# LECTURE: CYTOKINE AND CELLULAR THERAPY OF CANCER

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**Vice Chair Department of Surgery**

**Saturday, December 7th, 2013**

George Klein

Stephen Strom

# CLASSES OF MOLECULES THAT INITIATE THE INNATE IMMUNE RESPONSE – SIGNAL 0

Pathogen-associated Molecular Patterns (PAMPs):

Molecules expressed or released by invading microorganisms that are structurally unique to the pathogen.

Ruslan Medzhitov, 2000

Damage-associated Molecular Patterns (DAMPs):

Molecules expressed or released that are normally unavailable to the immune system but are released and recognized by immune cells following tissue injury [Danger].

Polly Matzinger, 1995

# DAMPs -Chronic Tumor Lysis Syndrome

## Cell Constituents:

HMGB1 – Cytochrome C

Heat shock proteins

Uric Acid, ATP, Adenosine; CpG DNA

s100 proteins

Hepatoma derived growth factor

LDH

DNA

**Acute Tumor Lysis Syndrome**

## Secreted molecules:

Fibrinogen domain A

Surfactant protein A

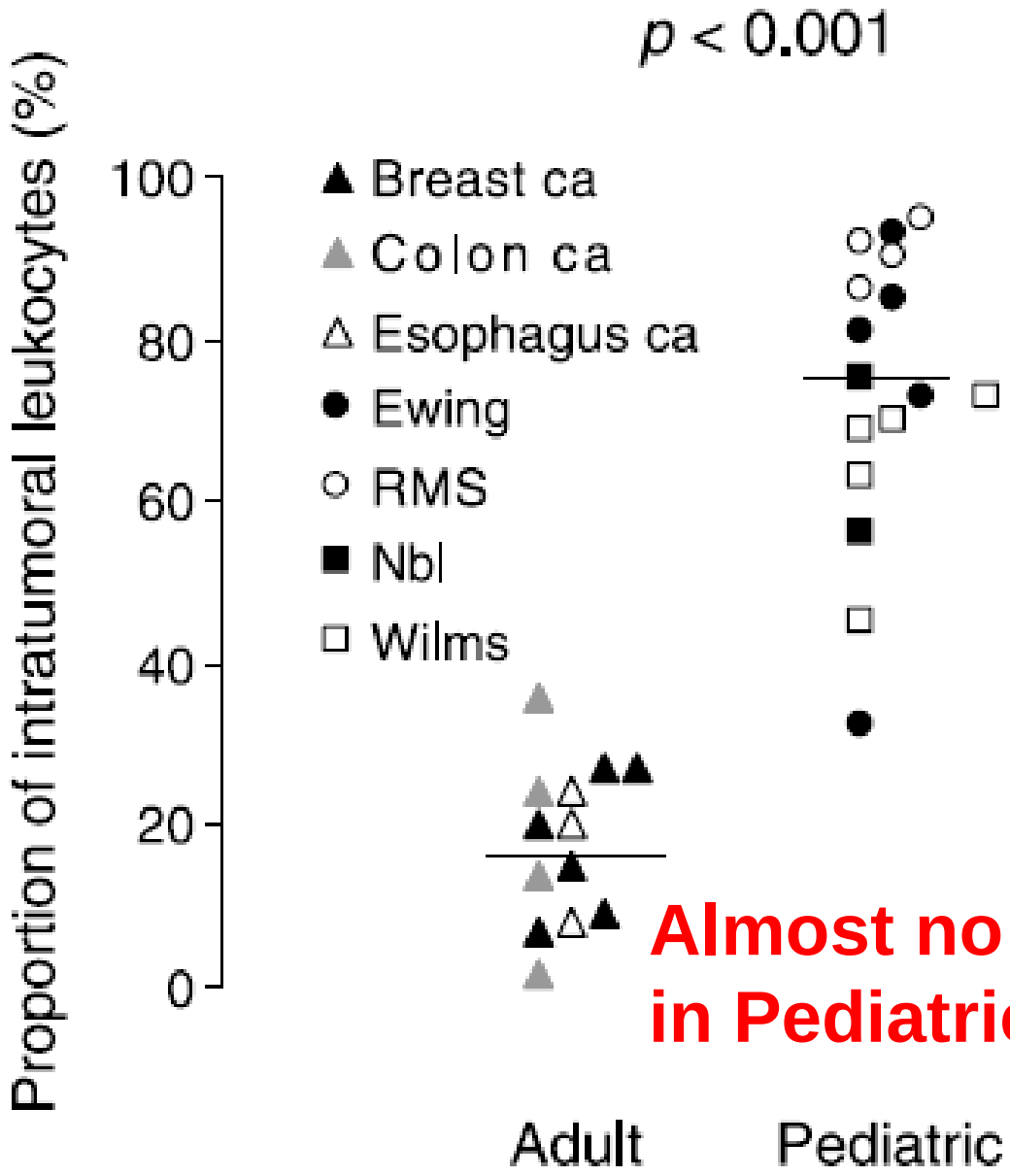
## Matrix elements:

Heparan sulfate

Soluble hyluranan

Fibronectin

# Increased Number of Intratumoral Leukocytes in Pediatric Cancers




Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

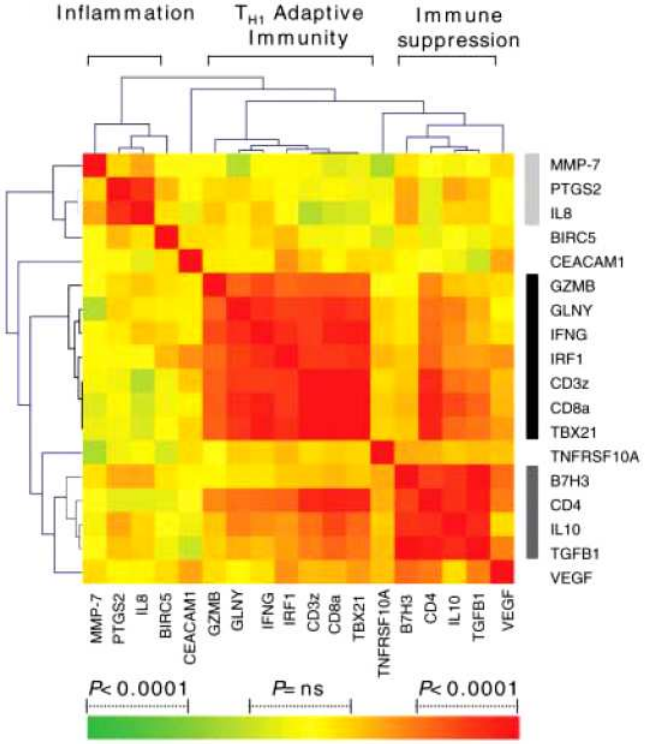
**Almost no DCs or T cells in Pediatric Cancers**

# Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,[1*†] Anne Costes,[1] Fatima Sanchez-Cabo,[2] Amos Kirilovsky,[1] Bernhard Mlecnik,[2] Christine Lagorce-Pagès,[3] Marie Tosolini,[1] Matthieu Camus,[1] Anne Berger,[4] Philippe Wind,[4] Franck Zinzindohoué,[5] Patrick Bruneval,[6] Paul-Henri Cugnenc,[5] Zlatko Trajanoski,[2] Wolf-Herman Fridman,[1,7] Franck Pagès[1,7†]

