Immune Potentiating Cytokines

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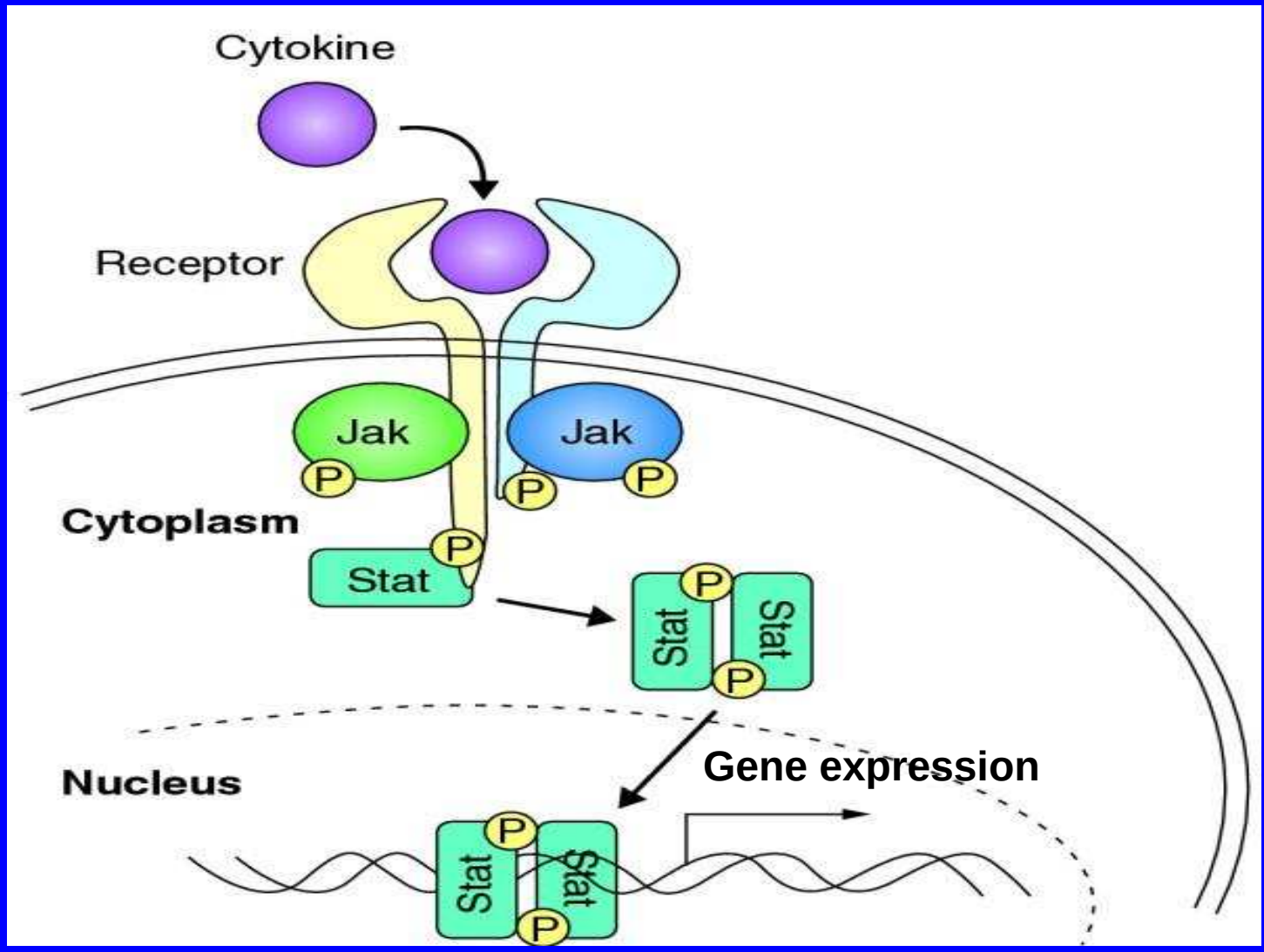
An Introduction to Cytokines

- General mechanisms of action
- FDA-Approved Cytokines for Cancer Immunotherapy
  - Interferon-α2b (Intron-A)
  - Pegylated interferon-α2b (Sylatron)
  - Interleukin-2 (Proleukin)
- Other Cytokines in Development

General mechanism of cytokine signaling

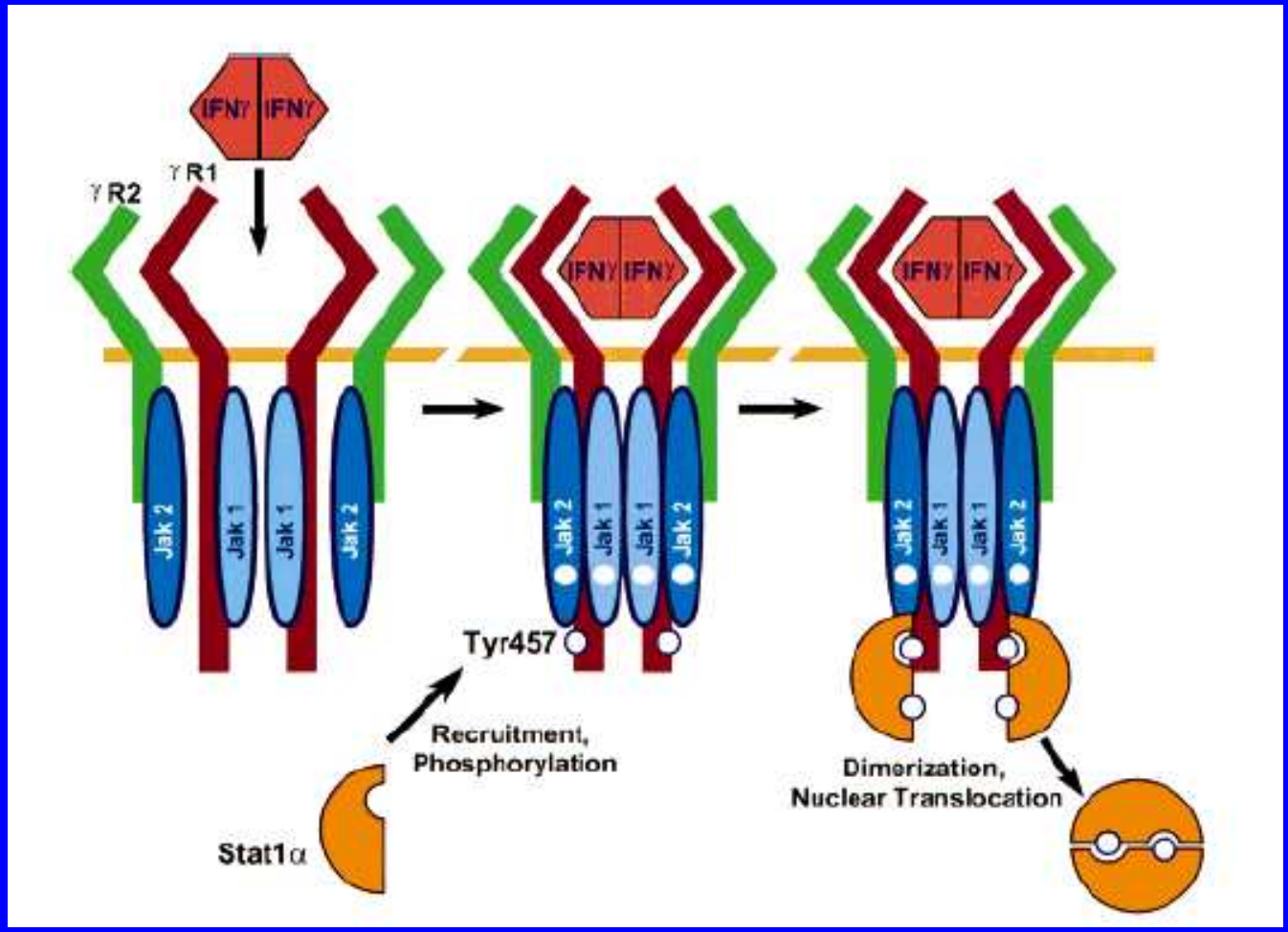


Interferons

- Type I
  - α: from neutrophils, m
  - β: from fibroblasts, epithelial cells
- Type II
  - : from T, NK cells

- Immunomodulatory effects
  - MHC class I/II upregulation
  - Modulation of T/NK cell cytolytic activity
  - Modulation of macrophage/DC function
  - Decreased Treg/increased Th1
  - Inhibition of angiogenesis

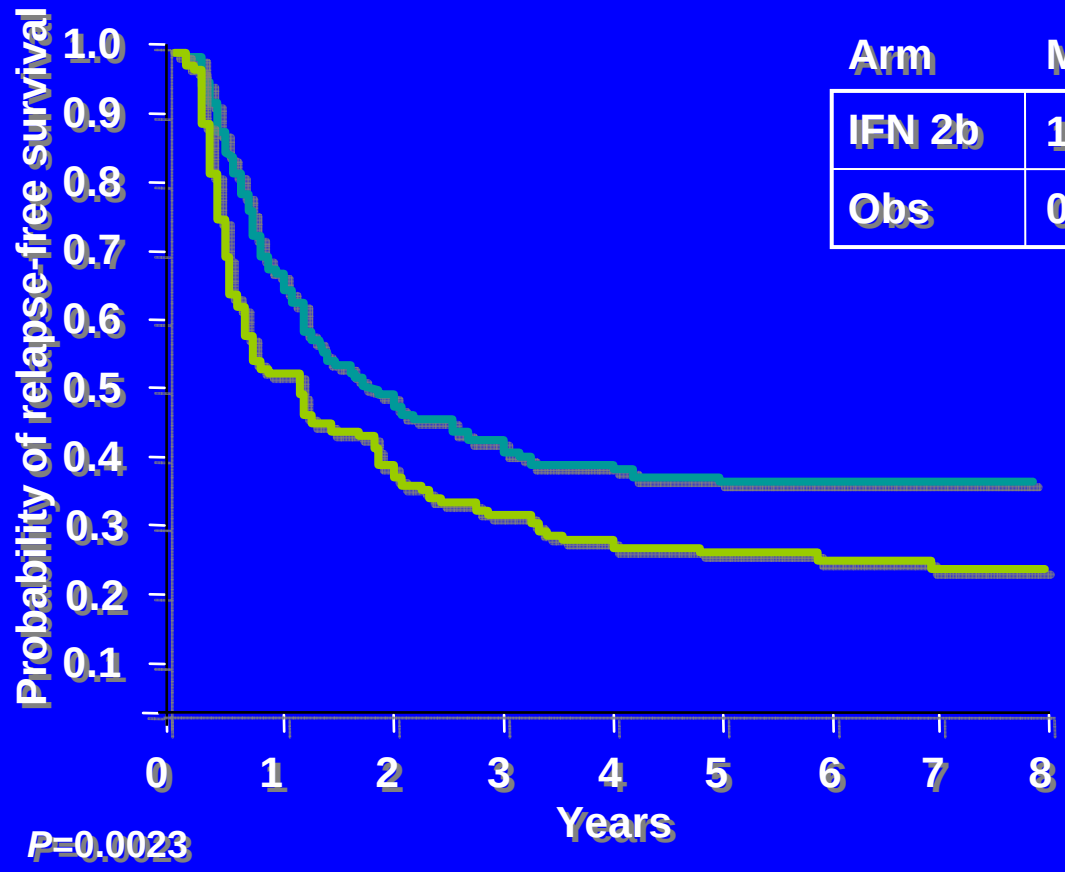
Interferon Signaling



Interferon Administration

- Induction Phase
  - 20 Million Units/m^2 IV Monday through Friday for 4 weeks
- Maintenance Phase
  - 10 Million Units/m^2 SQ M-W-F for 11 months
- Dose reductions or discontinuation for toxicity

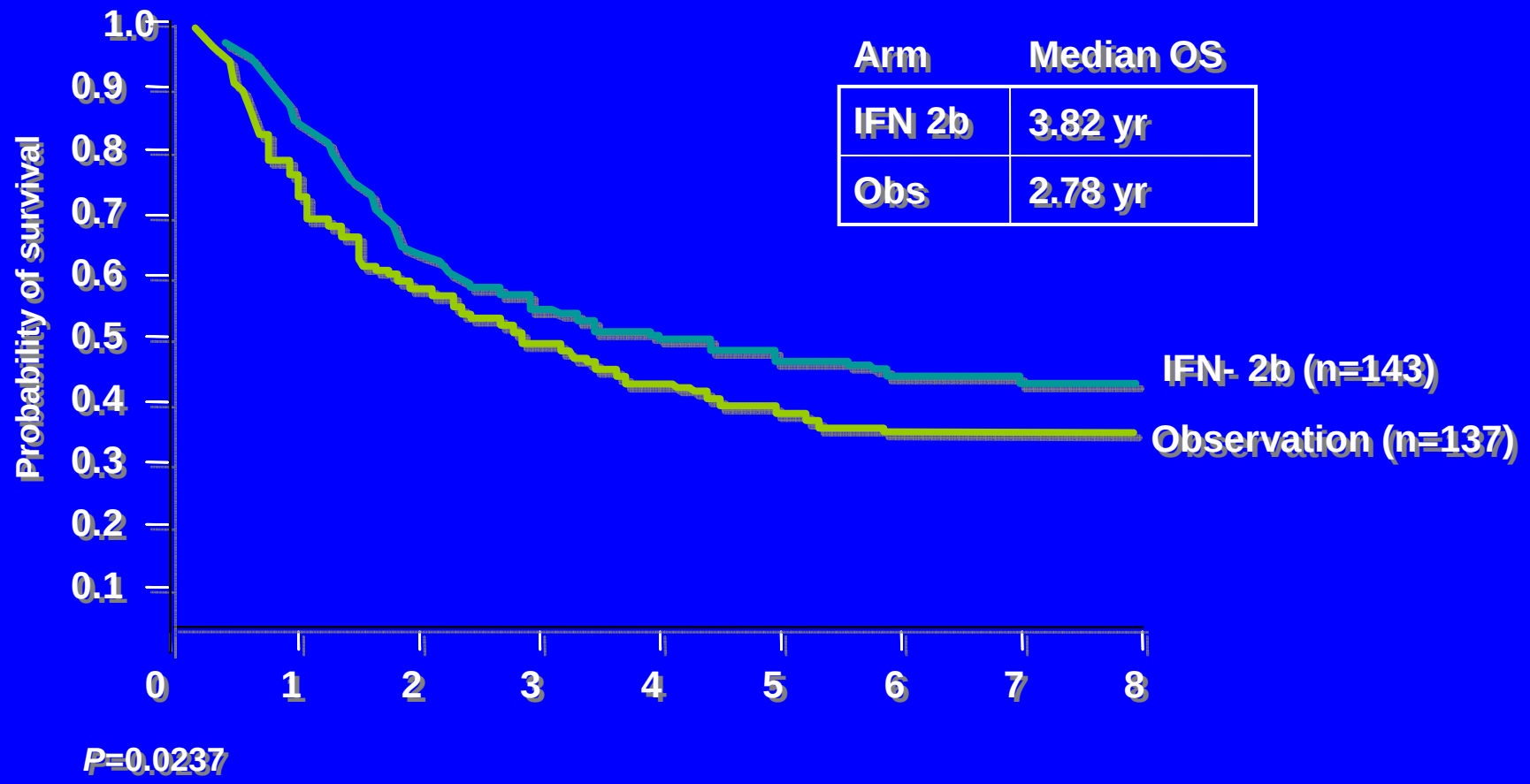
E1684: Estimated Relapse-Free Survival



Arm	Median RFS
IFN 2b	1.72 yr
Obs	0.98 yr

J Clin Oncol. 1996;14:7-17.

