

# Cytokines in Cancer Immunotherapy

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iSBTc Immunology Primer  
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# Presenter disclosure information

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  - BMS
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  - Roche
- DMC
  - Cougar/J and J
  - Medimmune

# Immunobiology of Cytokines

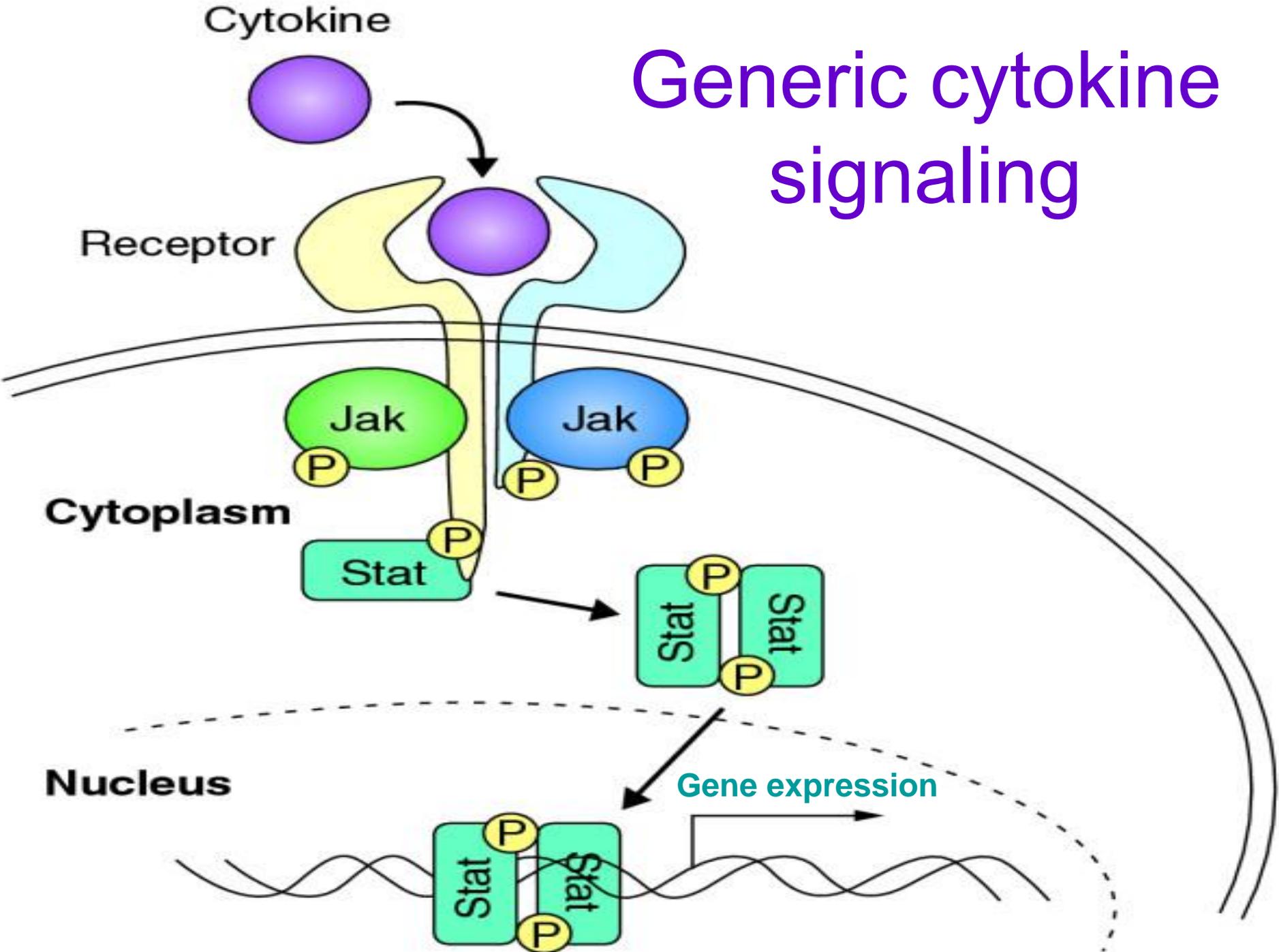
## For discussion

- Structure/function relationships
- Cellular source/stimuli for synthesis/secretion
- Target cell(s)/receptor structure
- Signaling induced by cytokine binding
- Preclinical applications
- Cytokine gene transfer/vaccine
- Clinical application/status

## Not for discussion

- Cytokines/polymorphisms in pathogenesis
  - Malignancy
  - Autoimmune disease
- Cytokine-directed therapies for nonmalignant conditions
- Complex interactive cytokine networks, innate
- Alternative structures (immunotoxins, immunocytokines)
- Intratumoral delivery

# Generic cytokine signaling



Receptor for:

IL-2

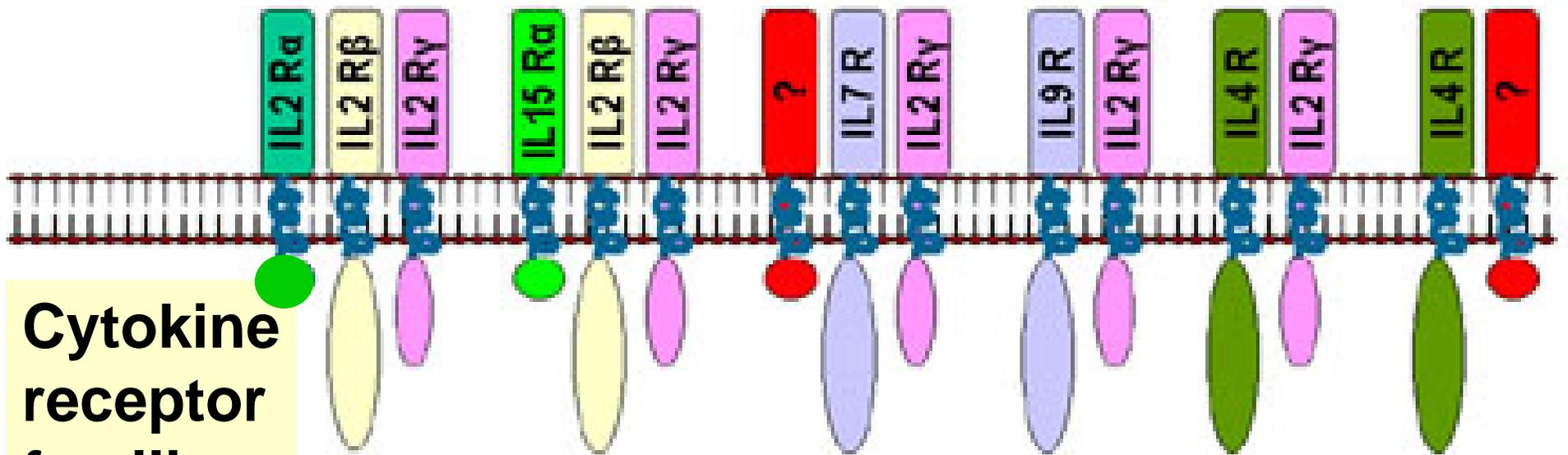
IL-15

IL-7

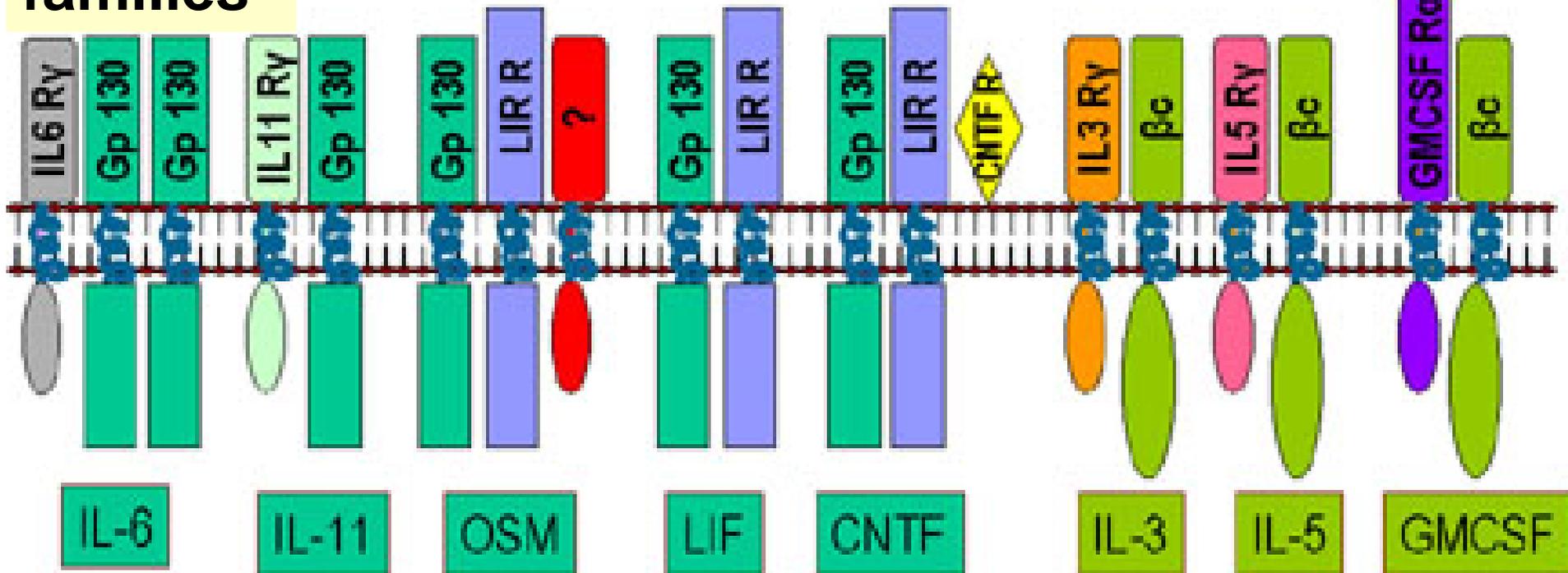
IL-9

IL-4

IL-13



Cytokine receptor families

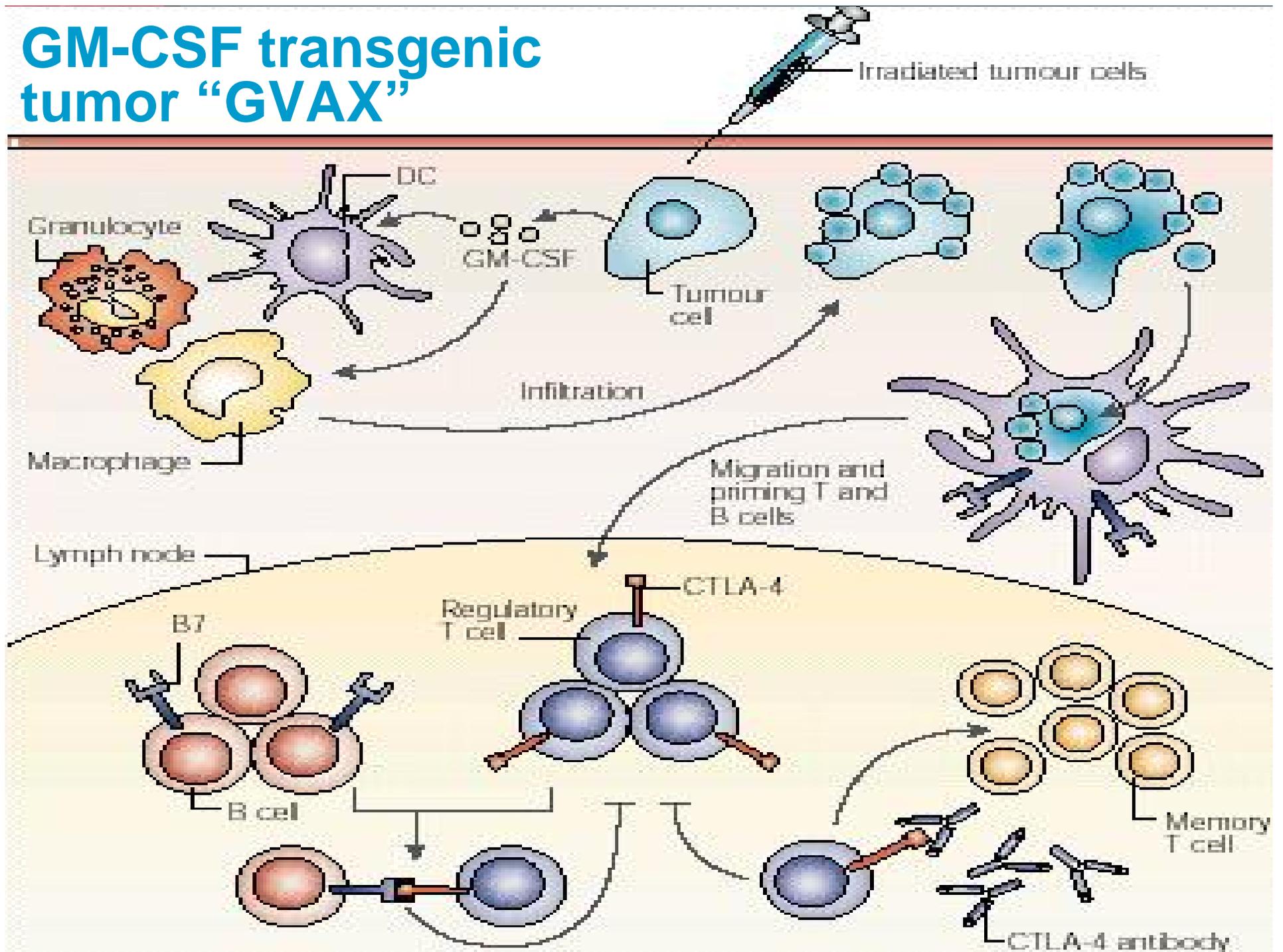


A vertical strip on the left side of the slide shows a microscopic image of cells, likely red blood cells, with a reddish hue. The main background of the slide is dark blue.

