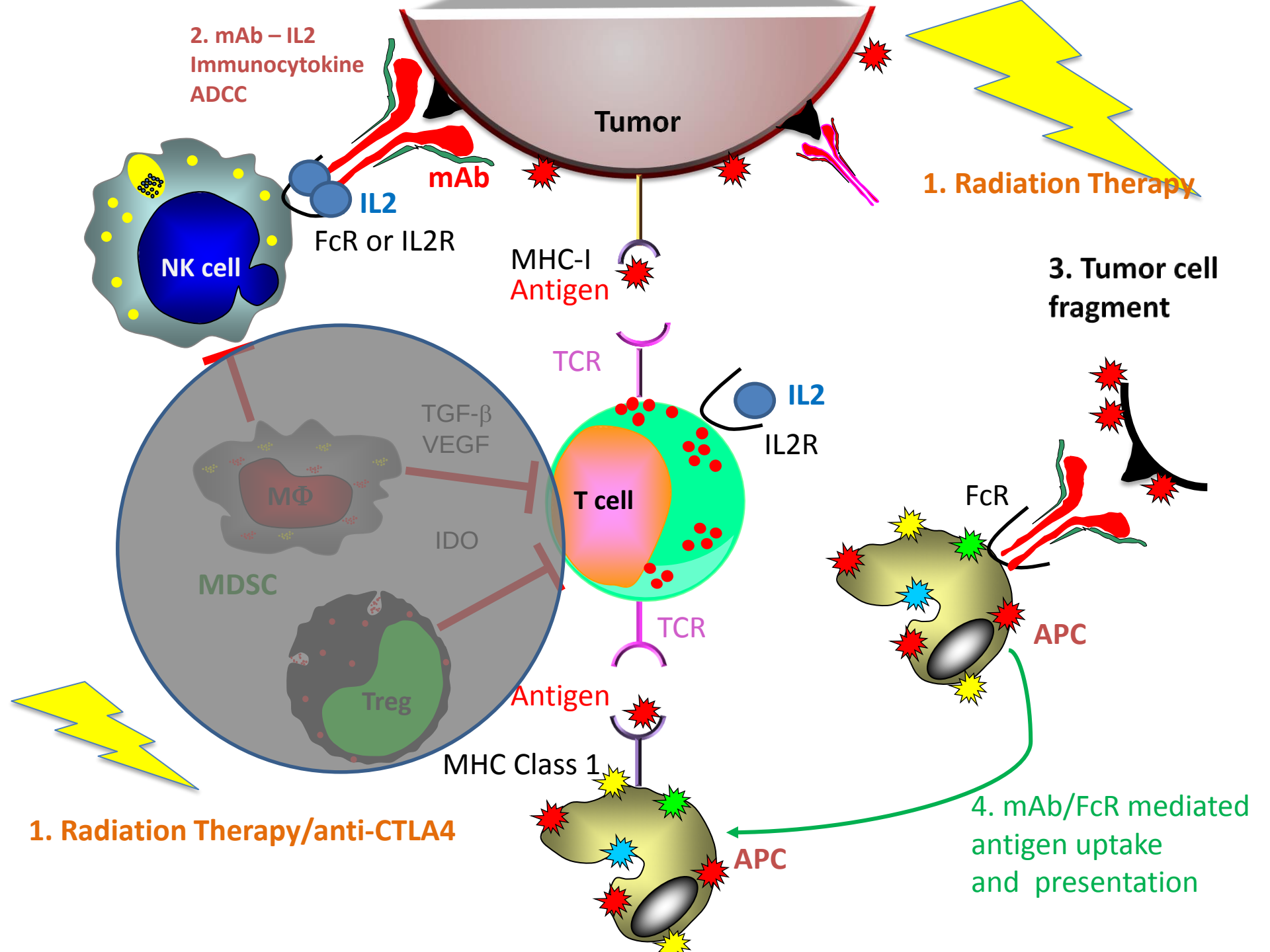
Radiation: 12 Gy x 1 – Day 1
hu14.18-IL2: 50 µg /mouse IT - daily Days 6-10
anti-CTLA-4: 200 µg /mouse IP – Days 3, 6, 9


mAb – IL2
Immunocytokine
Tumor
mAb
IL2
FcR or IL2R
NK cell
MHC-I
Antigen
Tumor cell
fragment
TCR
TGF-β
VEGF
IL2R
MΦ
T cell
FcR
IDO
MDSC
TCR
Tumor
Induced
Immune
inhibition
Treg
Antigen
MHC Class 1
APC
APC


Tumor
1. Radiation Therapy
mAb
IL2
FcR or IL2R
NK cell
MHC-I
Antigen
TCR
IL2
IL2R
TGF-β
VEGF
MΦ
T cell
IDO
MDSC
Treg
TCR
FcR
APC
Antigen
MHC Class 1
APC
1. Radiation Therapy/anti-CTLA4


2. mAb – IL2
Immunocytokine
ADCC
Tumor
1. Radiation Therapy
mAb
IL2
FcR or IL2R
NK cell
MHC-I
Antigen
TCR
IL2
IL2R
TGF-β
VEGF
MΦ
T cell
IDO
MDSC
Treg
FcR
APC
TCR
Antigen
MHC Class 1
APC
1. Radiation Therapy/anti-CTLA4