E1684: Estimated Overall Survival

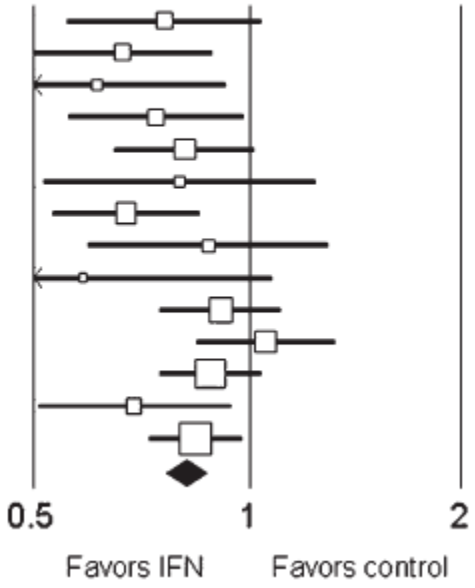


Arm	Median OS
IFN 2b	3.82 yr
Obs	2.78 yr

J Clin Oncol. 1996;14:7-17.

Meta-analysis of IFN effect on DFS

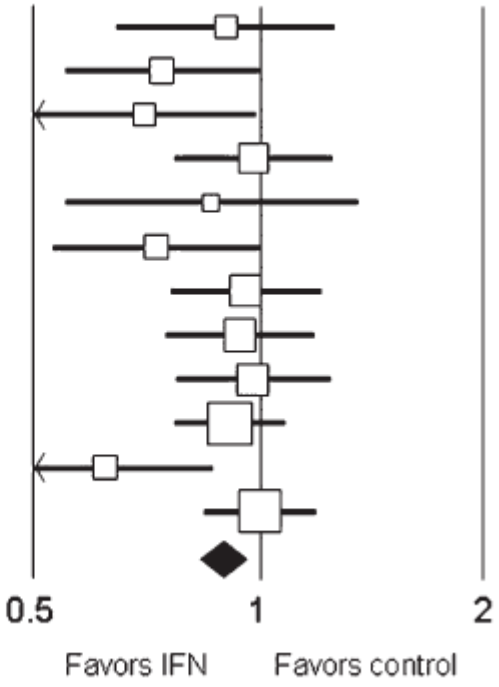
	HR	LL	UL	SE	Patients	Events (IFN/control)
NCCTG (Creagan, 1995)	0.76	0.56	1.04	0.16	264	77/85
E1684 (Kirkwood, 1996)	0.67	0.50	0.88	0.14	287	90/103
AMCG (Pehamberger, 1998)	0.61	0.40	0.93	0.21	311	37/57
FCGM (Grob, 1998)	0.74	0.56	0.98	0.14	499	100/119
E1690 (Kirkwood, 2000)	0.81	0.65	1.01	0.11	642	236/254
SMG (Cameron, 2001)	0.80	0.52	1.23	0.22	96	32/35
E1694 (Kirkwood, 2001)	0.67	0.53	0.85	0.12	880	98/151
WHO (Cascinelli, 2001)	0.88	0.60	1.28	0,20	444	162/158
E2696 (Kirkwood, 2001)	0.59	0.32	1.07	0.31	107	28/38
UKCCCR (Hancock, 2004)	0.91	0.75	1.10	0.10	674	211/215
EORTC18871 (Kleeberg, 2004)	1.05	0.84	1.31	0.11	484	159/218
EORTC18952 (Eggermont, 2005)	0.88	0.75	1.03	0.08	1388	596/328
DeCOG (Garbe, 2008)	0.69	0.51	0.94	0.16	296	84/102
EORTC18991 (Eggermont, 2008)	0.84	0.72	0.97	0.08	1256	322/361
	0.82	0.77	0.87	0.03		



Morcellin et al. JNCI 2010

Meta-analysis of IFN effect on OS

	HR	LL	UL	SE	Patients	Events (IFN/control)
NCCTG (Creagan, 1995)	0.90	0.64	1.25	0.17	264	68/72
E1684 (Kirkwood, 1996)	0.73	0.54	0.99	0.15	287	81/90
FCGM (Grob, 1998)	0.70	0.49	0.98	0.17	499	59/76
E1690 (Kirkwood, 2000)	0.98	0.76	1.24	0.12	642	194/186
SMG (Cameron, 2001)	0.86	0.54	1.35	0.23	96	31/36
E1694 (Kirkwood, 2001)	0.72	0.52	0.99	0.16	880	52/81
WHO (Cascinelli, 2001)	0.95	0.76	1.20	0.12	444	146/138
UKCCCR (Hancock, 2004)	0.94	0.74	1.17	0.12	674	151/156
EORTC18871 (Kleeberg, 2004)	0.98	0.77	1.23	0.12	484	137/202
EORTC18952 (Eggermont, 2005)	0.91	0.76	1.07	0.09	1388	534/292
DeCOG (Garbe, 2008)	0.62	0.44	0.86	0.17	296	65/88
EORTC18991 (Eggermont, 2008)	1.00	0.84	1.18	0.09	1256	256/257
	0.89	0.83	0.96	0.04		



Morcellin et al. JNCI 2010

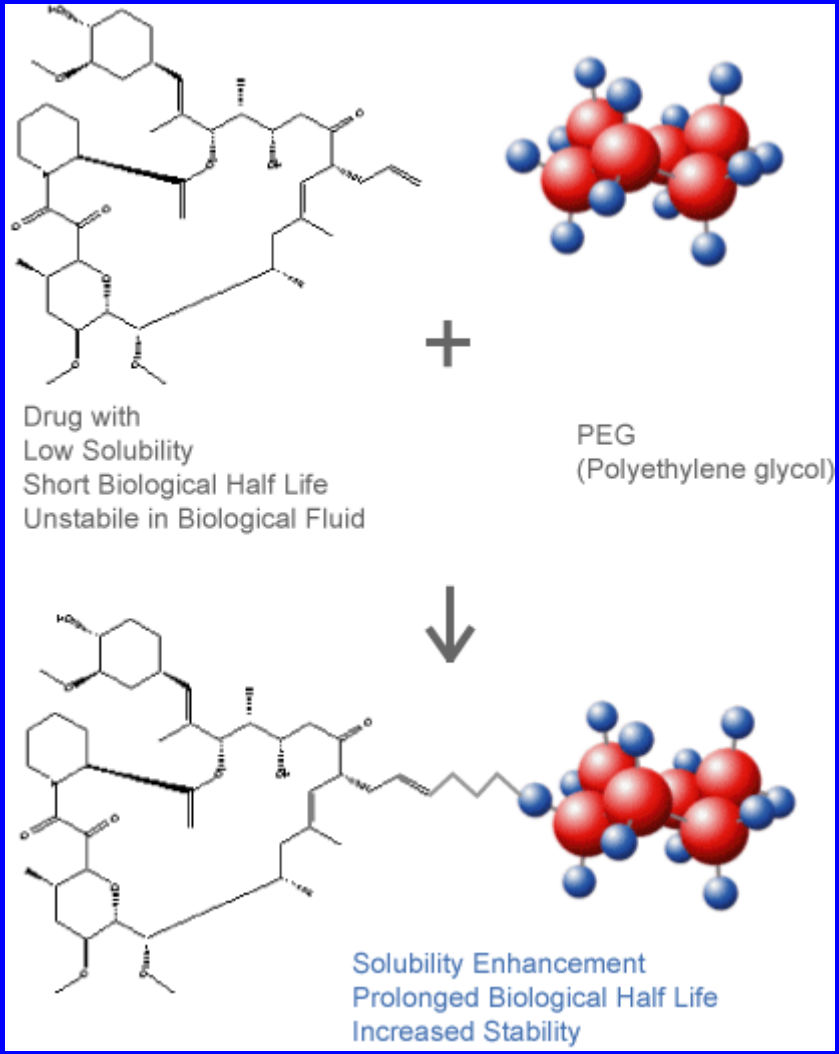
Common Interferon Toxicity

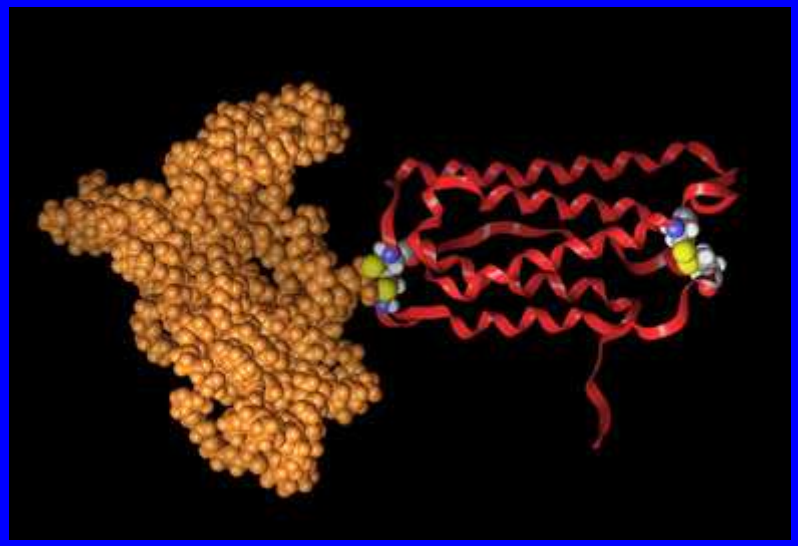
• Flu-like syndrome	Acetominophen/benadryl
• Fatigue	Dose reduction, if severe
• Cytopenias	Dose reduction, if severe
• Increased LFTs	Dose reduction, if severe
• Weight loss	Dose reduction, if severe
• Alopecia	

Less Common Interferon Toxicity

• Nausea/vomiting	Anti-emetics
• Diarrhea	Anti-diarrheals
• Hypotension	Fluids, dose reduce
• **Depression**	**Anti-depressants**
• Cough	Symptomatic
• Dry mouth	Fluids
• Skin rash	Moisturizers
• Irritability	Dose reduce

Pegylated Interferon

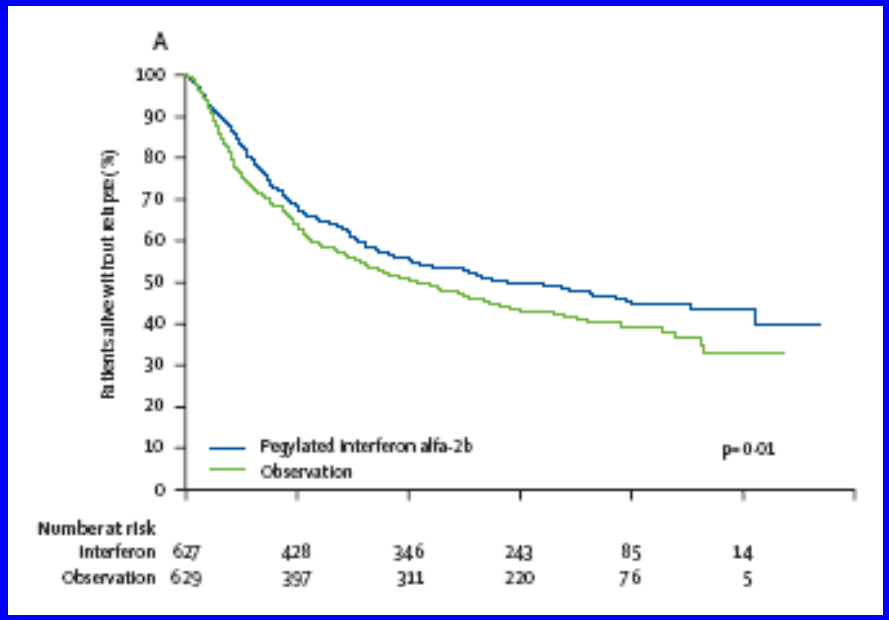




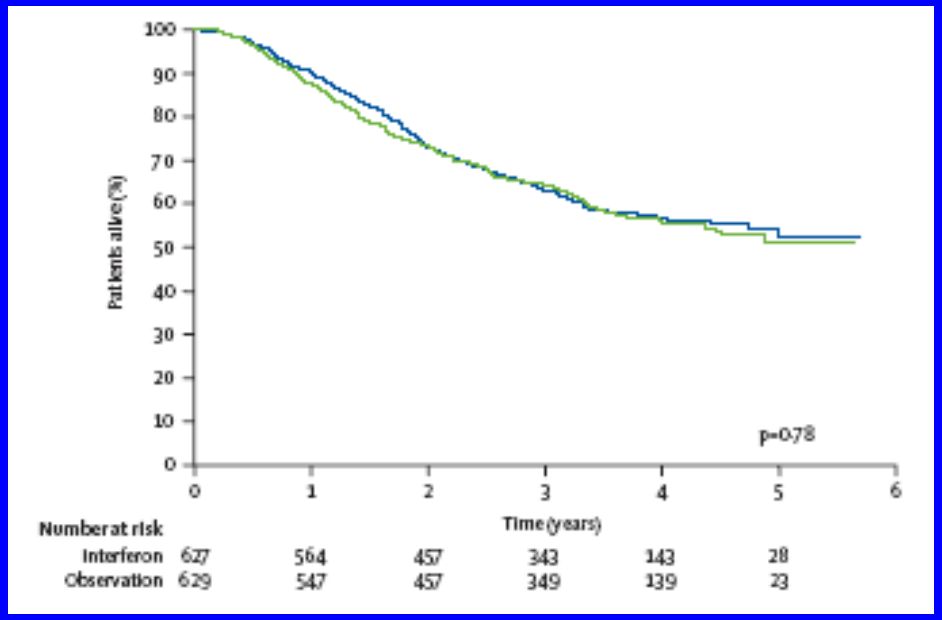
PEG-Interferon Administration

- Induction phase
  - 6 mcg/kg SQ weekly for 8 weeks
- Maintenance Phase
  - 3 mcg/kg SQ weekly for up to 5 years
- Dose reduction or discontinuation for toxicity