# GM-CSF as immunotherapy

- 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]

# GM-CSF transgenic tumor "GVAX"



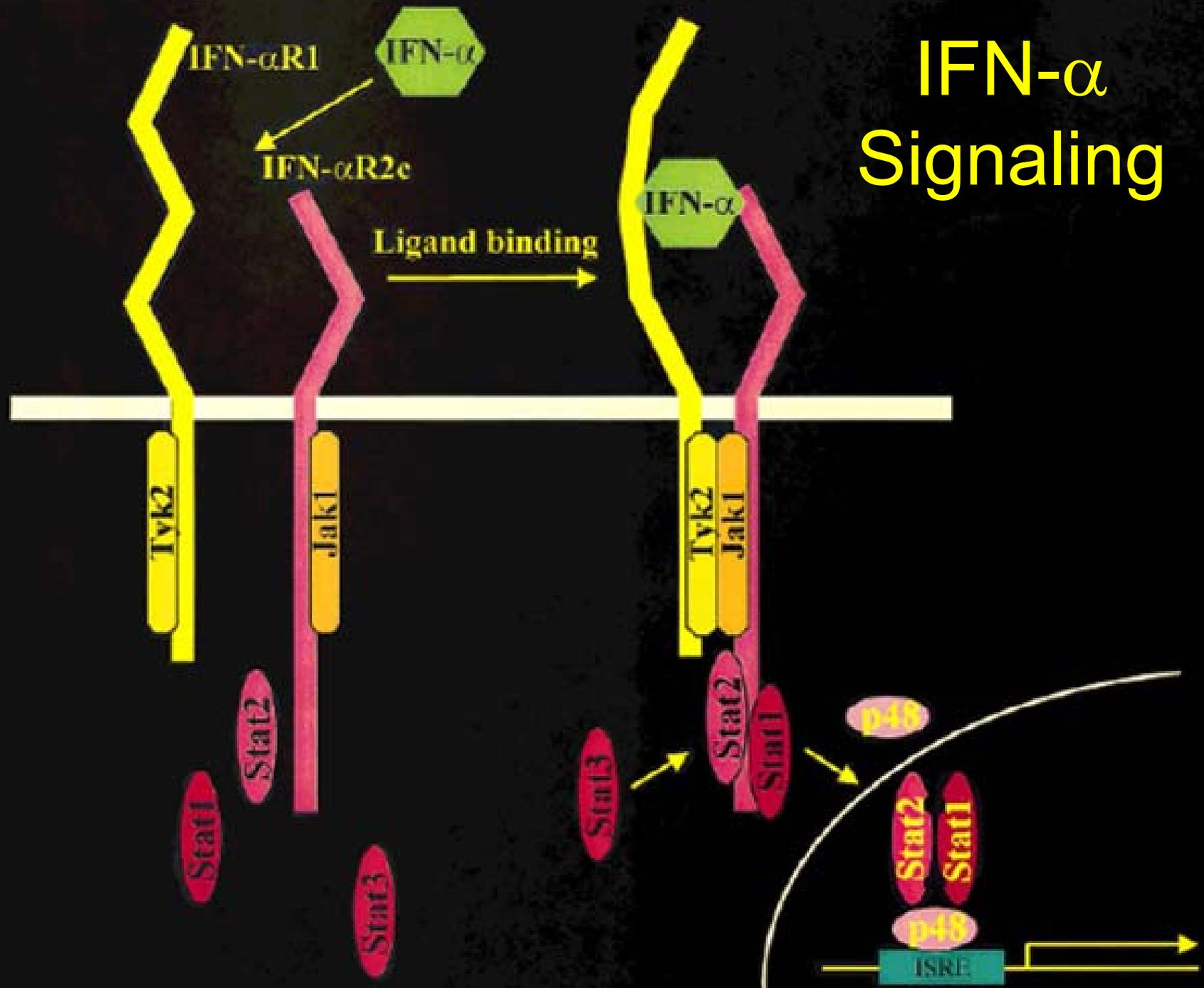
# Interferons

- Type I
  - $\alpha$ : from neutrophils,  $m\phi$
  - $\beta$ : from fibroblasts, epithelial cells
- Type II  $\gamma$ -IFN, type 1, 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

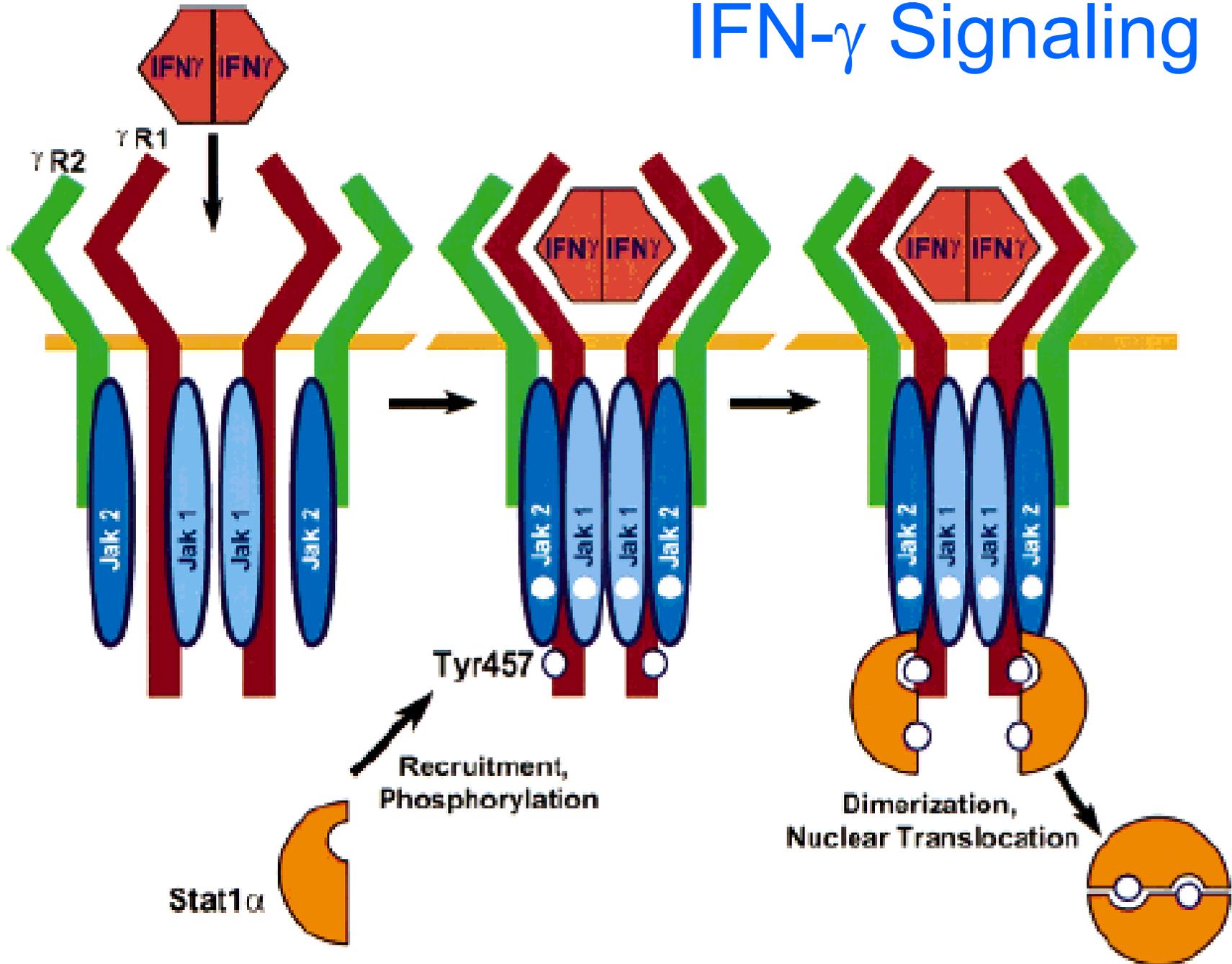
# Interferons (cont.)

- Direct effects on tumor cells
  - MHC upregulation
  - Antiproliferative/pro-apoptotic effects
- Anti-angiogenic effects
  - IP-10
  - Thrombospondin

# IFN- $\alpha$ Signaling

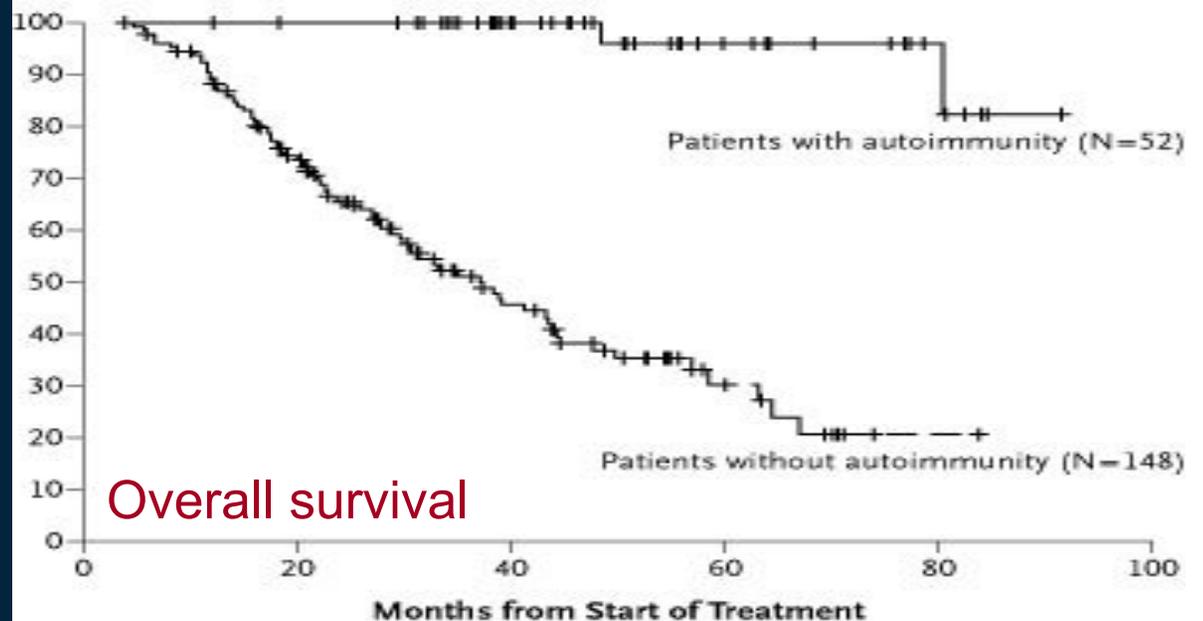
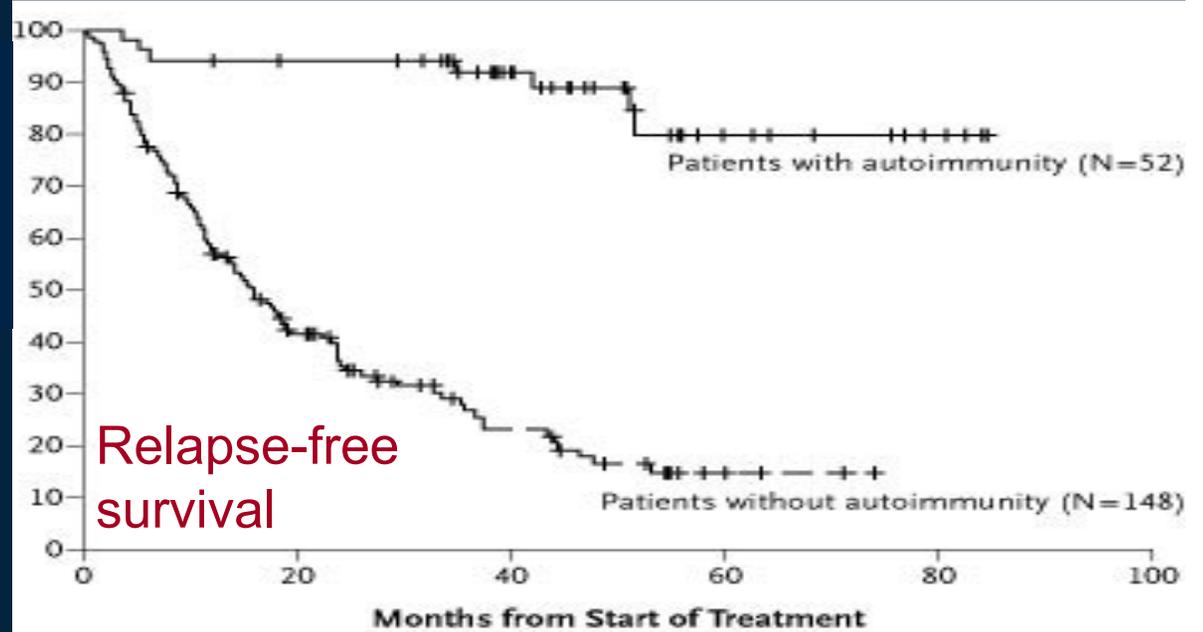