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

29 SEPTEMBER 2006 VOL 313 SCIENCE www.sciencem




**Tumor histopathology**

UICC-TNM Staging system

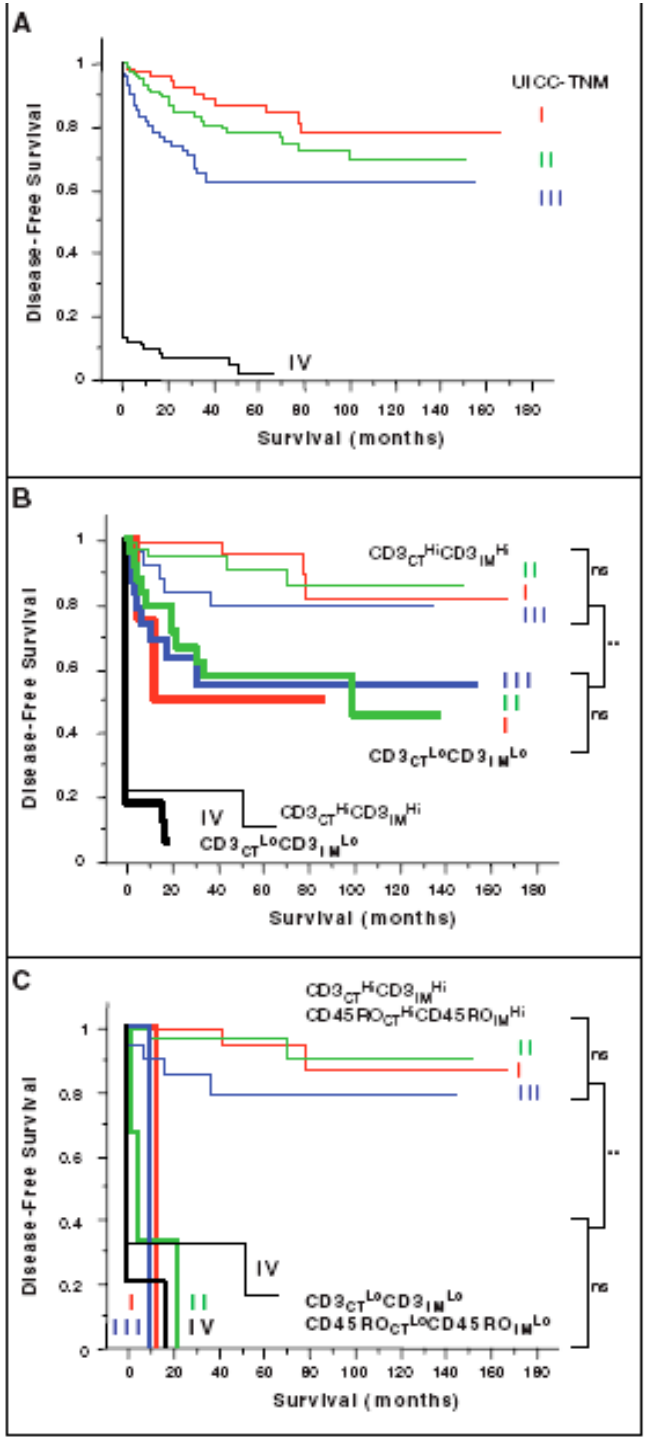
**Tumor infiltrating immune cells**

$CD3_{CT}CD3_{IM}$ evaluation

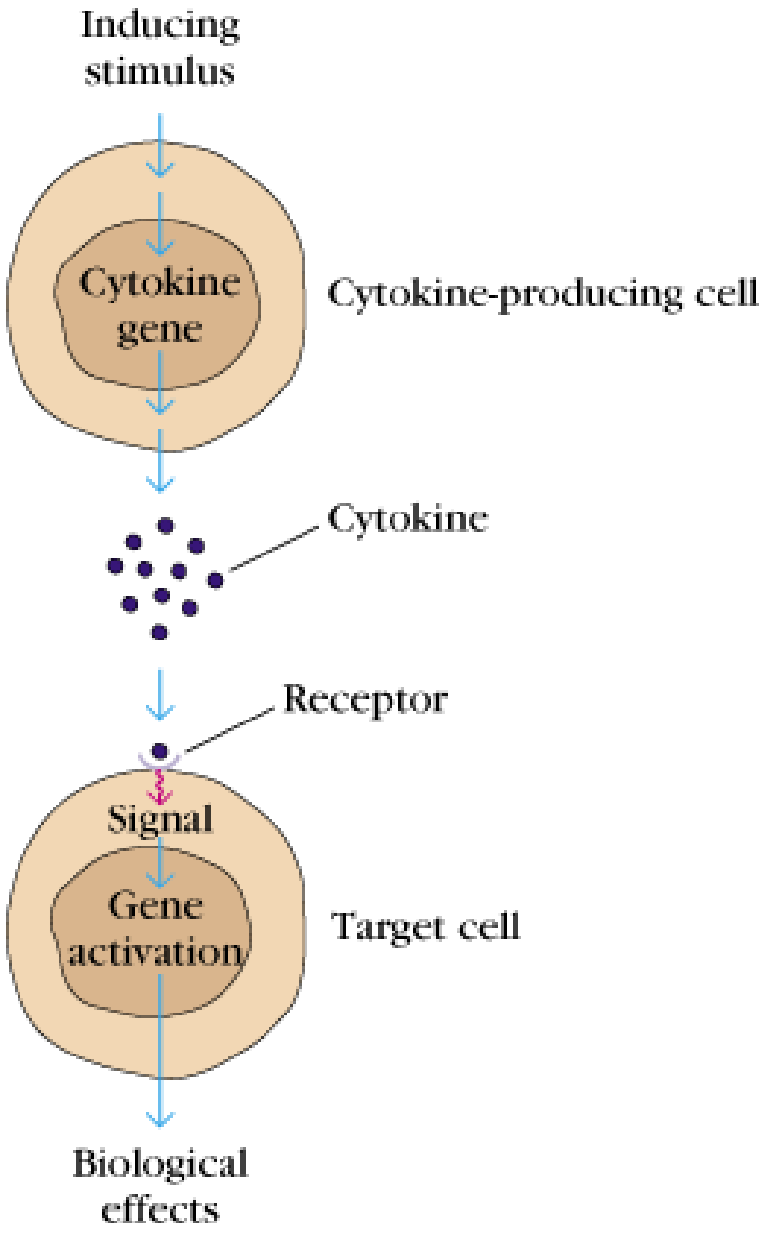
$CD3_{CT}CD3_{IM}$ evaluation

*plus*

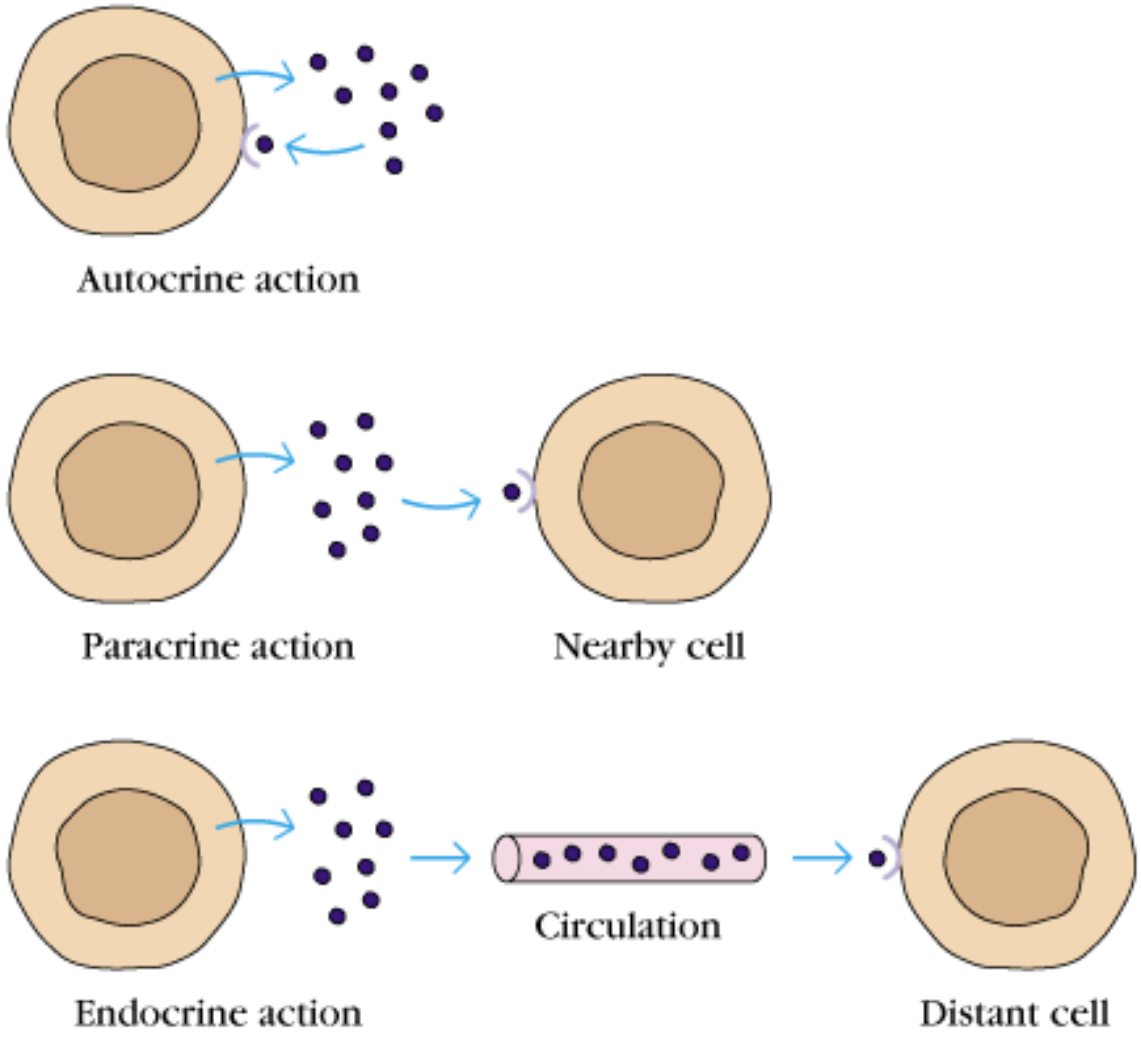
$CD45RO_{CT}CD45RO_{IM}$ evaluation



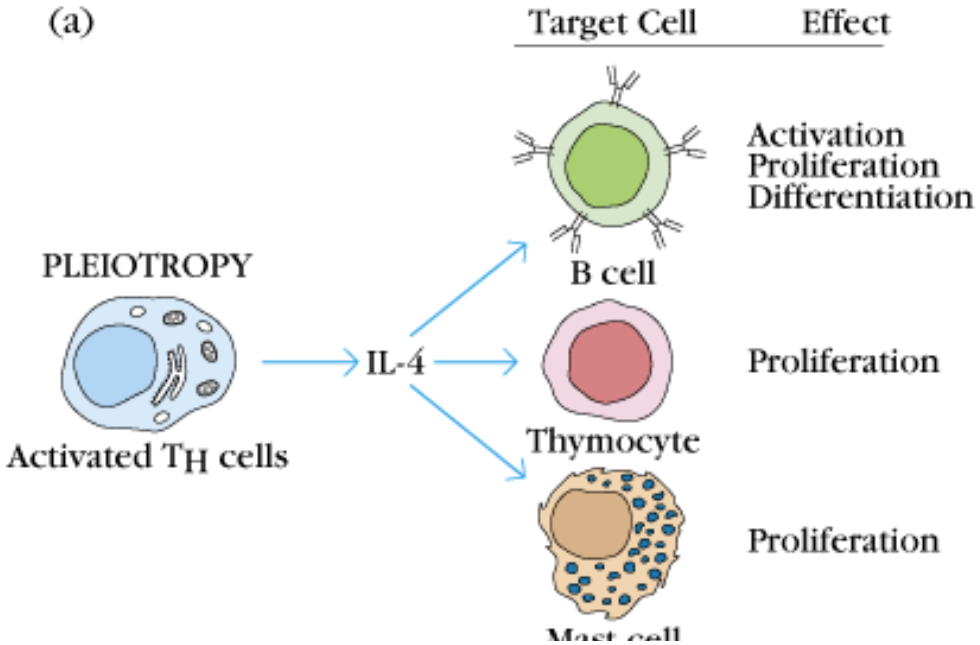
# General properties of cytokines



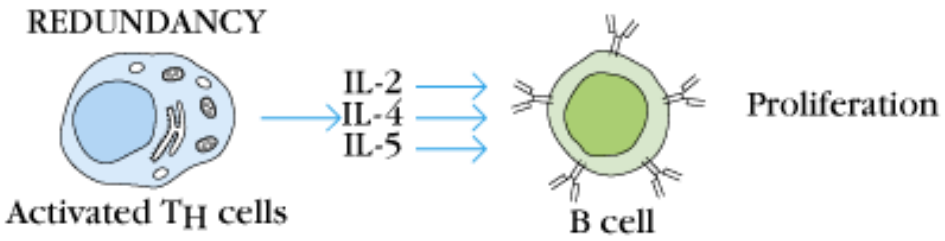
# General properties of cytokines



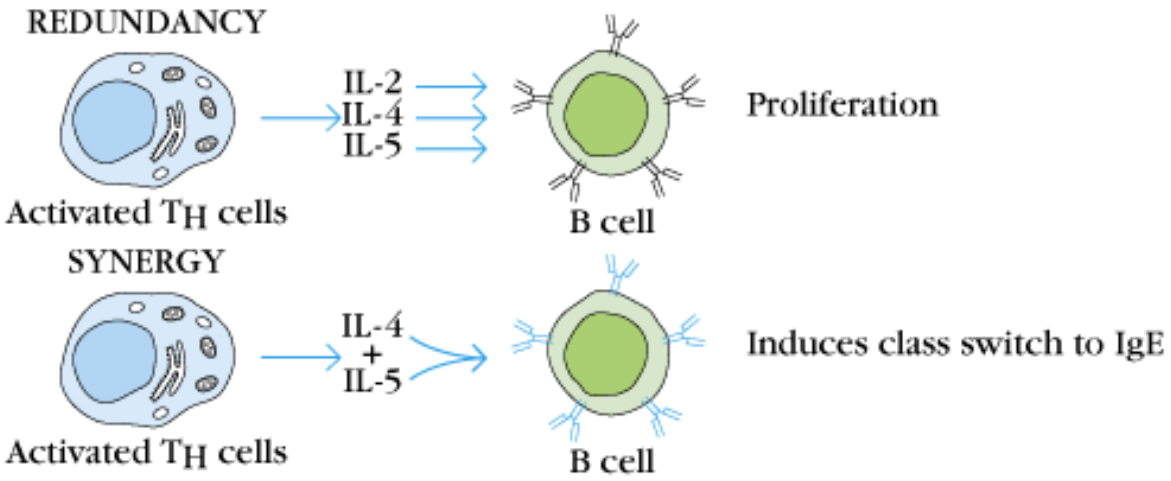
# General Properties Of Cytokines



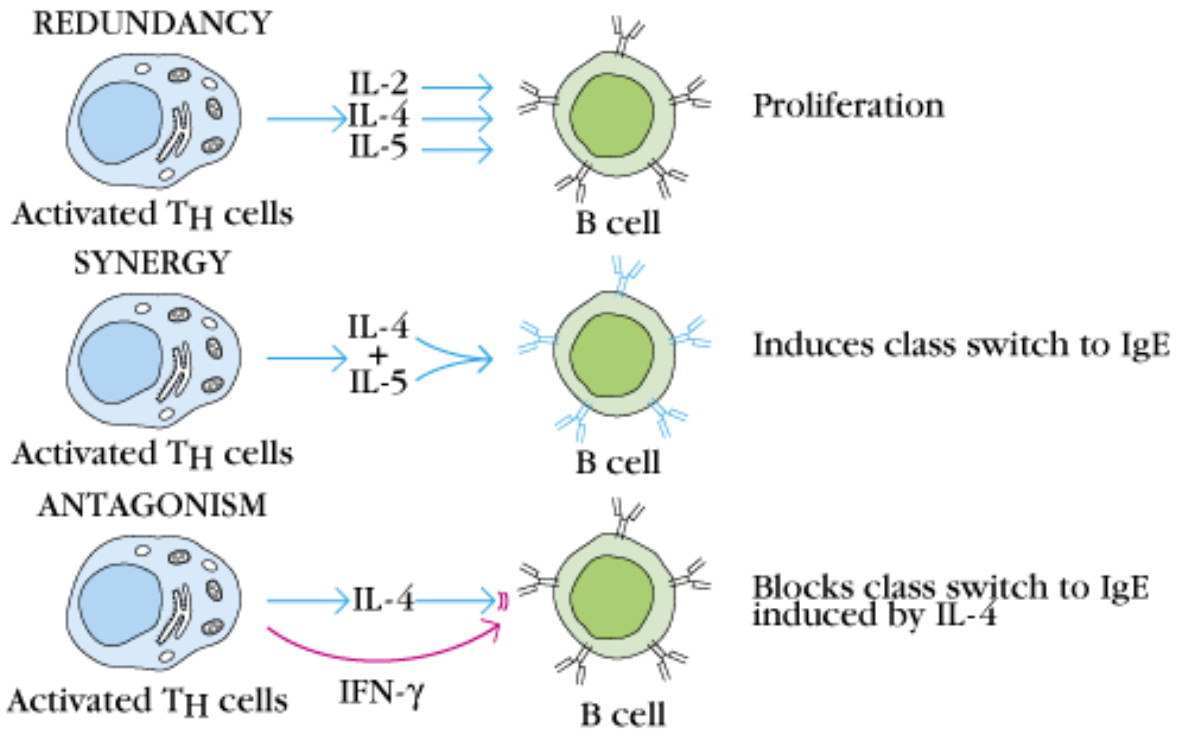
# General Properties Of Cytokines



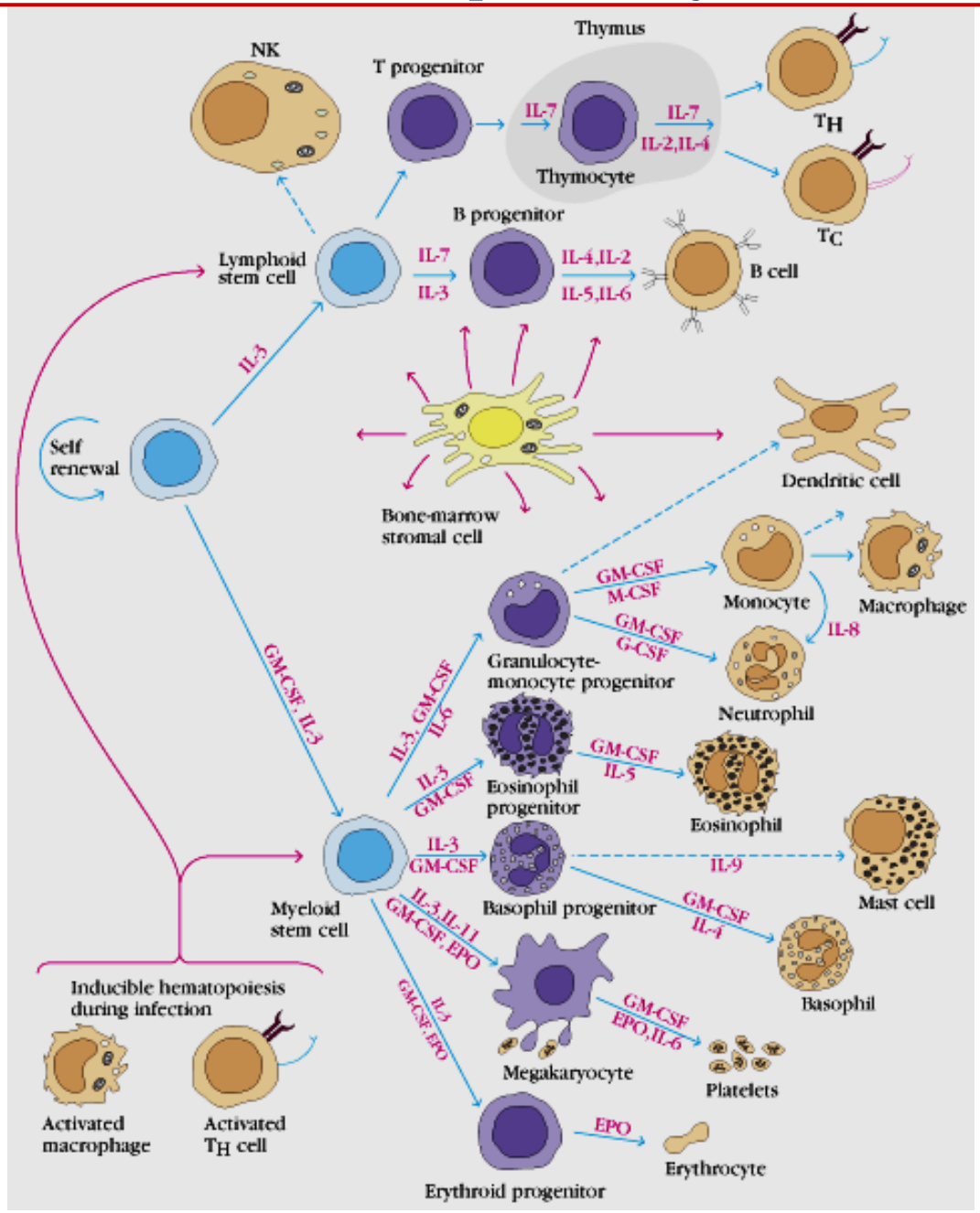
# General properties of cytokines



# General Properties Of Cytokines



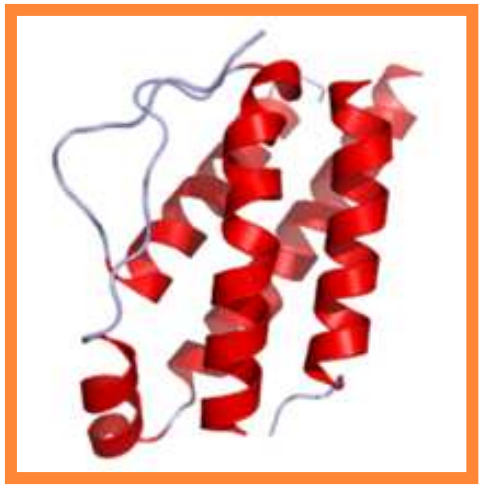
# Hematopoietic Cytokines




**IL-3**
**IL-11**
**EPO**
**M-CSF**
**G-CSF**
**GM-CSF**
**IL-5**
**IL-9**
**IL-4**
**IL-12, IL-18**
**IL-24**

# CYTOKINES IFNA, IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, IL-24….IL-38

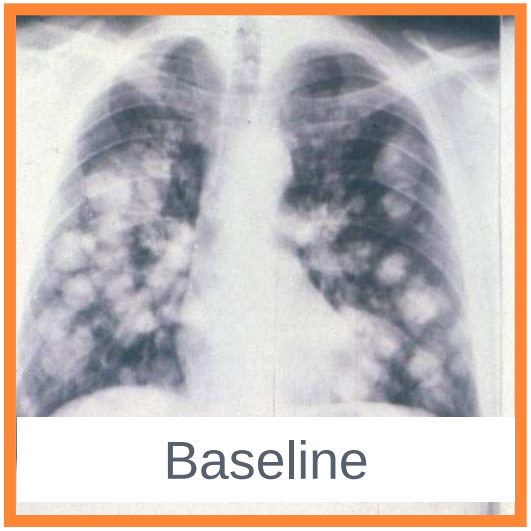
- IL-2 Discovered in 1976 and described as a protein that stimulated growth of T cells[1]
- Jurkat IL-2 in 1983 [Lotze]
- Recombinant IL-2 first cloned in 1983[1]
- First phase I studies of rIL-2 in malignant disease in 1984[2]
- Phase II clinical trials began in 1985[3]




1. Atkins MB, Lotze MT et al. *J Clin Oncol*. 1999:17;2105-2116.
2. Lotze MT et al *J Immunol.* 1985;134:157-166
3. Atkins MB et al. *J Clin Oncol.* 1986;4:1380-1391

# High Dose IL-2 Immunotherapy

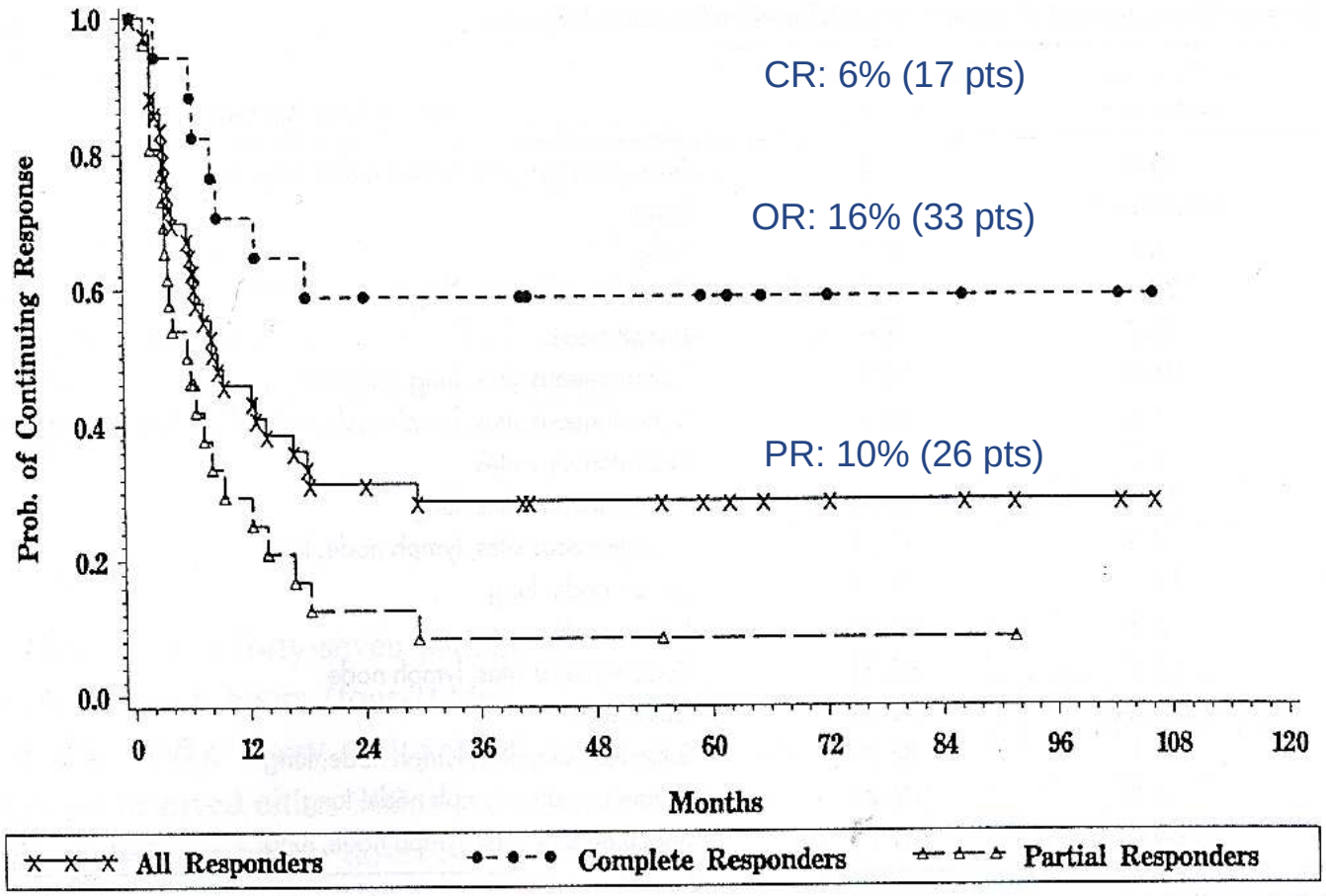
- Approved in patients with melanoma and kidney cancer.
- Significant ‘toxicity’.
- Associated with ‘cytokine storm’.
- iNOS blockers,  sTNF-R or IL-1Ra have yielded limited reduction in side effects.
- IL-2 treatment is associated with a ‘systemic autophagic syndrome’ and temporally limited tissue dysfunction.