Pegylated IFN vs. Observation in Resected Stage III Melanoma



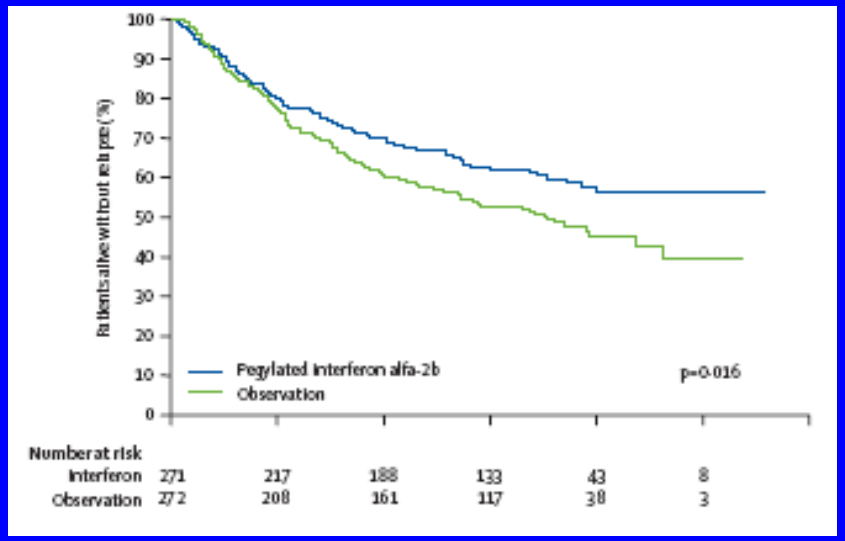
Recurrence-free Survival



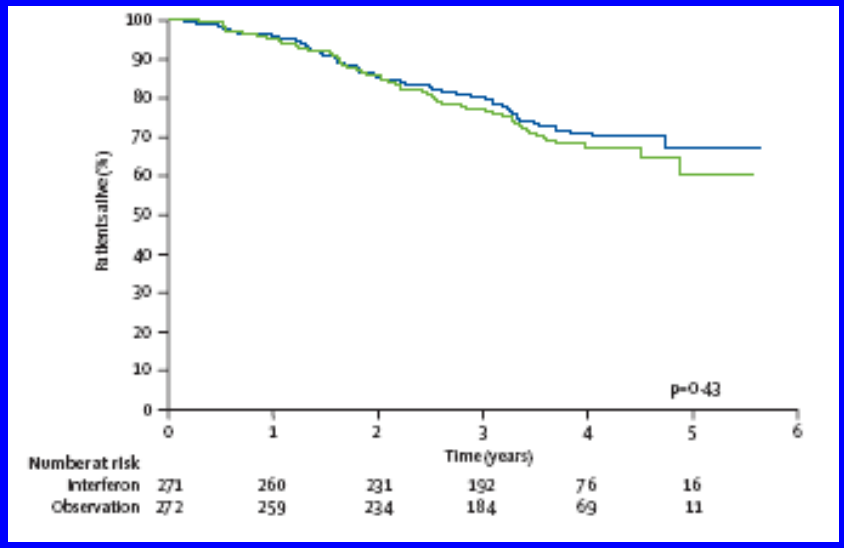
Overall Survival

Eggermont et al. Lancet 2008

PEGylated IFN in N1a Disease



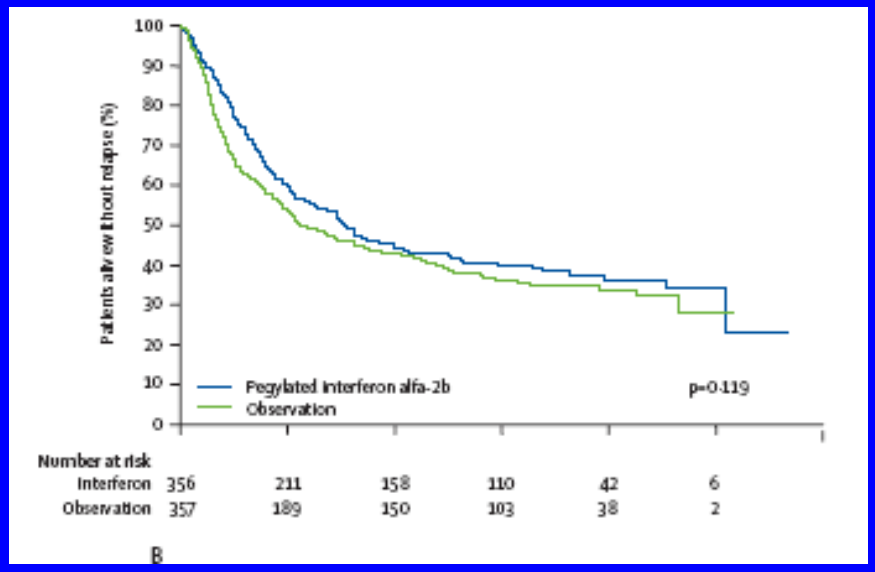
Recurrence-free Survival



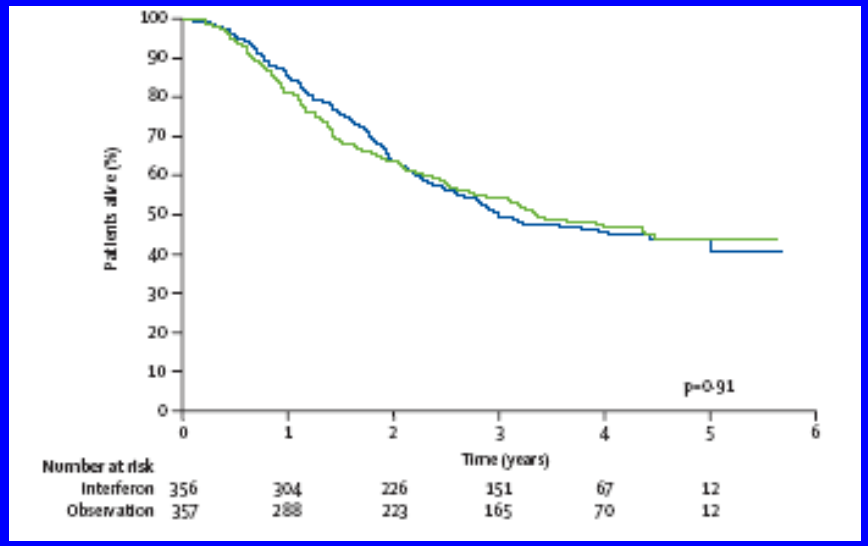
Overall Survival

Eggermont et al. Lancet 2008

PEGylated IFN in N1b Disease



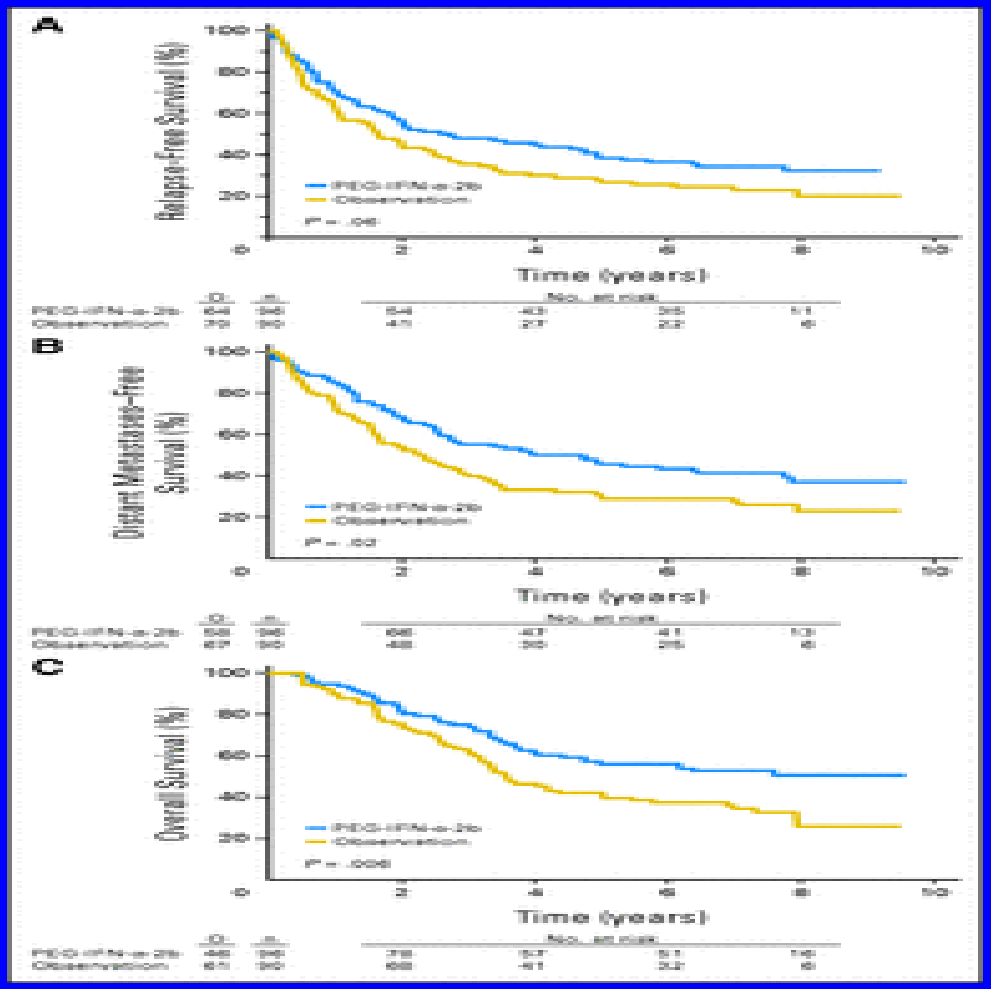
Recurrence-free Survival



Overall Survival

Eggermont et al. Lancet 2008

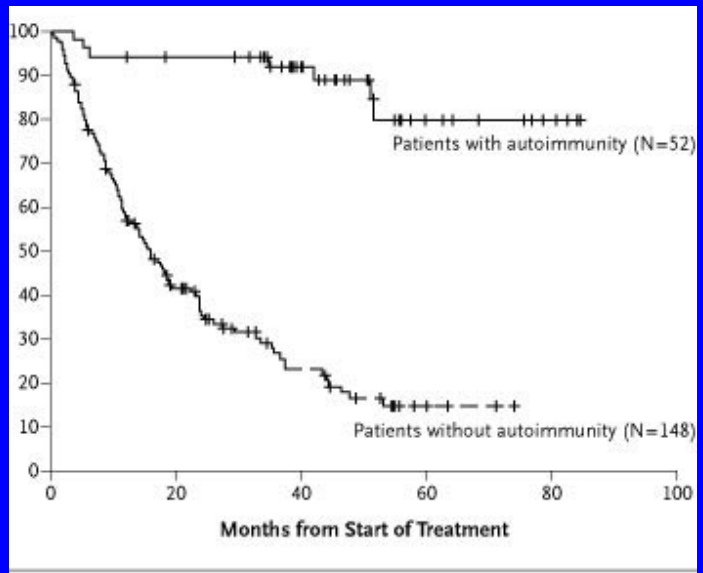
PEGylated IFN in patients with ulcerated primary melanomas



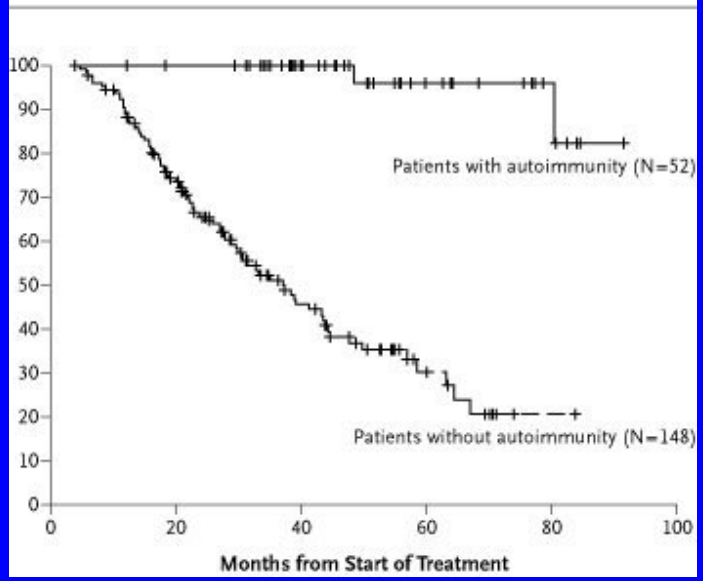
Eggermont et al. JCO 2012

Induction of autoimmunity correlates with survival in IFN-α treated patients

Relapse-free Survival



Overall Survival

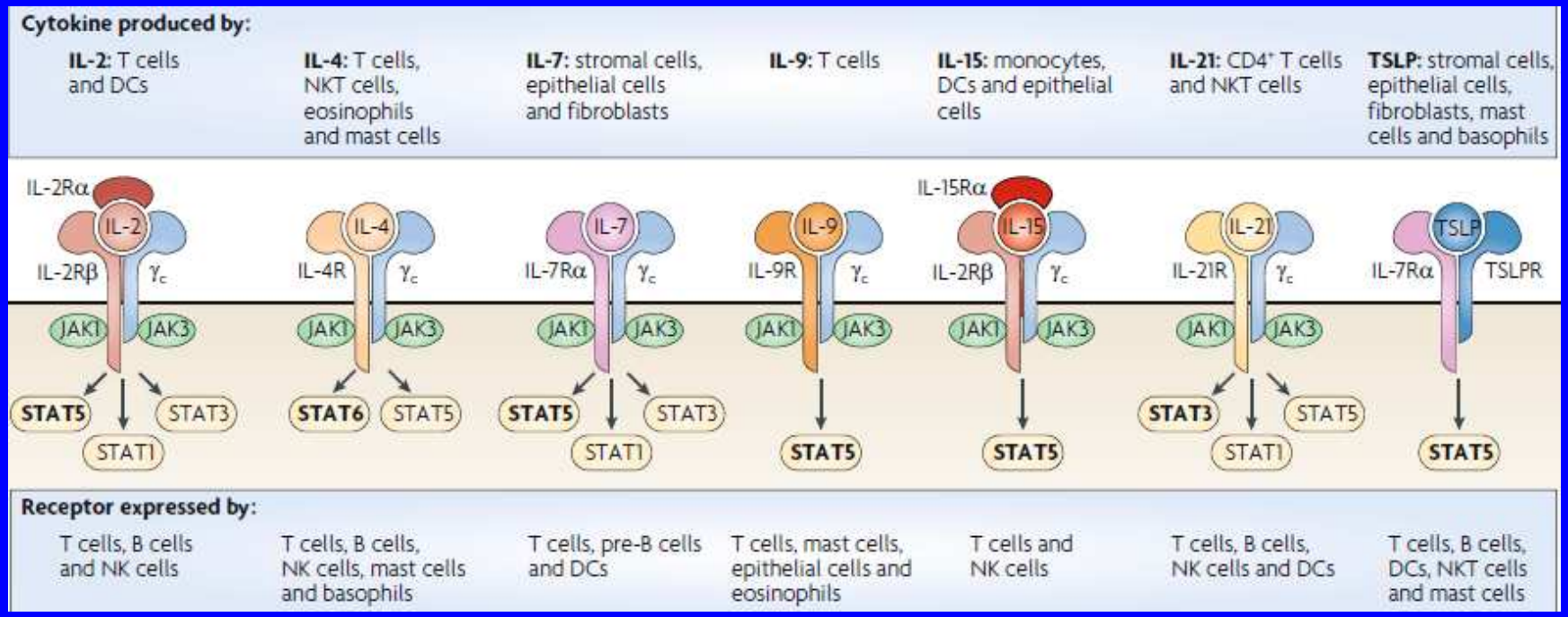


Gogas et al. NEJM 2006

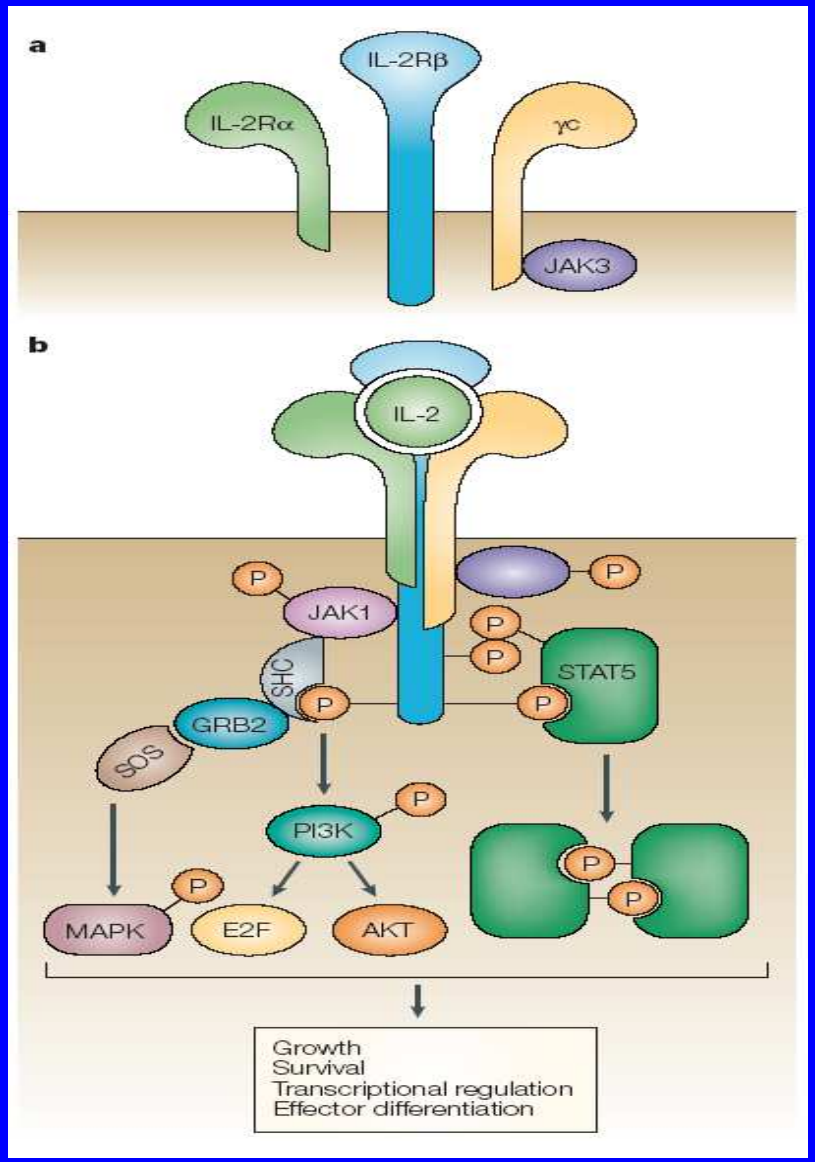
Interleukin-2 (IL-2)