# IFN- $\gamma$ Signaling



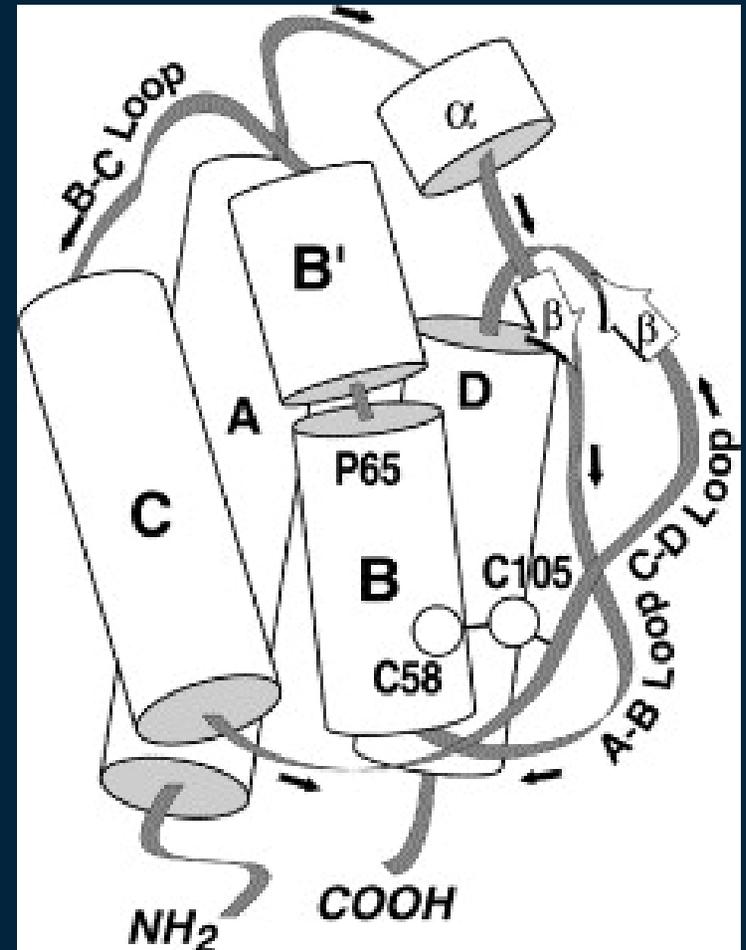
# IFN autoimmunity/ adjuvant benefit in melanoma

IFN in  
melanoma,  
other  
malignancies  
remains a work  
in progress



# Interleukin-2

- Short chain type I cytokine
- Four  $\alpha$ -helical bundles
- Produced by activated T cells
- TCR/CD3 engagement plus CD28 ligation required
- Main targets are T, NK cells
- Stimulates immune responses and prevents tolerance
- Also downregulates immune response: role in  $T_{reg}$  development/activity



# Interleukin-2

“T cell growth factor”

Produced by Th1 cells

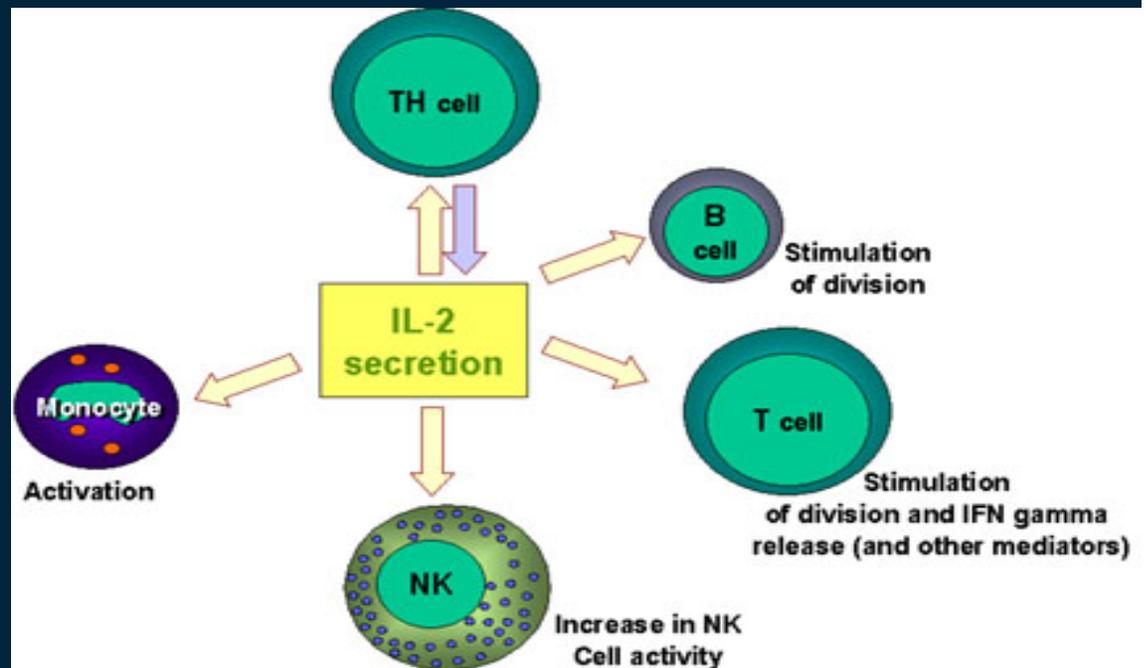
Many cell types express IL-2R

- B, NK/NKT, monocytes
- Affinity, functions depend on subunit  $\alpha\beta\gamma$  expression

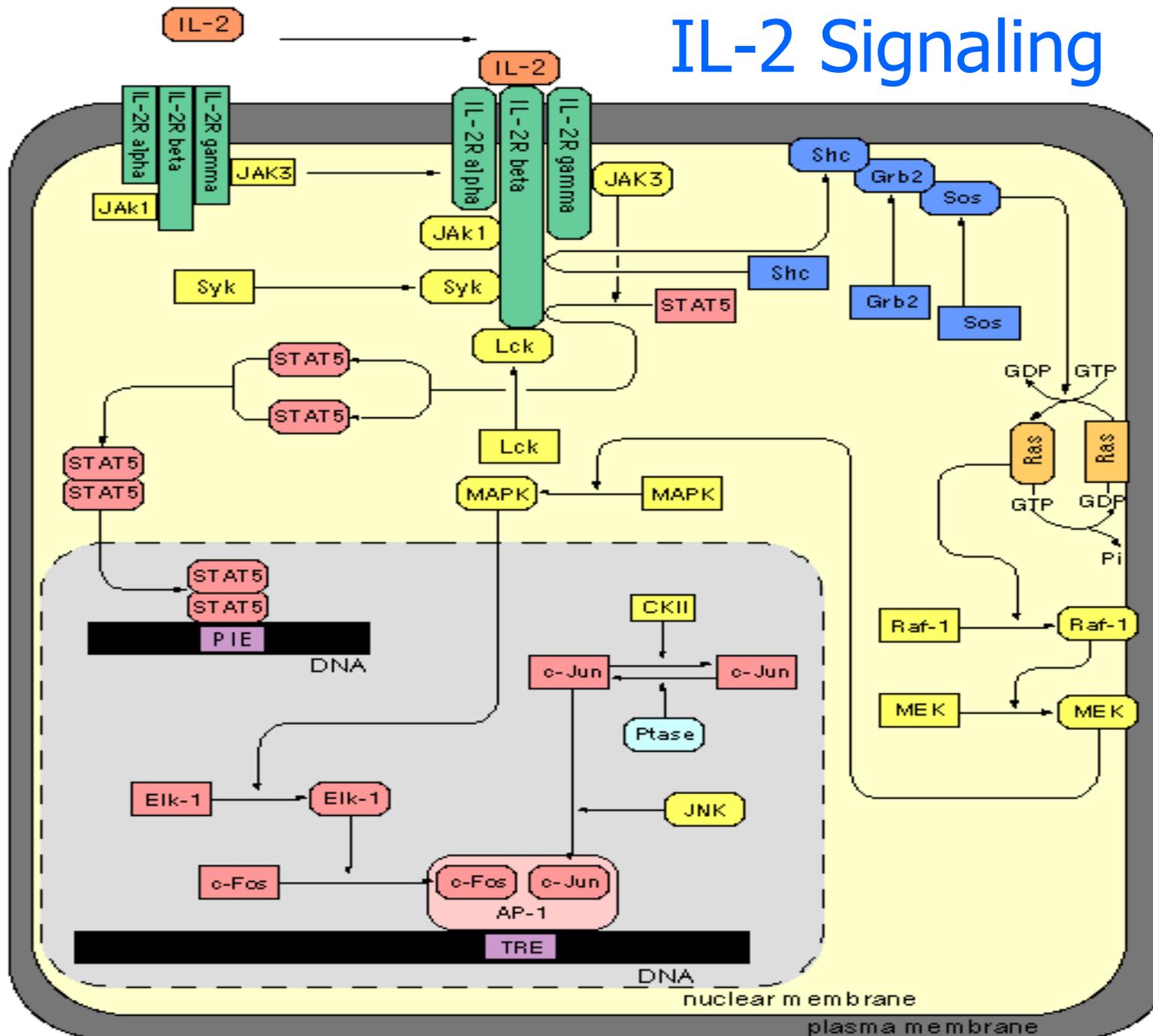
Signaling

- JAK-STAT>
- MAPK
- PI3K

Proliferation,  
cytotoxicity



# IL-2 Signaling





# *In Vivo* Effects of IL-2

- Induction of multiple cytokines
  - TNF, interferon-gamma, GM-CSF, M-CSF, G-CSF, IL-4, IL-5, IL-6, IL-8, IL-10
- Increase in soluble IL-2r
- Lymphopenia followed by rebound
- Increased NK activity (during rebound)
- Increased CD25, HLA-DR expression on T-cells
- Decreased PBMC proliferative responses
- Tissue infiltration by lymphocytes
- Eosinophilia
- Neutrophil chemotaxis defect

# Pioneering NCI studies

# Extramural IL-2 studies

## Biology/source

- T cell growth factor
- Jurkat source
- Recombinant E. coli

## Preclinical models

- Toxicities from capillary leak
- Toxicities vary by species
- Dose-dependent activity

## Early clinical studies+LAK

Supportive role in adoptive cell-Rx strategies

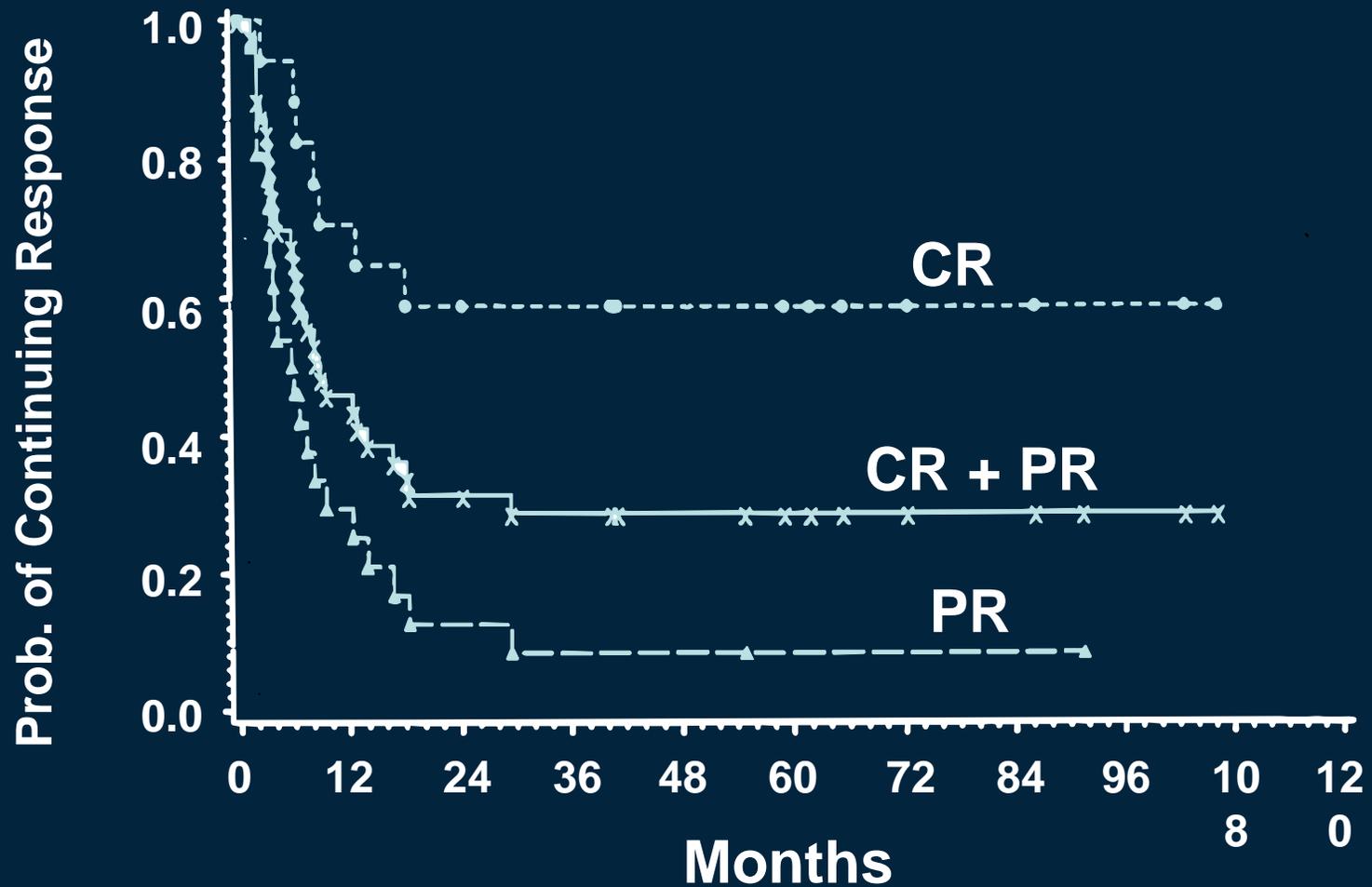
## In solid tumors

- With LAK cells
- Single agent high/low doses
- With  $\alpha$ -IFN
- With other cytokines
- With chemotherapy
- Toxicity modulation
- Biological predictors of benefit