Baseline


After Treatment

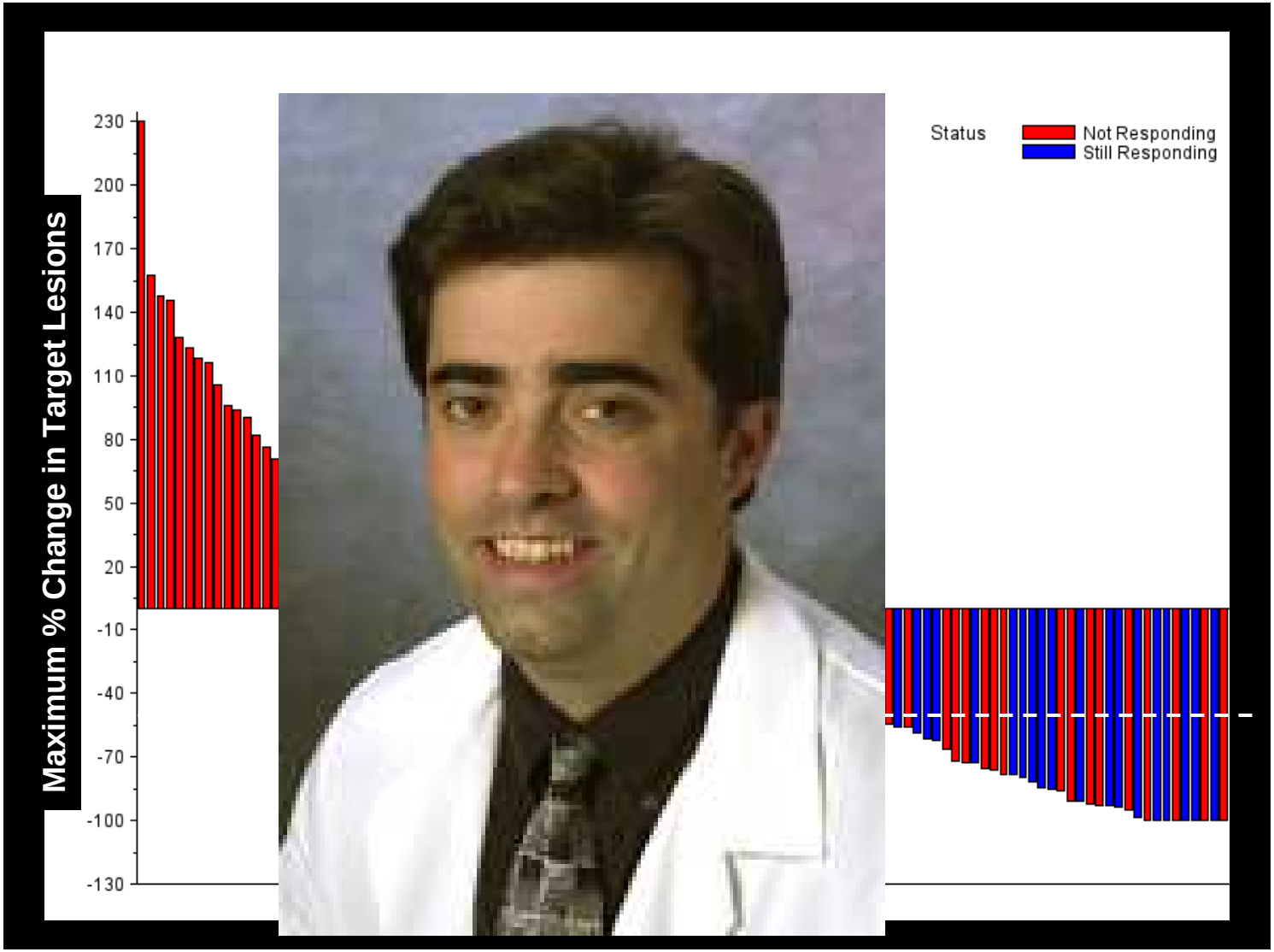
*AR. Chavez, X Liang, MT Lotze. Ann. N.Y.Acad.Sci.1182:14-27 (2009)*

# THE HALLMARK OF IL-2 THERAPY




Atkins MB, Lotze MT, et al. J Clin Oncol 1999

# Renal Cancer Response Rate=25% (n=118)



**May 27, 2010** — Two white-coated cancer researchers are among the luminaries picked for *TIME* magazine's 2010 list of the 100 most influential people in the world. Larry Kwak, MD, PhD, and Doug Schwartzentruber, MD, FACS, join Sarah Palin, James Cameron, Steve Jobs, & Lady Gaga on this year's "influentials" list.

# Dr. Doug Schwartzentruber


Melanoma
gp100 2092M
+IL-2

BiovaxID
patient-specific
vaccine for
follicular
lymphoma

Dr. Larry Kwak

# N ENGL J MED 2011; JUNE 2; 364:2119-27.

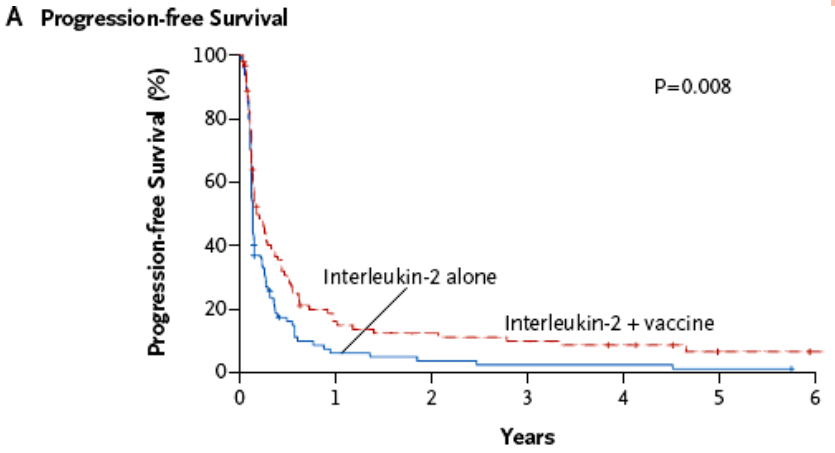
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

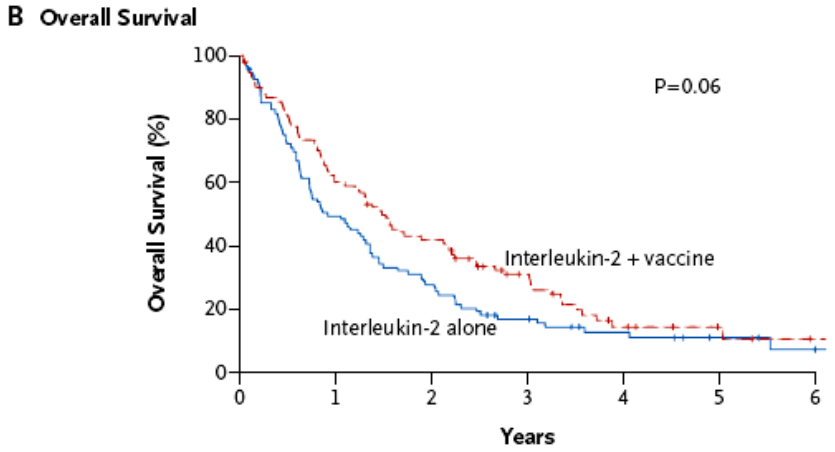
## gp100 Peptide Vaccine and Interleukin-2 in Patients with Advanced Melanoma

Douglas I. Schwartzentruber. M.D.. David H. Lawson. M.D..



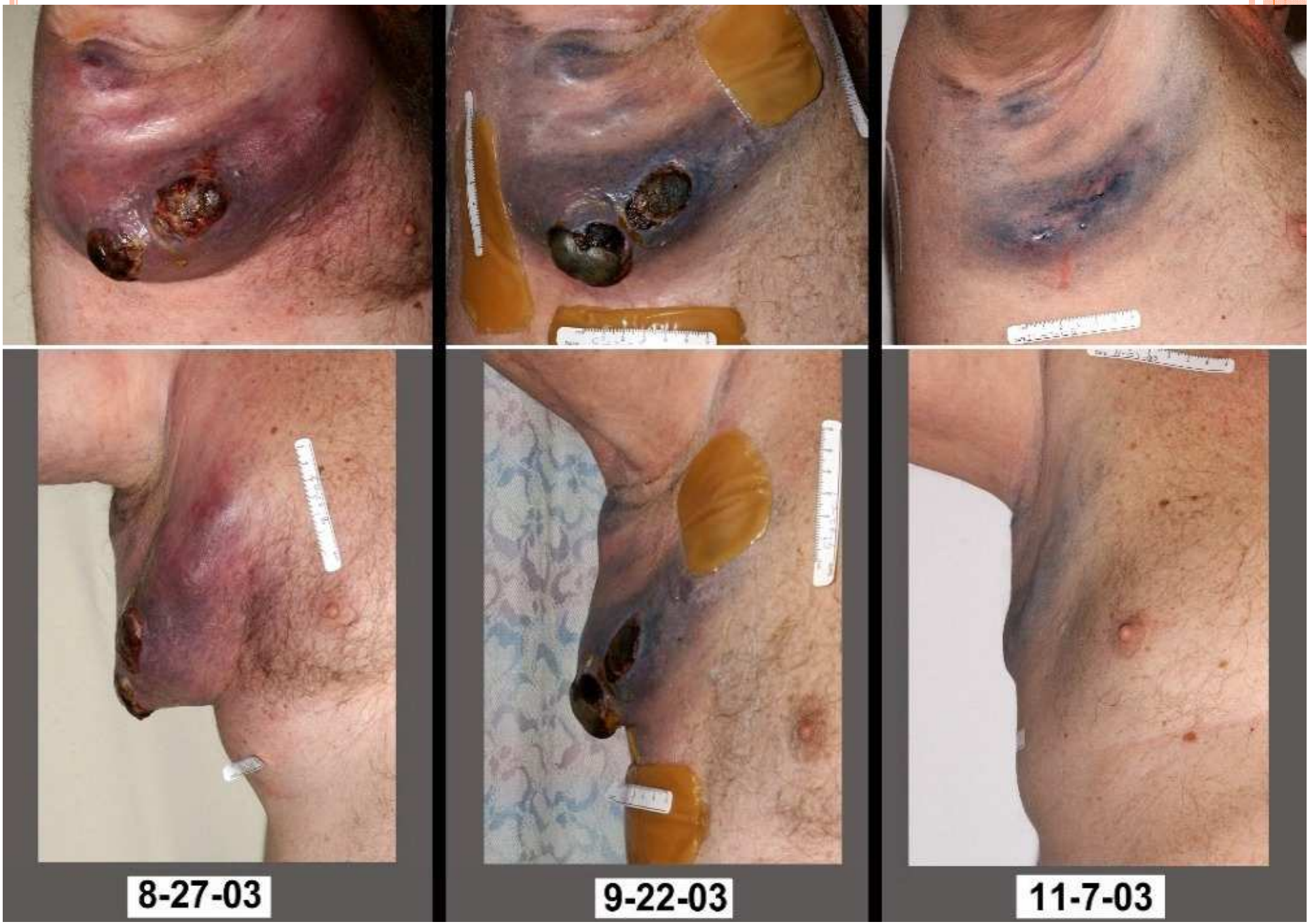


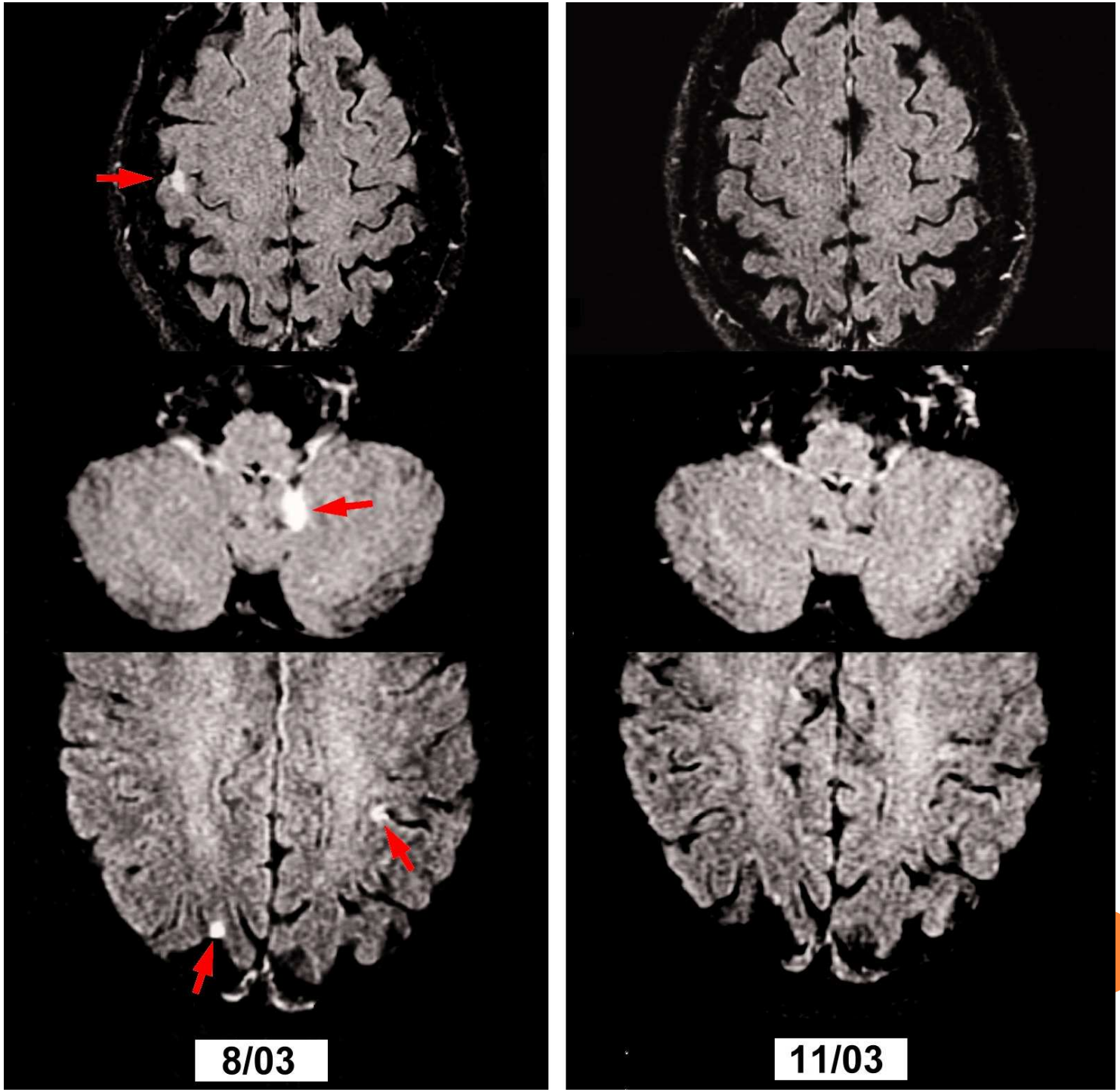

| No. at Risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Interleukin alone | 94 | 5 | 3 | 2 | 2 | 1 | 0 |
| Interleukin-2 + vaccine | 91 | 13 | 10 | 8 | 6 | 2 | 1 |