- Natural biologic immunomodulatory agent
- T cell growth factor
- Proliferation of T cells and NK cells
- Promotes the killing activity of these cells
- Powerful anti-tumor effects in animal studies
- Extensively evaluated in patients with cancer
- FDA approved for metastatic renal cell in 1992
- FDA approved for metastatic melanoma in 1998

Interleukins and Their Receptors



Margolin, Lazarus and Kaufman 2013

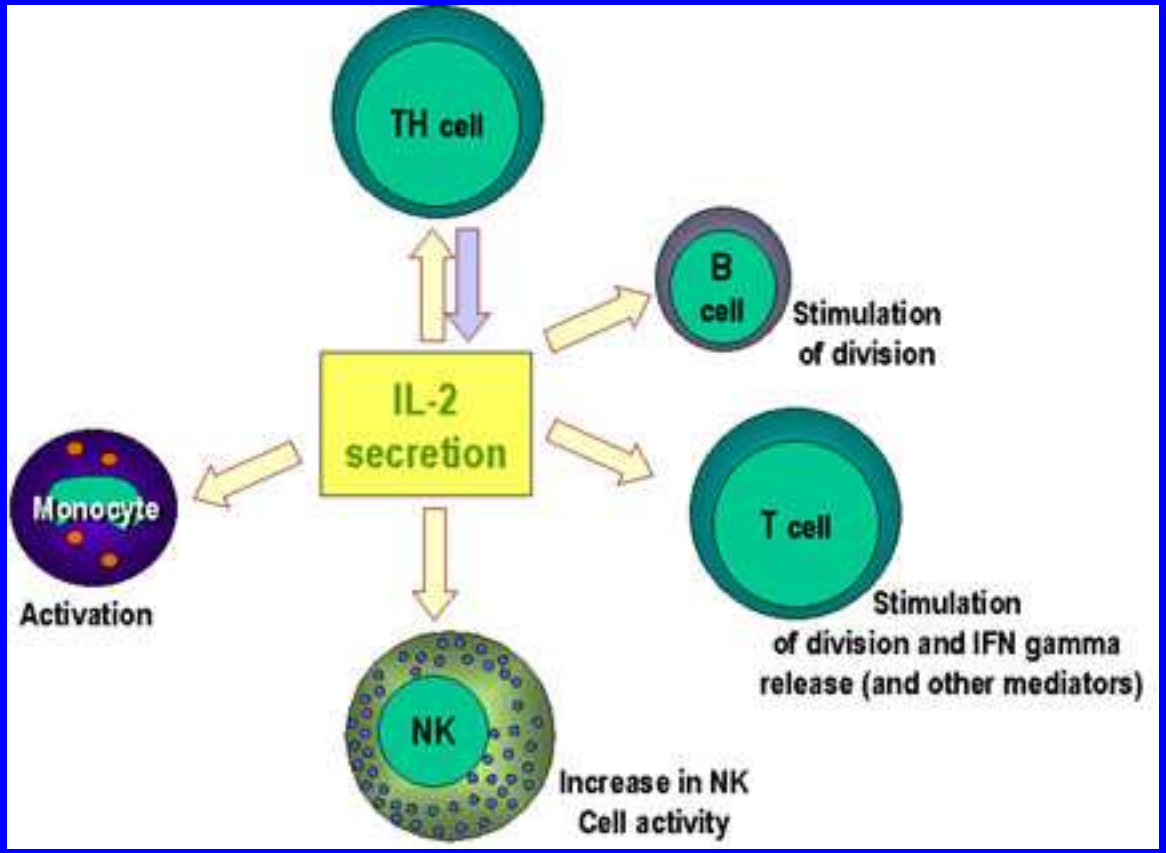


IL-2 Receptor

- Binds chain
- Forms heterotrimeric complex
- Signals through and c chains
- Induces T cell growth and promotes survival

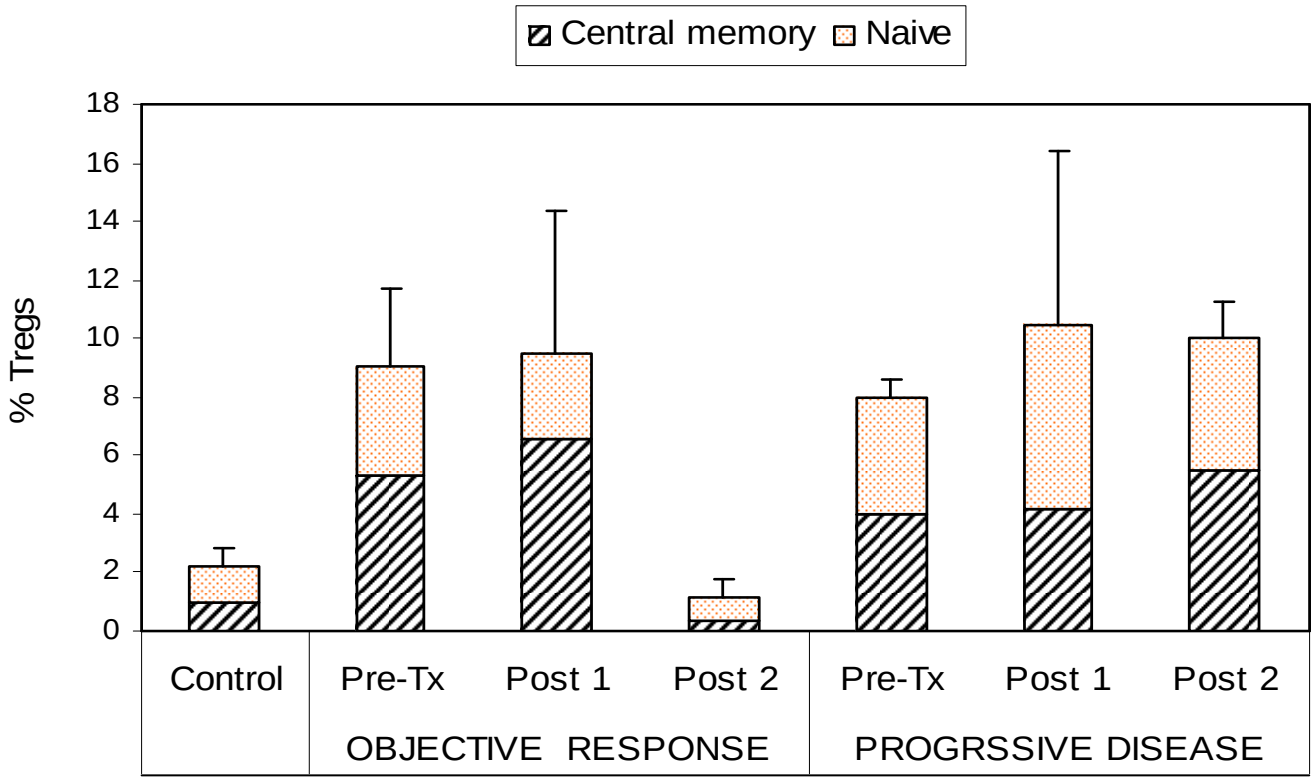
Malek and Bayer, Nature Immunol Rev, 2004

Mechanism(s) of IL-2 anti-tumor activity



Malek and Bayer, Nature Rev Immunol 2004

Tregs exhibit a paradoxical response to IL-2 treatment



High-dose Bolus rIL-2 Regimen

- IL-2 600,000 IU/kg every 8 hours by 15-minute IV infusion for a maximum of 14 doses
- 9-16 day rest period
- Repeat schedule for another 14 doses
- Maximum 28 doses per course of therapy
- No dose reductions are performed during high-dose IL-2 therapy
- Excessive toxicity treated by withholding dose or discontinuing treatment for that cycle

Proleukin® (aldesleukin) prescribing information.

Patient Selection for IL-2 Treatment

- ECOG performance status 0 or 1
- Adequate pulmonary function
  - FEV1 and FVC ε75% of predicted
  - No evidence of symptomatic pulmonary disease
- Normal cardiac function
  - For patients >50 years of age or with ischemic symptoms, consider stress thallium or other stress tests
- Adequate renal function
  - Creatinine levels should be δ1.6 mg/dL

Schwartzentruber DJ. In: Rosenberg SA, ed. *Principles and Practice of the Biologic Therapy of Cancer*. 2000:32-50.

Patient Selection (cont)

- Adequate hepatic function
  - Bilirubin δ 2.0 g/dL
  - SGOT <3 · ULN, unless due to liver metastases
- Adequate hematologic function
  - ANC ε1500/mm3
  - Platelets >100,000
  - Hemoglobin ε9.0 gm/100 mL
- No CNS metastases (unless adequately treated)
  - MRI brain within one month
- No corticosteroids

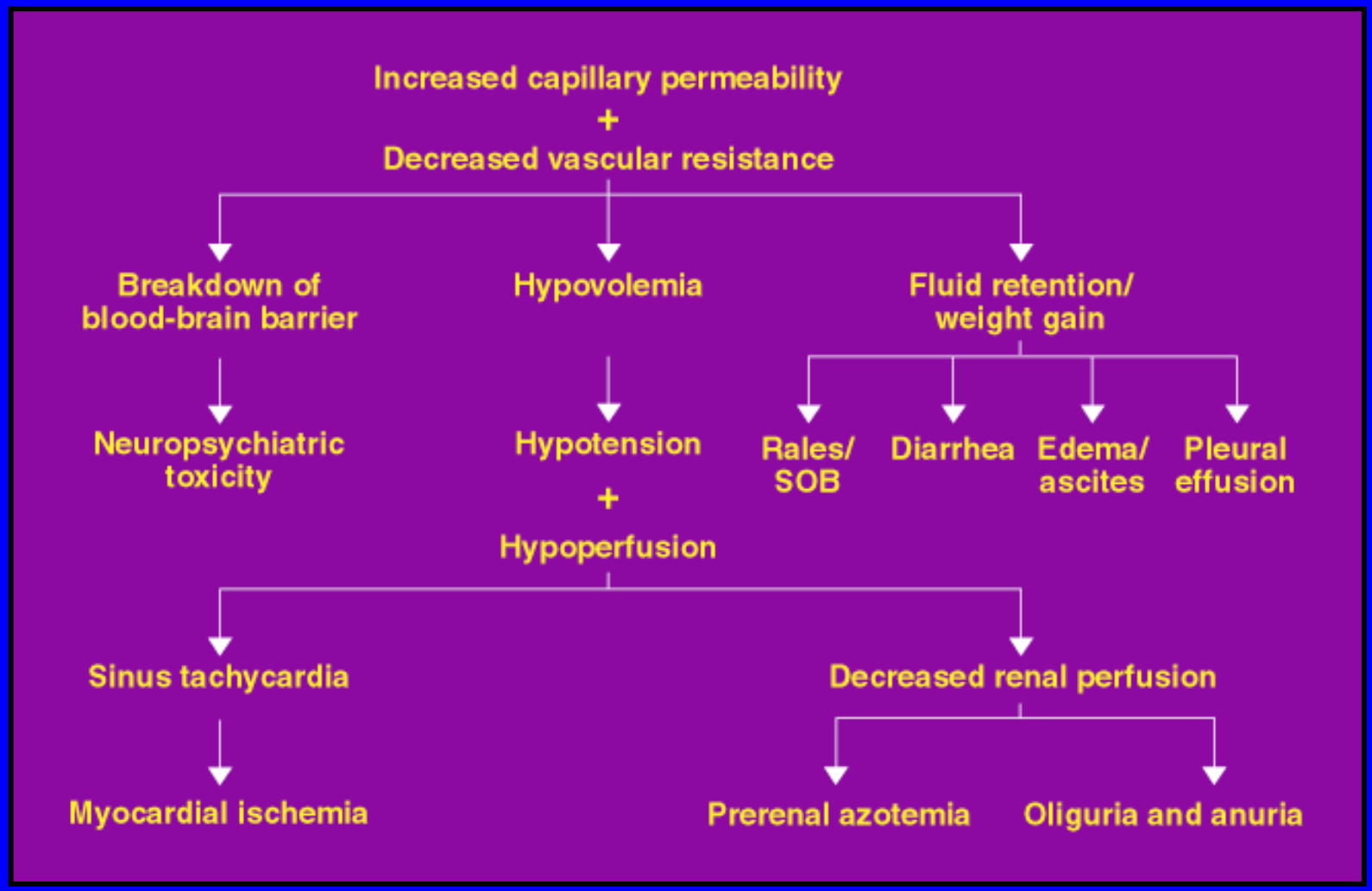
Schwartzentruber DJ. In: Rosenberg SA, ed. *Principles and Practice of the Biologic Therapy of Cancer.* 2000:32-50.