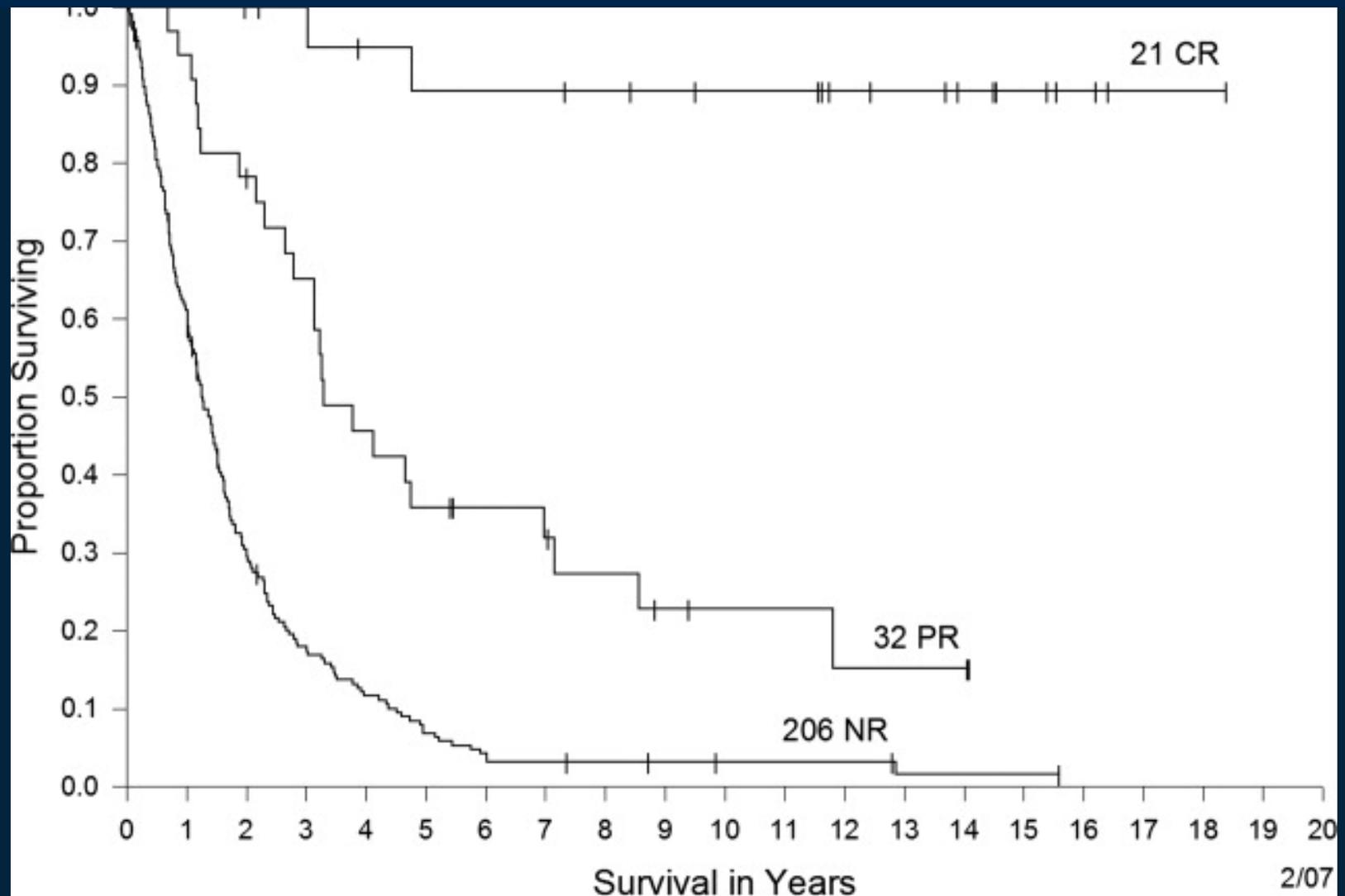
## In heme malignancies

- Preclinical activity
- Clinical benefits not achieved

# Responses in melanoma



# Responses in renal cancer



Klapper, JA et al. Cancer (2008)

## IL-2 Grade 3-4 Toxicity

	NCI-SB HD IL-2	CWG HD IL-2
<b>Median Doses per Course</b>	12 (28)	68% (19 doses)
<b>Death</b>	0	1%
<b>Hypotension</b>	36.4%	56.8%
<b>Pulmonary</b>	4.2%	13.7%
<b>CNS orientation</b>	10.2%	14.7%
<b>CNS consciousness</b>	2.5%	
<b>Infection</b>	2.8%	3.2%
<b>Nausea/vomiting</b>	13.4%	9.5%
<b>Diarrhea</b>	9.2%	
<b>Hyperbilirubinemia</b>	3.2%	11.6%
<b>ALT</b>	3.2%	
<b>Creatinine &gt; 8.0 mg/dL</b>	1.1%	13.7% (gr 3-4)
<b>Oliguria (&lt; 80 ml/8h)</b>	12%	
<b>Atrial Arrhythmia</b>	4.2%	8.4% (all cardiac)
<b>Malaise</b>	20.5%	3.2%

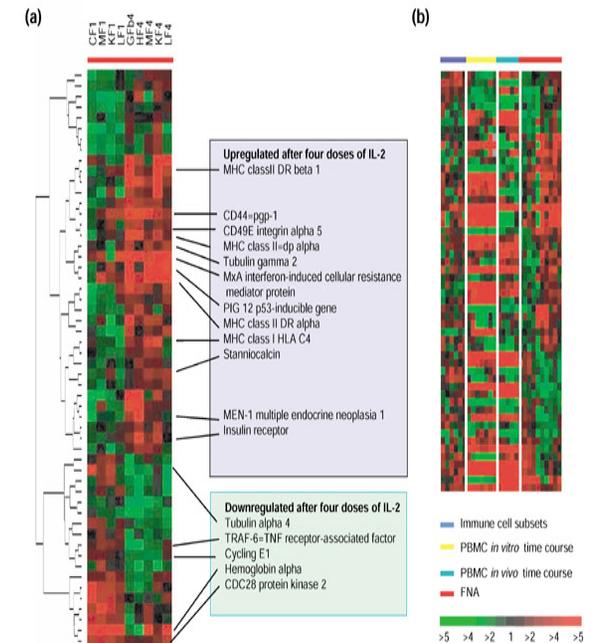
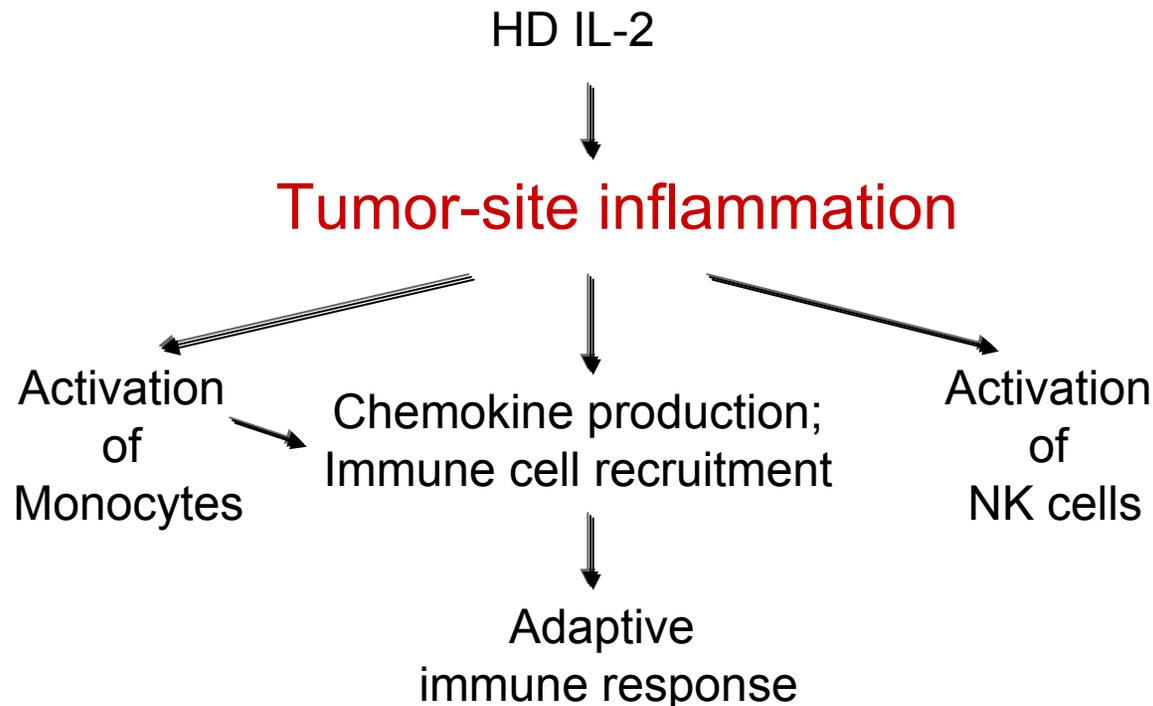
# Severe toxicities of high-dose IL-2

Grade 3 or 4 Toxicity	Incidence	
	Fyfe et al (1995) N = 255 (% of patients)	Rosenberg et al (1994) N = 149 (% of IL-2 courses)
Hypotension	74	57
Pulmonary (dyspnea)	17	10
Renal (creatinine elevation)	14	10
Hepatic (hyperbilirubinemia)	21	19
CNS	32	28
Myocardial injury (ischemia, infarction, myocarditis)	6	2
Arrhythmias (all grades)	14	5
Infection	6	3
Thrombocytopenia	21	29
Death	4	1

# How does high-dose IL-2 work?

Multiple hypotheses from animal data, but rigorous human data lacking.

Gene expression profiling on FNA of tumor, serial PBMC in melanoma patients on HD-IL-2



# Phase III Trial of High-Dose IL-2 ± Peptide Vaccine in Patients With Metastatic Melanoma

## Eligibility Criteria

- Pts with stage IV or locally advanced stage III cutaneous melanoma
  - HLA-A\*0201 positive
- (N=185)

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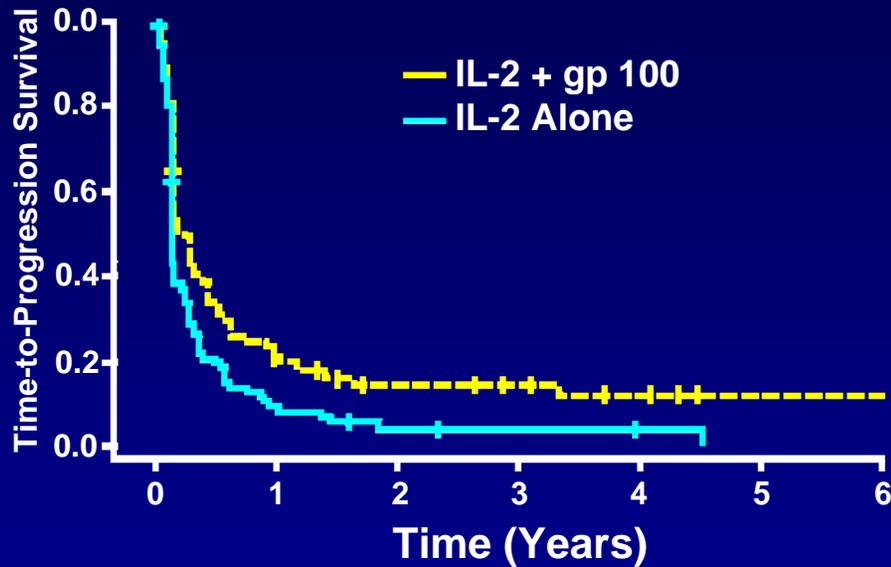
**High-dose IL-2** 720K IU/kg/dose IV every 8 hours x 12  
Repeat every 3 weeks  
(N=94)

**Vaccine** ([gp100:209-217(210M)] subcutaneous d1 q3w) +  
**High-dose IL-2** (as above)  
(N=91)

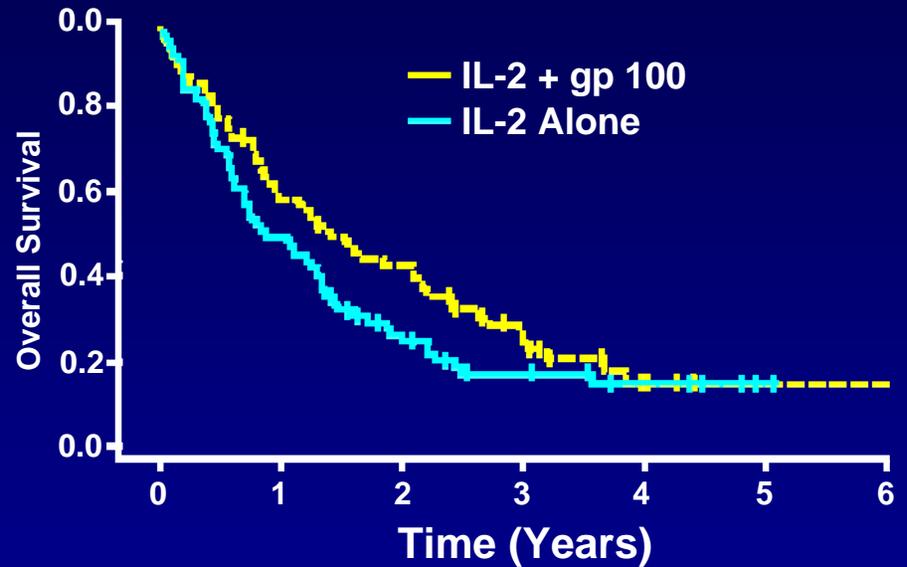
- Primary endpoint: RR
- Secondary endpoints: toxicity, PFS, quality of life, and immunologic monitoring
- Central HLA typing, pathology review, and blinded response assessment performed at the National Institutes of Health