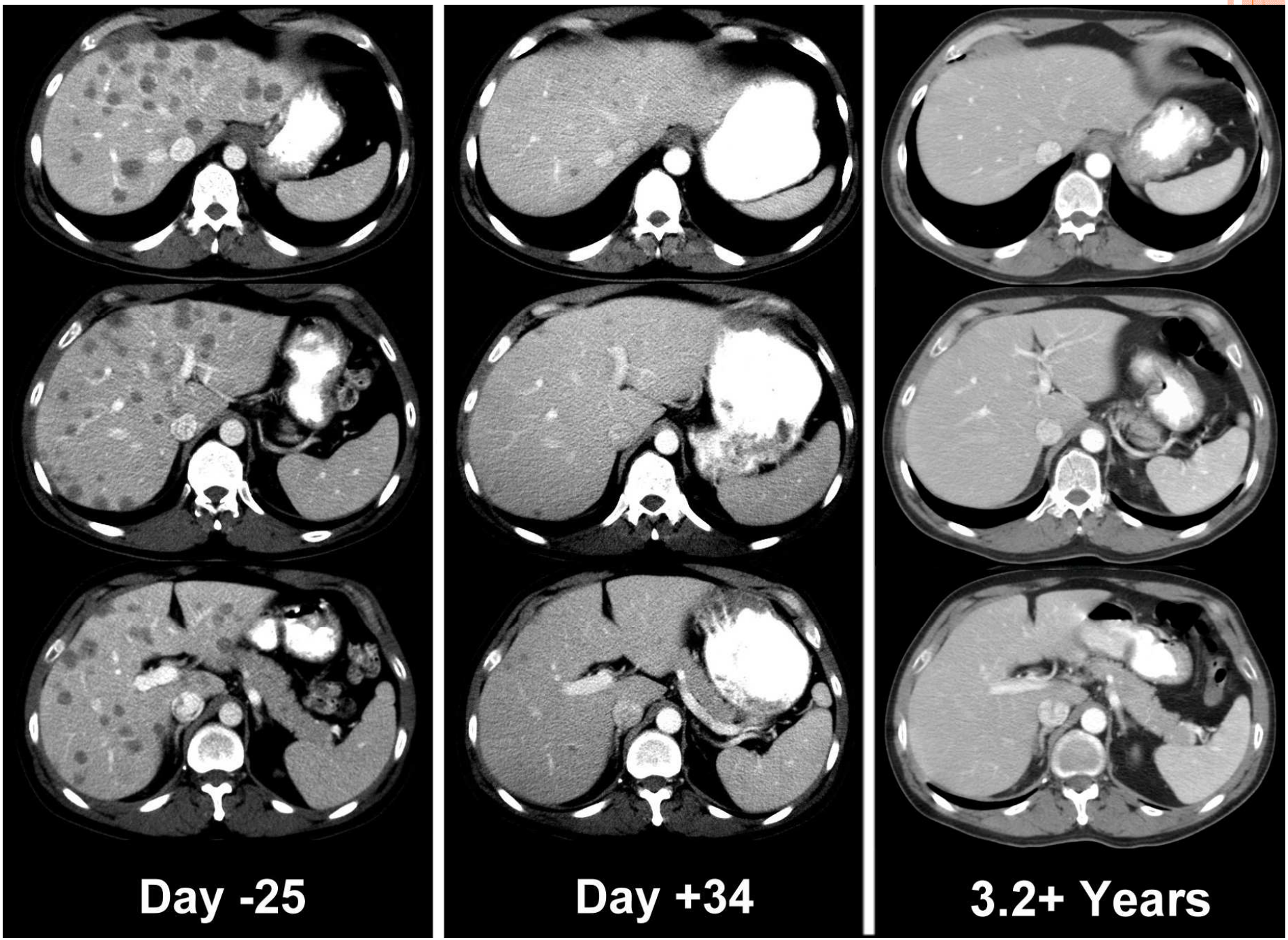


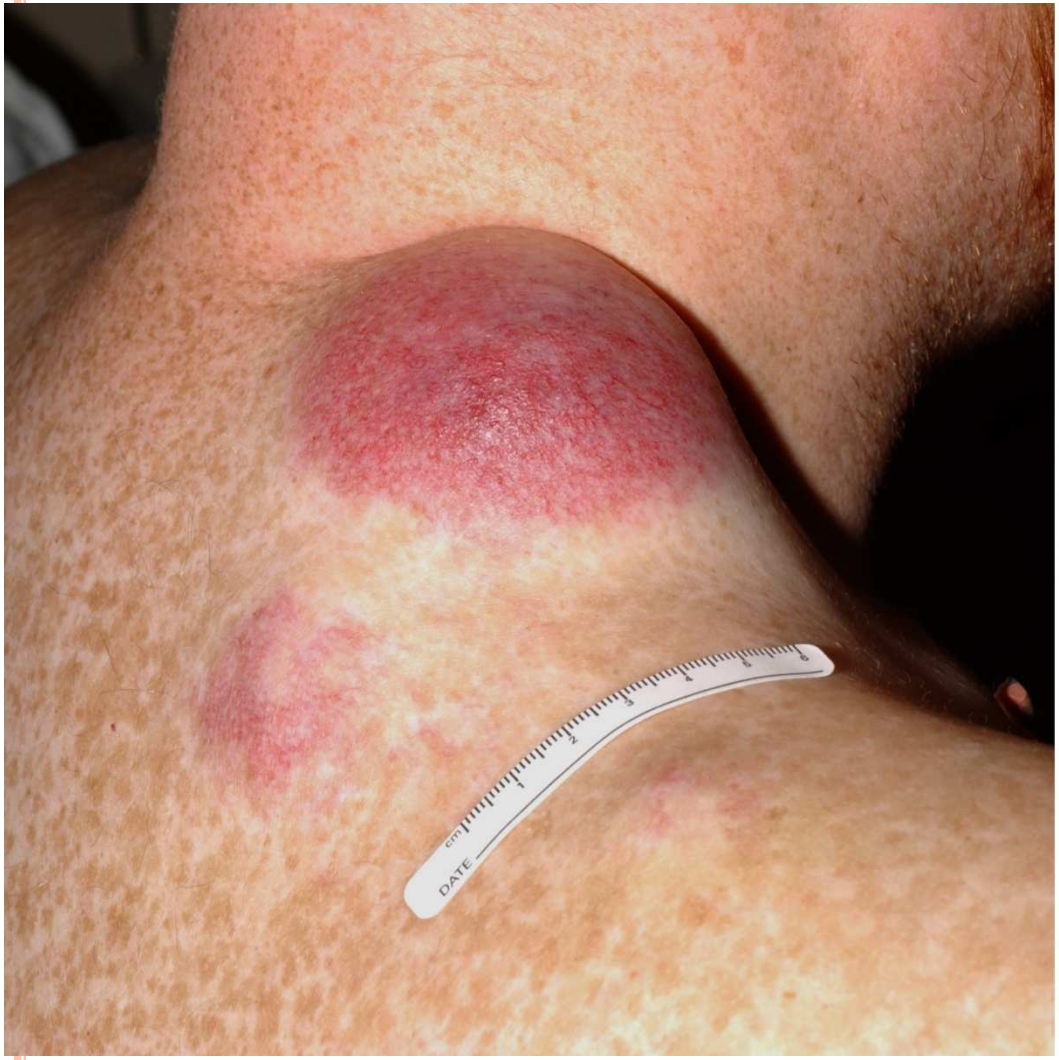

| No. at Risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| Interleukin alone | 94 | 46 | 26 | 14 | 8 | 4 | 1 |
| Interleukin-2 + vaccine | 91 | 54 | 37 | 20 | 8 | 4 | 1 |

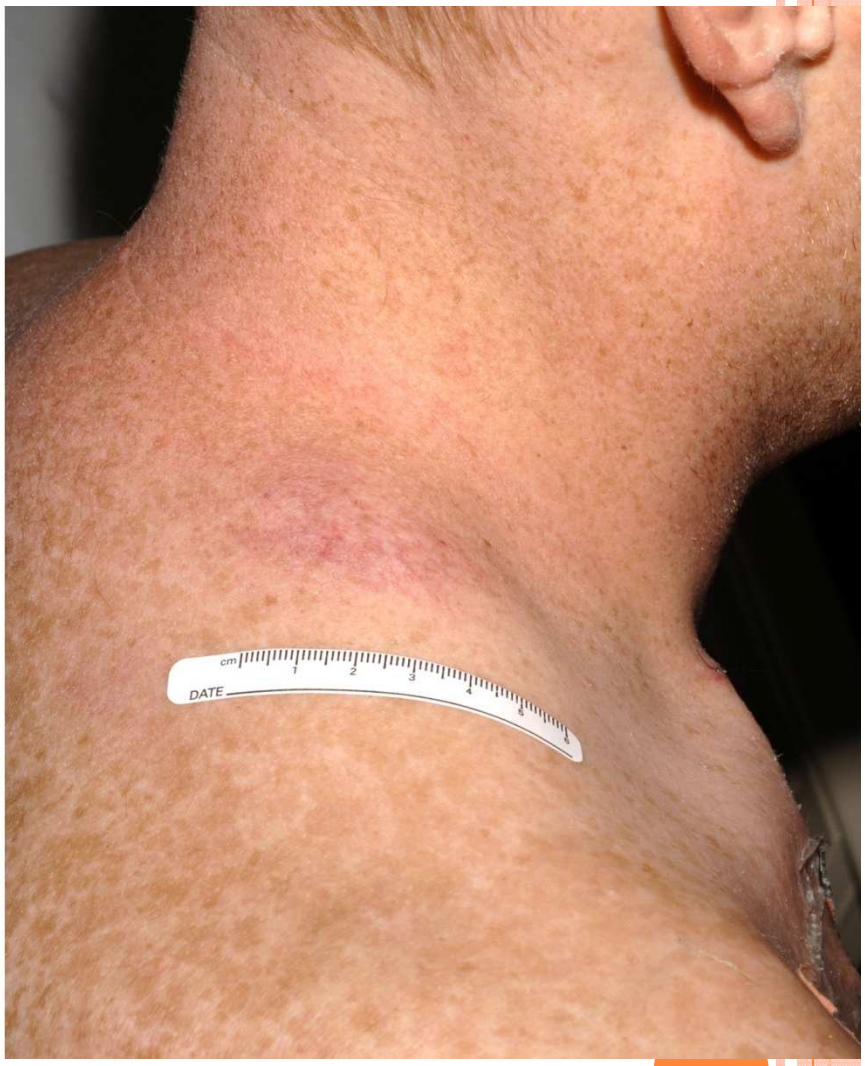
# Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2


8-27-03
9-22-03
11-7-03


8/03
11/03


Day -25
Day +34
3.2+ Years


cm
DATE


cm
DATE

Inhibi lrome –
A Phase I/II Aldesleukin in
R C)