IL-2 Toxicity

- Most side effects are preventable
- Nearly all side effects are reversible
- All side effects can be managed by qualified physicians and nurses

- Management begins with pre-treatment screening

Vascular Leak Syndrome

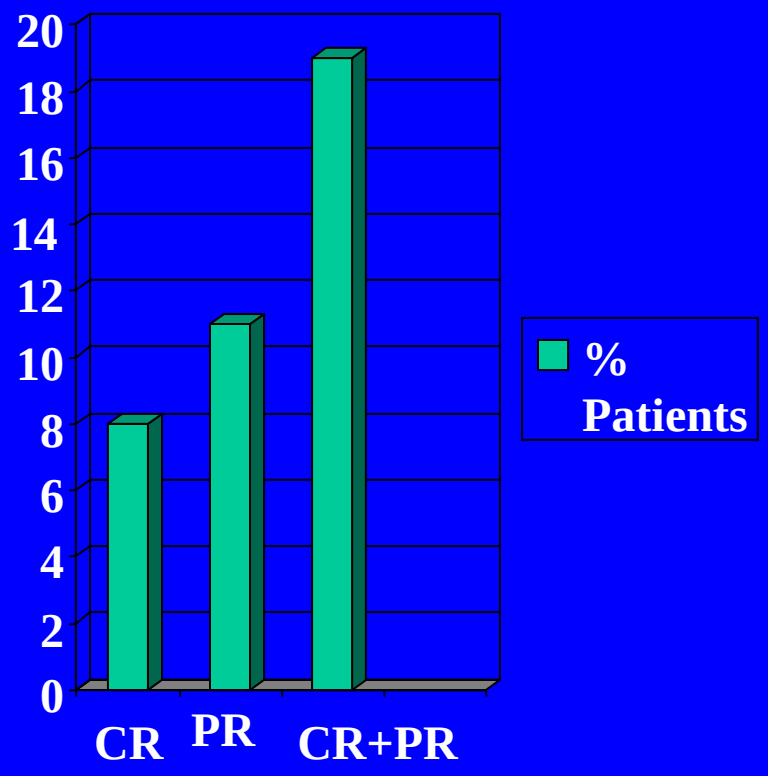


Pivotal High Dose IL-2 Trials: The NCI Experience

	N	No. of Patients (%)	
		CR	PR
Melanoma	182	12(6.6)	15(8.2)
Renal Cell	227	21(9.3)	22(9.7)
Total	409	33(8.1)	37(9.0)

Rosenberg et al. Ann Surg 1998

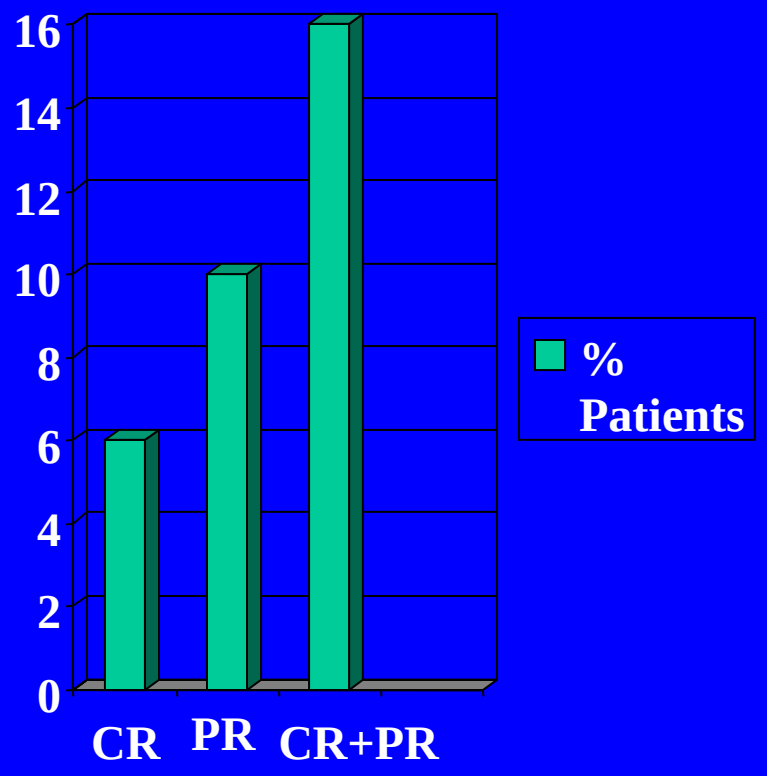
High-dose IL-2 for Renal Cell Carcinoma



- 115 patients
- Renal cell carcinoma
- 720,000 IU/kg (147)
- Median follow-up 52 months
- No mortality

Yang, Cancer J, 1997

High-dose IL-2 for Melanoma

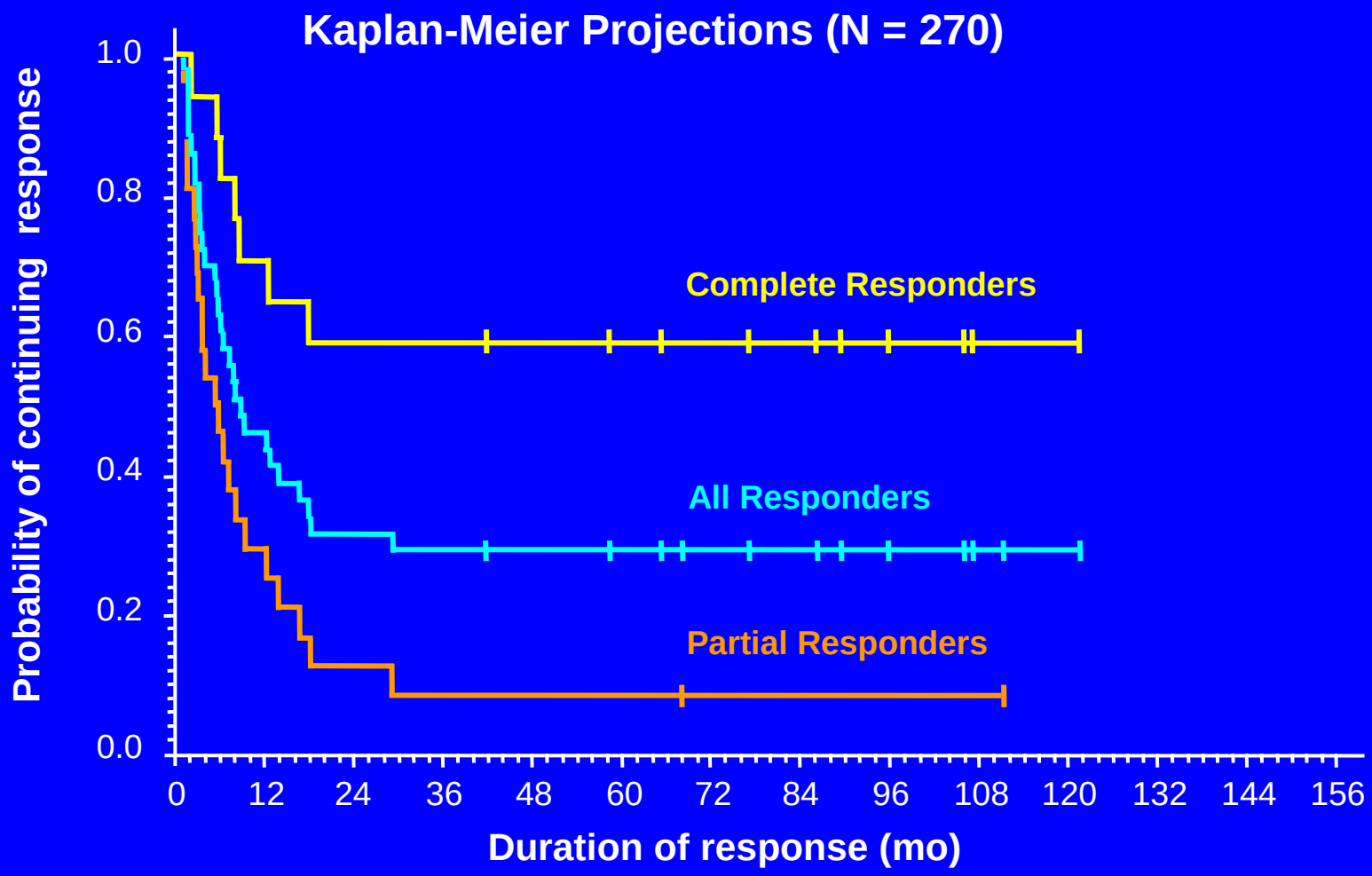


- 270 patients, 1985-93
- Melanoma
- 600,000 IU/kg (118)
- 720,000 IU/kg (147)

- 6 (2.2%) mortality
  - 5/6 had ECOG PS 1
  - Bacterial sepsis in all 6
  - No prophylactic antibiotics
  - No deaths after 1990

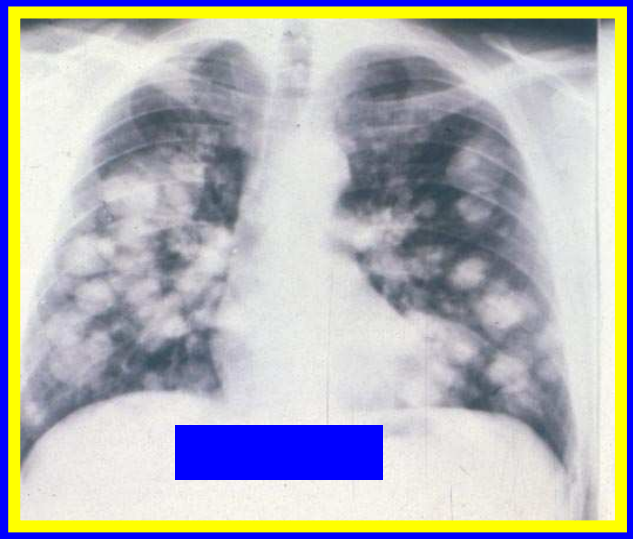
Atkins et al, J Clin Oncol, 1999

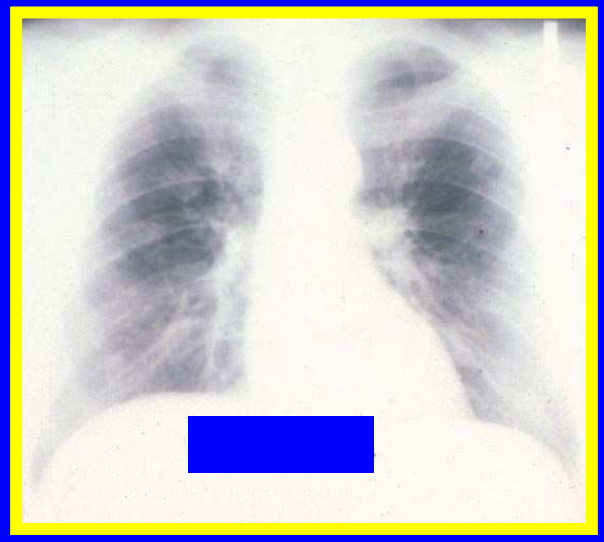
High-dose IL-2 induces durable objective clinical responses in 15-20%



Data on file. Chiron Corporation.

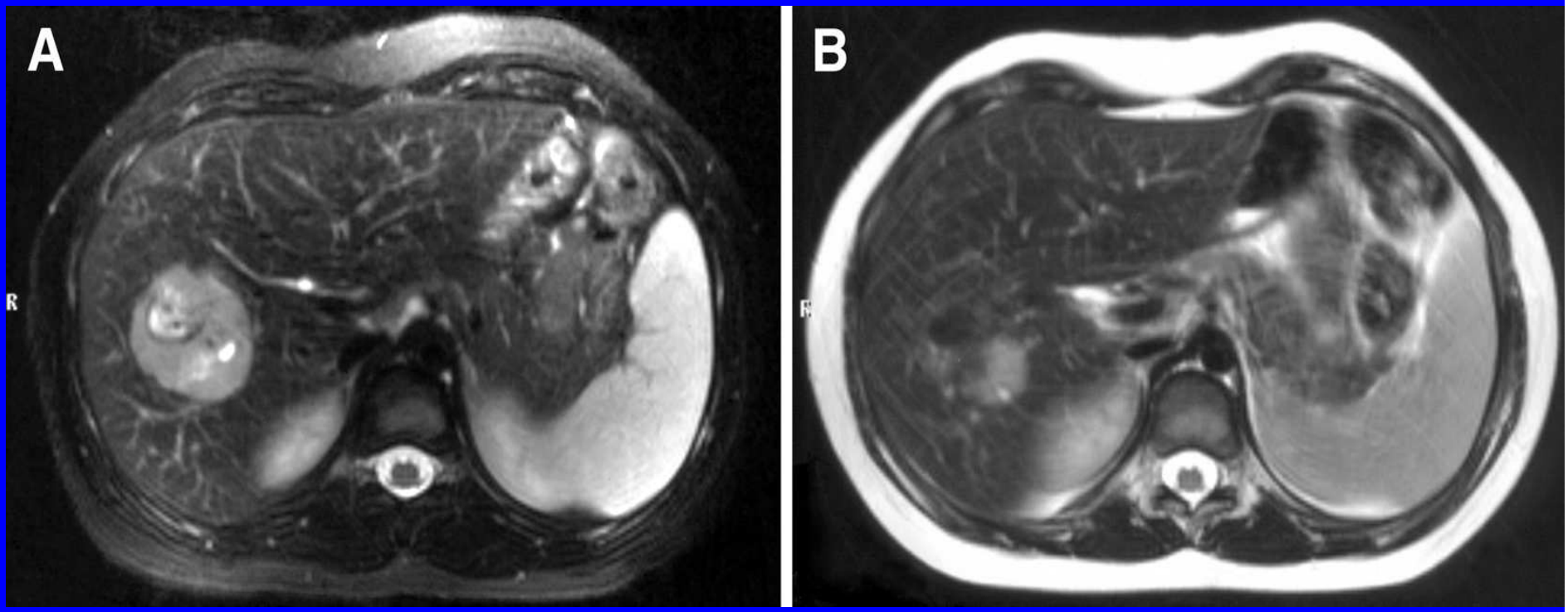
Case Example of Melanoma Patient Treated With High-Dose IL-2





Provided by D Schwartzentruber MD.