# High-Dose IL-2 ± Peptide Vaccine: Results

**PFS**



**OS**

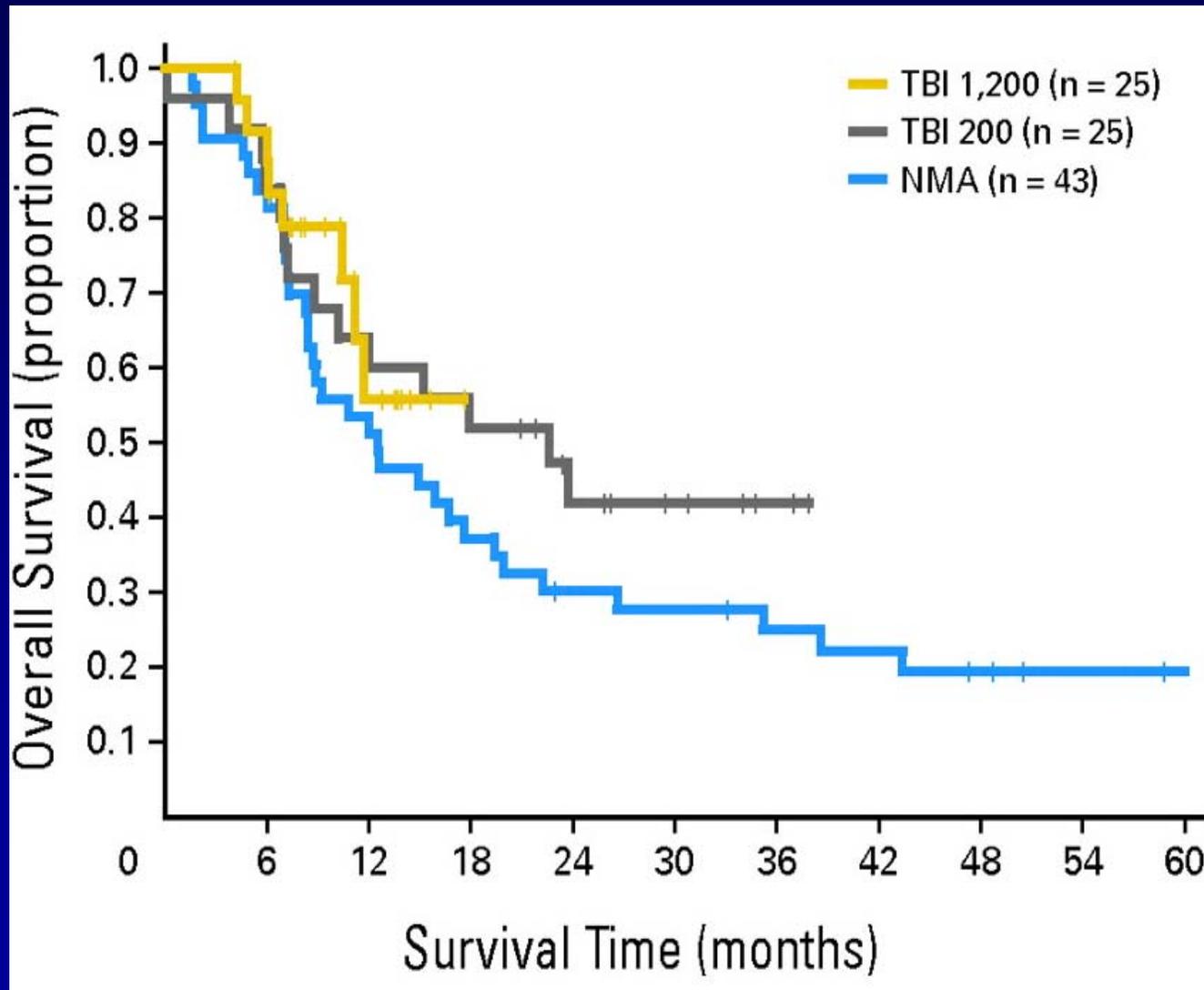


Treatment	RR* (%)	PFS (mos)	OS (mos)
High-dose IL-2	9.7	1.6	12.8
High-dose IL-2 + vaccine	22.1	2.9	17.6

\*Investigator assessment.

Reproduced with permission from Schwartzentruber. ASCO. 2009 (abstr CRA9011).

# Adoptive T-Cell Therapy and Intensive Myeloablative Chemoradiation: Results



Regimen	RR (%)
TBI 12 Gy	72
TBI 2 Gy	52
NMA	48.8



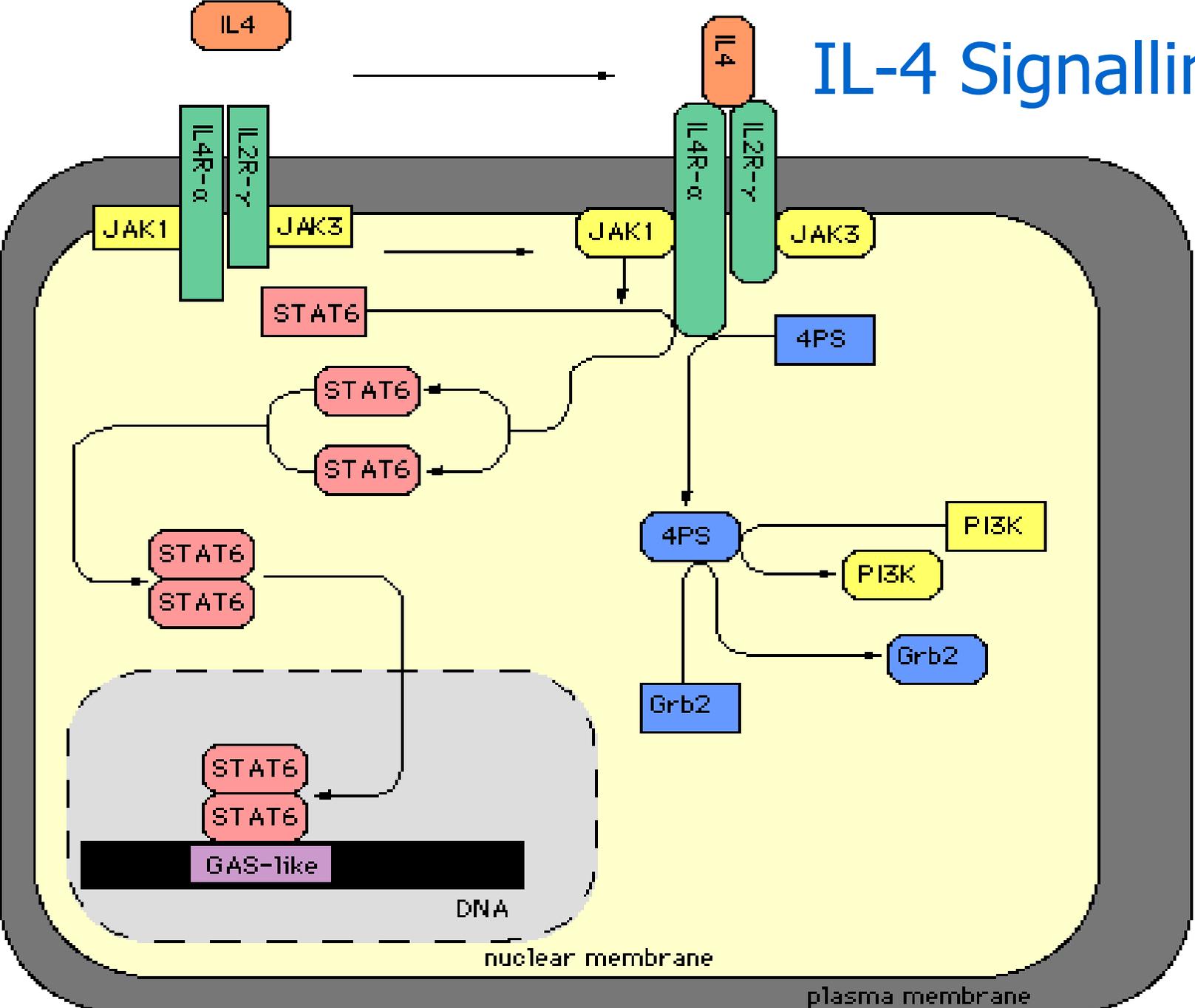
## IL-2: Current and future

- Structural alterations
- Toxicity modulation without loss of activity
- Combinations
  - Anti-angio/cytotoxics/STIs/other cytokines
  - Vaccines (melanoma peptides enhanced IL-2)
- Greater insight into mechanisms
  - Renal “Select” trial to validate prior observations re: histology, hypoxia genes
  - Melanoma “Selection” trial to study immune polymorphisms, tumor gene expression
  - Autophagy? (Lotze)

# Interleukin-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 m $\Phi$  tumorcidal activity

# IL-4 Signalling



# Interleukin-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<sub>2</sub> responses
- Partially common receptor
- Promotes Ig class switch
- Used w/ GM-CSF → moDCs