2. mAb – IL2
Immunocytokine
ADCC
Tumor
1. Radiation Therapy
mAb
IL2
FcR or IL2R
NK cell
MHC-I
Antigen
3. Tumor cell
fragment
TCR
IL2
IL2R
TGF-β
VEGF
MΦ
T cell
IDO
MDSC
TCR
FcR
APC
Treg
Antigen
MHC Class 1
APC
1. Radiation Therapy/anti-CTLA4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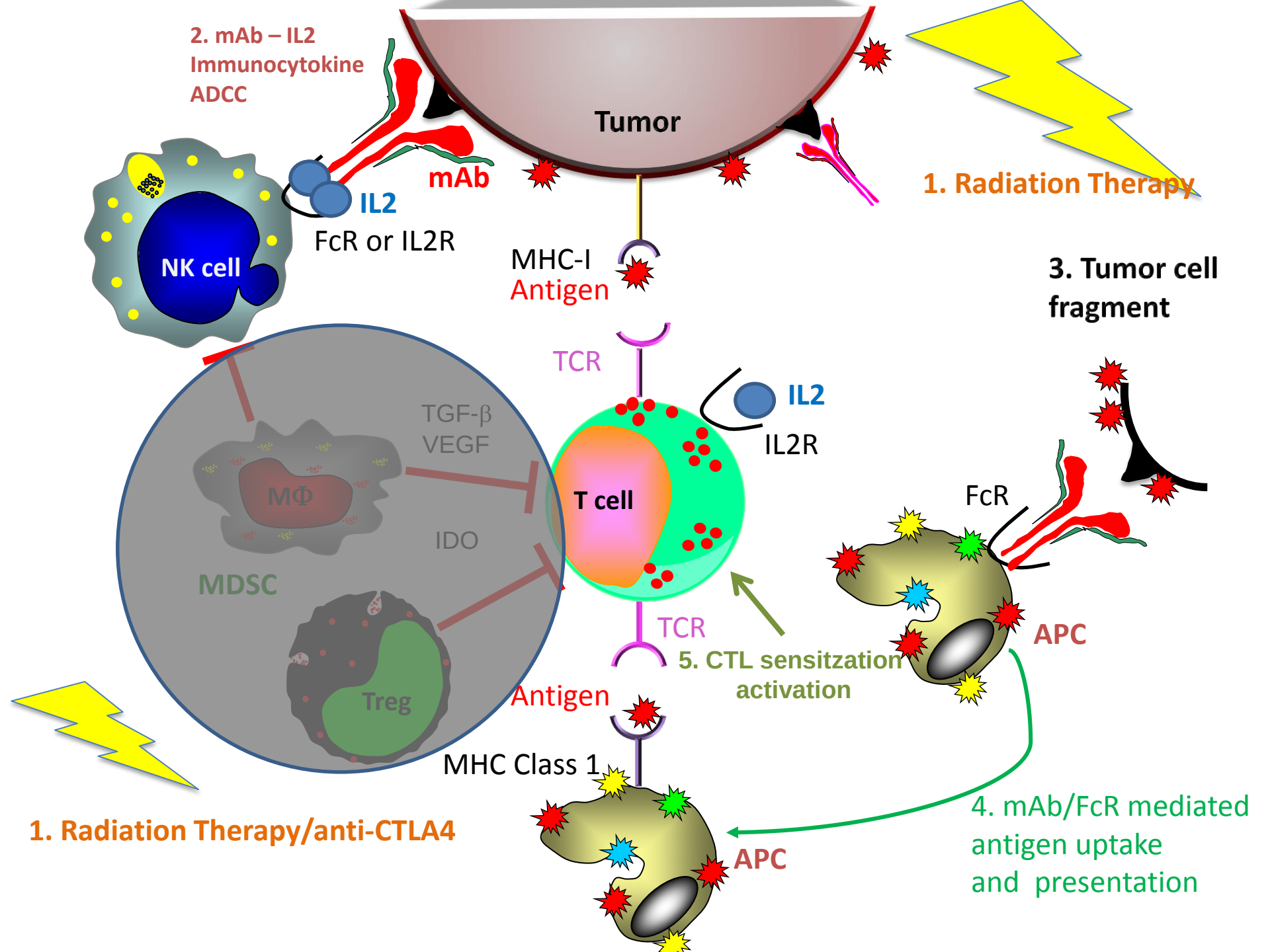
Treg
1. Radiation Therapy/anti-CTLA4
APC
4. mAb/FcR mediated
antigen uptake
and presentation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IDO
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TCR
APC
5. CTL sensitzation
activation
Antigen
Treg
MHC Class 1
1. Radiation Therapy/anti-CTLA4
APC
4. mAb/FcR mediated
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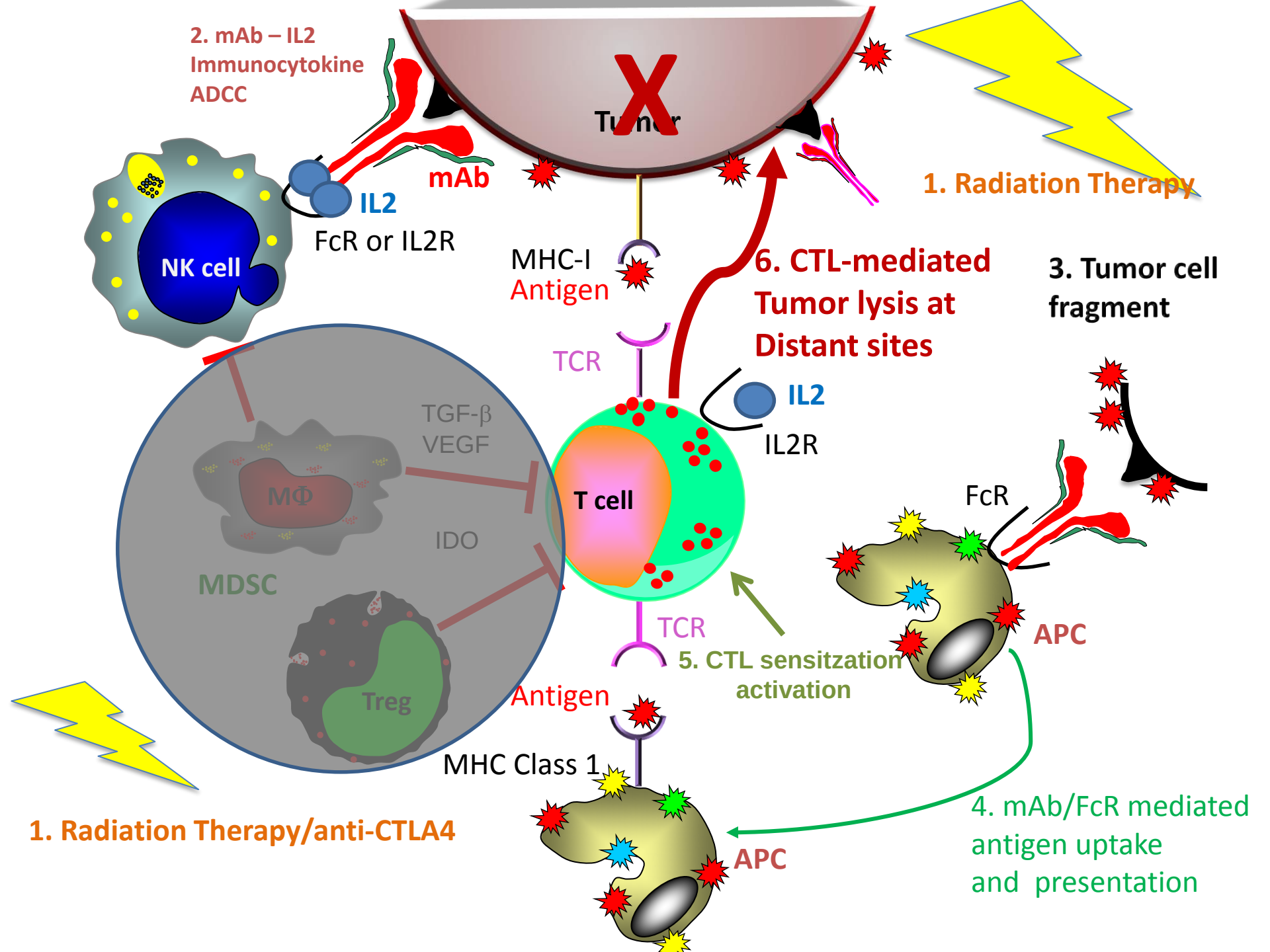
2. mAb – IL2
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Tumor lysis at
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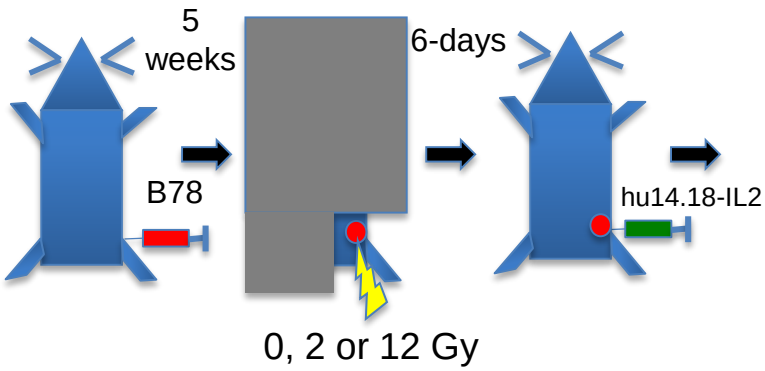
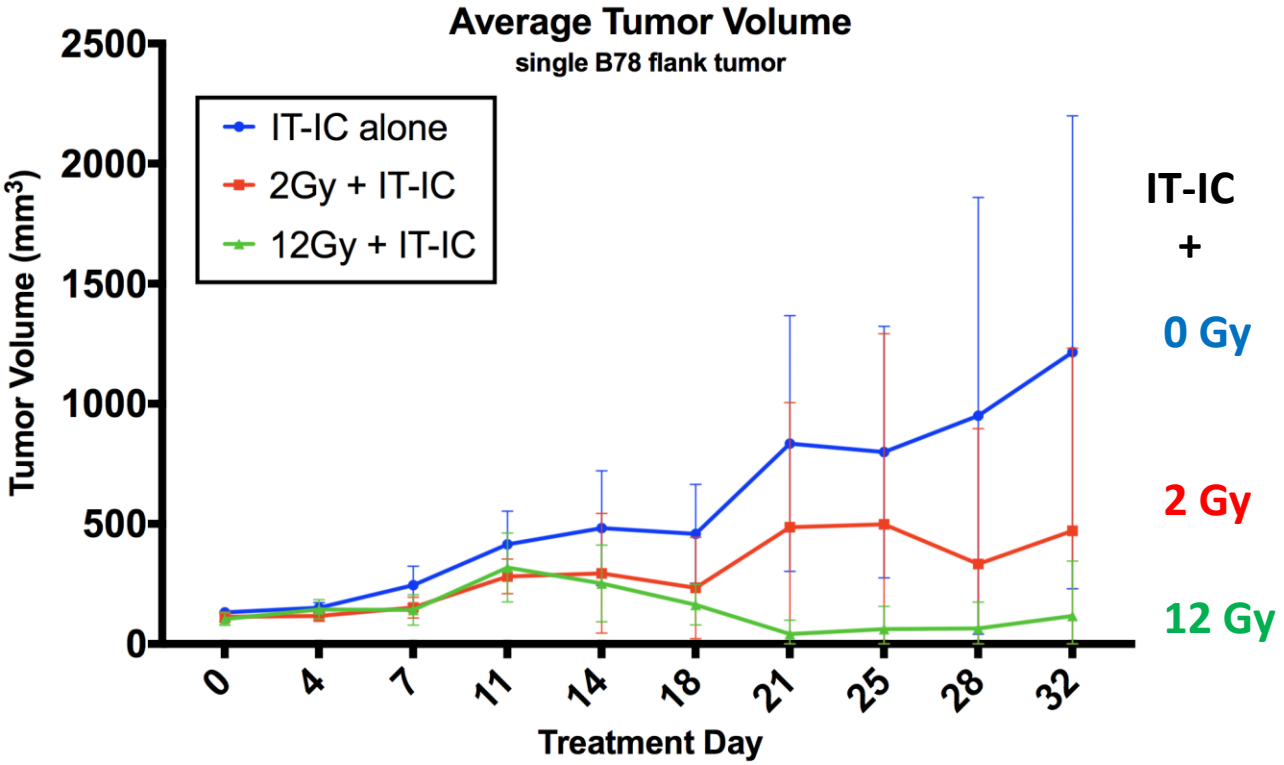
1. Radiation Therapy/anti-CTLA4

How much RT is needed to get the local RT + IT-IC anti-tumor effect?