Case Study: 8 year old girl with melanoma spread to the liver

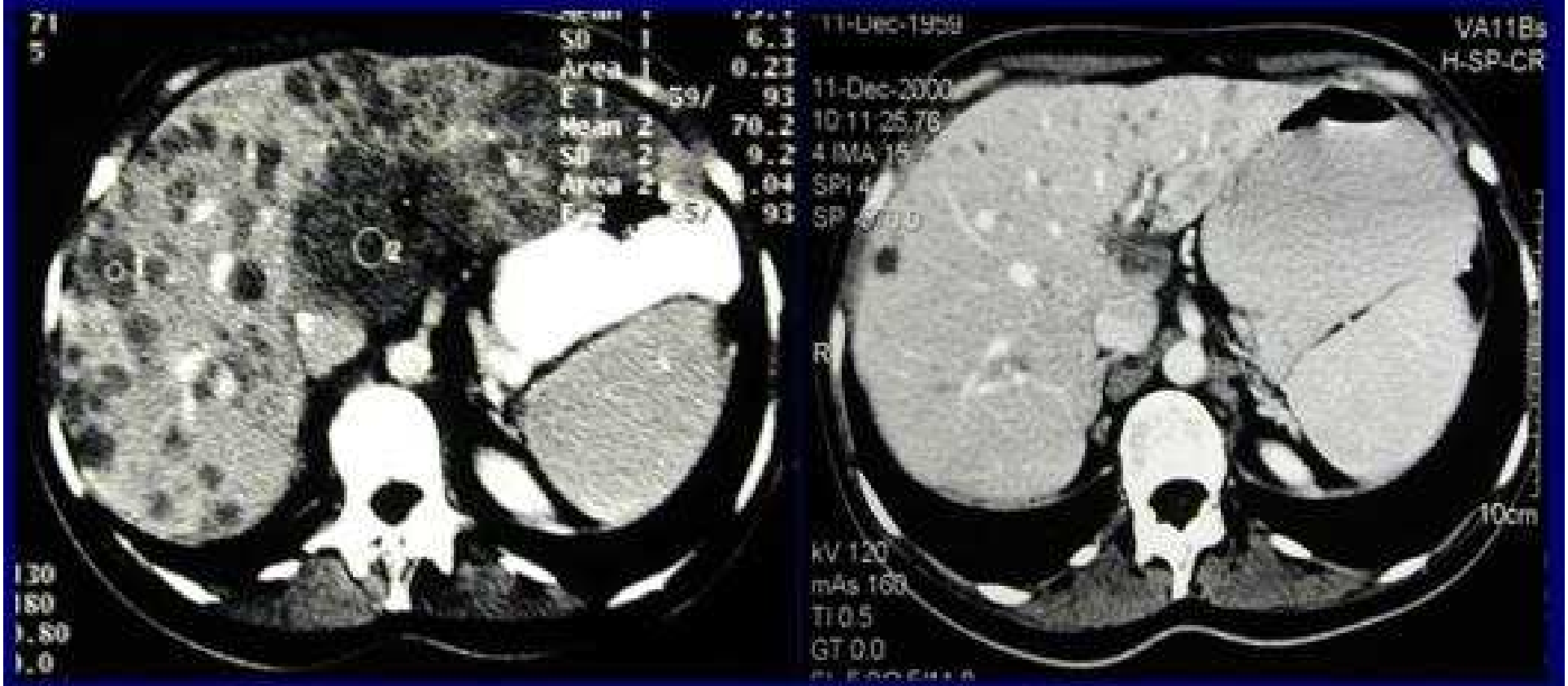


Before Treatment

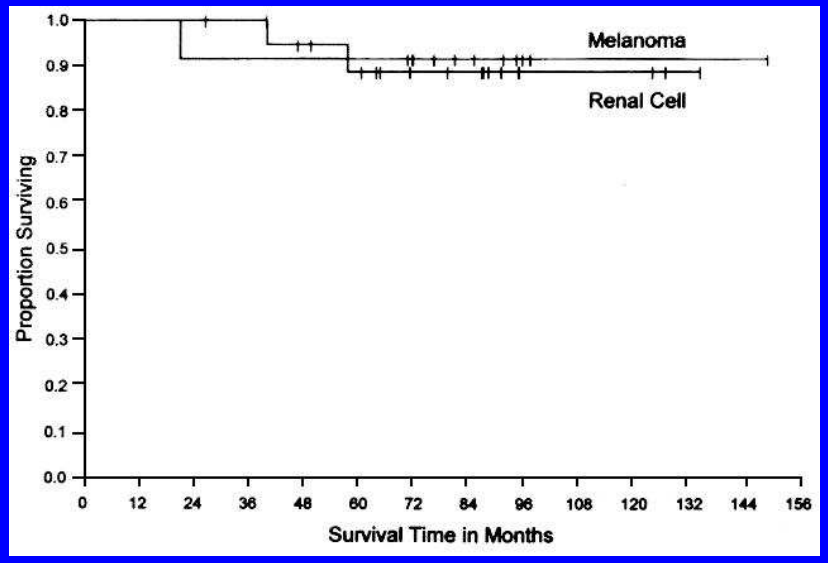
After Treatment

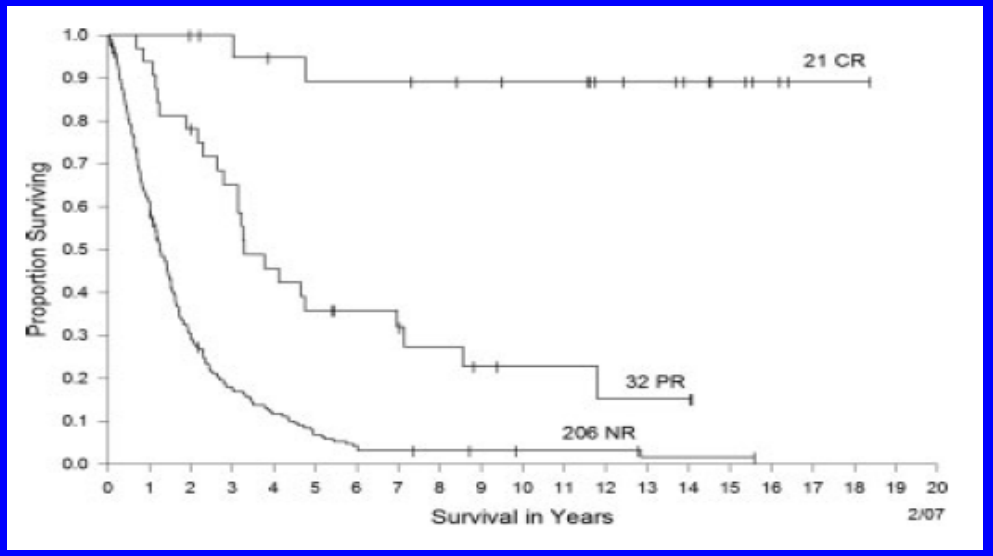
Soni et al. J Pediatr Hematol Oncol 2002

High-dose IL-2 after No Response on Biochemotherapy



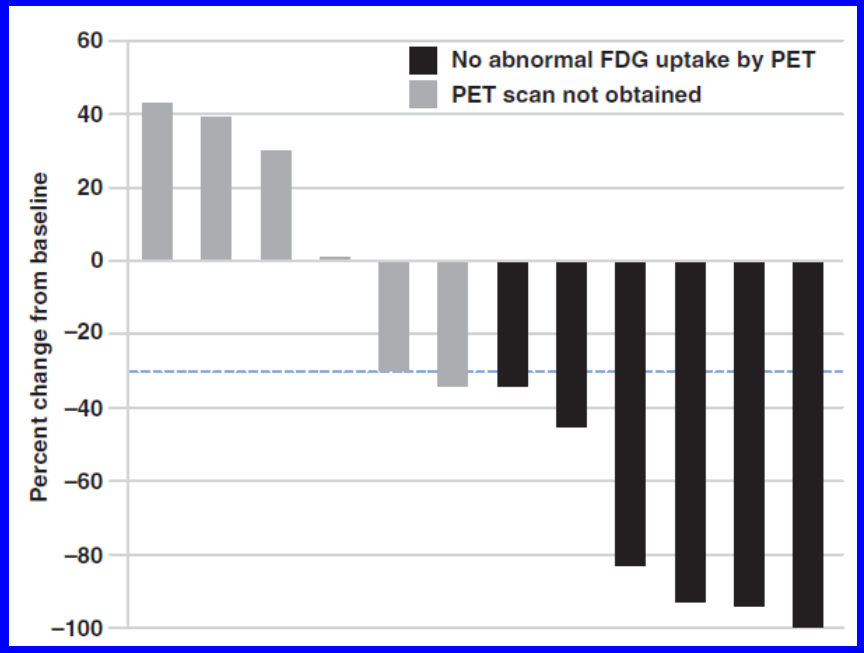
High-dose IL-2 promotes durable disease free survival in responders



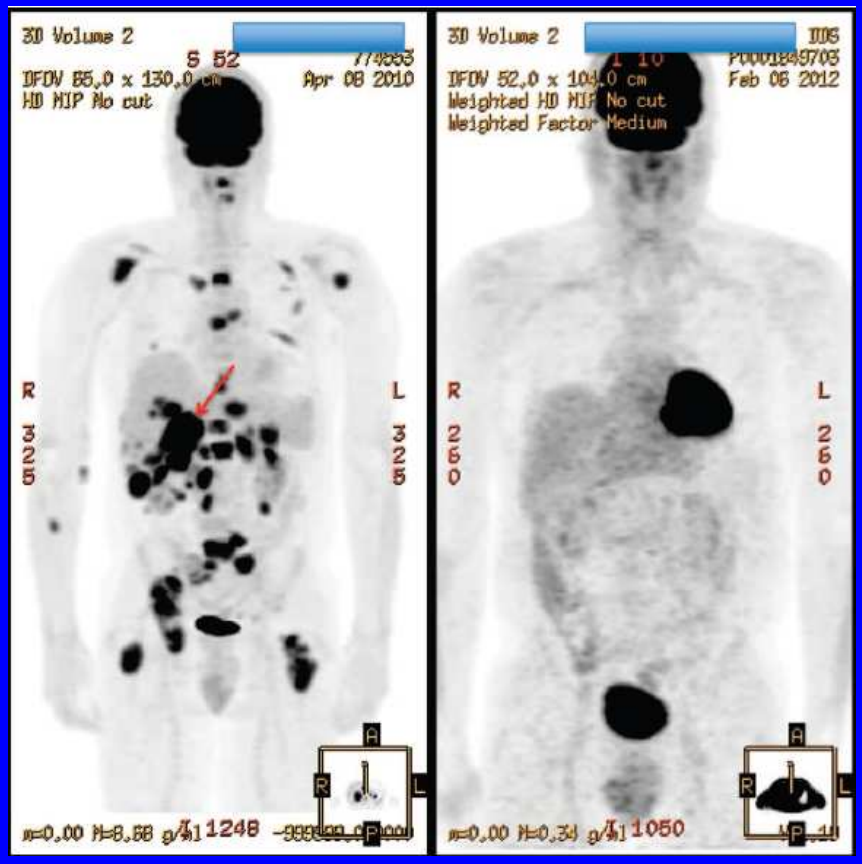


Rosenberg et al. Ann Surg 1998
Klapper et al. Cancer 2008

IL-2 and radiation therapy: Abscopal effect?



66% objective response rate



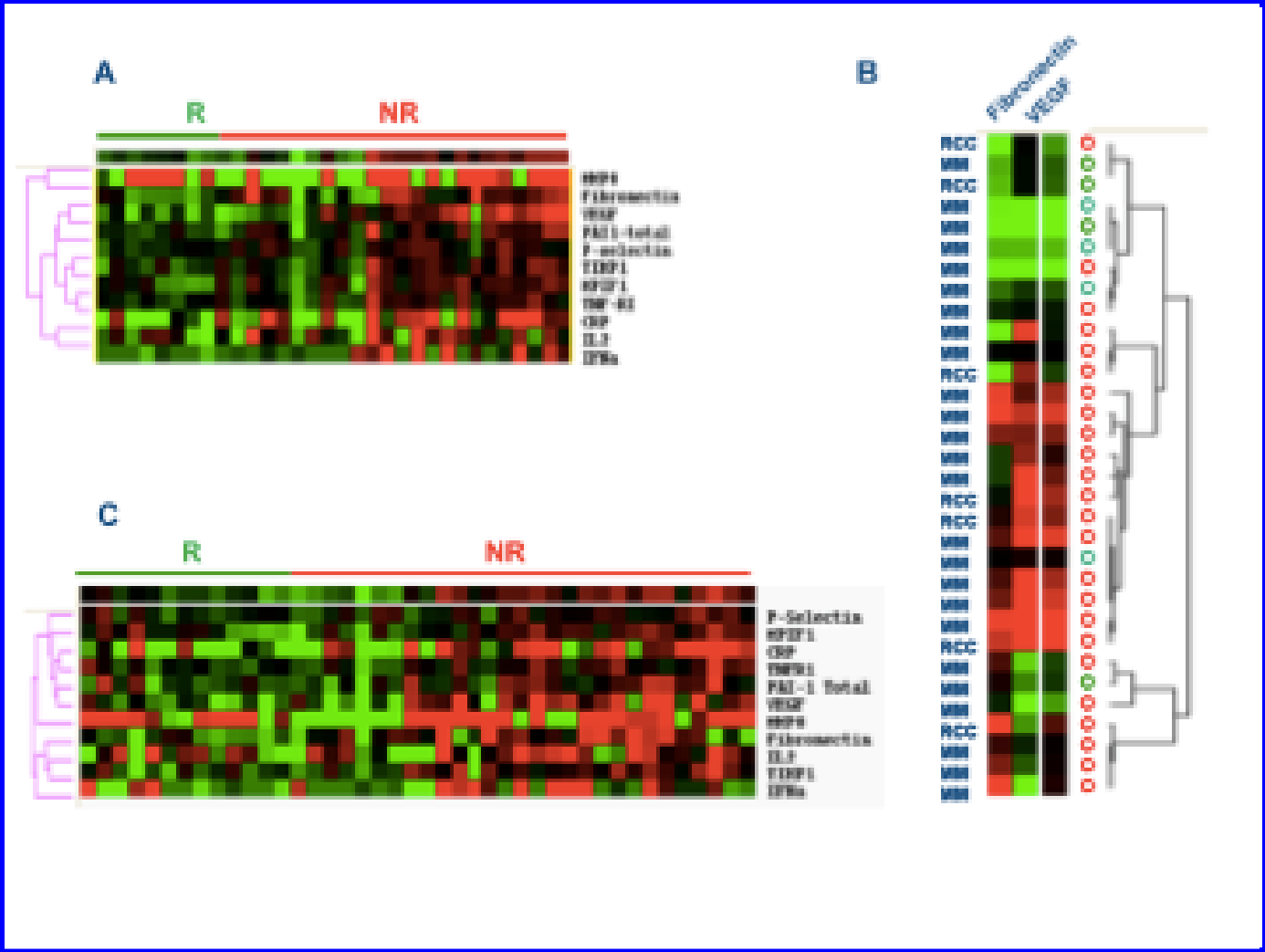
Seung et al. Sci Transl Med 2012

What correlates with response to IL-2 treatment?

- Performance status
- Development of autoimmunity
  - Autoimmune thyroiditis
  - Vitiligo
- Amount of IL-2 given during first course
- Height of the rebound lymhocytosis
- CA IX (in renal cell carcinoma)?
- Pre-treatment VEGF/fibronectin levels?