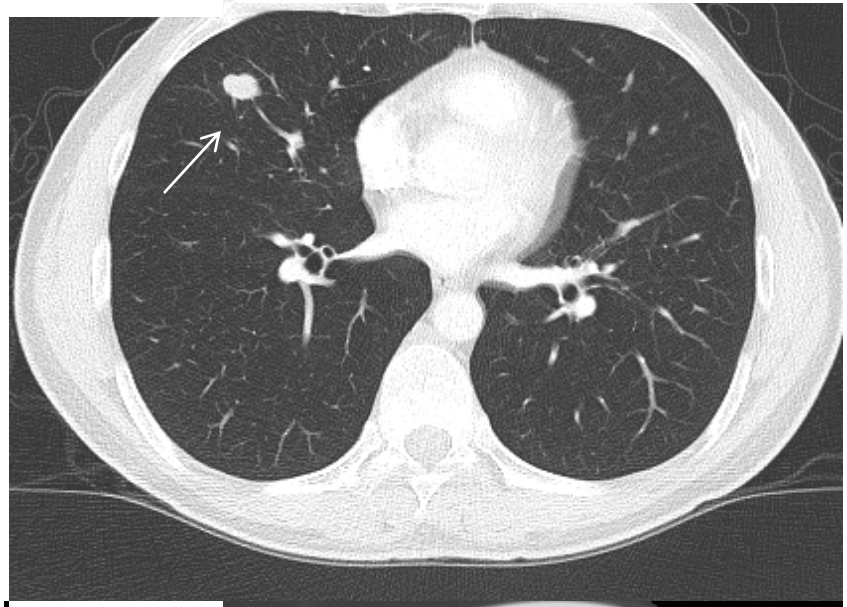
A Cyt ) Study
Princip otze, MD

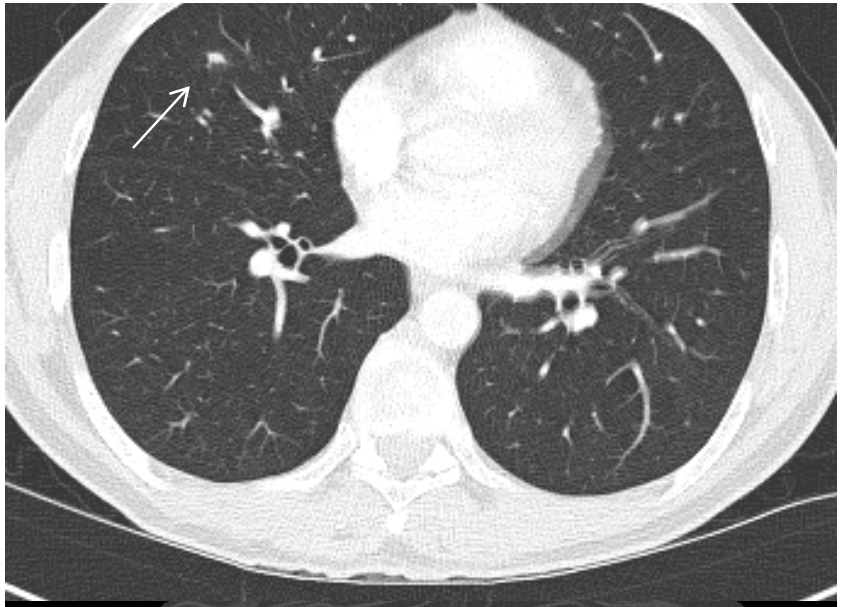
16 Patients

Pre-Therapy

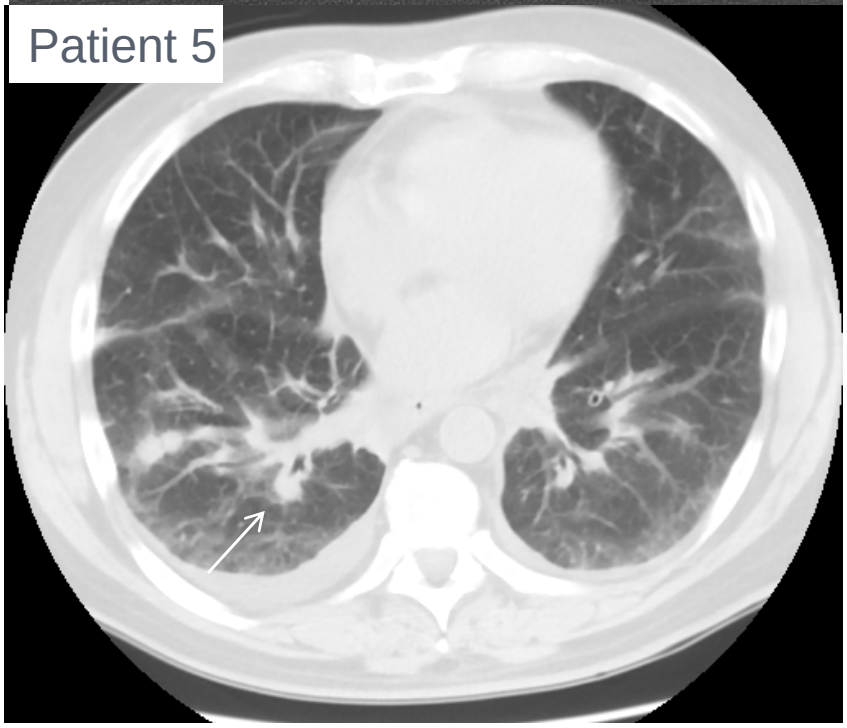
Post-therapy

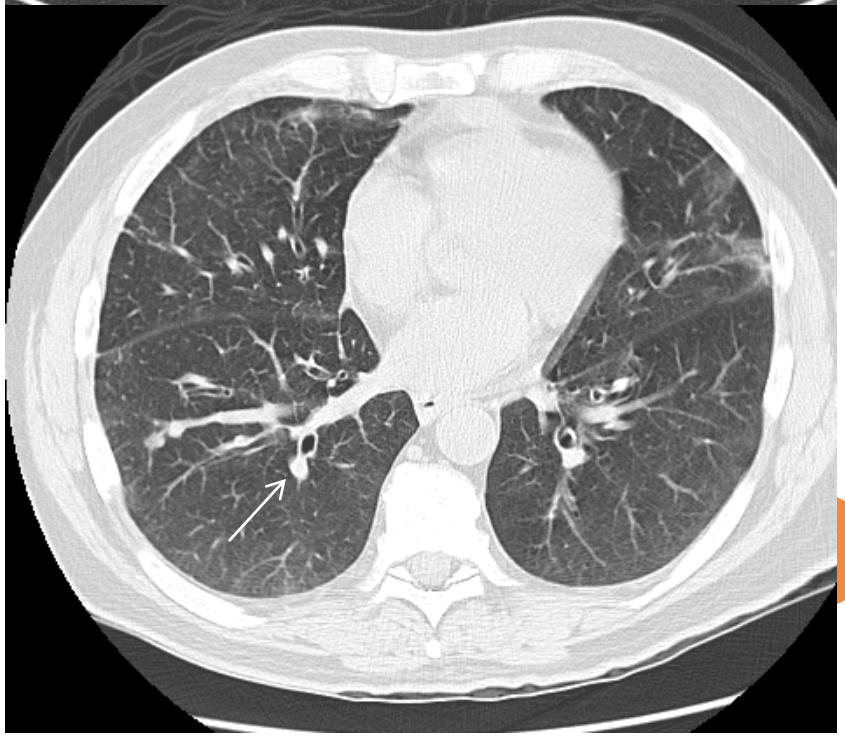
Patient 3





Patient 5

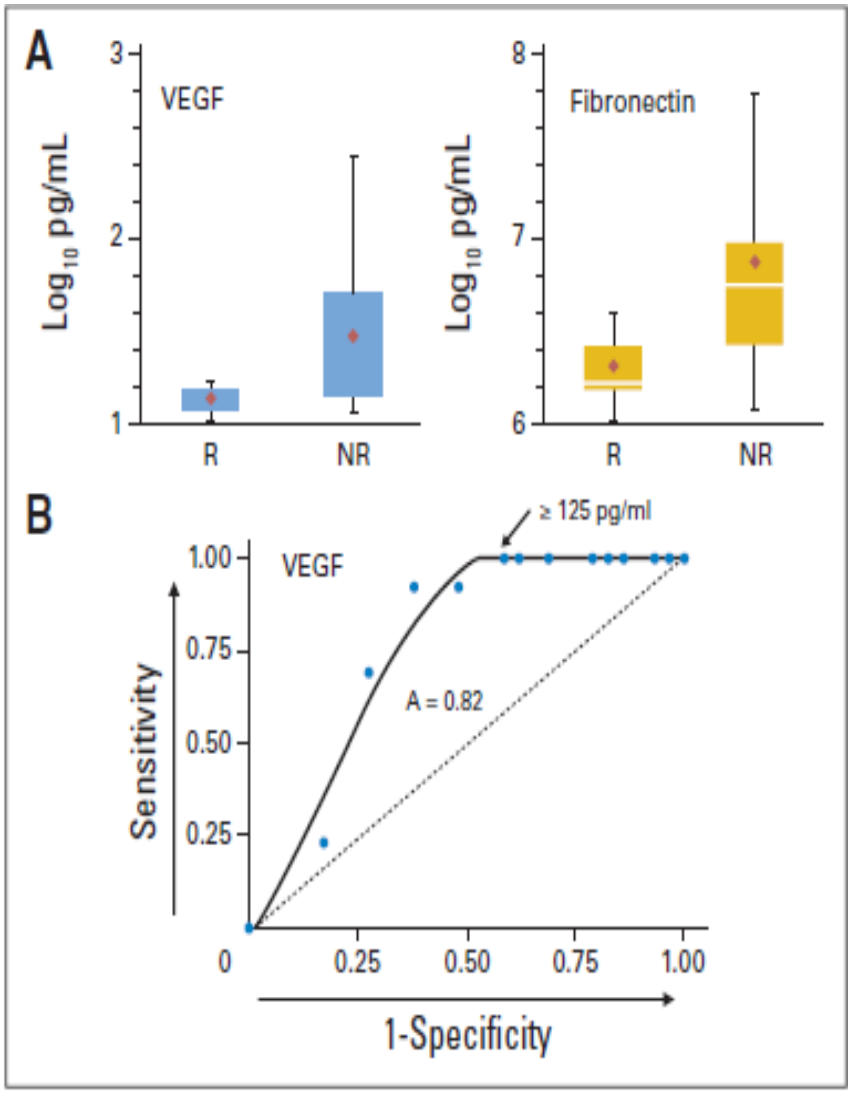


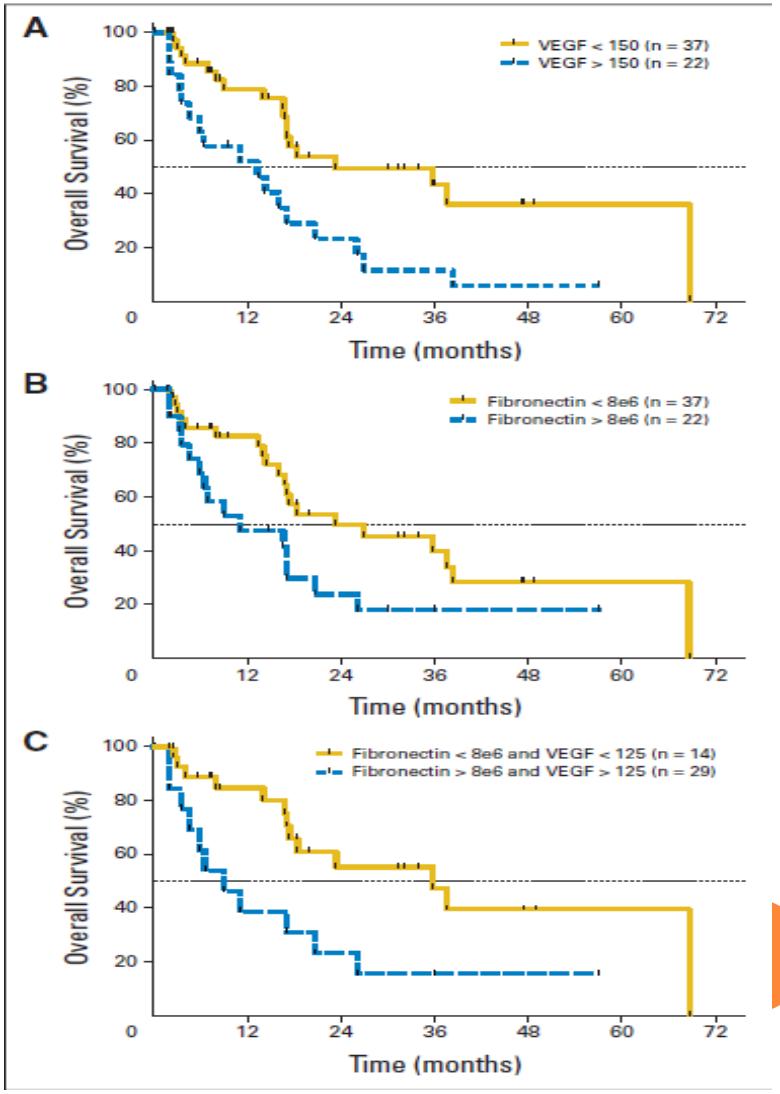


VOLUME 27 · NUMBER 16 · JUNE 1 2009
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

# Serum Vascular Endothelial Growth Factor and Fibronectin Predict Clinical Response to High-Dose Interleukin-2 Therapy

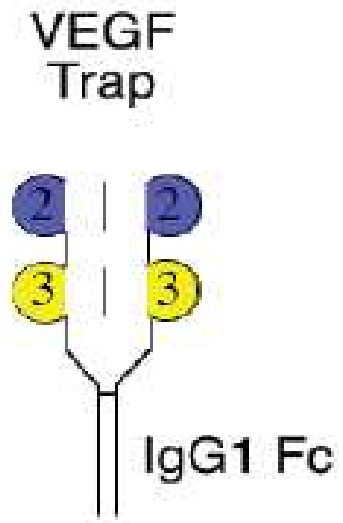
*Marianna Sabatino, Seunghee Kim-Schulze, Monica C. Panelli, David Stroncek, Ena Wang, Bret Taback, Dae Won Kim, Gail DeRaffele, Zoltan Pos, Francesco M. Marincola, and Howard L. Kaufman*



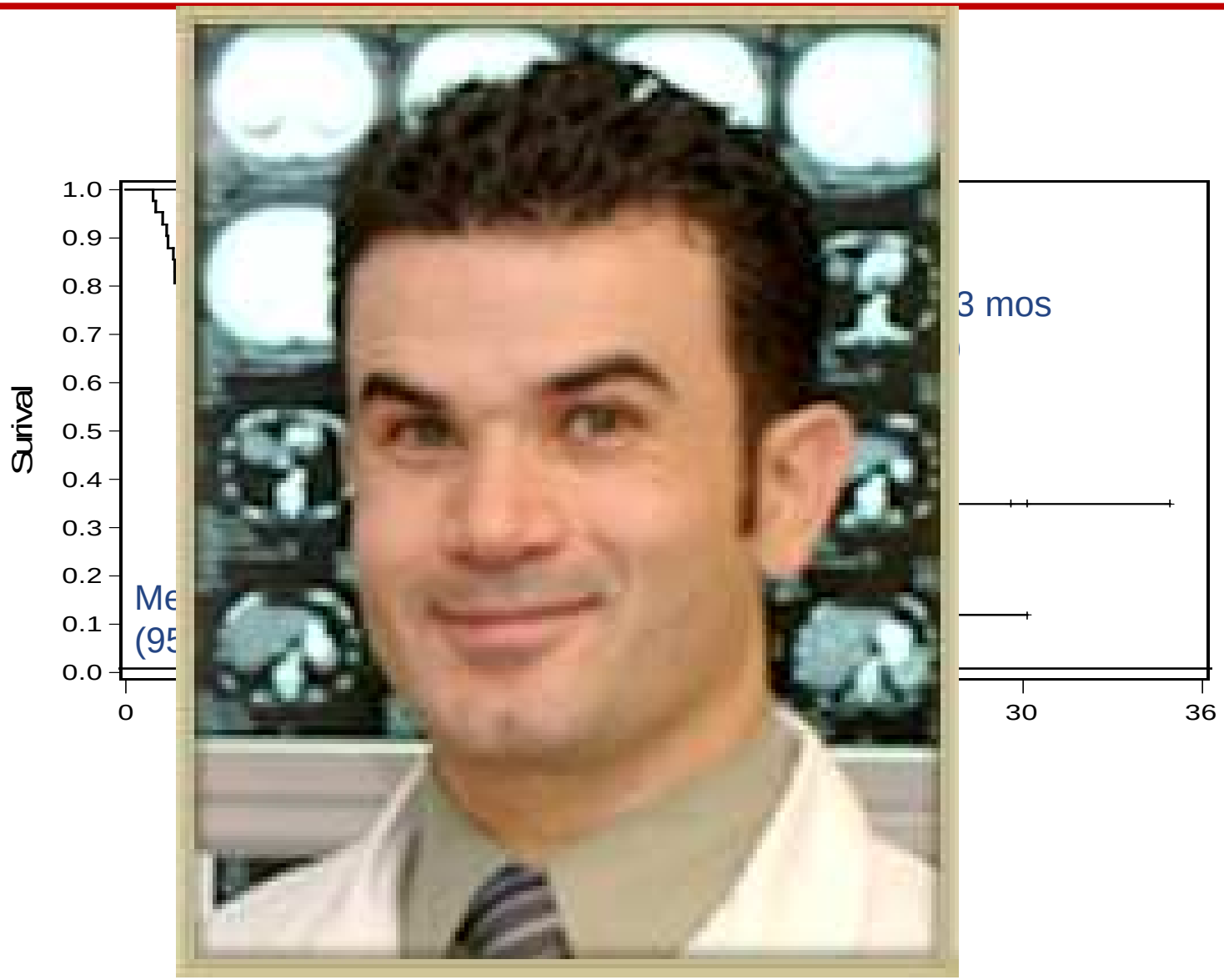
# VEGF TRAP

- Aflibercept (VEGF Trap) is a fusion protein combining the Fc portion of human IgG1 with the principal extracellular ligand-binding domains of human VEGFR1 & VEGFR2




- Acts as a high-affinity soluble decoy VEGF receptor and potent angiogenesis inhibitor
- Aflibercept has highest binding affinity for VEGF described to date. Dissociation constant 0.5 pM

# Kaplan – Meier plots of the probability of OS and PFS (N=40)




Tarhini et. al. Clin Cancer Res 2011

# IL-2 AND IPILIMUMAB ARE FDA APPROVED DRUGS FOR THE TREATMENT OF MELANOMA

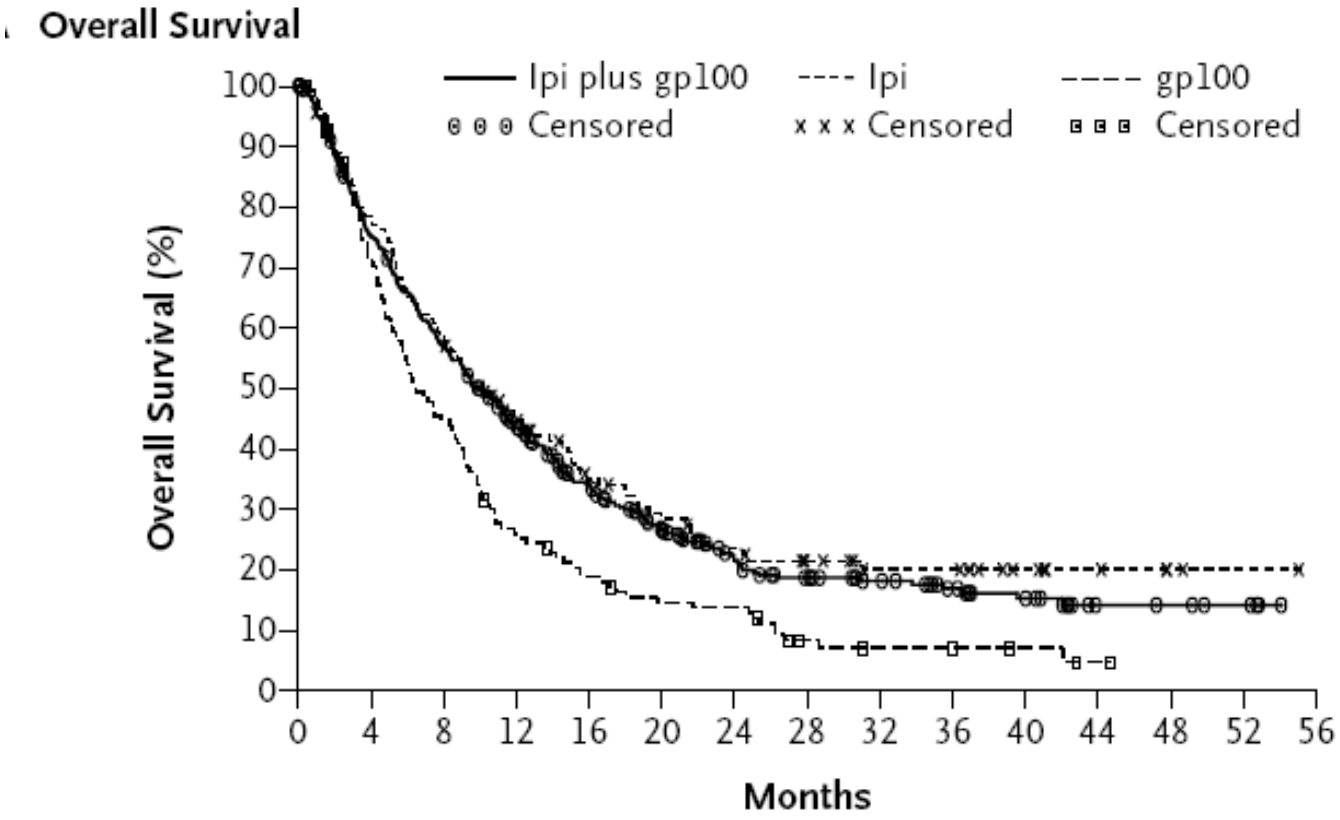
## Proleukin (IL-2)

- Cytokine that promotes proliferation and cytotoxicity of T cells and NK cells
- Extensively evaluated in patients with cancer
- Results in durable objective responses in 16-17%
- FDA approved for metastatic melanoma in 1998

## Ipilimimab (αCTLA-4)

- Monoclonal antibody that blocks CTLA-4 binding to B7
- Promotes anti-tumor activity through T cells
- Demonstrated improved overall survival in Phase III trial
- FDA approved for metastatic melanoma in 2011

# IPILUMIMAB IMPROVES OVERALL SURVIVAL



# PHASE I/II TRIAL OF IL-2 AND IPILUMIMAB

- NCI Surgery Branch trial
- 36 patients with metastatic melanoma
- 3 patients treated with Ipilumab at 0.1, 0.3, 1.0 and 2.0 mg/kg every 3 weeks X 3
- 24 patients treated with Ipilumimab at 3.0 mg/kg every 3 weeks X 3
- All patients received IL-2 (720,000 IU/kg) after the 2nd and 3rd dose of Ipilumimab
- 8/36 (22%) had an objective response
  - 3 CR
  - 5 PR
  - 6/8 ongoing >11-19 months
- 5/36 (14%) developed grade III/IV Ipi-related toxicities
- No correlation between Ipi dose and response or toxicity-all patients recovered