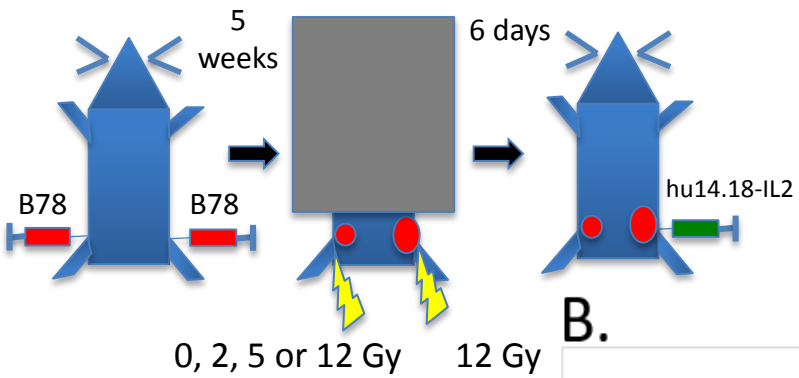


Peter Carlson
MD PhD Student



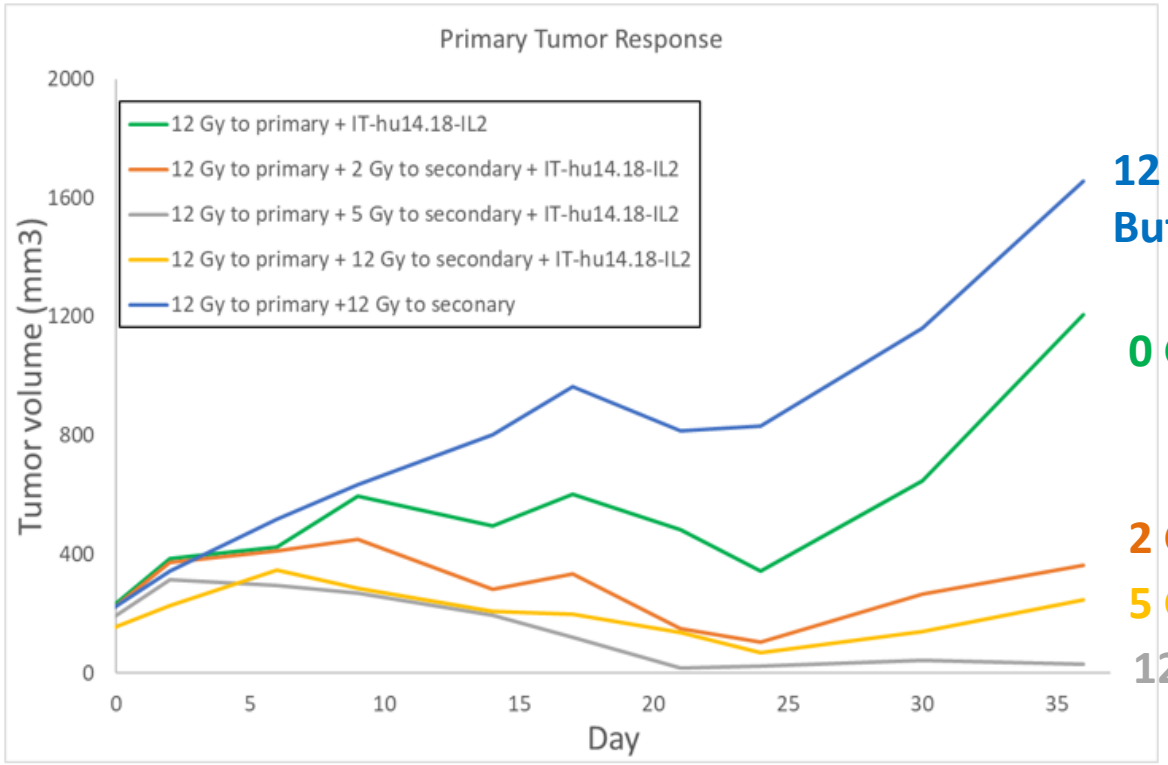
Carlson P et al,
SITC poster P454 2017

How much RT to the 2nd tumor is needed to inhibit Concomitant Immune Tolerance?



B.

IT-IC + 12 Gy to primary.
RT to secondary:



12 Gy But NO IC

0 Gy +IC

2 Gy +IC

5 Gy +IC

12 Gy +IC

Carlson P et al,
SITC poster P454 2017

Clinical translation:
How can you deliver 2 Gy RT to ALL sites of distant disease? Targeted molecular RT?

First clinical testing of the combination of:
1. Radiation; 2. Anti-GD2 Immunotherapy; 3. Checkpoint blockade:
[1. 131-I-MIBG; 2. ch14.18/CHO mAb; 3. Nivolumab]
Neuroblastoma Protocol IND in Preparation
["MiNivAn" trial]

Supported by INBRACED/SKC (a USA/UK Charitable foundation), Stand Up to Cancer, St. Baldrick's , and BMS (USA), Apeiron (Austria), EUSA (UK)

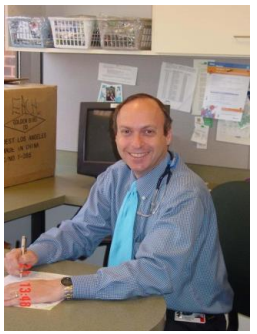
Trans-Atlantic clinical trial for relapsed neuroblastoma
Radiation delivered by 131-I-MIBG
Immunotherapy using 10-day LTI of ch14.18/CHO developed by Lode et al
Anti-PD1 as "checkpoint blockade" **(Siebert N, …, Lode H et al, Oncoimmunology, 2017)**
To open at 4 sites:

Dr. J Gray MD (Study Chair)
Southampton UK

Dr. M. Gaze
London UK

Dr. H. Lode
Greifswald Germany

Dr. K. DeSantes
Madison USA



Pilot Study: RT + IT-IC + anti-CTLA4 + Nivolumab for Advanced Melanoma (adult)

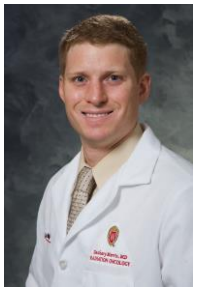
A UWCCC Clinical Trial (IND being prepared) with collaboration from Apeiron and BMS and NCI support

Goals:

A. **First in human Phase-I testing of IT-IC with an IC that can bind to tumor and mediate ADCC**
B. **First in human IT-IC of such an IC immunologically timed after local RT**
C. **First in human testing of this in combination with anti-CTLA4 and or anti-PD1**
D. **Toxicity/Tolerance/Anti-tumor effects**
E. **Serial biopsies of the same lesions, to look for the changes seen in murine tumors**

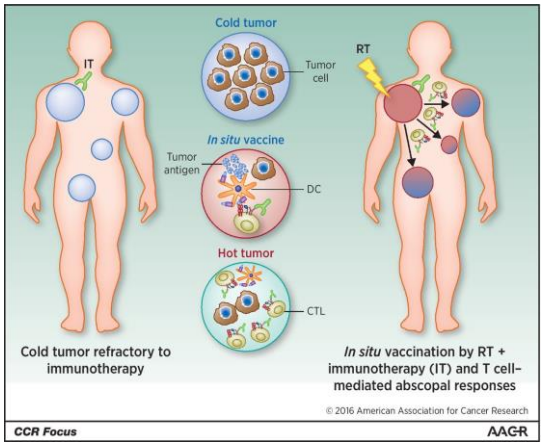
Mark Albertini MD

Zach Morris MD PhD



Could become PCDT- PedCITN trial for GD2+ NBL, Osteogenic or Ewing's Sarcoma.

This work with anti-GD2 mAb *should translate to other available tumor-specific mAbs and newer mAbs in development*



Whiteside, Demaria et al, CCR 2016

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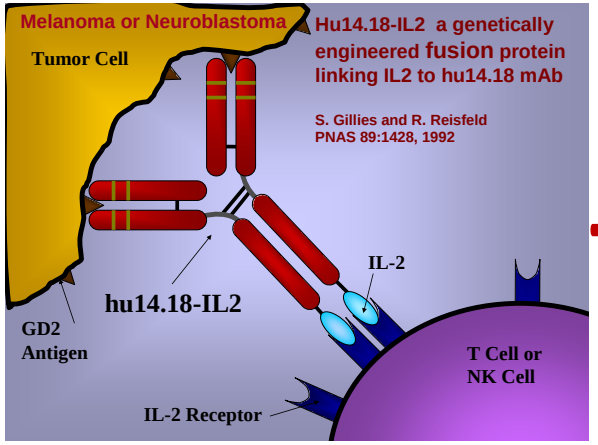

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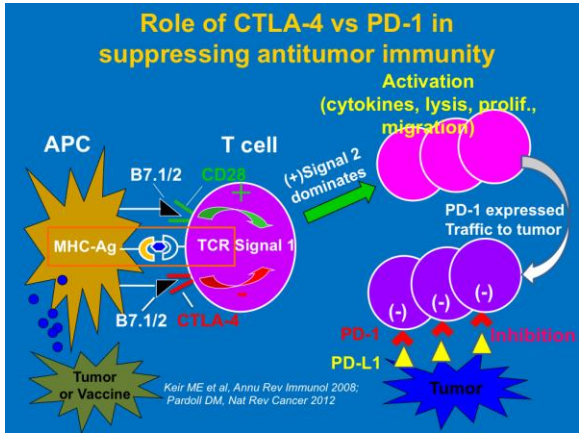


Pardoll DM, Nat Rev Cancer 2012

RT

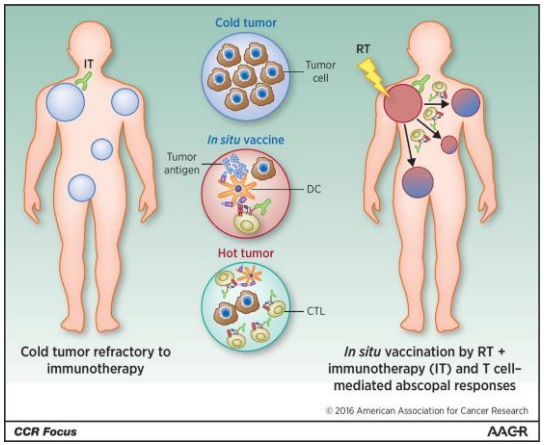
IT-IC

(tumor-reactive mAb + cytokine)

Checkpoint Blockade

Potential mechanisms in this *in situ* vaccine effect

1. RT increases tumor immune susceptibility, accessibility, blocks immune suppression
2. IC induces ADCC, attracts immune cells, activates cytokine pathways
3. IC-coated tumor cells and cell membrane fragments are taken up by APCs
4. IL2 (from IC) in microenvironment enhances induction of adaptive T cell response
5. Checkpoint blockade expands adaptive response, blocks immune suppression (Tregs)



Whiteside, Demaria et al, CCR 2016

RT

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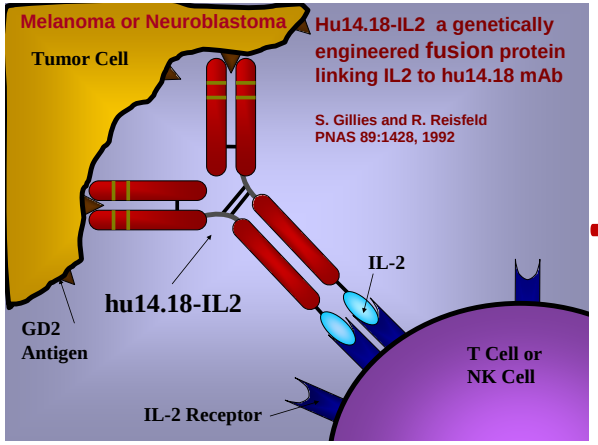

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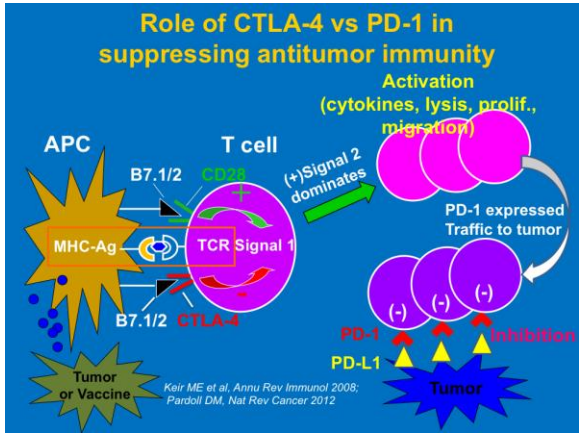

Pardoll DM, Nat Rev Cancer 2012

Checkpoint Blockade

Can IC be replaced by tumor-reactive mAb+ IL2 if given IT (instead of IV)?