Komenaka et al. Clin Dermatol. 2004

Biomarkers of IL-2 response: Proteomic analysis



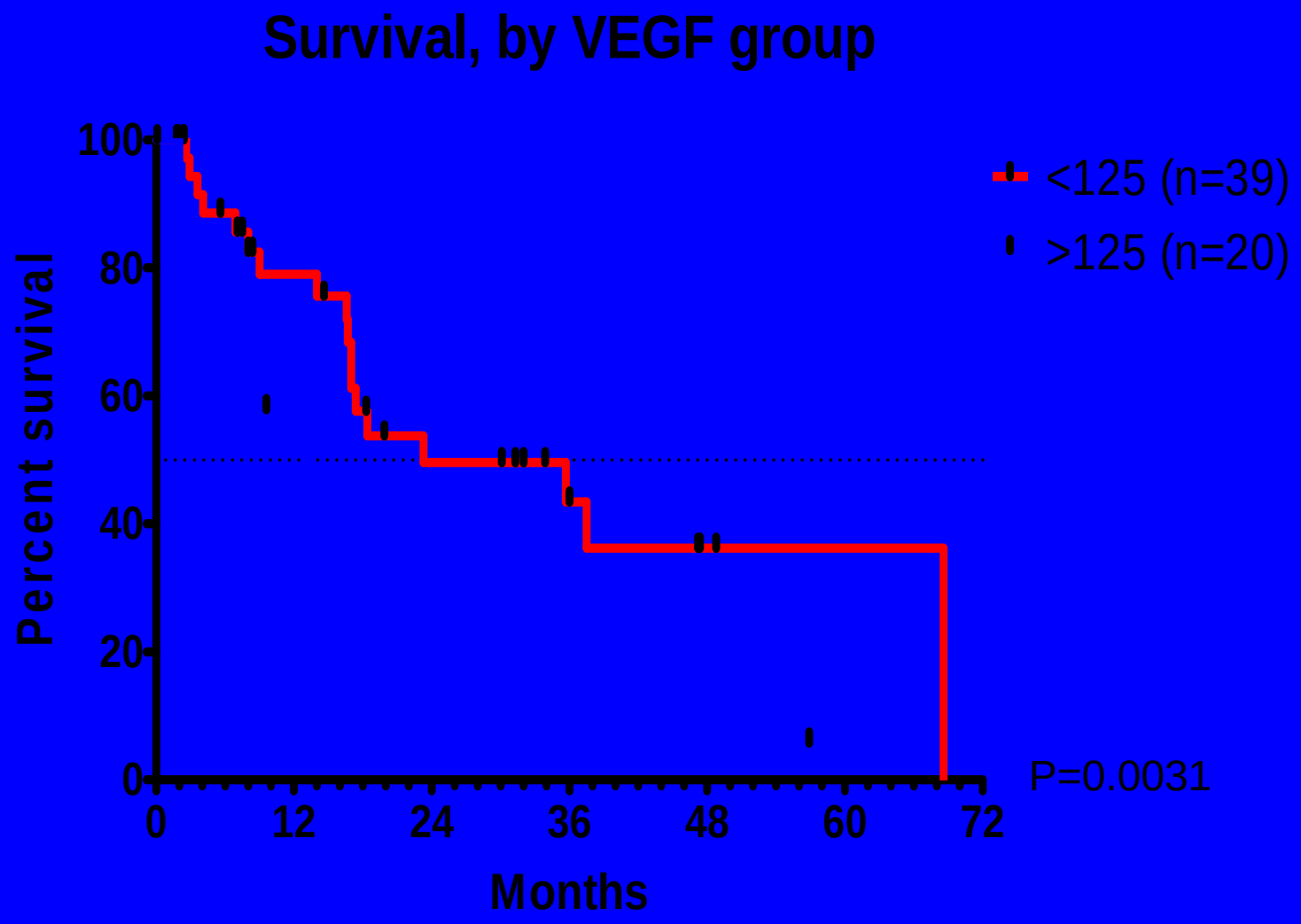
A Validation set

B VEGF and Fibronectin map

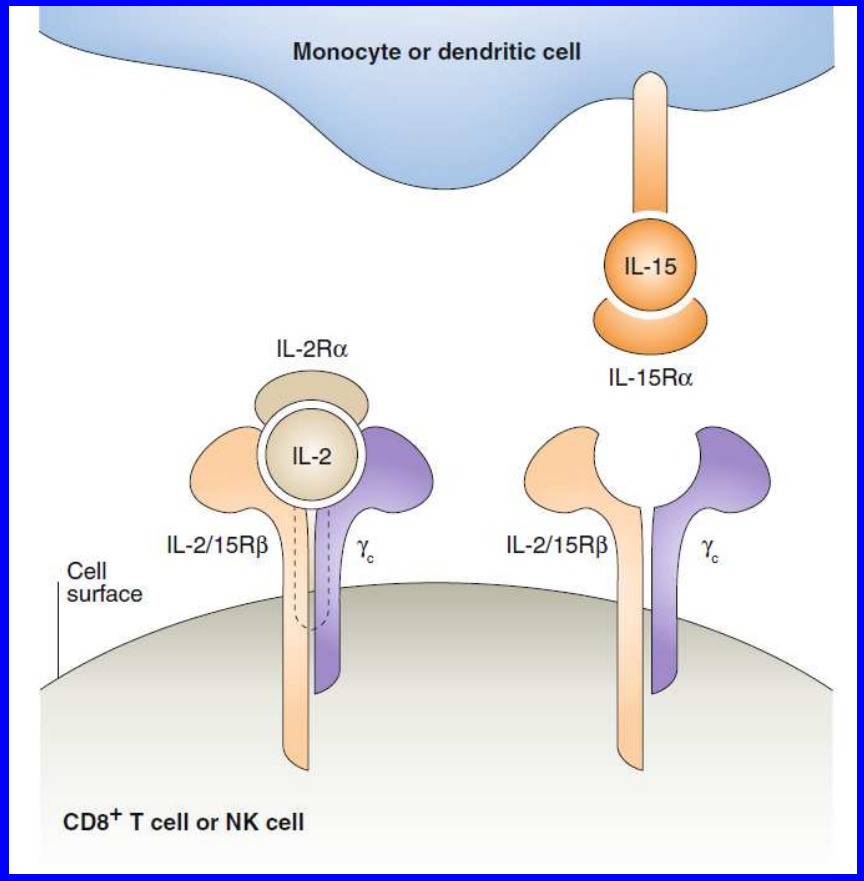
C Training and Validation set

Sabatino et al. JCO 2009

VEGF predicts survival following IL-2 treatment



IL-15 Signaling



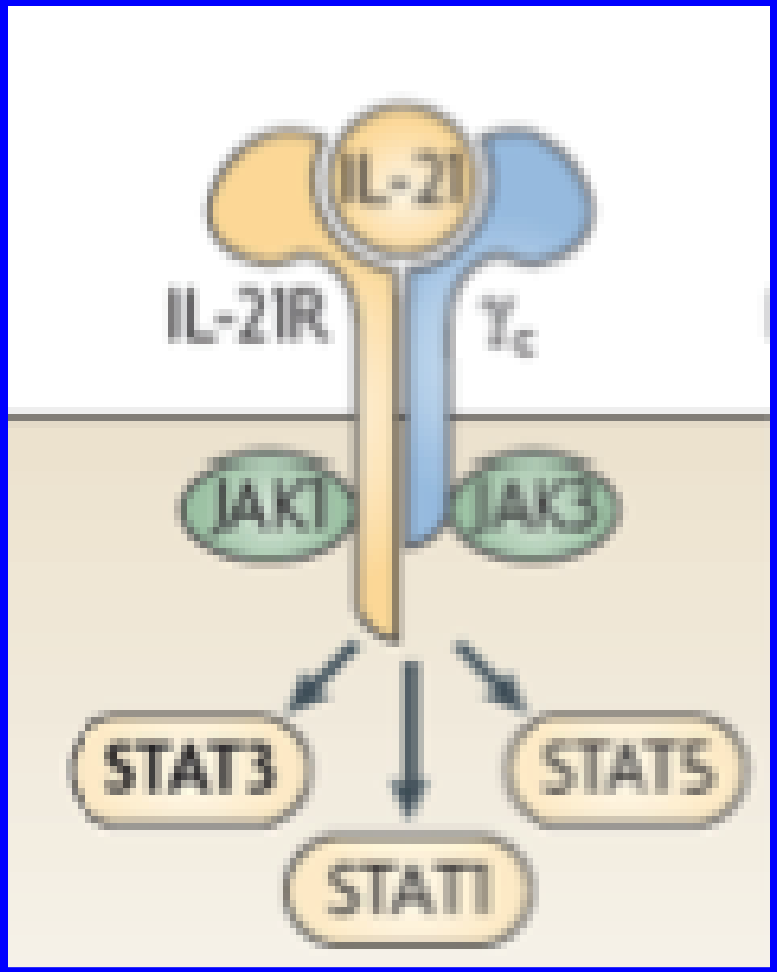
- Unique γ_c cytokine that complexes with receptor from cell of origin, then signals target cell
- With IL-2 and IL-7 in γ_c cytokine family promoting T cell growth and differentiation but may not expand Tregs
- Clinical trials starting

Margolin, Lazarus and Kaufman 2013

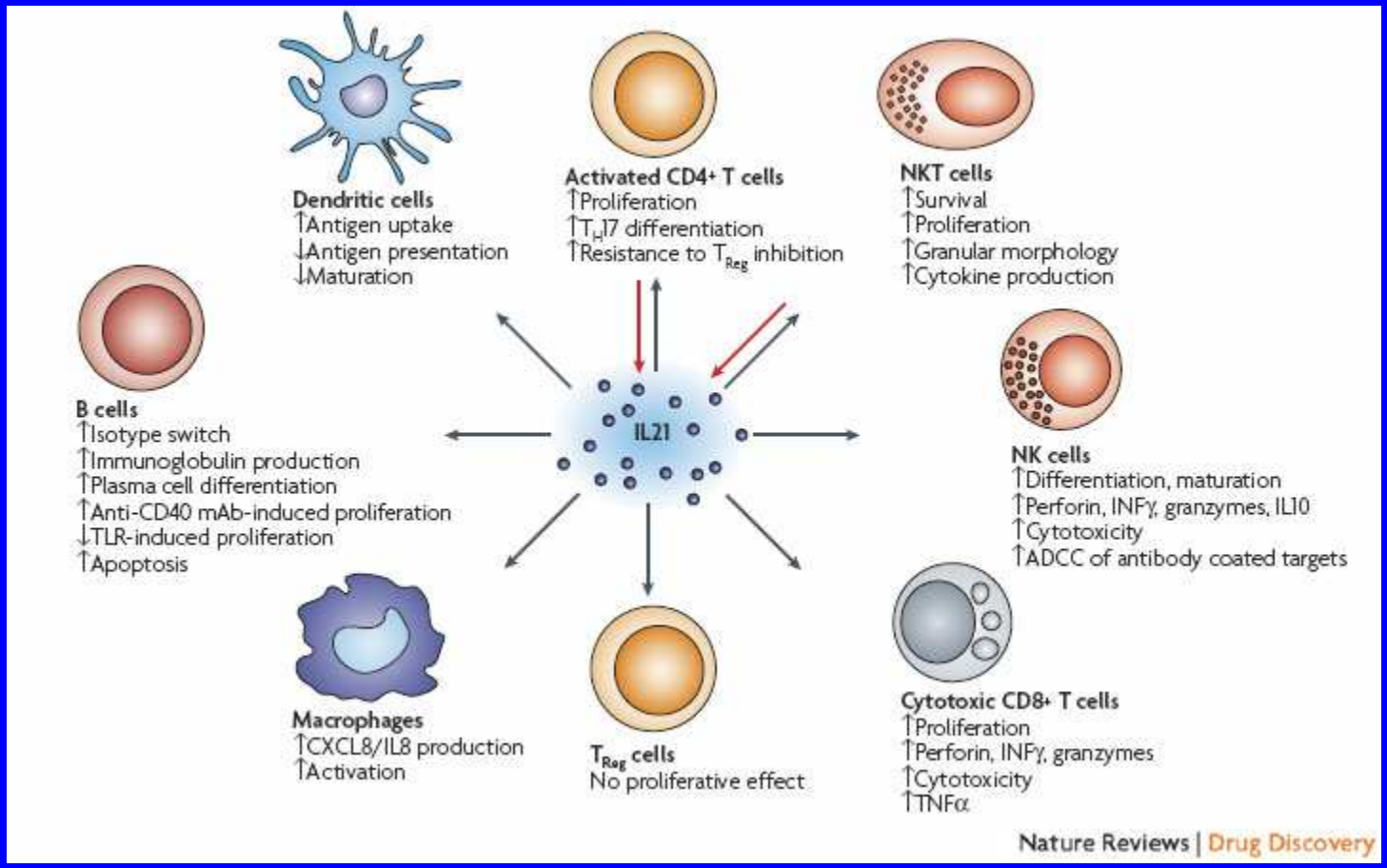
Comparing IL-2 and IL-15

- IL-2
  - Activated T, B express high-affinity receptor
  - Prolif/differentiation of NK, T, and B cells
  - Promotes activation-induced cell death
  - Maintenance of Treg
  - -/- KO develops autoimmunity
- IL-15
  - Produced by DC, monos
  - Surface-bound on DC/mono receptors on NK, CD8a1 T cells
  - Promotes proliferation NK, T, B, and memory CD8 T cells
  - Inhibits AICD
  - Does not support Treg
  - -/- KO is lymphopenic

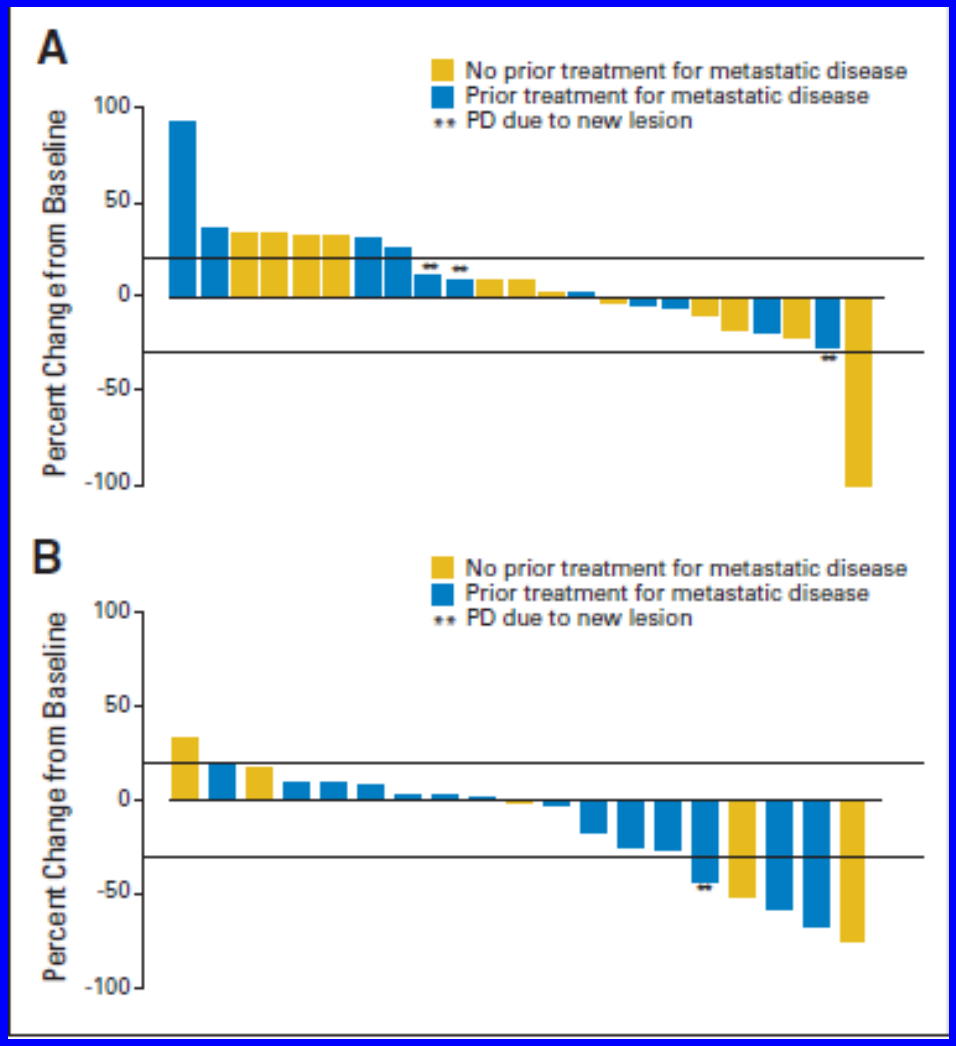
Interleukin-21



IL-21


Dendritic cells
↑Antigen uptake
↓Antigen presentation
↓Maturation
Activated CD4+ T cells
↑Proliferation
↑T_H17 differentiation
↑Resistance to T_{Reg} inhibition
NKT cells
↑Survival
↑Proliferation
↑Granular morphology
↑Cytokine production
B cells
↑Isotype switch
↑Immunoglobulin production
↑Plasma cell differentiation
↑Anti-CD40 mAb-induced proliferation
↓TLR-induced proliferation
↑Apoptosis
IL21
NK cells
↑Differentiation, maturation
↑Perforin, INFγ, granzymes, IL10
↑Cytotoxicity
↑ADCC of antibody coated targets
Macrophages
↑CXCL8/IL8 production
↑Activation
T_{Reg} cells
No proliferative effect
Cytotoxic CD8+ T cells
↑Proliferation
↑Perforin, INFγ, granzymes
↑Cytotoxicity
↑TNFα
Nature Reviews | Drug Discovery

Phase I IL-21 clinical trial



Melanoma

Renal cell carcinoma

Thompson et al. JCO 2008

IL-4

- Pleomorphic Th2 cytokine
- Net effects depend on milieu
  - Mainly a B cell-stimulator
  - Inhibits non-specific NK activity
  - Enhances other adaptive immune functions
    - Growth factor for Th2
    - Promotes proliferation, cytotoxicity of CTL
    - Stimulates MHC class II expression
    - Contributes to DC maturation
    - Enhances mΦ tumorcidal activity

IL-4

- Promising preclinical data, especially transgenic secretion by tumor
- Clinical experience limited
  - Studied like IL-2 at MTD
  - Unfavorable therapeutic index
- Used routinely to elicit i-moDC from PBMC
  - Used *ex vivo* w/GM-CSF
  - Shares some structure, function with IL-13

IL-4 and IL-13

- Similarities
  - Predominantly anti-inflammatory effects
  - Favor Th_2 responses
  - Partially common receptor
  - Promotes Ig class switch
  - Used w/ GM-CSF moDCs
- Differences
  - IL-13 activity on monocyte/mΦ cells
  - IL-13 lacks B, T cell effects
- IL-13 receptors on tumor cells, especially glioma
  - Immunotoxins
  - Chimeric T cell Ag receptor