Assortment of receptor subunits depend on cell type

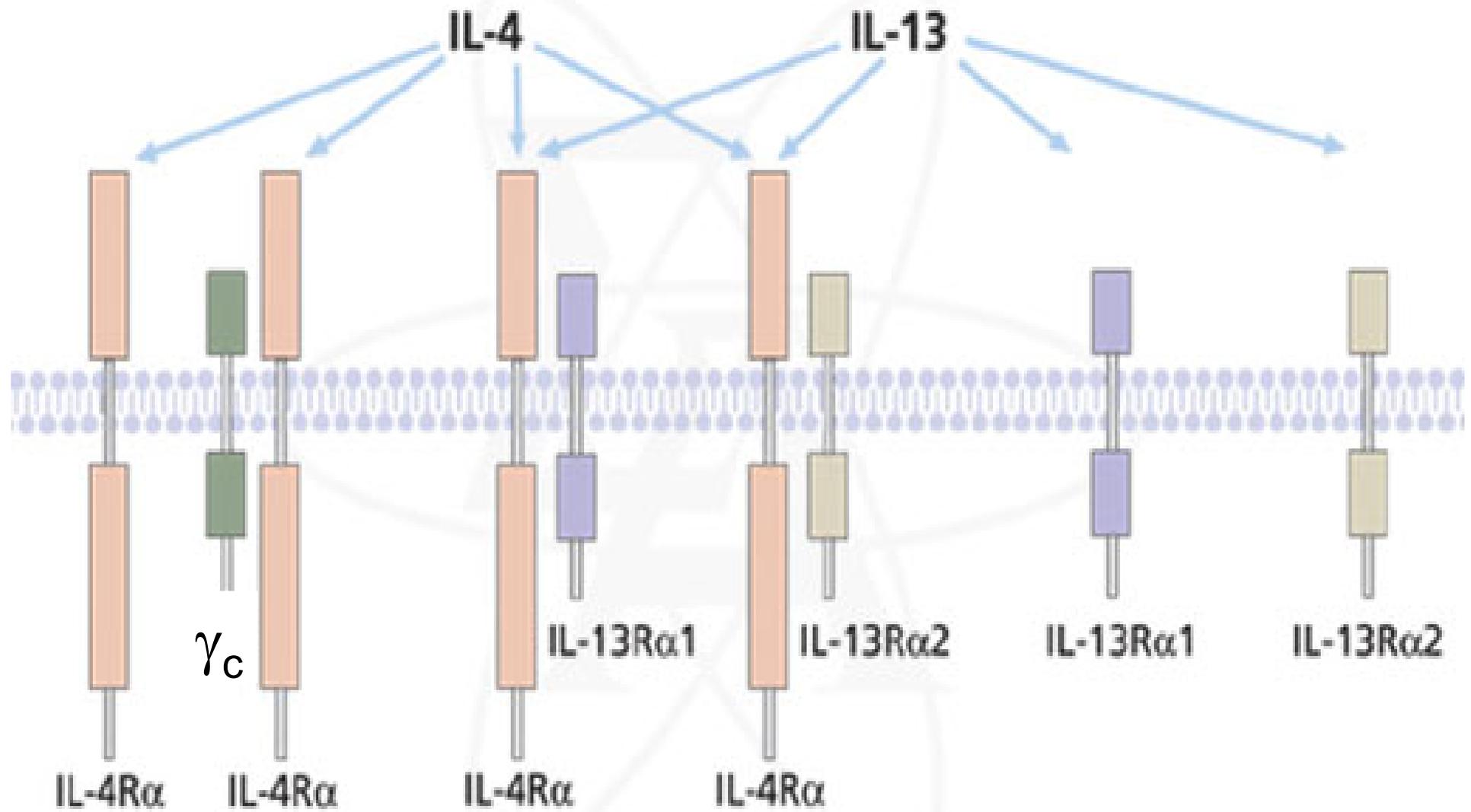
## ■ Differences

- IL-13 activity on monocyte/m $\Phi$  cells
- IL-13 lacks B, T cell effects

## ■ IL-13 receptors on tumor cells, especially glioma

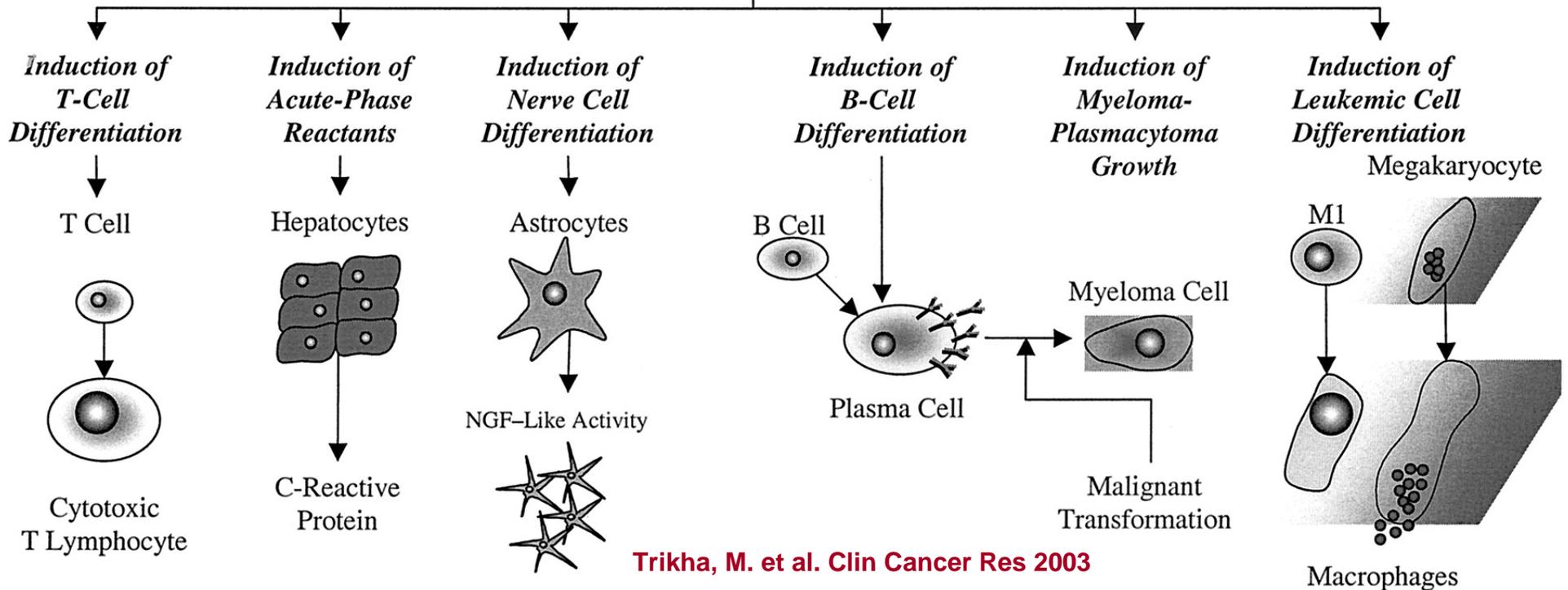
- Immunotoxins
- Chimeric T cell Ag receptor

## IL-13 and IL-4 receptor combinations and binding



*Inflammation*      *Immunity*      *Reproduction*      *Hematopoiesis*      *Neural Development*      *Bone Metabolism*

# IL-6 is pleiotropic

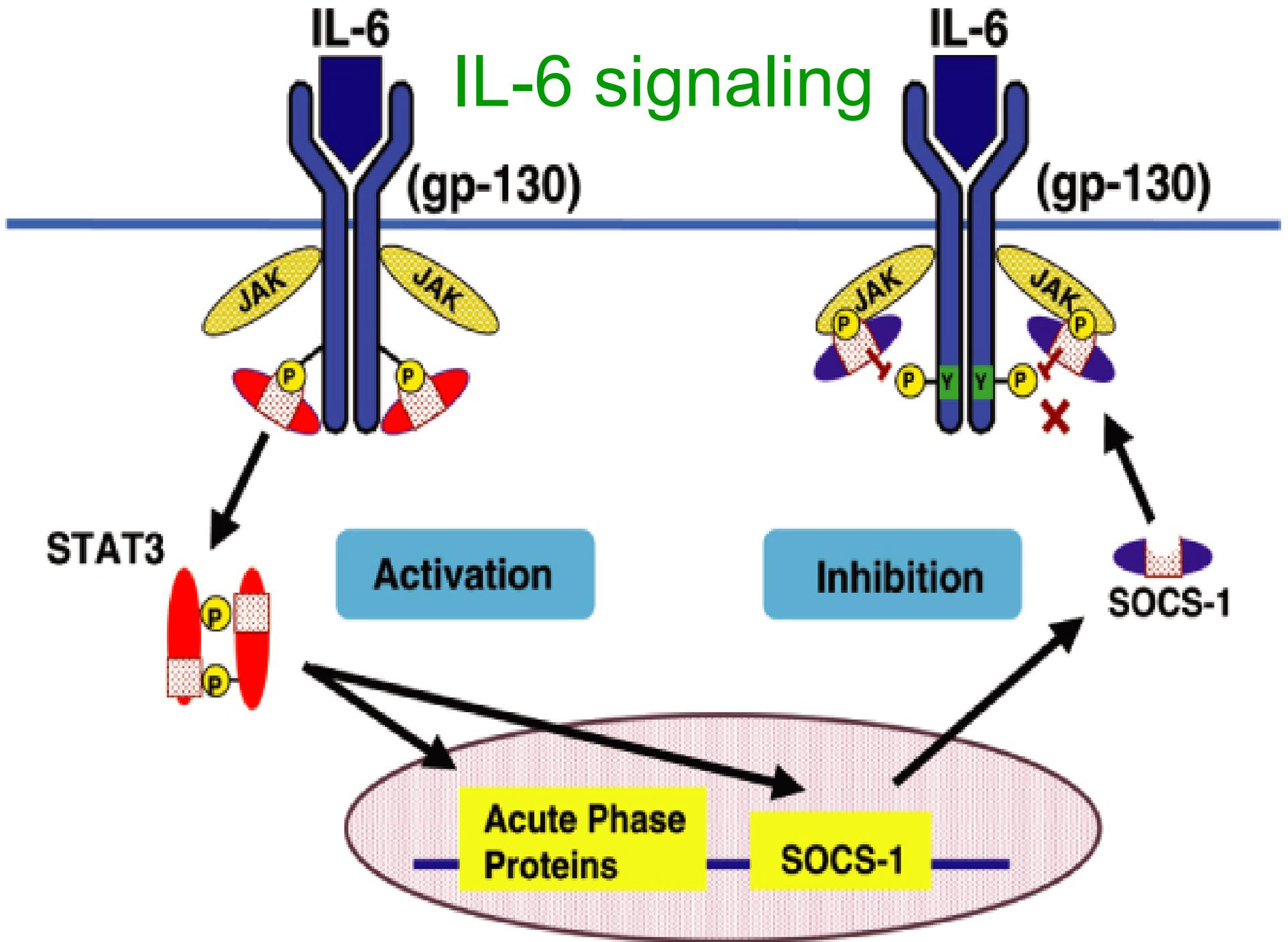


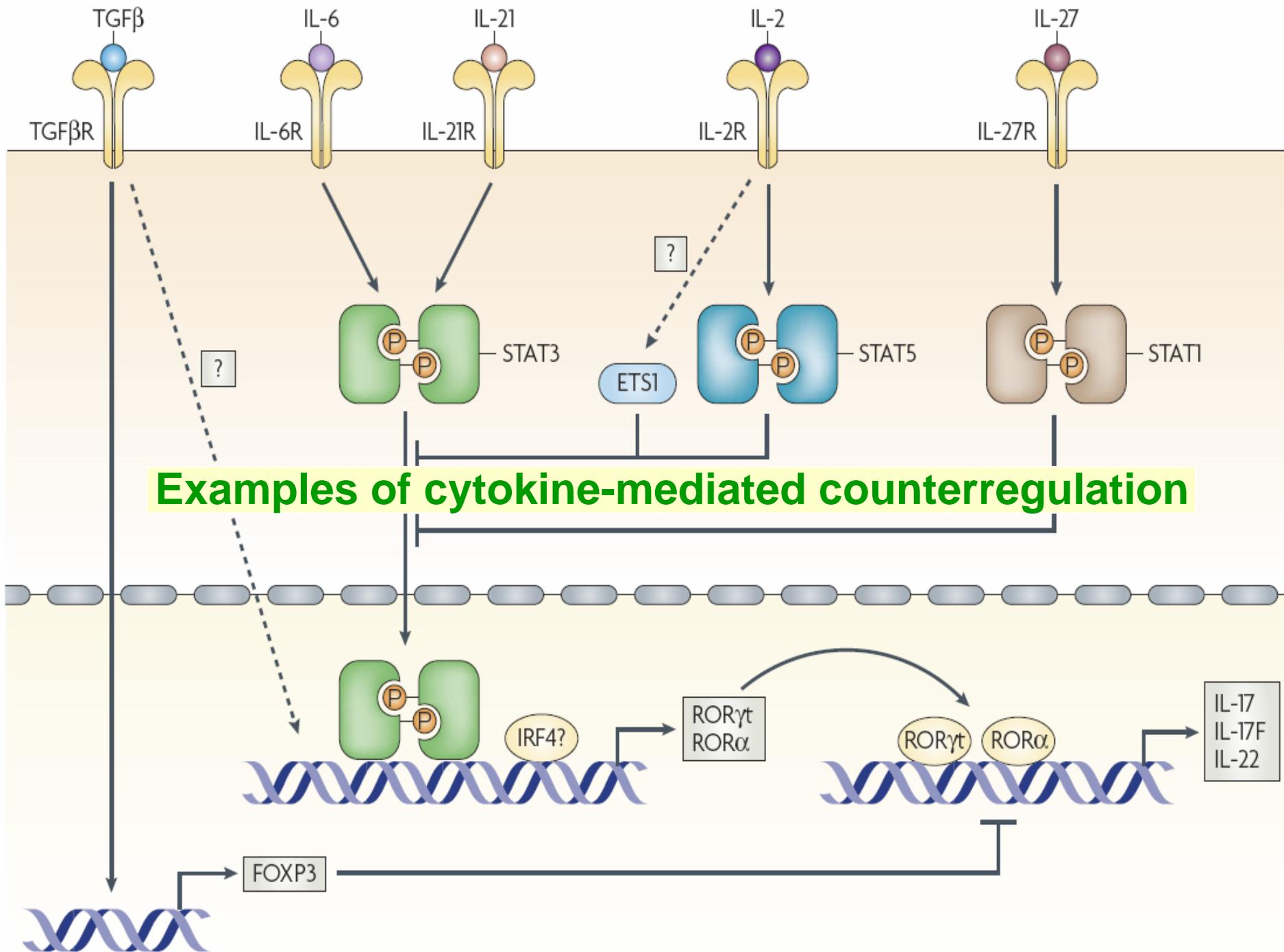
**Trikha, M. et al. Clin Cancer Res 2003**

# IL-6

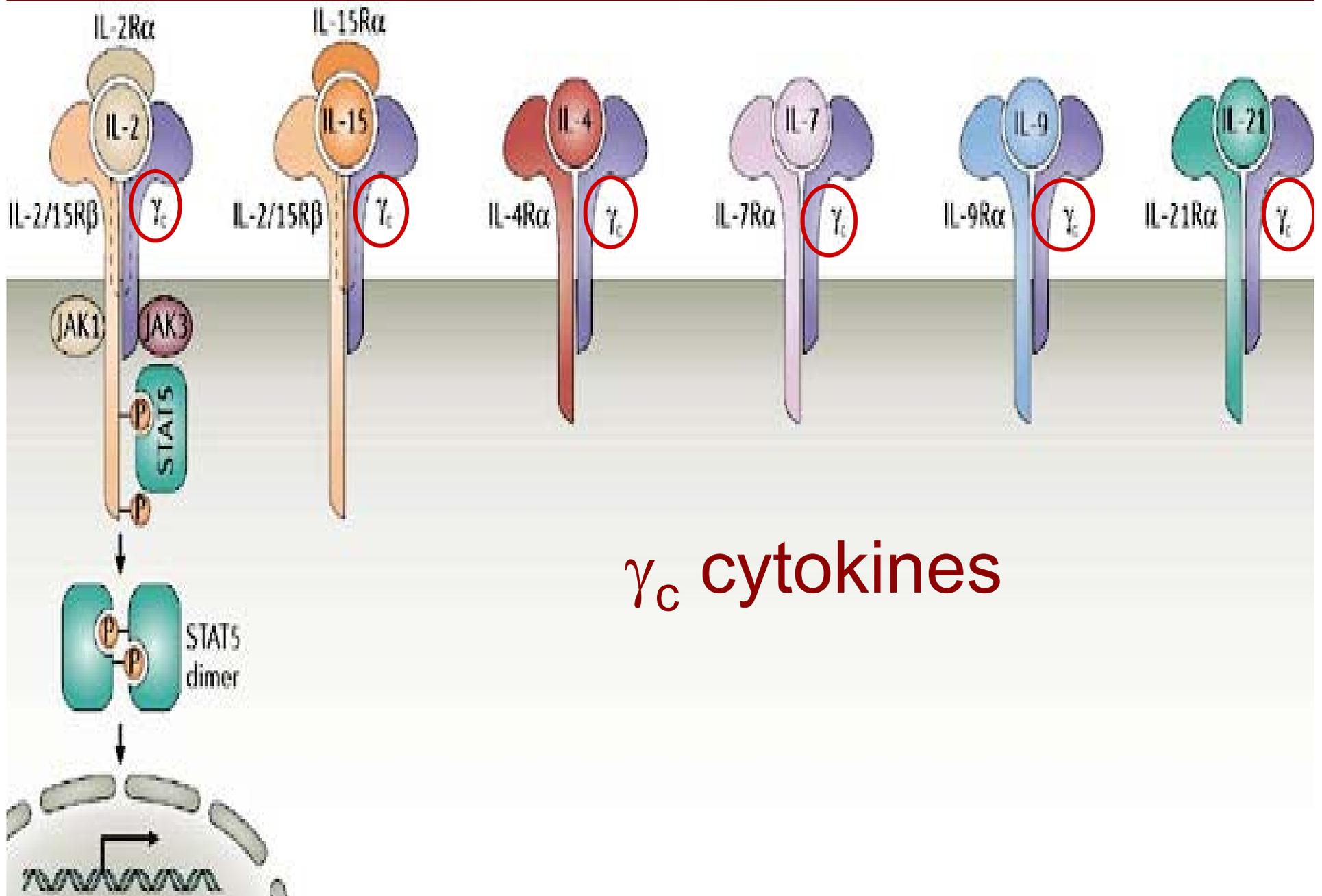
- Tumor source
  - Unfavorable prognostic for renal cancer
  - Important growth factor for myeloma
  - Mediates paraneoplastic thrombocytosis
- Adaptive system
  - B cell growth/differentiation
  - CTL differentiation, Type 2 responses
- Preclinical data suggested antitumor activity
- Clinical data
  - Too toxic
  - ?tumor promotion → Blockade may be therapeutic