Potential mechanisms in this *in situ* vaccine effect

1. RT increases tumor immune susceptibility, accessibility, blocks immune suppression
2. IC induces ADCC, attracts immune cells, activates cytokine pathways
3. IC-coated tumor cells and cell membrane fragments are taken up by APCs
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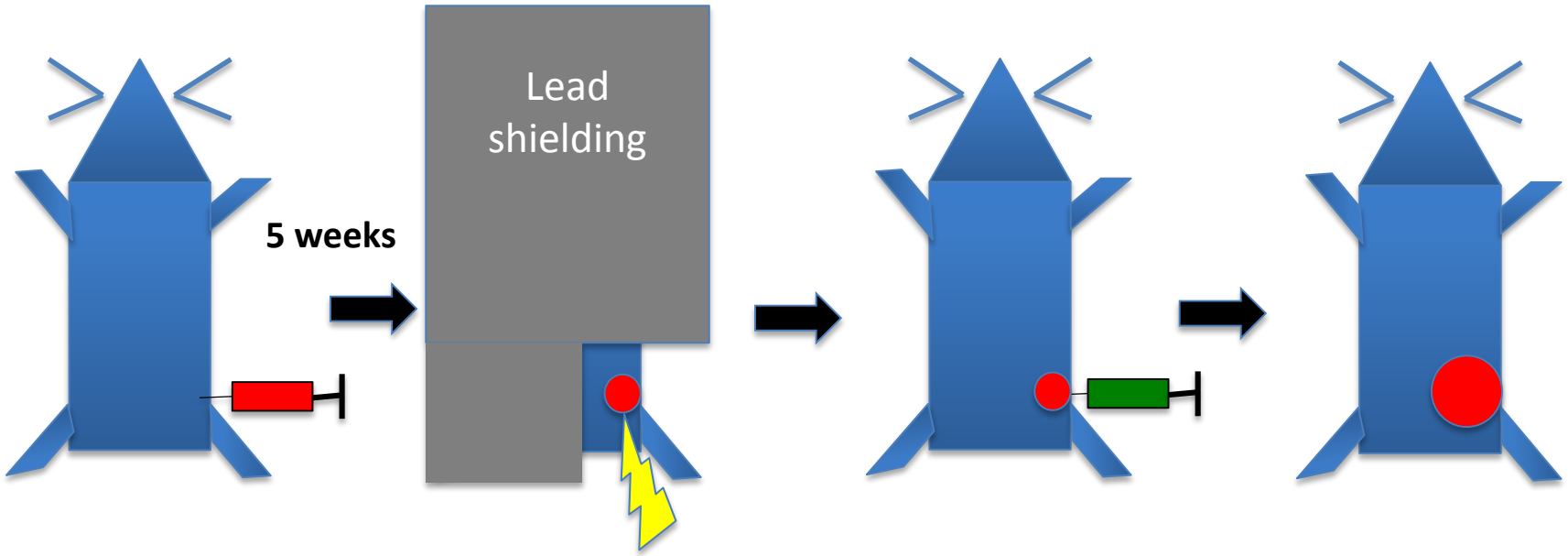
Can **macroscopic disease (200mm^3)** be controlled by RT +

IT mAb + IL2?



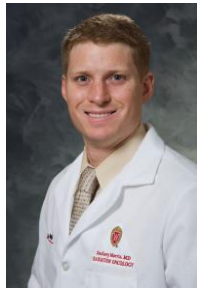
Tumor cells injected in syngeneic mice

DAY 1. Macroscopic tumors radiated to 12 Gy

DAY 6-10. Daily intra-tumor injections of **IL2 + anti-GD2 mAb**

Tumor growth and animal survival monitored

Z. Morris MD PhD

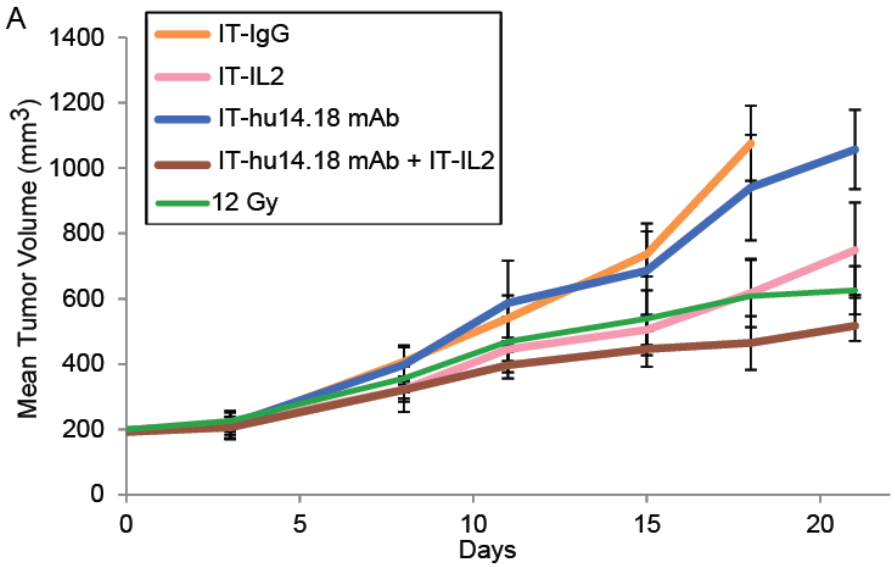


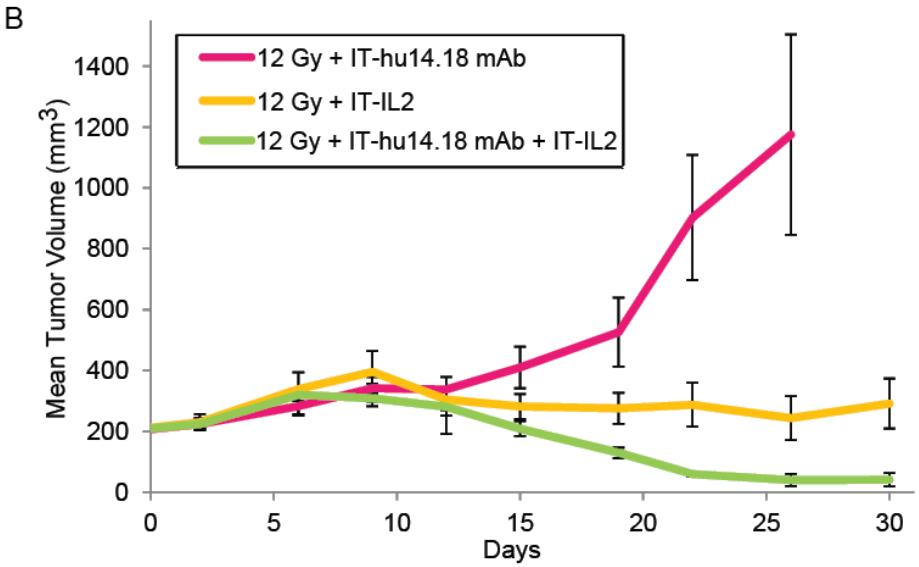
Tumor cells

B78 melanoma – poorly immunogenic B16 melanoma that expresses GD2

Guy E. et al
SITC Poster P327, 2017.

Can **macroscopic disease (200mm³)** be controlled by RT + **IT mAb + IL2**?

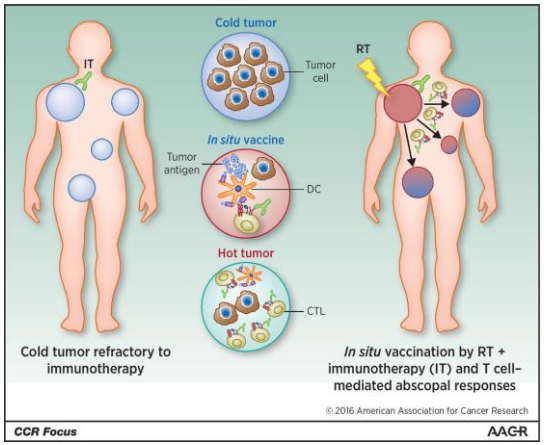




1. **Some benefit from RT + IL2 (no mAb)**
2. **Greater benefit, with tumor elimination, from RT + mAb + IL2**
    1. **(consistent with prior data showing need for GD2 on the tumor)**

Guy E. et al. SITC Poster P327, 2017
Erbe A. et al. SITC Poster P322, 2017

See related work comparing IT mAb with IT (IL2 vs. IL15)
Rakhmilevich A. et al. SITC Poster P286, 2017.



Whiteside, Demaria et al, CCR 2016

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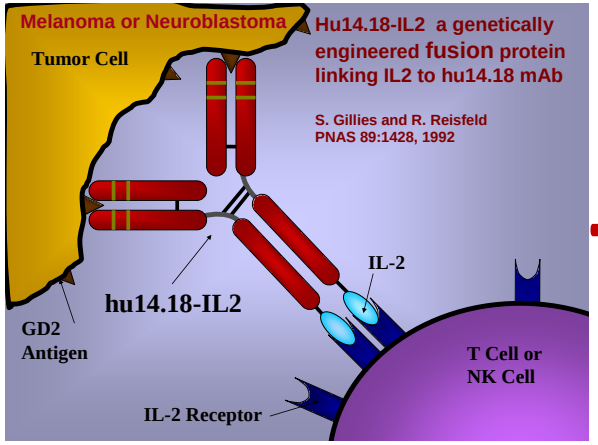

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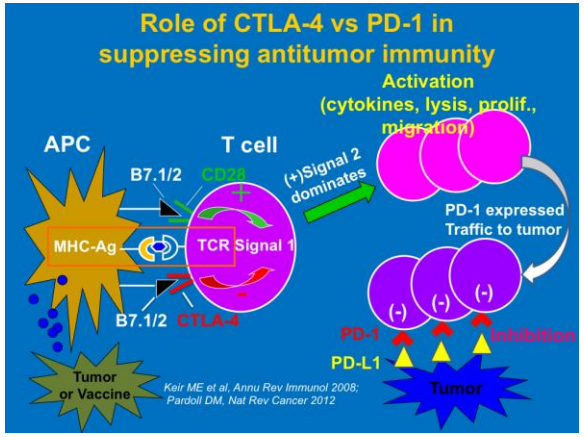


Pardoll DM, Nat Rev Cancer 2012

RT

IC CAN be replaced by tumor-reactive mAb + IL2 in *in situ vaccine*!