# STUDY UPDATE

- Median follow-up of 71 months

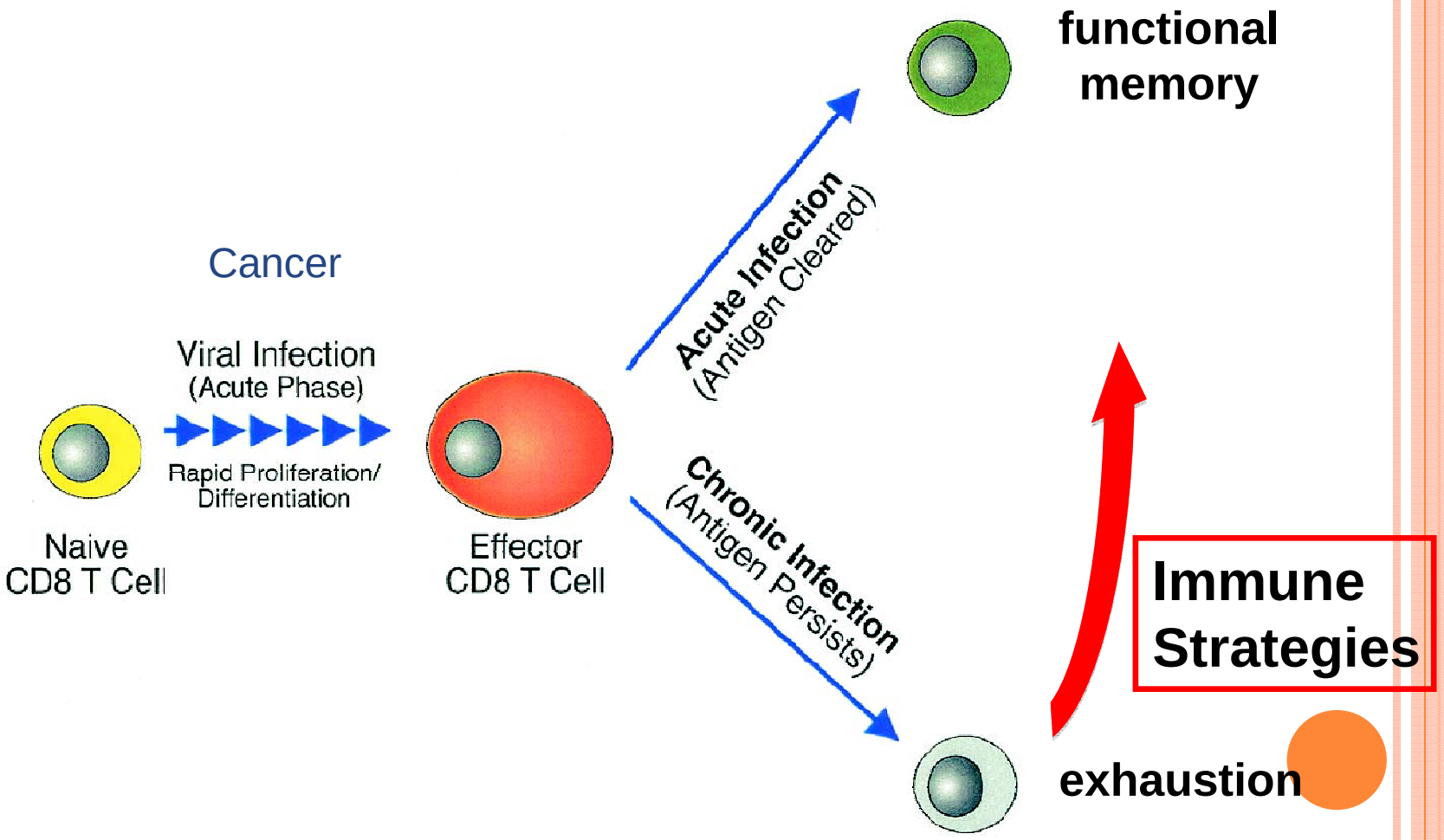
- 25% objective response rate
- 17% complete response
- Median survival of 16 months

# PROPOSED STUDY DESIGN

- Single arm, open-label trial
  - Arm … weeks x 4 cycles (day … 00 IU/kg) q 3 wee … )
  - At w … b (10 mg/kg) q 3 mon …

Howard I …
Cytokine Working Group

# CD8 T CELL DIFFERENTIATION DURING ACUTE VS. CHRONIC VIRAL INFECTION - CANCER




Kendall Smith, Rafi Ahmed

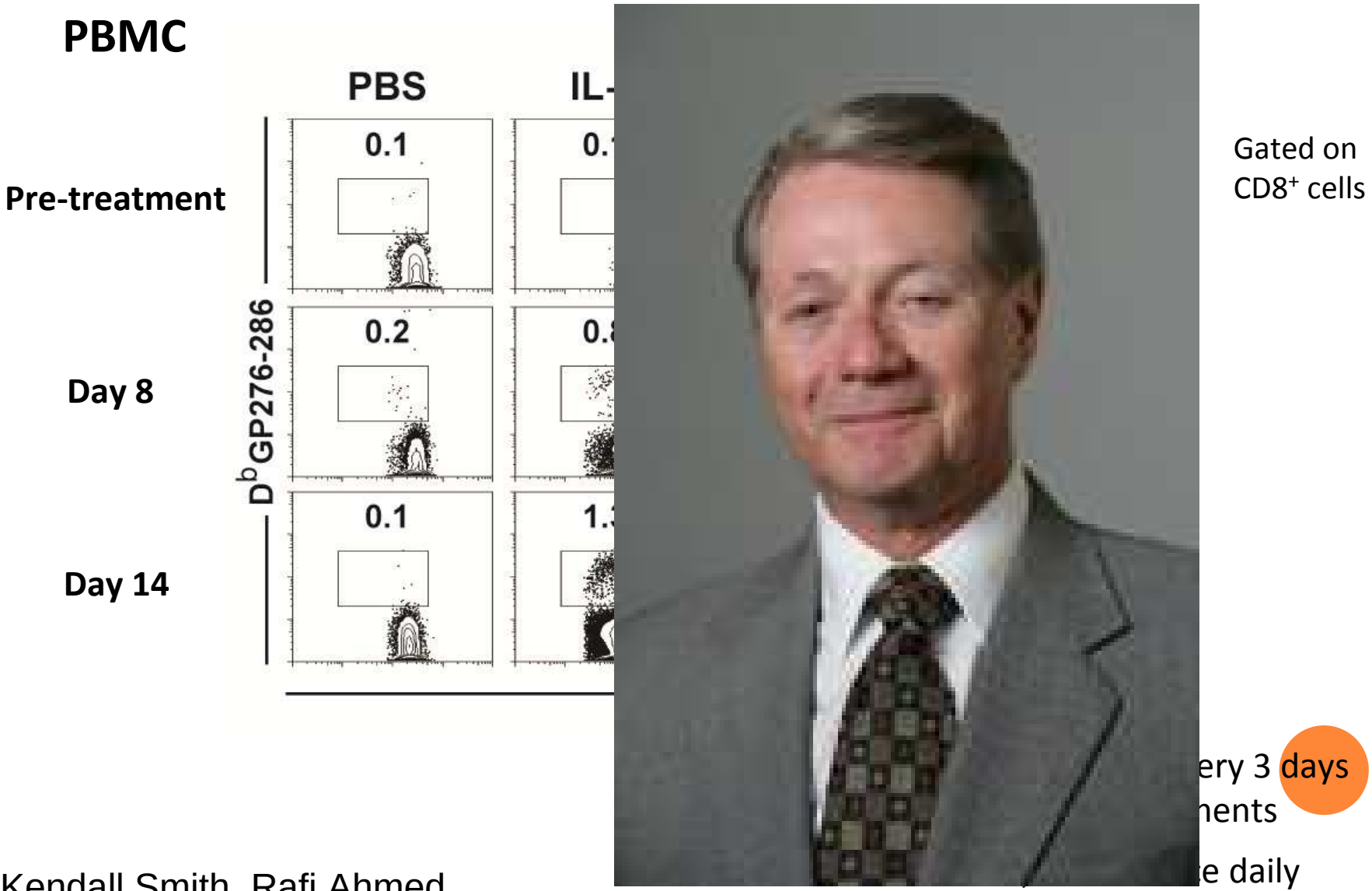
# COMBINATION THERAPY WITH PD-1 BLOCKADE :

- Therapeutic vaccination
- Antiviral / Cancer drugs
- Blockade of other inhibitory receptors and molecules
  - CTLA-4, LAG-3, Tim-3, 2B4
- Cytokines
  - IL-7, IL-15, IL-2

*West et al. JCI. 2013*

Kendall Smith, Rafi Ahmed

# RESCUE EXHAUSTED CD8 T CELLS DURING CHRONIC INFECTION




Kendall Smith, Rafi Ahmed

# INTERLEUKIN 7 (IL-7)

- Rec
  mer
- Enh
  mod
  pro
  cell
- Dos
  init
  infe


*Alpdogan et al, Blood 2001;98:2256-226; Alpdogan et al, J. Clin. Invest. 2003; 112:1095–1107; Rosenberg et al, J Immunother 2006;29:313–319; Sportes et al, J Exp Med 2008; 205: 1710-1714; Levy et al, J. Clin. Invest. 2009; 119:997–1007; Sereti et al, Blood 2009: 113:6304-6314; Sportes et al, Clin Cancer Res 2010; 16: 727–735.*

TRANSPLANTATION

# Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease

Onder Alpdogan, Cornelius Schmaltz, Stephanie J. Muriglan, Barry J. Kappel, Miguel-Angel Perales, Jimmy A. Rotolo, Jens A. Halm, Benjamin E. Rich, and Marcel R. M. van den Brink

*Alpdogan et al, Blood 2001;98:2256-226*

# IL-7 enhances peripheral T cell reconstitution after allogeneic hematopoietic stem cell transplantation

Önder Alpdogan, Stephanie J. Muriglan, Jeffrey M. Eng, Lucy M. Willis, Andrew S. Greenberg, Barry J. Kappel, and Marcel R.M. van den Brink

Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

*Alpdogan et al, J. Clin. Invest. 2003; 112:1095–1107.*

# IL-7 – INITIAL CLINICAL TRIALS WITH CYT99 007

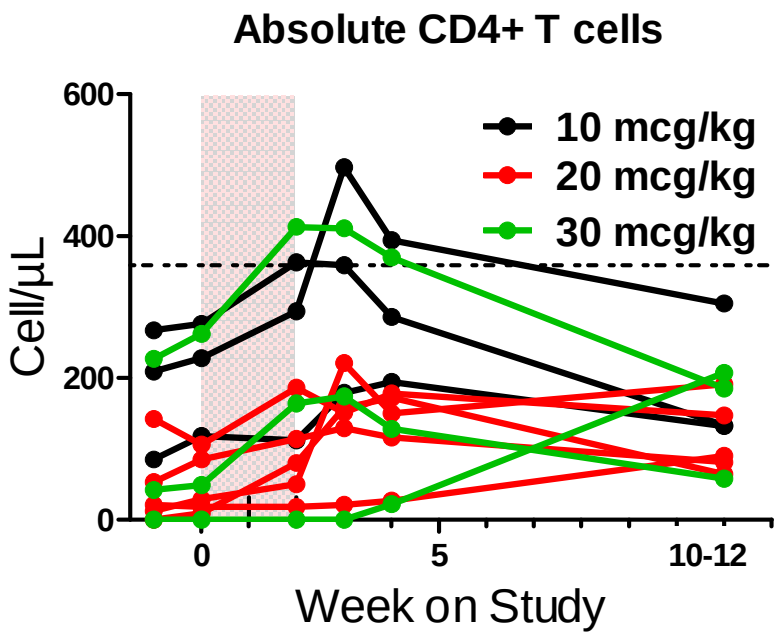
## Table - 62 patients treated on 5 clinical trials with CYT 99 007

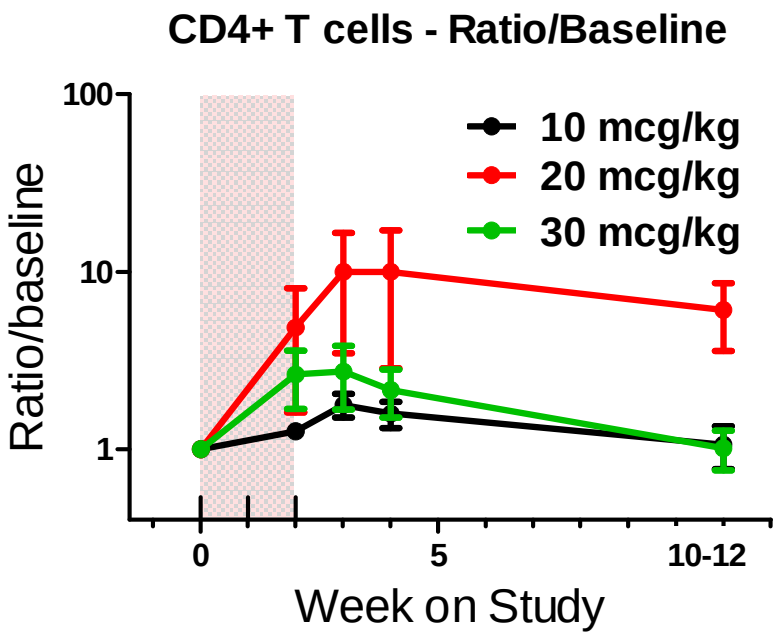
| Study | Indication | N | IL-7 Dose | Outcome | *Ref* |
|---|---|---|---|---|---|
| 1 | Solid tumor | 12 | 3 – 60 mcg/kg x8 + gp100 & MART1 pept vaccine | Rise in CD4 and CD8 T cells<br>Decrease in Tregs | *1* |
| 2 | Solid tumor | 16 | 3 – 60 mcg/kg x8 | Rise in CD4 and CD8 T cells<br>No objective tumor responses | *2,3* |
| 3 | HIV | 19 | 3 – 30 mcg/kg x1 | Rise in CD4 and CD8 T cells<br>Transient rise in HIV RNA | *4* |
| 4 | HIV | 14 | 3 – 10 mcg/kg x8 | Rise in CD4 and CD8 T cells<br>Transient rise in HIV RNA<br>Rise in HIV-spec CD4 T cells | *5* |
| 5 | | | | | |

*[1]Rosenberg et al, J Immunother 2006;29:313–319; [2]Sportes et al, J Exp Med 2008; 205: 1710-1714; [3]Sportes et al, Clin Cancer Res 2010; 16: 727–735; [4]Sereti et al, Blood 2009: 113:6304-6314; [5]Levy et al, J. Clin. Invest. 2009; 119:997–1007; [6]Perales et al, unpublished.*

# A multicenter, open-labeled, controlled, randomized study of recombinant Interleukin-7 (CYT107) treatment to achieve and maintain CD4 T-lymphocyte counts above 200 cells/μL in recipients of HLA-Matched ex vivo T cell depleted peripheral blood stem cell transplant.