IL-7

Signaling/gene expression

- JAK 1,3 STAT 5
- PI3K mTOR activation

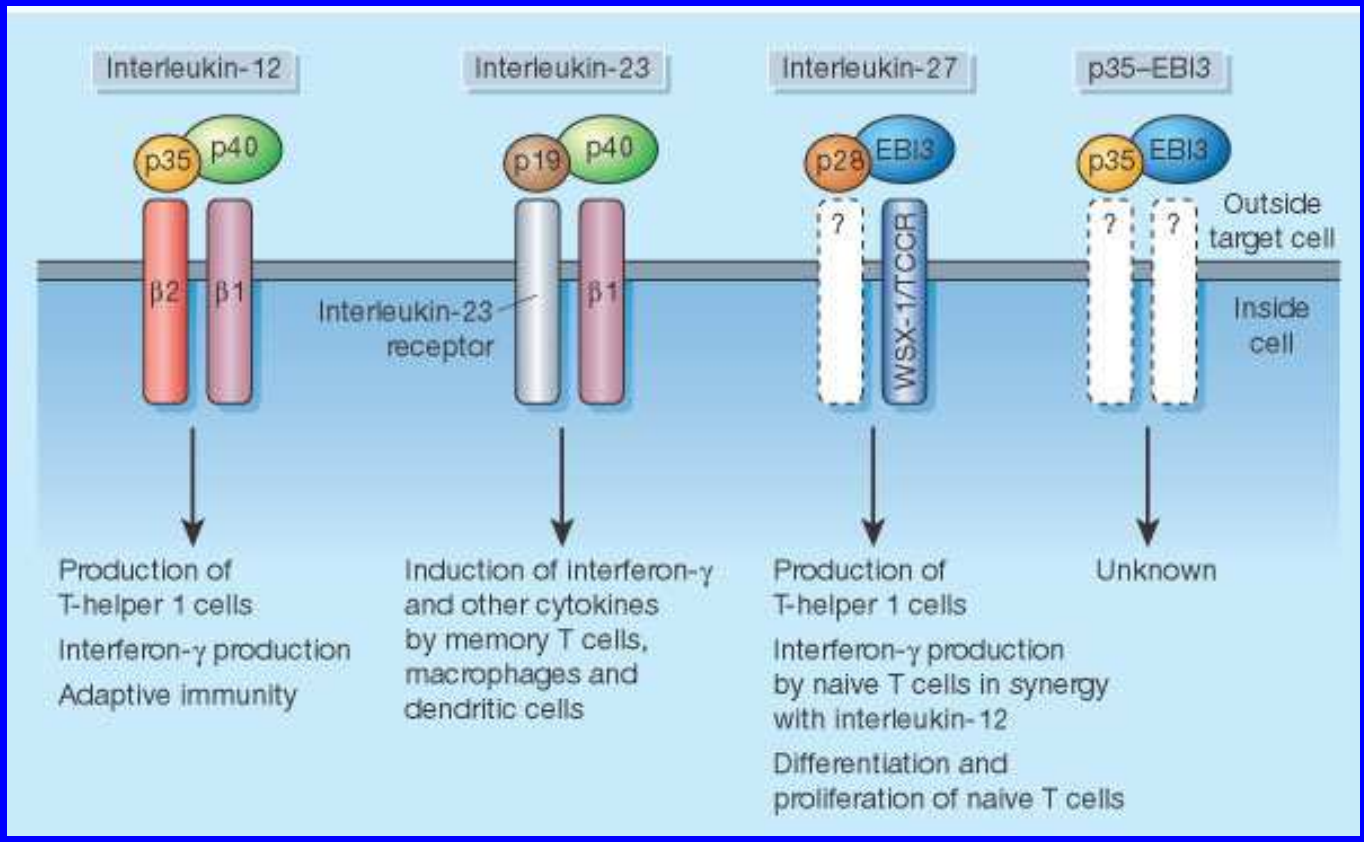
Regulation contrasts with IL-2, IL-15

- Unique to IL-7 is receptor downregulation
- IL-7 accumulates during lymphopenia due to utilization

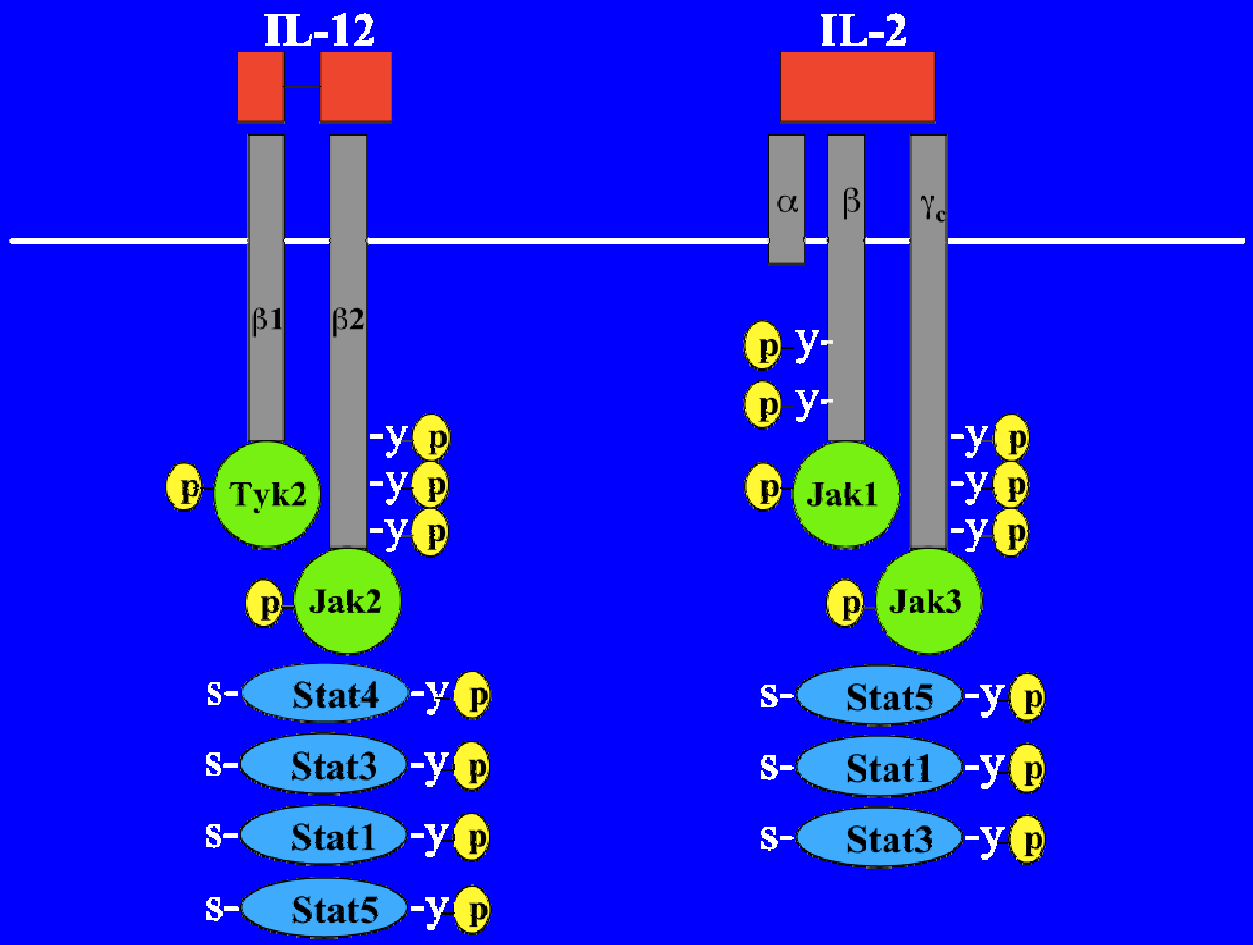
Mediates homeostatic expansion of naïve cells during lymphopenia

- May have clinical potential, possibly with IL-15, IL-21)

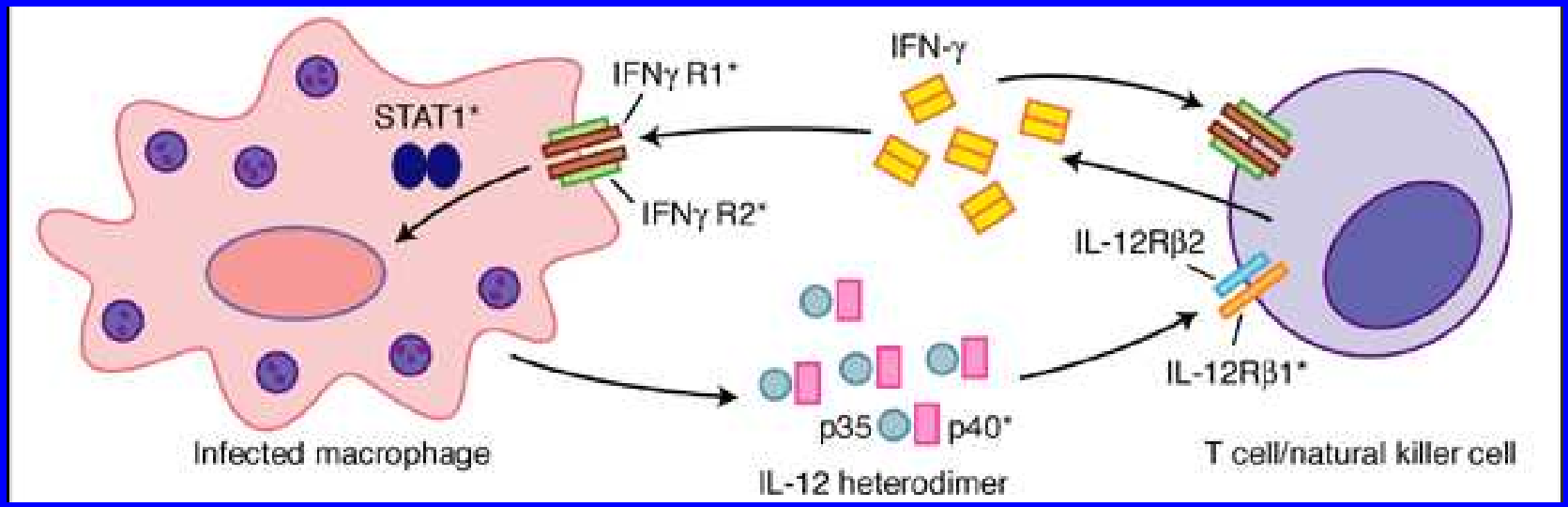
IL-12 Cytokine Family



IL-2 vs. IL-12 Signaling



IL-12 links innate and adaptive immune responses



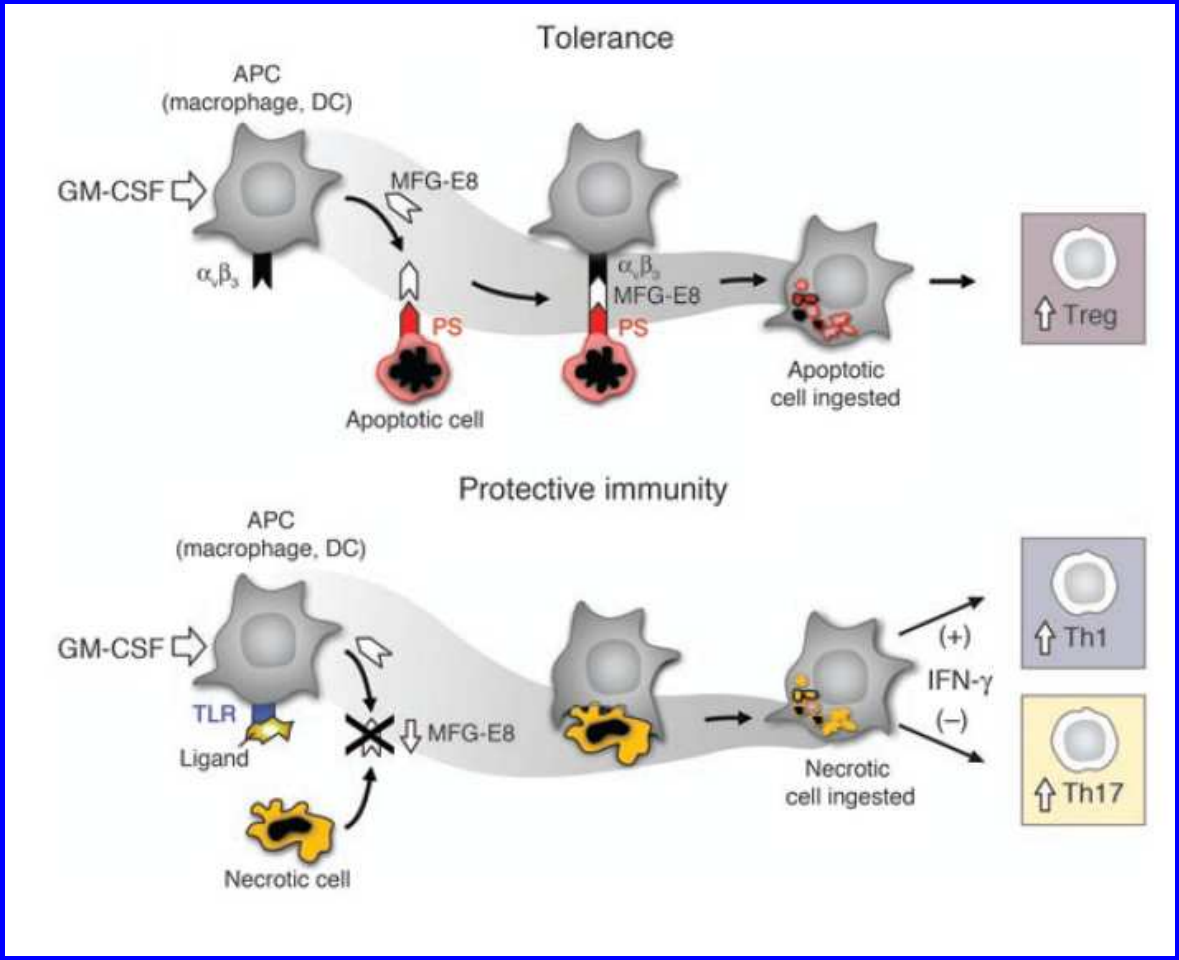
IL-12

- Link between innate, adaptive immune response
  - Receptors on variety of immune cells
  - Induces IFN- , a prototypical type I cytokine
- Potent inducer of counterregulatory type 2 cytokines
  - Emerged in clinical trials for advanced malignancy
  - Schedules and doses may be manipulated
- Clinical potential
  - Vaccine adjuvant
  - Induction of anti-angiogenesis
  - In combinations e.g. w/ -IFN, IL-2?

GM-CSF

- Cells of origin
  - Th1, Th2
  - Others include epithelial, fibroblast, *tumor*
- Target cell: immature DC (& myeloid progenitor)
- Biological functions
  - Stimulation of T cell immunity via effect on APC
  - Myeloid cell proliferation, differentiation
- Clinical development
  - Hematopoietic support
  - Not a potent stand-alone cytokine in cancer
  - Adjuvant for melanoma: (-) results+/- peptide vaccine
  - Immunocytokine in prostate cancer DC product
  - Transgenic expression (GVAX) [and other cytokines]

Mechanism of GM-CSF anti-tumor activity?



Jinushi et al. JCI 2007

Non-immune potentiating cytokines

- IL-6
- IL-10
- IL-17??
- TGF-β
- VEGF

Conclusions

- Immune-potentiating cytokines have shown clinical benefit in patients with cancer
  - IL-2
  - Interferon-α
- The mechanism of cytokine-mediated tumor regression is unclear
- Several cytokines are in clinical development
- Combination studies are in progress
  - Cytokines and immunotherapy (e.g. anti-CTLA-4, PD-1)
  - Cytokines and targeted therapy (e.g. BRAF inhibitors)
  - Cytokines and radiation (e.g. absocopal effect)
- Predictive biomarkers are in development
  - Autoimmunity
  - VEGF