Checkpoint Blockade

Potential applications of this *in situ* vaccine effect

Should allow testing of RT + IT (mAb + IL2) + checkpoint blockade using a variety of tumor-reactive mAbs for many distinct tumor types

Collaborators in our Immunotherapy Research: 2017

UWCCC (partial list)

- J Hank
- A Rakhmilevich
- A Erbe
- Z Morris
- KM Kim
- M Albertini
- E Ranheim
- M Patankar
- K DeSantes
- C Capitini
- M Otto
- J Weichert
- B Bednarz
- J Kuo
- R Yang
- P Harari
- K McDowell
- W Wang
- Z Perez-Horta
- A Hoefges
- M Merdler
- J Weiland
- J Goldberg
- P Carlson
- J Voeller
- A Pieper
- V Subbotin
- E Guy
- C Baniel

- Energetic Undergrads

INBRACED Consortium

J. Gray, M. Gaze, H. Lode

University of Wisconsin Paul P. Carbone Comprehensive Cancer Center

UW Health American Family Children's Hospital

WISCONSIN UNIVERSITY OF WISCONSIN–MADISON

Our UWCCC Lab Research Team

UW Pediatric Heme-Oncology-BMT Team

- **C.O.G. (Pediatric Oncologists)**
  - S Shusterman
  - A Yu
  - J Maris
  - J Park
  - W London
  - R Seeger
  - C Mackall
  - Many Others
- **SU2C- St. Baldrick's**
  - PCDT
- **St. Jude**
  - F Navid
  - V Santana
  - W Furman
  - S Federico
- **Provenance**
  - S Gillies
- **BMS**
  - Alan Korman
  - Mark Selby
  - Clinical Trials
- **Apeiron**
  - H Loibner
  - O Mutschlechner
- **Scripps**
  - R Reisfeld
- **Nektar**
  - D Charych
- **Invenra**
  - R Green

Support for our Immunotherapy Research: 2017

ICTR
UW Institute for Clinical and Translational Research

University of Wisconsin Paul P. Carbone Comprehensive Cancer Center

UW Health American Family Children's Hospital

WISCONSIN UNIVERSITY OF WISCONSIN-MADISON

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

STAND UP TO CANCER

St. Baldrick's FOUNDATION
Conquer Childhood Cancers

ADMINISTERED BY AACR American Association for Cancer Research FINDING CURES TOGETHER
SCIENTIFIC PARTNER OF STAND UP TO CANCER

Solving Kids' Cancer™
every kid deserves to grow up

Alex's Lemonade Stand

Hyundai Hope On Wheels®
Helping Kids Fight Cancer

CHILDREN'S ONCOLOGY GROUP

Crawdaddy Founda

MACC FUND
Hope for Kids

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

hhmi
Howard Hughes Medical Institute

American Cancer Society®

University of Wisconsin's Childhood Cancer Reunion

KIDS WITH COURAGE V

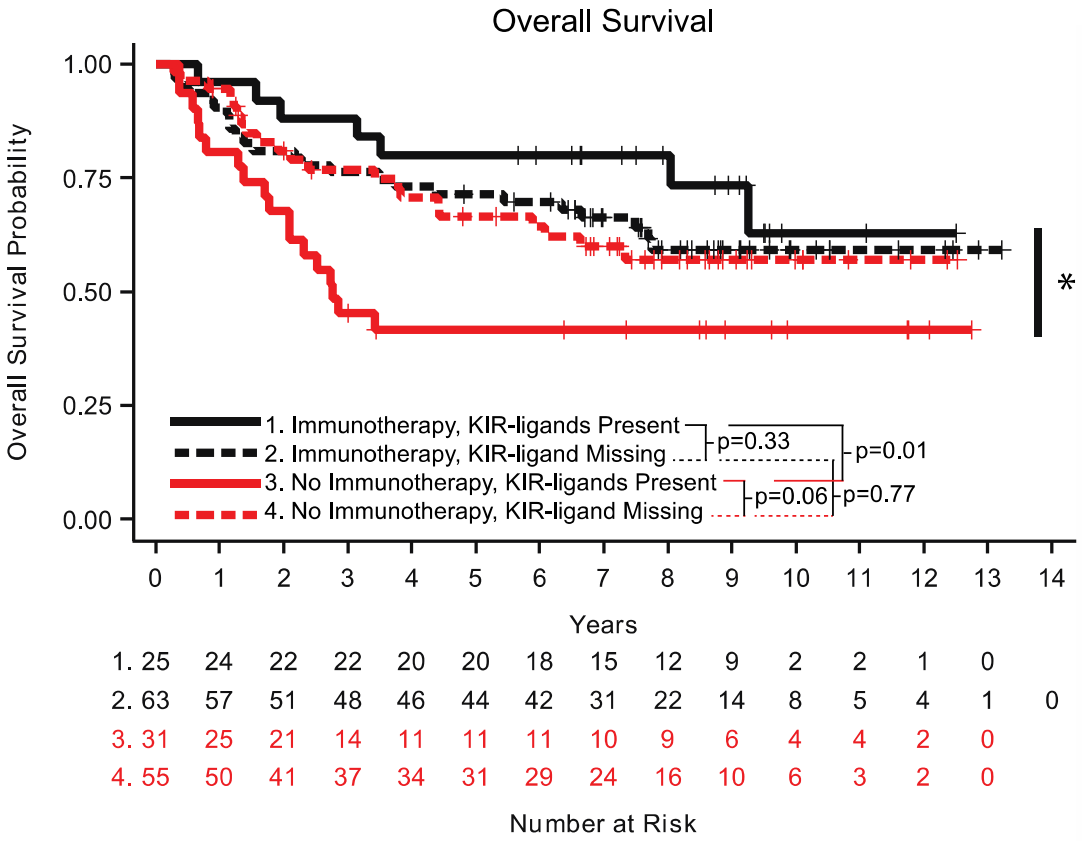
PROOF THAT CANCER RESEARCH MAKES A DIFFERENCE!

Our Goal: Use Improved Therapy (like Immunotherapy) to help cure Cancer, with less toxicity for many more children (and adults)!

UW Health
American Family Children's Hospital

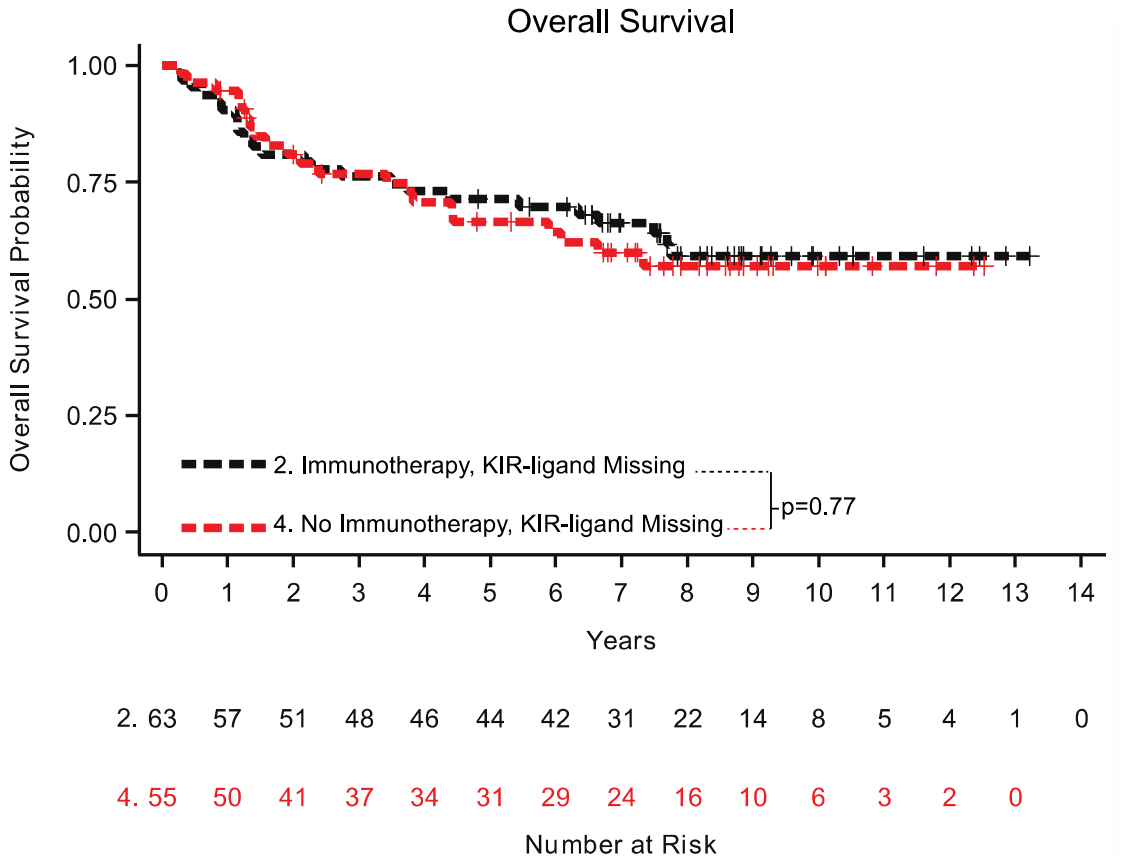
Carbone Cancer Center
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Overall Survival for 174 pts: based on **Immunotherapy** vs. **No Immunotherapy** & KIR-Ligands Present (——) or missing(- - - - -).



Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

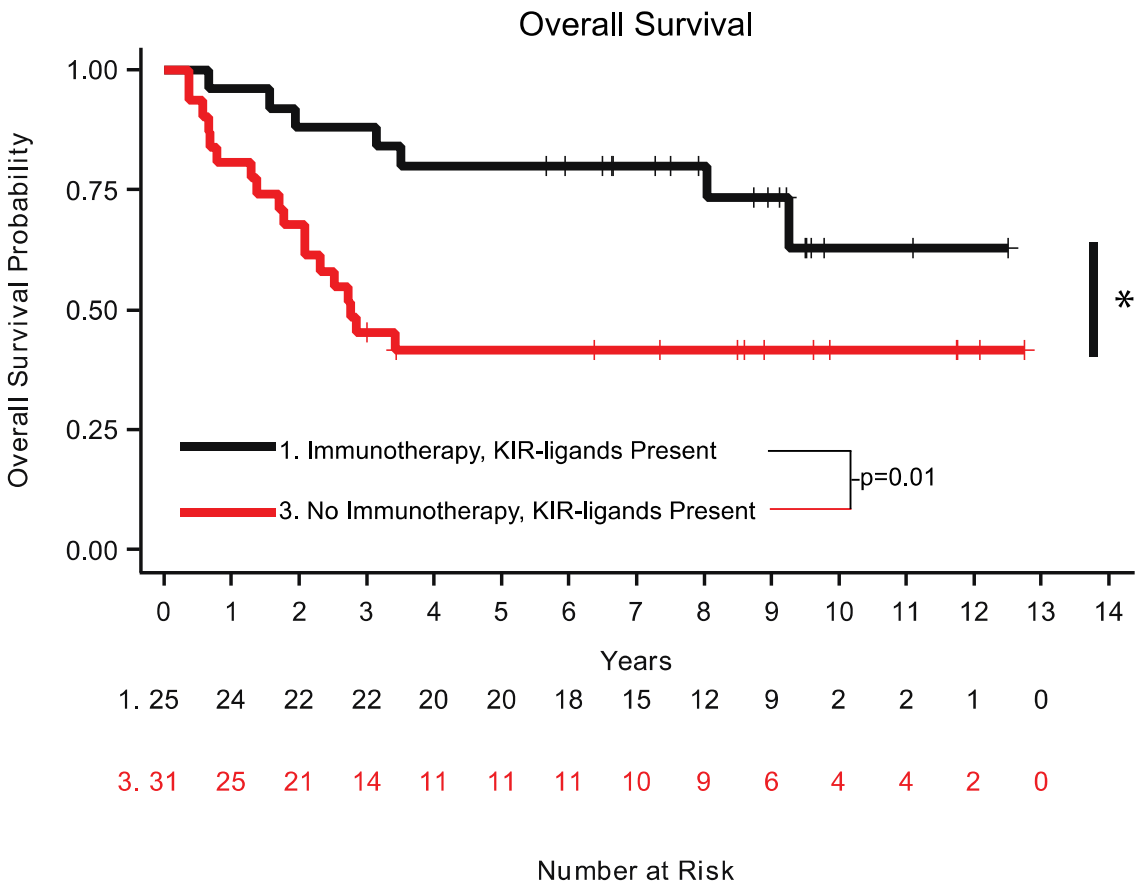
Overall Survival for **118** of 174 pts with KIR-Ligand missing : **Immunotherapy** vs. **No Immunotherapy** (------).



p = 0.77

Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

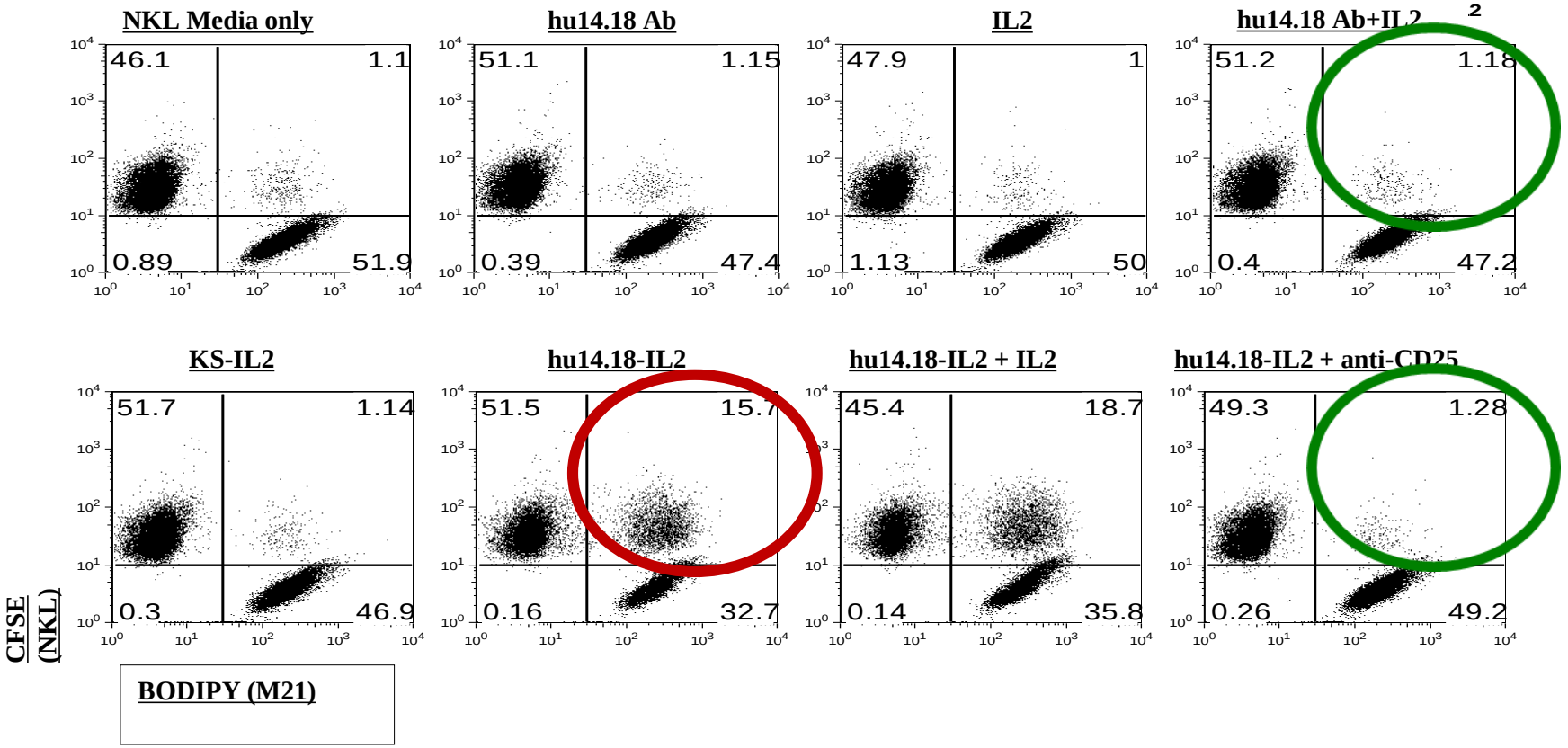
Overall Survival for **56** of 174 pts with KIR-Ligands present : **Immunotherapy** vs. **No Immunotherapy** & (—).



p = 0.01

Erbe, Wang et al, SITC Poster P26 2017; Clin. Canc. Res. 2017

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

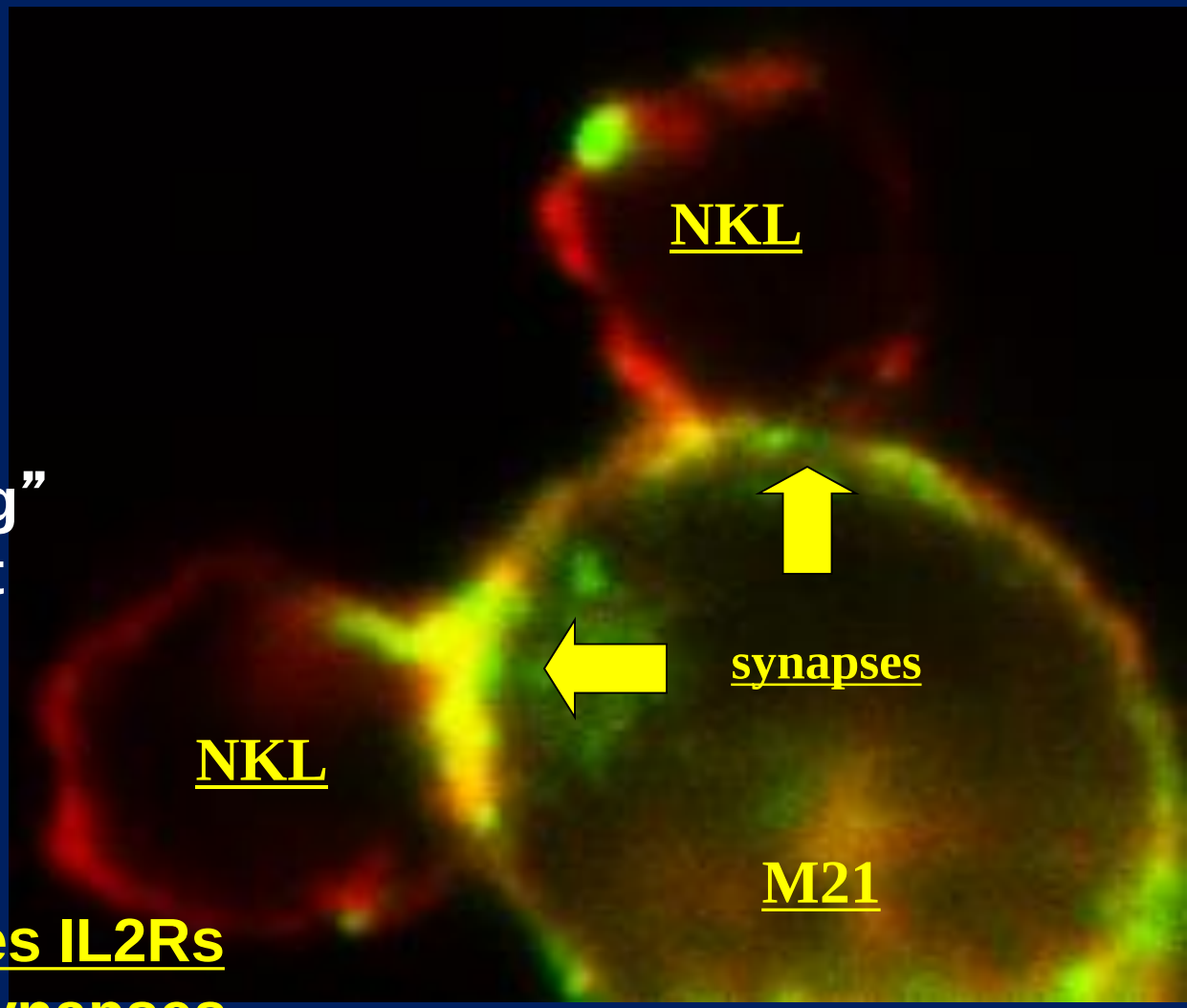


Buhtoiarov IN, Neal ZC, Jan J, Buhtoiarova TN, Hank JA, Yamane B, Rakhmilevich AL, Patankar MS, Gubbels JAA, Reisfeld RA, Gillies SD, Sondel PM. J. Leukocyte Bio. 2011

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.