# IL-6 signaling





**Examples of cytokine-mediated counterregulation**



# 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 (greatest clinical potential, possibly with IL-15, IL-21)

# IL-15

- Unique  $\gamma_c$  cytokine—complexes with receptor from cell of origin, then signals target cell
- With IL-2 and IL-7 in  $\gamma_c$  cytokine family promoting T cell growth, differentiation

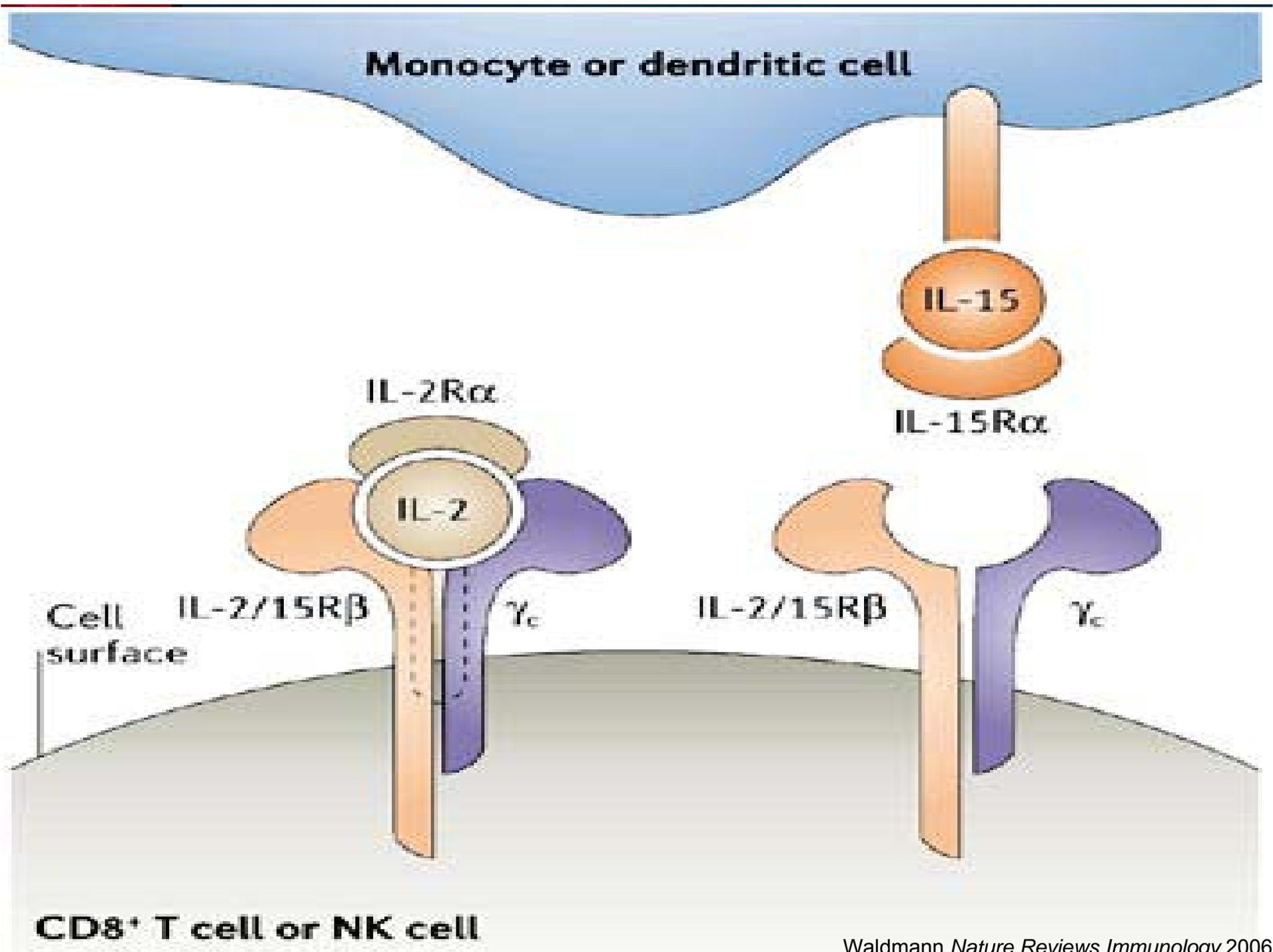
# IL-2 and IL-15 compared

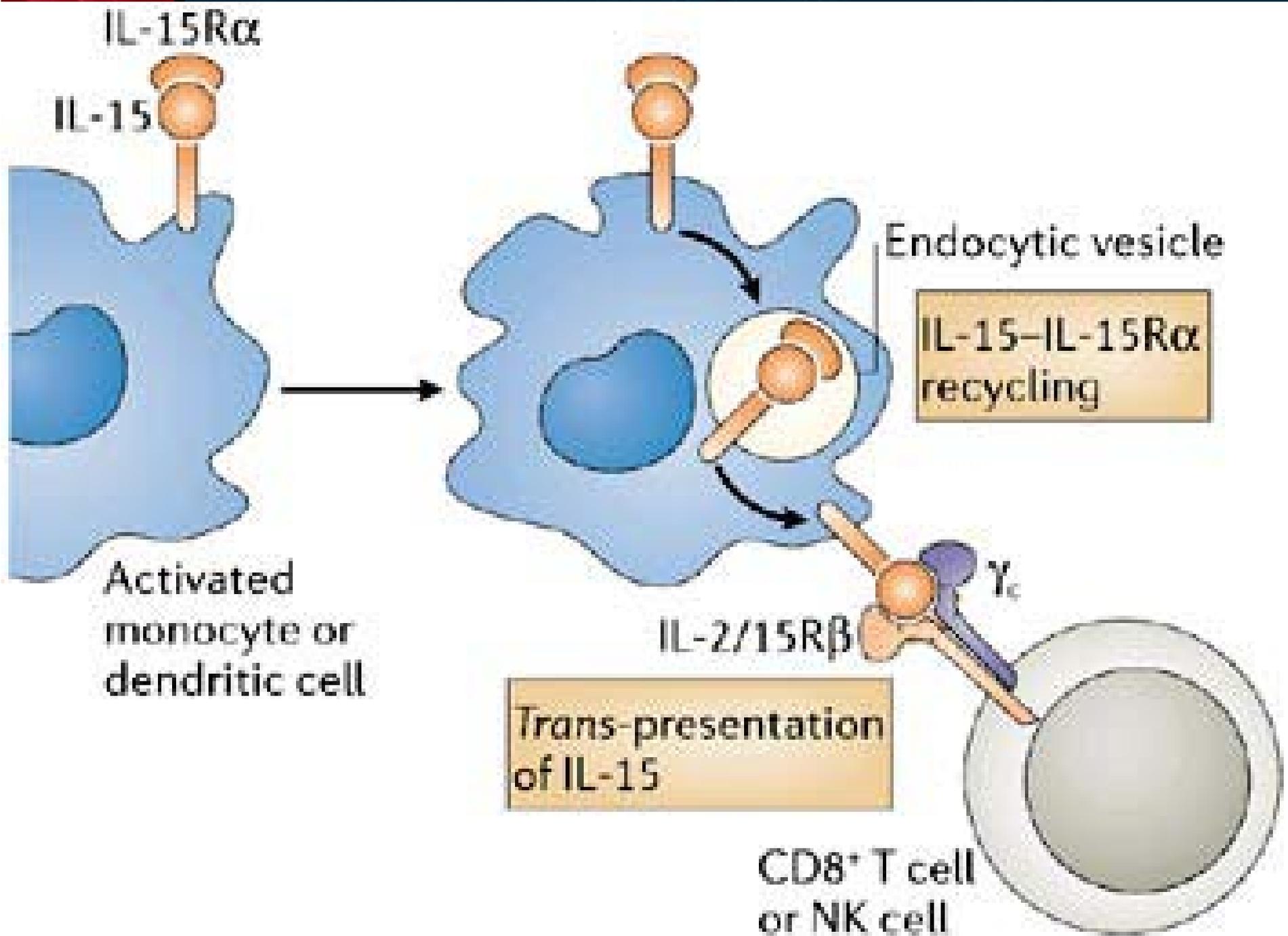
## IL-2

- Activated T, B express high-affinity  $\alpha\beta\gamma$  receptor
- Prolif/differentiation NK, T, B
- Promotes activation-induced cell death
- Maintenance of Treg
- -/- KO develops autoimmunity

## • IL-15

- Produced by DC, monos
- Surface-bound on DC/mono  $\leftrightarrow$  receptors on NK, CD8a1 cells
- Promotes proliferation NK, T, B, memory CD8
- Inhibits AICD
- Does not support Treg
- -/- KO is lymphopenic

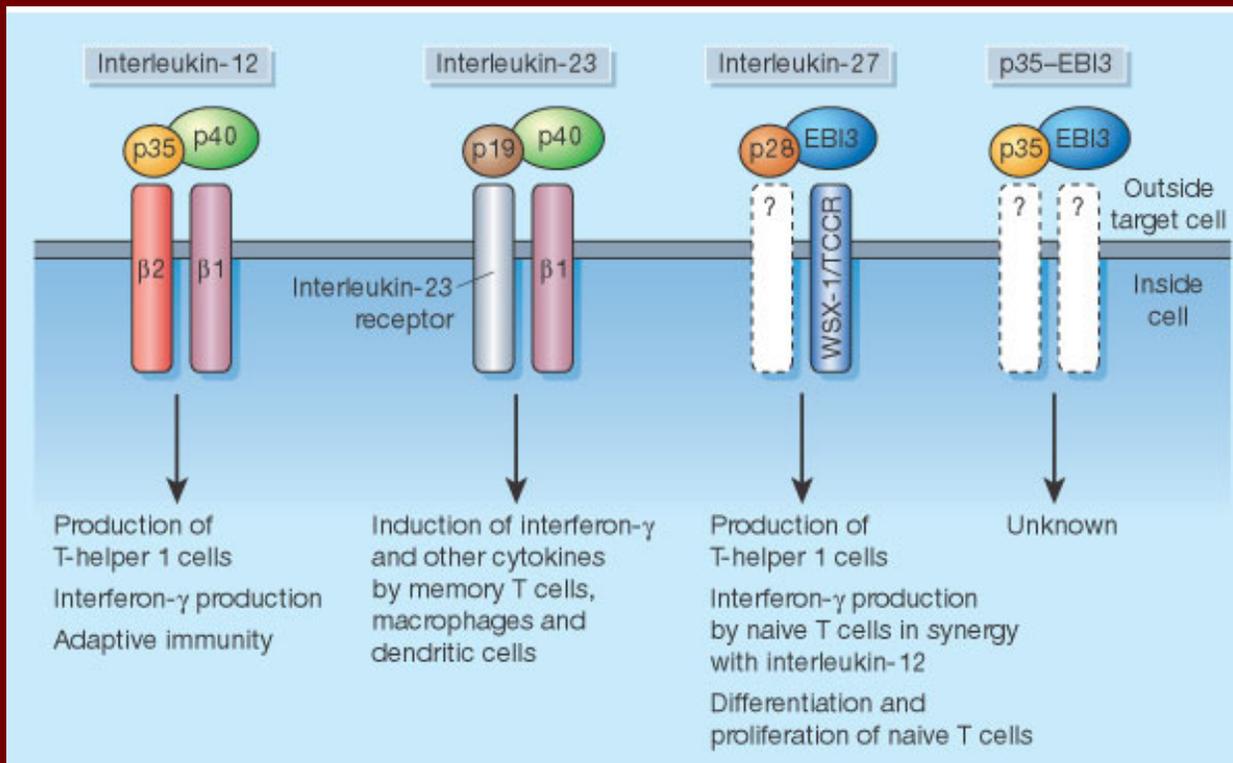
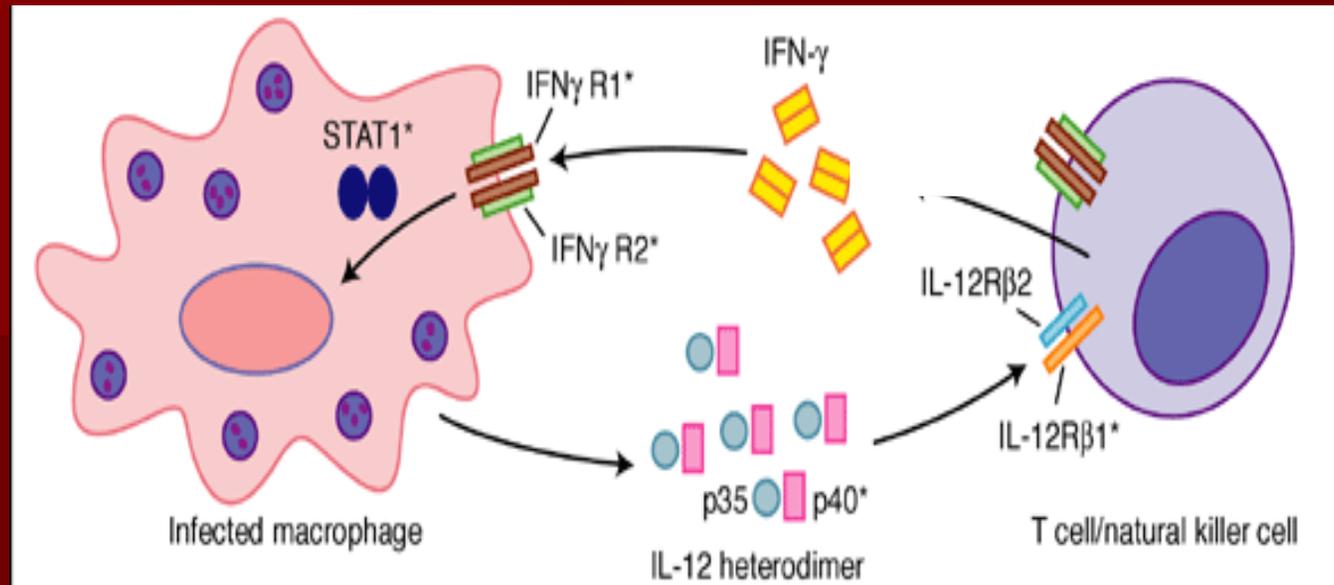




# IL-12

- Link between innate, adaptive immune response
  - Receptors on variety of immune cells
  - Induces IFN- $\gamma$ , 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/ $\alpha$ -IFN, IL-2?