# RHIL-7 (CYT107) INCREASES CD4+ T CELL COUNTS POST TCD ALLO-HSCT



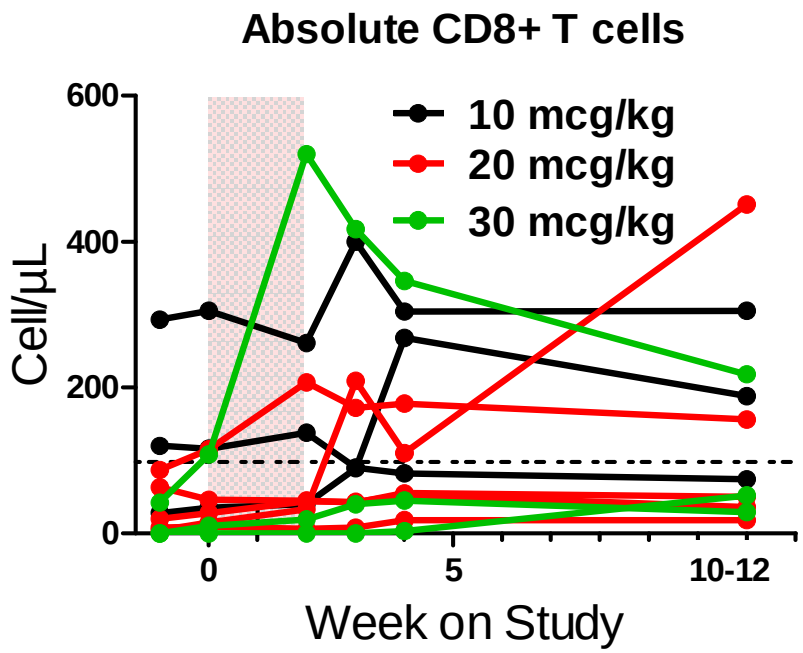



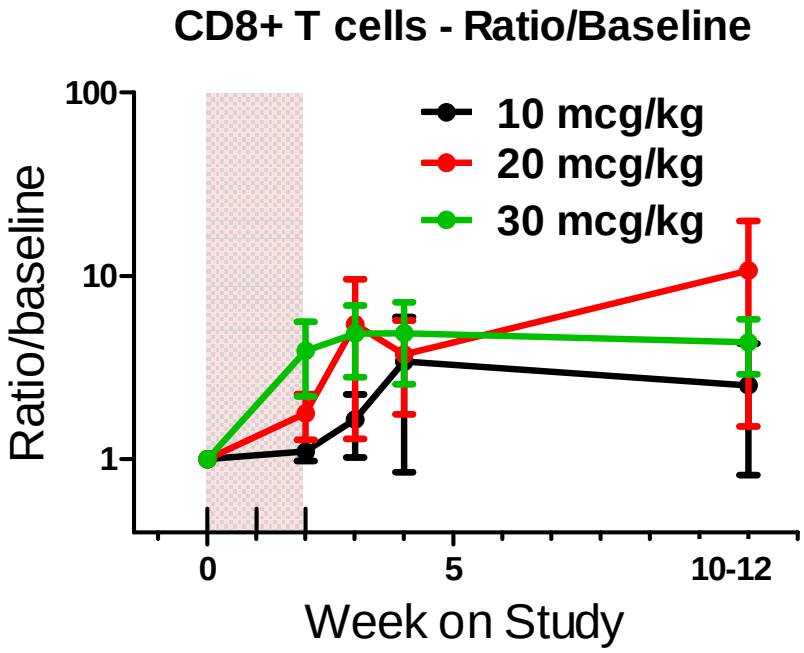

**107.4/mm³ average increase at day 21, p=0.002**
**(range 0 to 35-fold increase)**

*Perales, CITN Investigator Meeting – Nov 2013*

*Perales, Blood. 2012 Dec 6;120(24):4882-91*

# RHIL-7 (CYT107) INCREASES CD8+ T CELL COUNTS POST TCD ALLO-HSCT



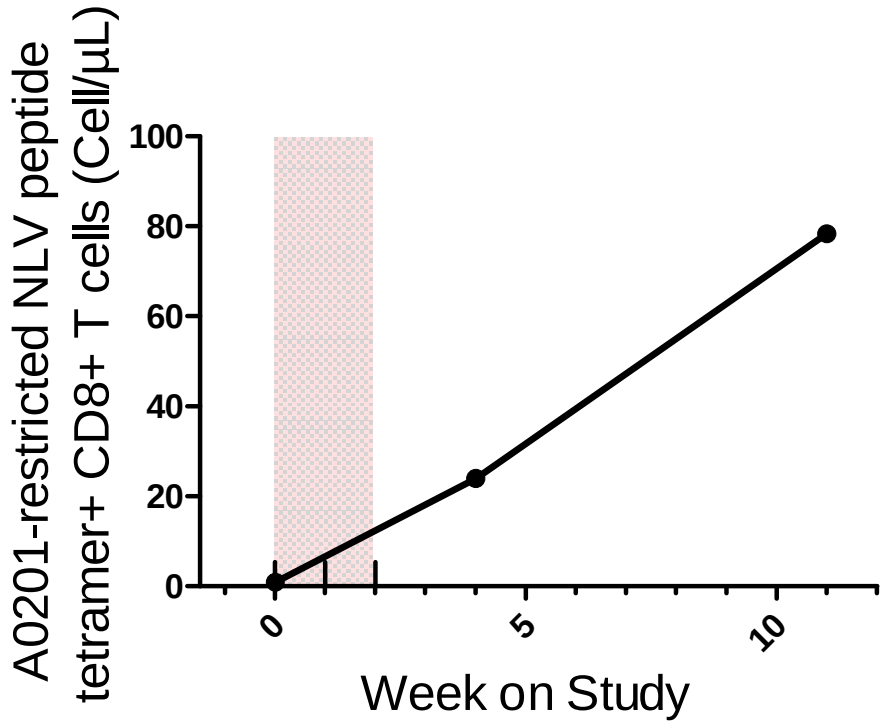




**66.9/mm$^3$ average increase at day 28, p=0.05**
**(range 0 to 11-fold increase)**

*Perales, CITN Investigator Meeting – Nov 2013*

*Perales, Blood. 2012 Dec 6;120(24):4882-91*

# CMV-SPECIFIC RESPONSES WERE INCREASED IN A PATIENT WITH A HISTORY OF CMV VIREMIA

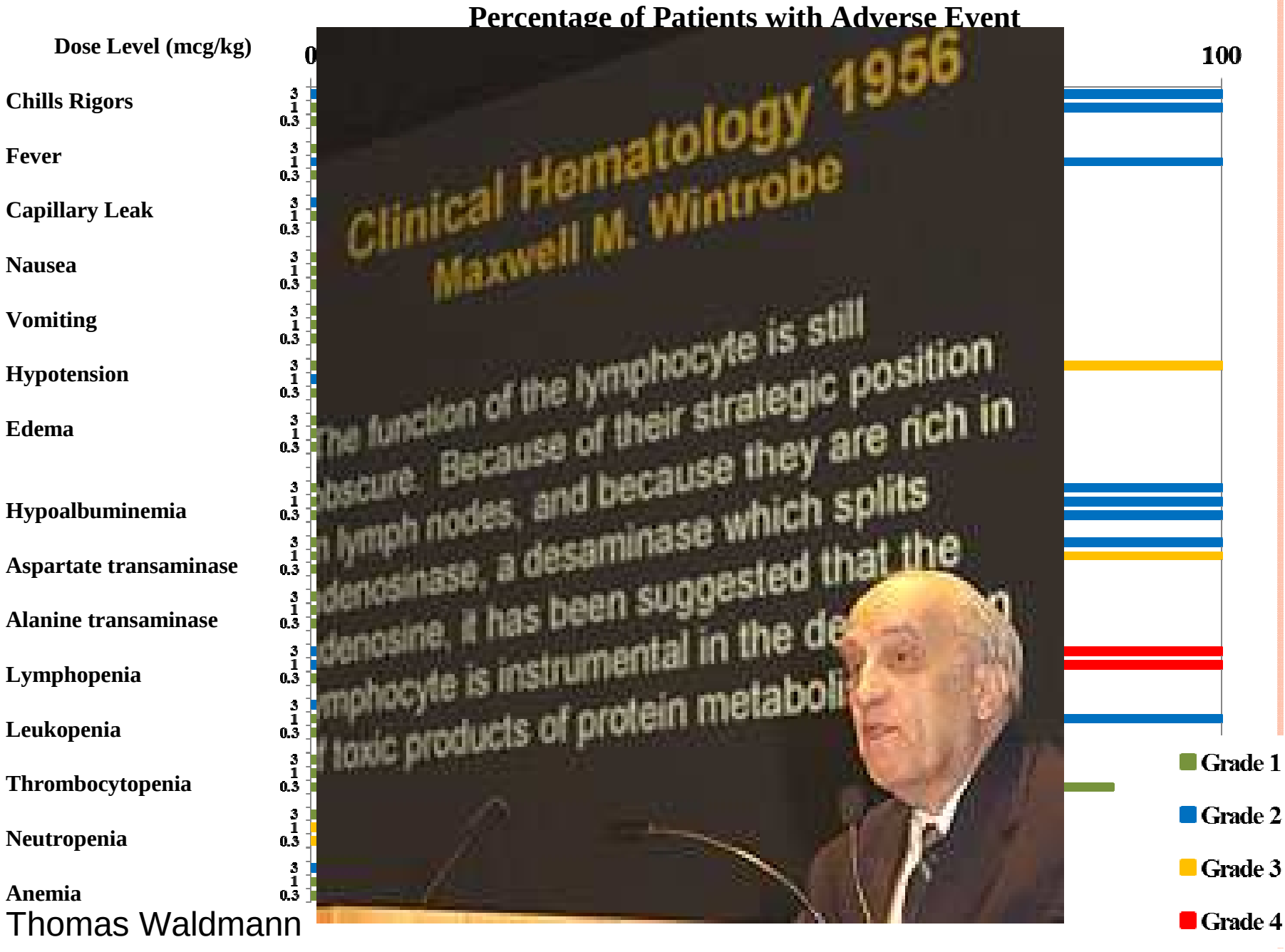



CMV responses were also detected after rhIL-7 injection in 2 other CMV-seropositive patients



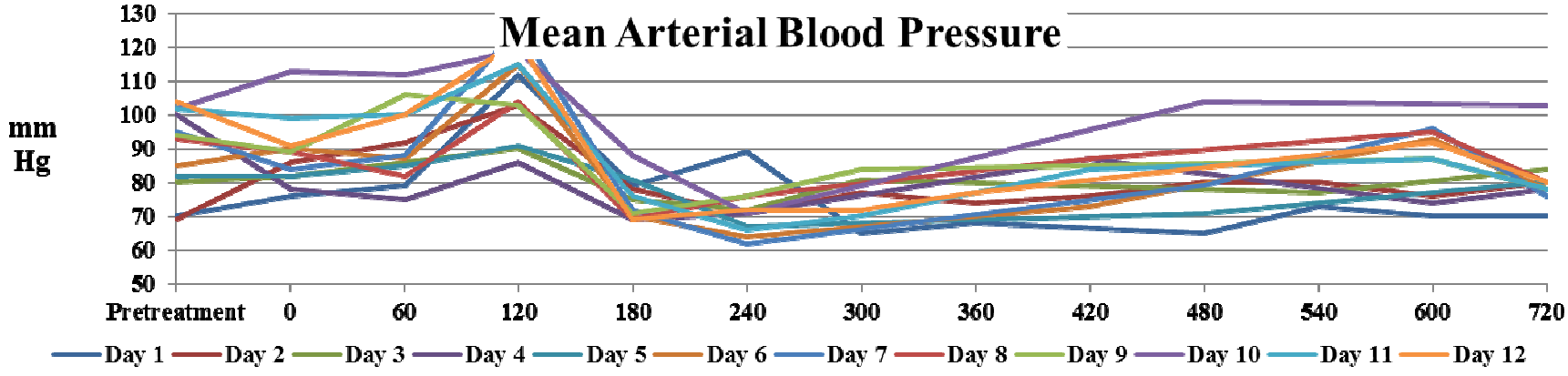
*Perales, Blood. 2012 Dec 6;120(24):4882-91*

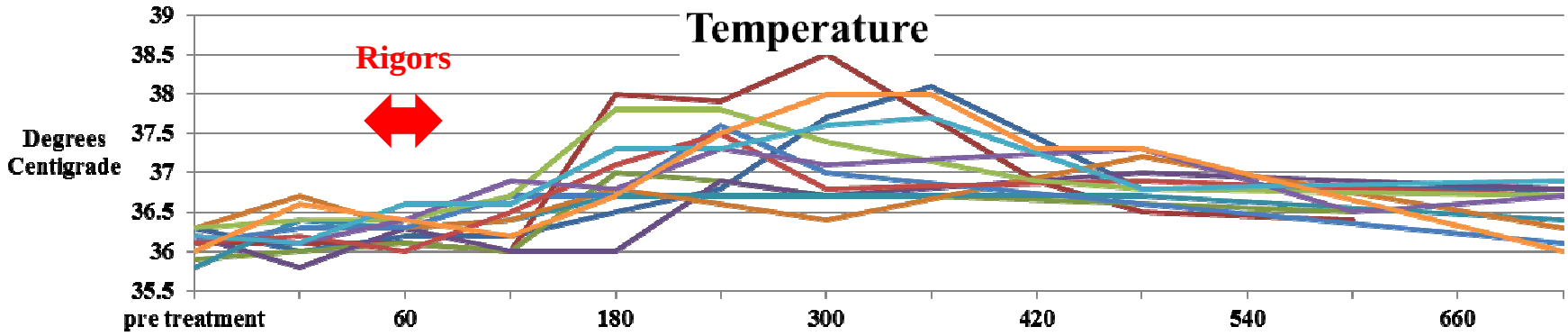
# Interleukin 15 Adverse Event Summary

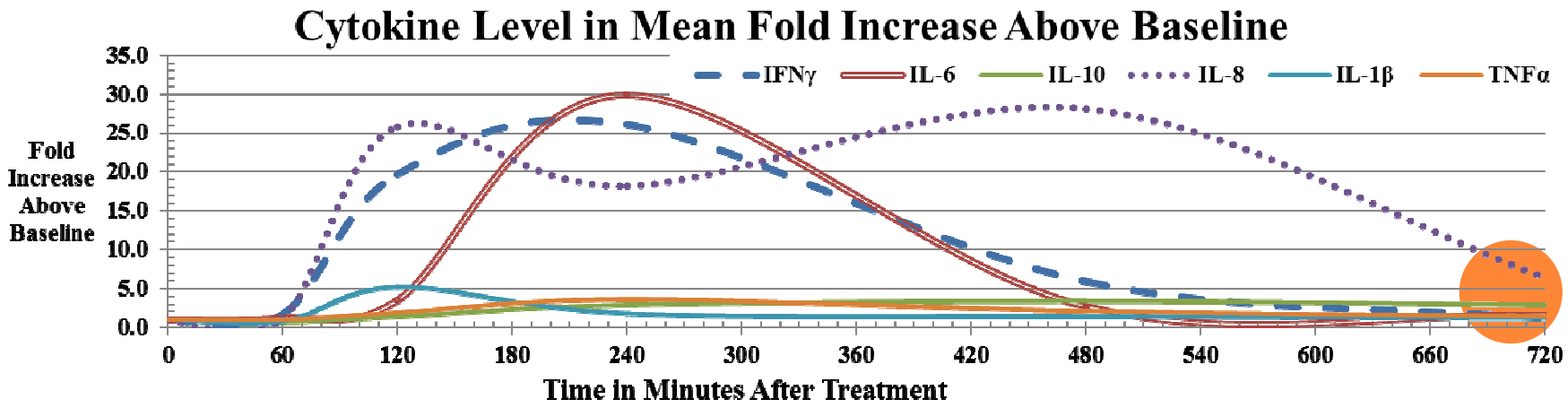



Thomas Waldmann

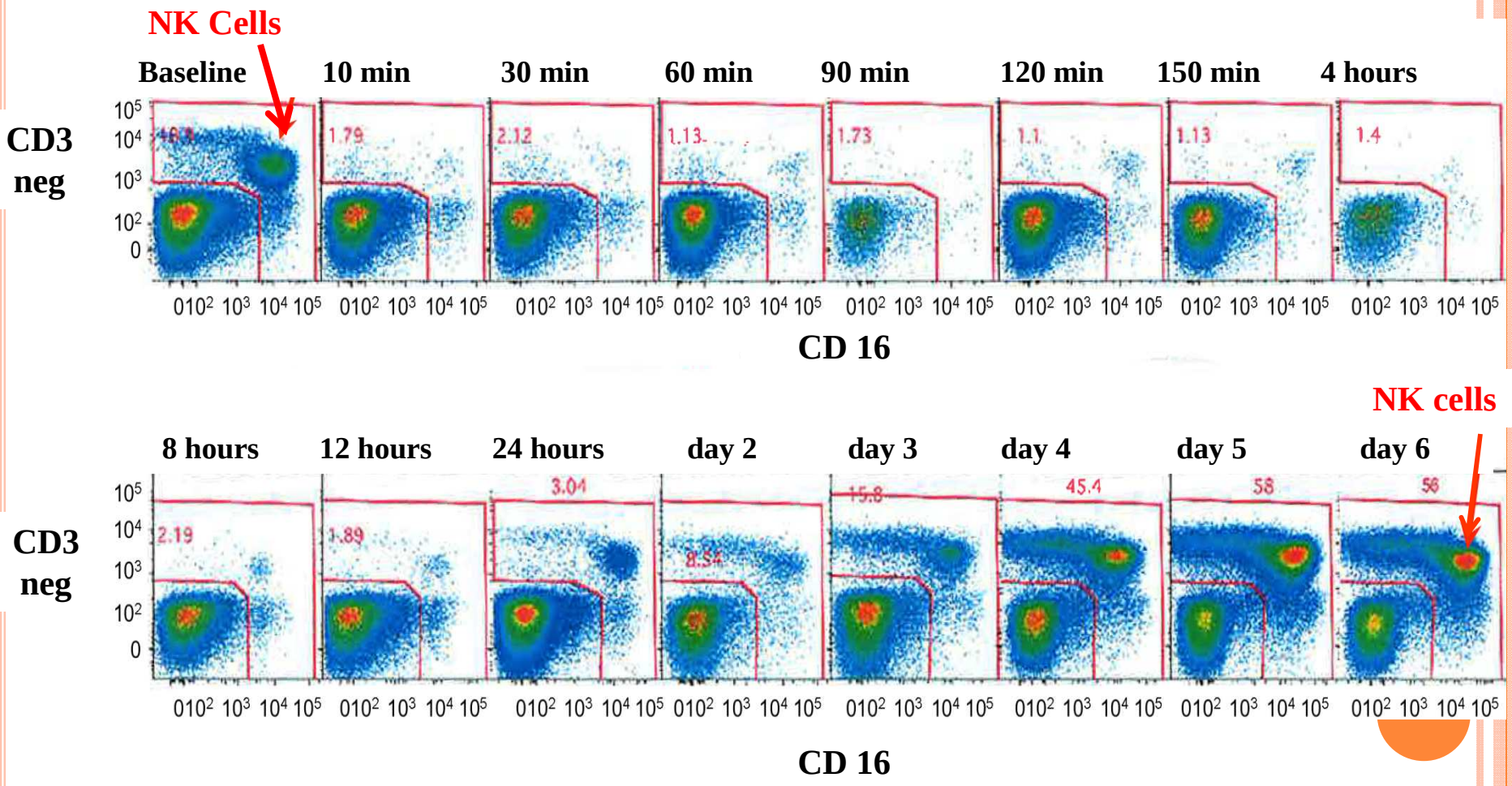
# PATIENTS


Mean Arterial Blood Pressure
mm Hg
130
120
110
100
90
80
70
60
50
Pretreatment
0
60
120
180
240
300
360
420
480
540
600
720
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12