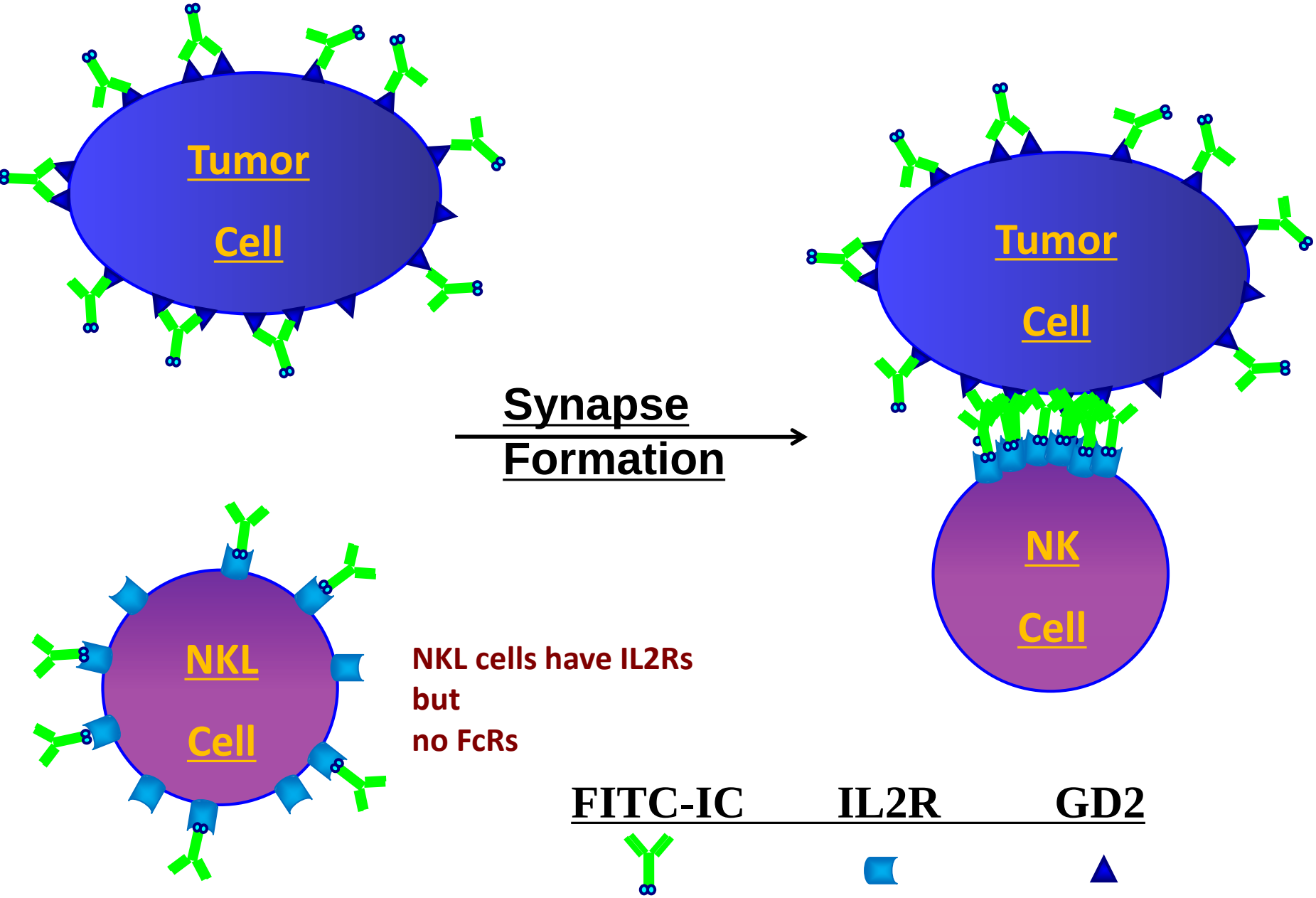
IC gives "ring staining" On M21 (via GD2), but localizes to synapse on NKL (CD25-pos., CD16-neg.)



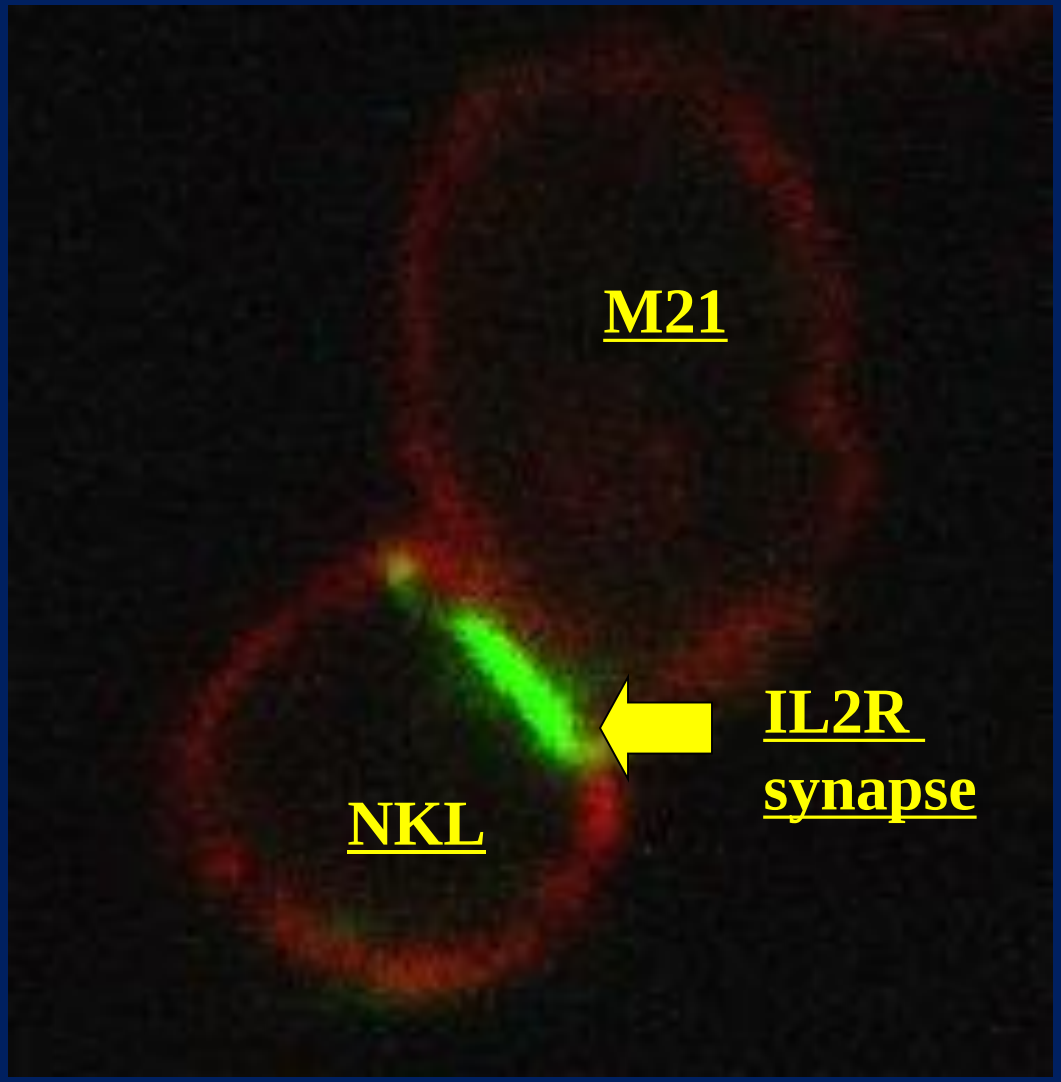
Cell-bound IL2 induces IL2Rs To cause activating synapses.

Gubbels et al: CII , 2011

IC is a bifunctional targeting agent via IL2Rs



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

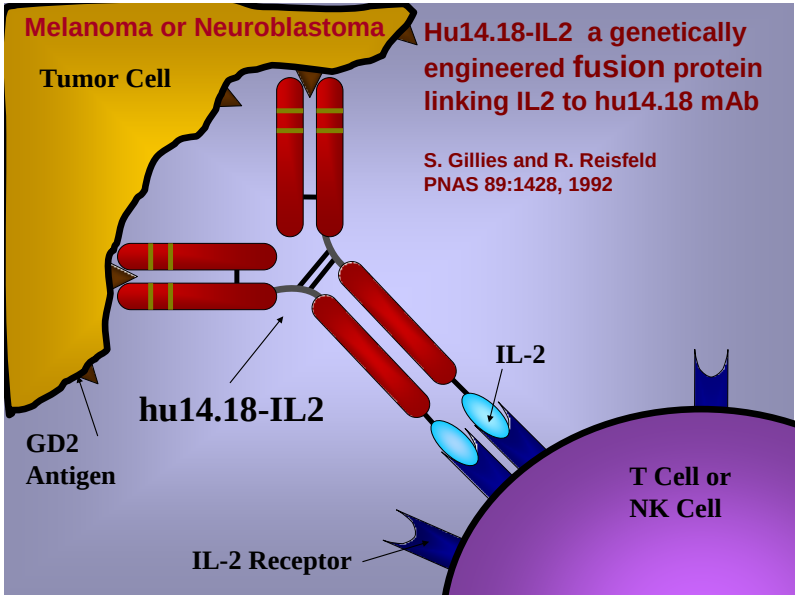
Gubbels, Buhtoiarov et al: CII, 2011

hu14.18-IL2 (next-gen of FDA approved dinutuximab anti-GD2)