Temperature
Rigors
Degrees Centigrade
39
38.5
38
37.5
37
36.5
36
35.5
pre treatment
60
180
300
420
540
660

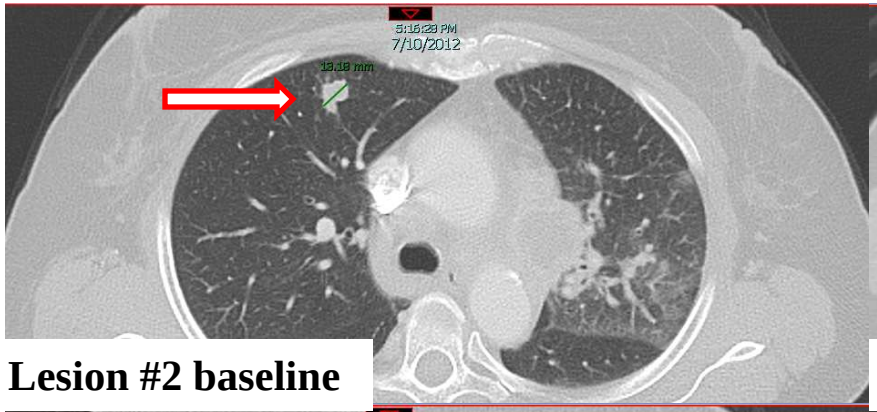

Cytokine Level in Mean Fold Increase Above Baseline
IFNγ
IL-6
IL-10
IL-8
IL-1β
TNFα
Fold Increase Above Baseline
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
60
120
180
240
300
360
420
480
540
600
660
720
Time in Minutes After Treatment

# Rapid Disappearance Of NK Cells rhIL-15 Treatment

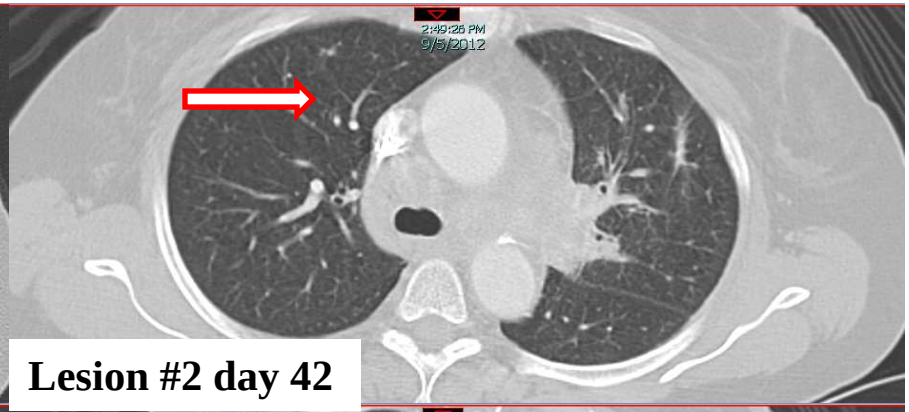


# CLINICAL ACTIVITY

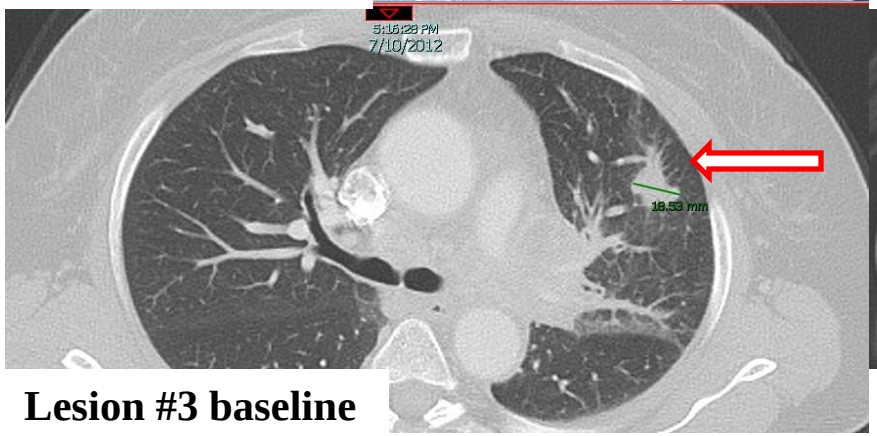
**Patient #16 Unconfirmed PR at day 42 restaging**



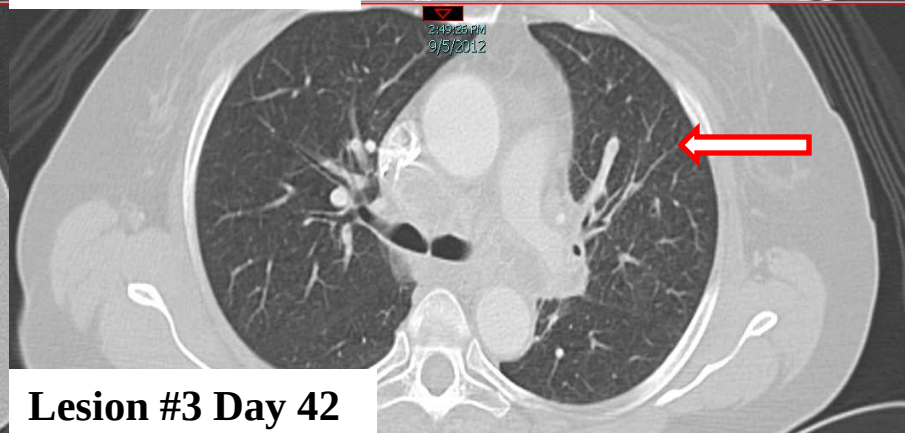

**Lesion #2 baseline**




**Lesion #2 day 42**

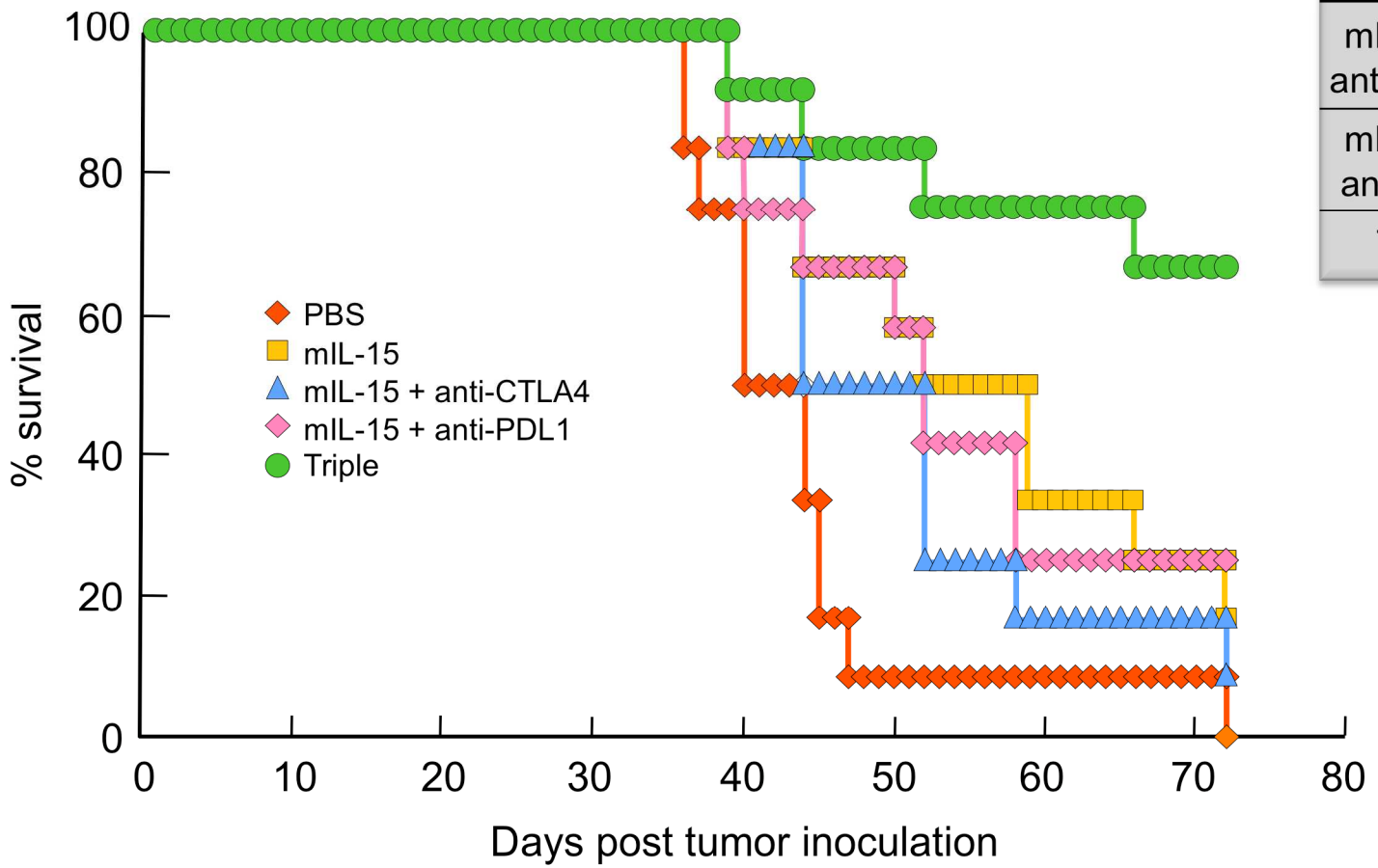



**Lesion #3 baseline**




**Lesion #3 Day 42**

# The Combination of mIL-15, Anti-CTLA4 and Anti-PDL1 Enhances Survival of TRAMP-C2 Tumor Bearing Animals




| Compare to mIL-15 | p value |
|---|---|
| mIL-15 + anti-CTLA4 | 0.4197 |
| mIL-15 + anti-PDL1 | 0.7729 |
| Triple | **0.0405** |

10 mice/group

George Pavlakis, Thomas Waldmann; NIH

# IMPACT UPON RELAPSE-FREE * AND OVERALL SURVIVAL**


E1684: IF … N vs Observation*

HR=1.24
$P_2$=0.09

Proportion Alive and Relapse Free

1.0 0.8 0.6 0.4 0.2 0.0

0 2 4 … 4 5 6 7 8 9 10

Time (Years)

Interval (Years)

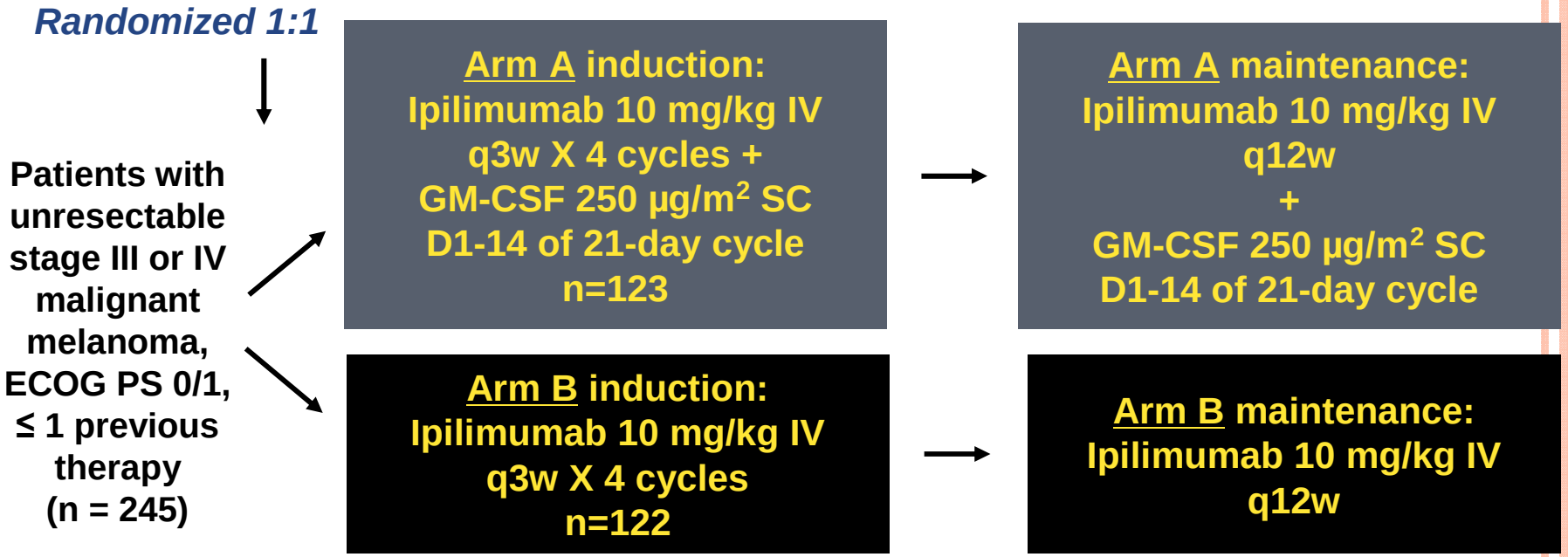
| | 0-2 | 2-4 | … | 2-4 | 4-6 | 6-8 | 8-10 |
|---|---|---|---|---|---|---|---|
| Observ. | 89/140 | 12/51 | 3… | 16/94 | 5/72 | 2/44 | 0/13 |
| IFN | 73/146 | 14/68 | 3… | 15/108 | 5/85 | 2/53 | 0/20 |

(No. events/No. at risk) … /No. at risk)

Meta-anal … S impact

Kirkwood. *Clin Cancer Res*. 2004;10:1670; Wheatley, Ives et al., 2007, 2008

# RANDOMIZED PHASE II STUDY OF GM-CSF + IPILIMUMAB VS. IPILIMUMAB




- Primary endpoint: OS
- Therapy continuation permitted with ≤ doubling of sum of target lesion diameter or ≤ 4 new lesions in absence of declining PS

Hodi FS, et al. ASCO 2013. CRA 9007.

# RANDOMIZED PHASE II STUDY OF GM-CSF + IPILIMUMAB: RESULTS

| Efficacy, n (%) | GM-CSF + Ipilimumab (n = 123) | Ipilimumab (n = 122) | HR | *P* Value |
|---|---|---|---|---|
| ORR | 19 (15.5%) | 18 (14.8%) | ---- | .880 |
| CR | 2 (1.6%) | 0 | ---- | NR |
| PR | 17 (13.8%) | 18 (14.8%) | ---- | NR |
| SD | 26 (21.1%) | 23 (18.9%) | ---- | NR |
| Median PFS | 3.1 mos | 3.1 mos | 0.92 | .569 |
| Median OS | 17.5 mos | 12.7 mos | 0.64 | .014 |
| 1-year Survival Rate | 68.9% | 52.9% | NR | NR |

Hodi FS, et al. ASCO 2013. CRA 9007.

# CONCLUSIONS

- Tumors release DAMPs which promote an immune response
- Cytokines are characterized by pleiotropy, redundancy, synergy, and antagonism
- IFNa and IL-2 remain our most effective cytokines for use in patients
- Novel combinations with GM-CSF and checkpoint inhibitors are on the horizon
- IL-15 appears promising in single agent studies and may be combined with antibodies and/or checkpoint inhibitors (CITN)

# 1. WHICH OF THE FOLLOWING IS NOT A DAMP?

- A. HMGB1
- B. IL-1
- C. Histone H1
- D. LPS
- E. DNA

# 2. WHICH OF THE FOLLOWING CYTOKINES ARE NOT APPROVED FOR CLINICAL USE?

- A. Erythropoietin
- B. GM-CSF
- C. IFNa
- D. IL-12
- E. IL-2

# 3. WHICH OF THE FOLLOWING COMBINATIONS ARE NOT BEING TESTED WITH IL-2?

- A. IL-12
- B. Hydroxychloroquine
- C. Ipilimumab (CTLA-4 antibody)
- D. VEGF-TRAP
- E. Axitinib (TKI; VEGFR1, VEGFRII)

# 4 (EXTRA CREDIT)WHICH OF THE FOLLOWING PROCESSES ANTAGONIZE APOPTOSIS?

- A. Necrosis
- B. Autophagy
- C. Necroptosis
- D. Pyroptosis
- E. Excitotoxicity