1. Anti-GD2/IL2 fusion protein14.18-IL2
2. More effective than 14.18 + IL2
3. NK cells involved (ADCC)
4. Efficacy in minimal disease setting**

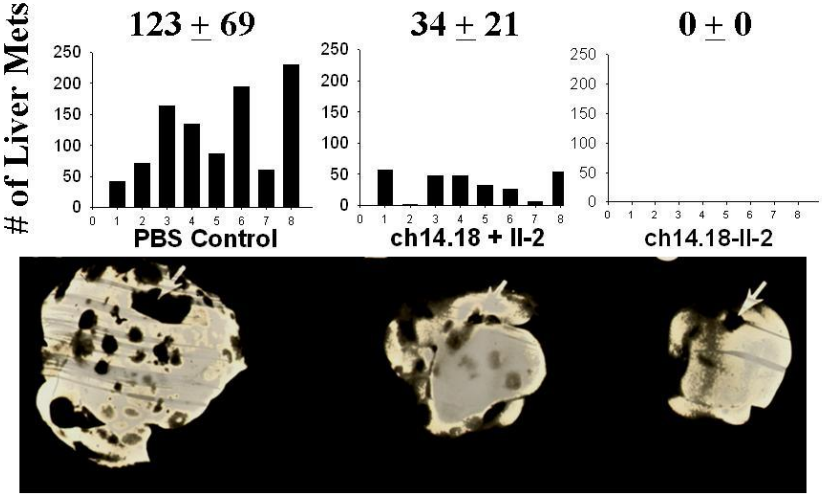
(mouse)*Neal ZC, et al Clin. Cancer Research 2004

(human)*Shusterman S. et al, J. Clin. Onc., 2010

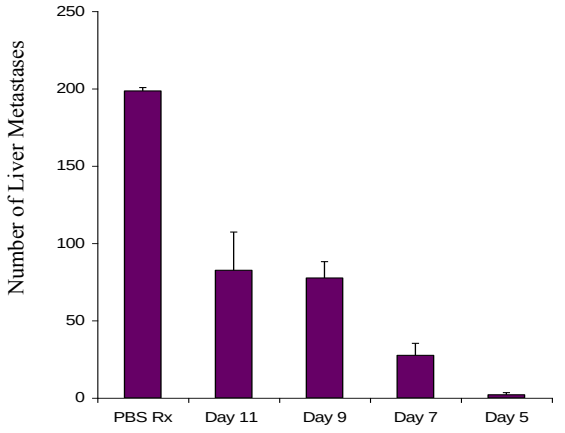


Efficacy of ch14.18-IL2 Immunocytokine against Murine Neuroblastoma Liver Metastases

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



Effective anti-GD2 Immunotherapy: Dependence on Minimal Tumor Status



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.

Neal ZC, et al Clin. Cancer Research 2004

HOW DOES BMT CURE LEUKEMIA? The Graft vs. Leukemia (GVL) effect (immune mediated)

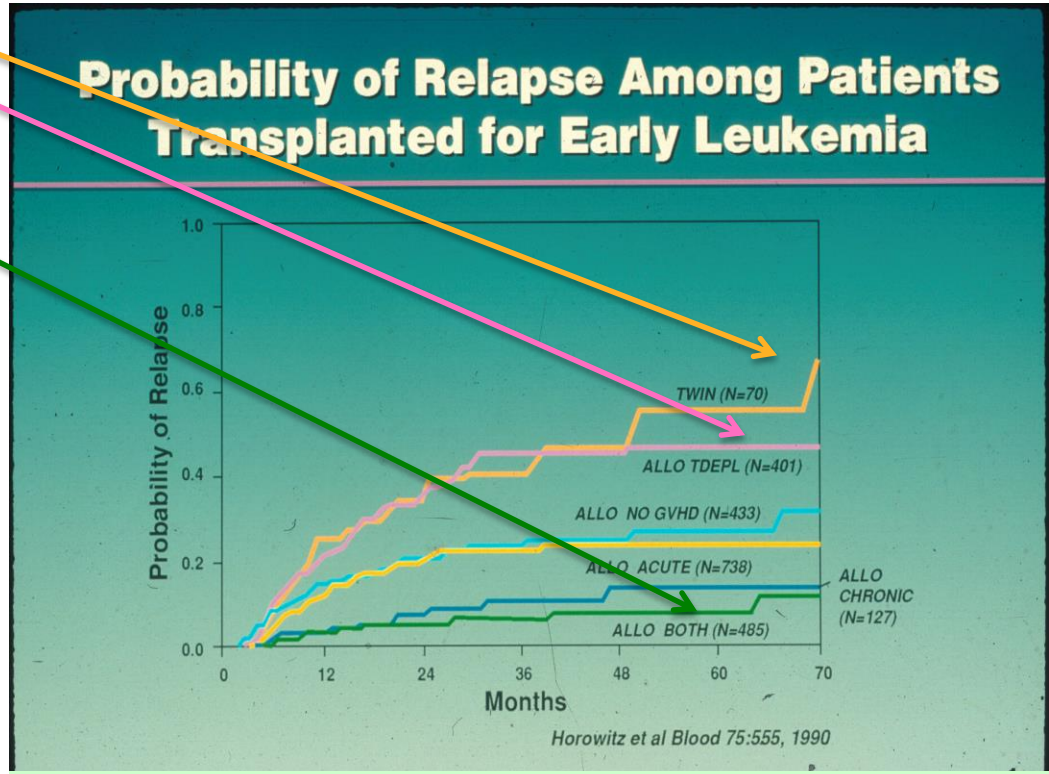
1. Relapse most likely after transplant
   From twin or after
   Immune-cell elimination

2. Relapse least likely after transplant
   showing some immune reaction
   (GVHD)

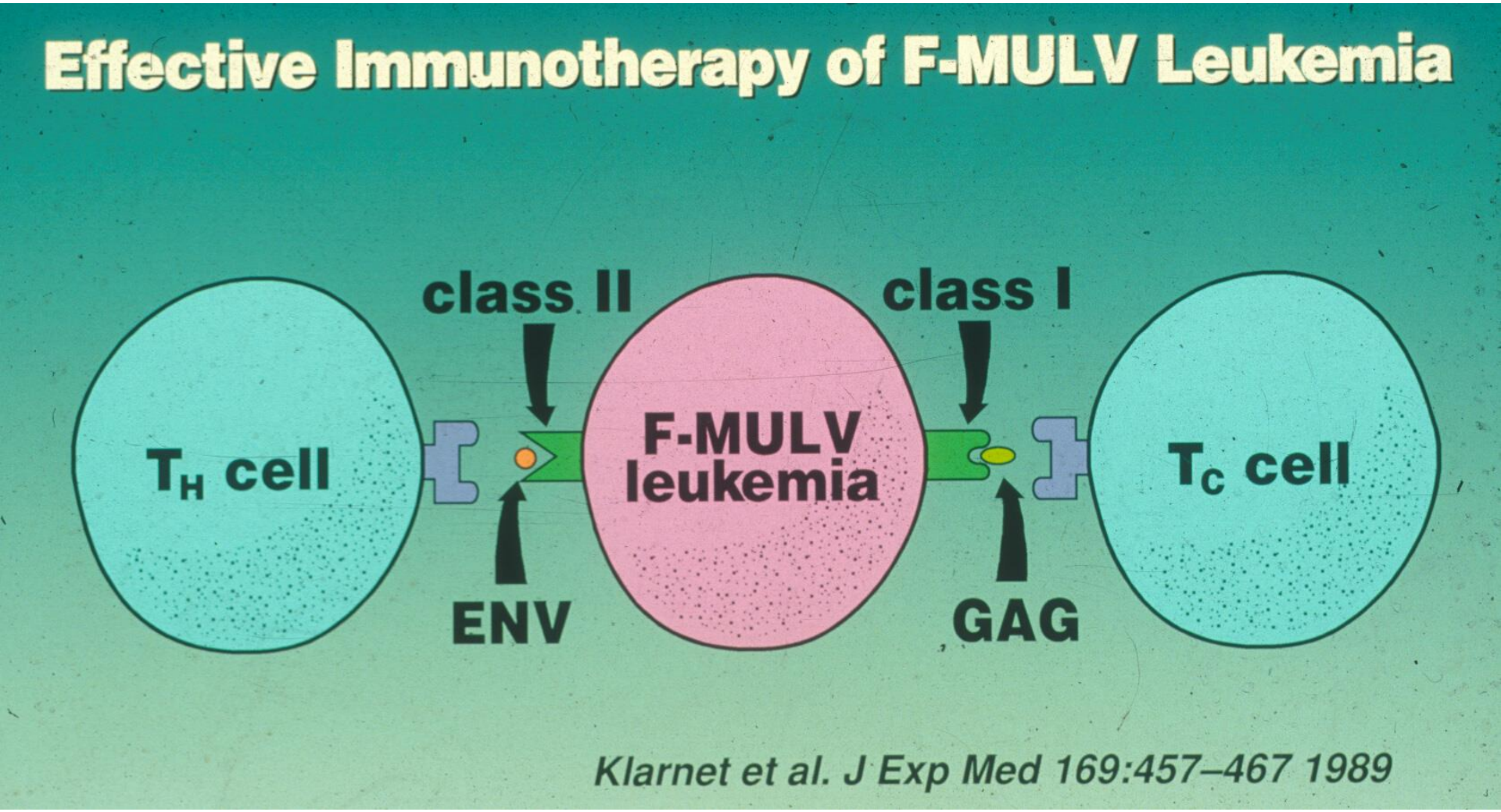
Thus *cure from leukemia* by BMT involves immune mechanisms *(immunotherapy):*
How to separate the cells that cause "graft vs. tumor" from the cells that cause "graft vs. host disease"?

2 paths forward:
1. ***Use cells from a healthy donor, or***
2. ***Use cells from the patient***

RESULTS FROM >2100 BMT PATIENTS: WHO RELAPSES?



Horowitz, Gale, Sondel et al; Blood, 75:555, 1990:
Demonstration of immunotherapeutic Graft vs. Leukemic Effect



1970s-80s: Pioneering preclinical work on ADOPTIVE transfer of tumor-reactive T cells with ADAPTIVE tumor recognition

Fefer, Greenberg, Cheever and colleagues; Rosenberg and colleagues; several others