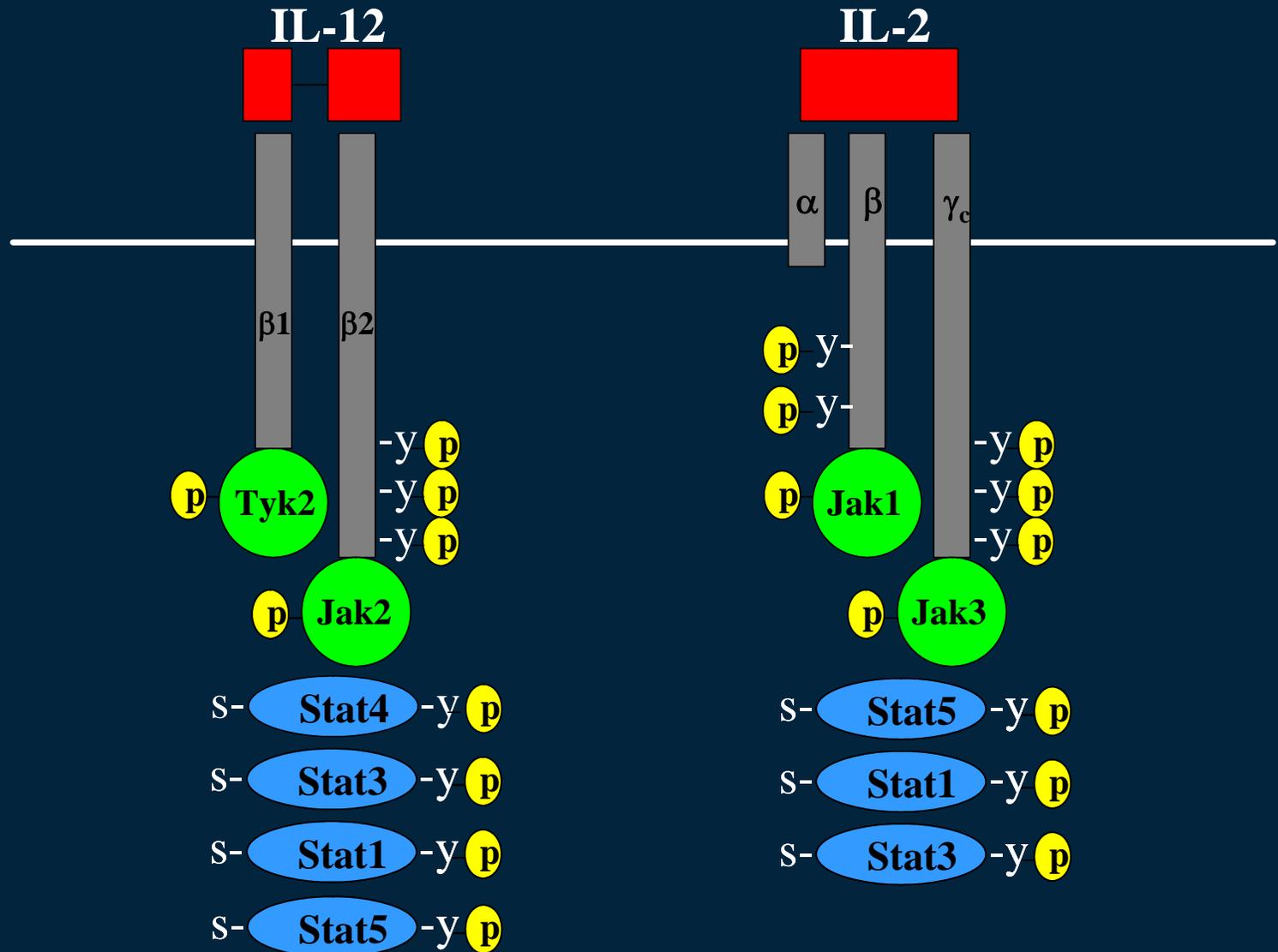
# IL-12 and innate/adaptive → immune system



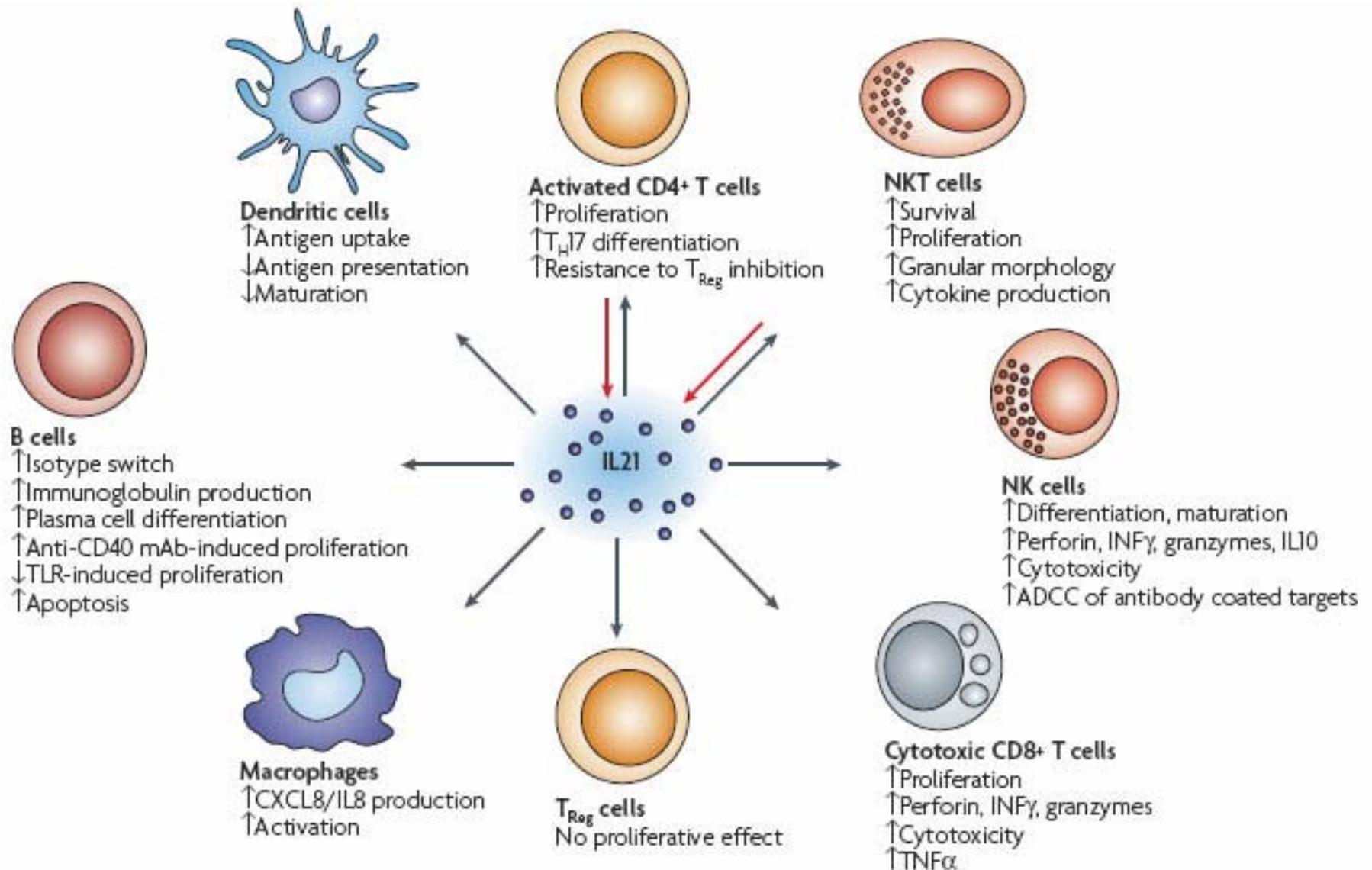
## IL-12 family



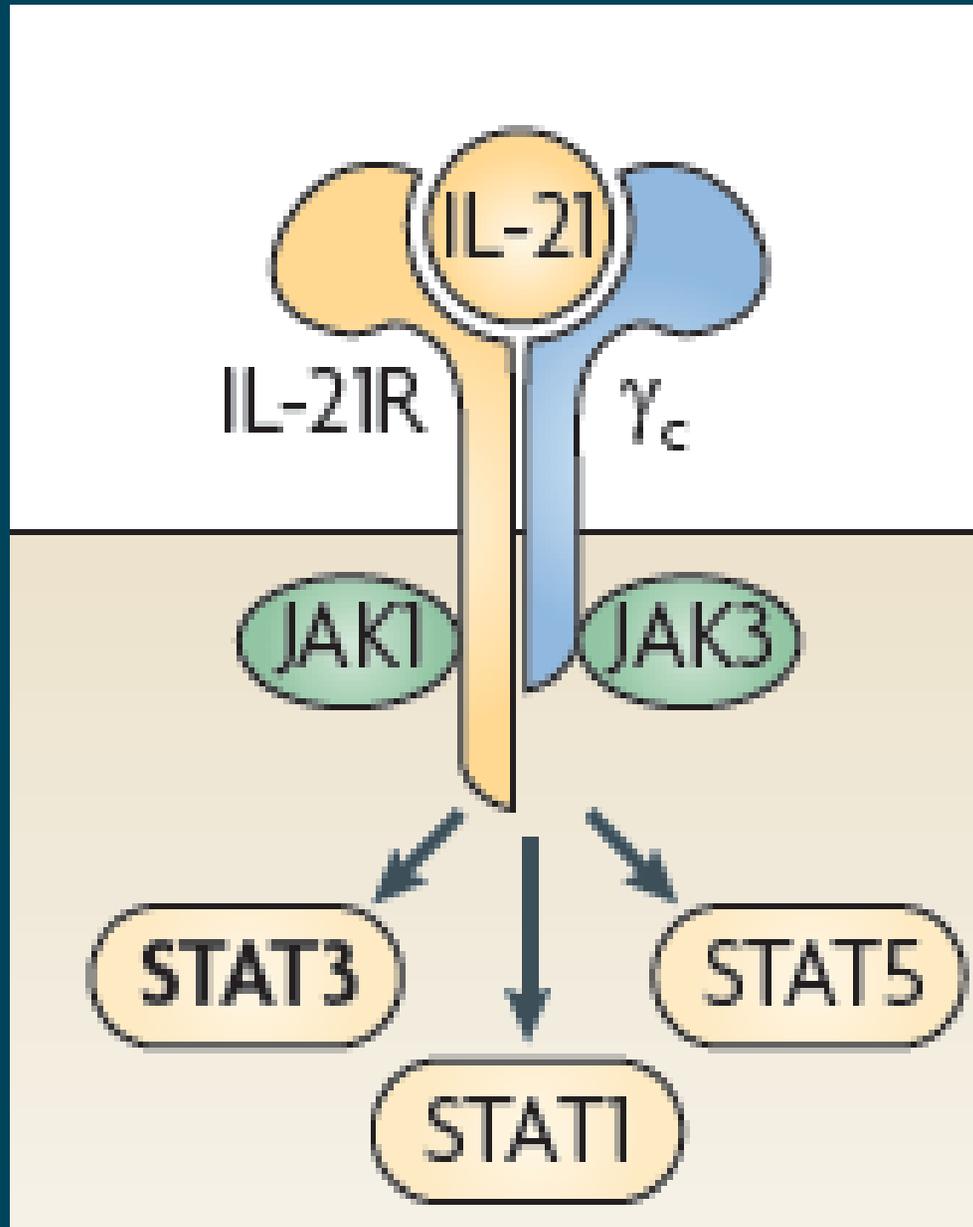
# Jak/Stat Signaling: IL-12 versus IL-2



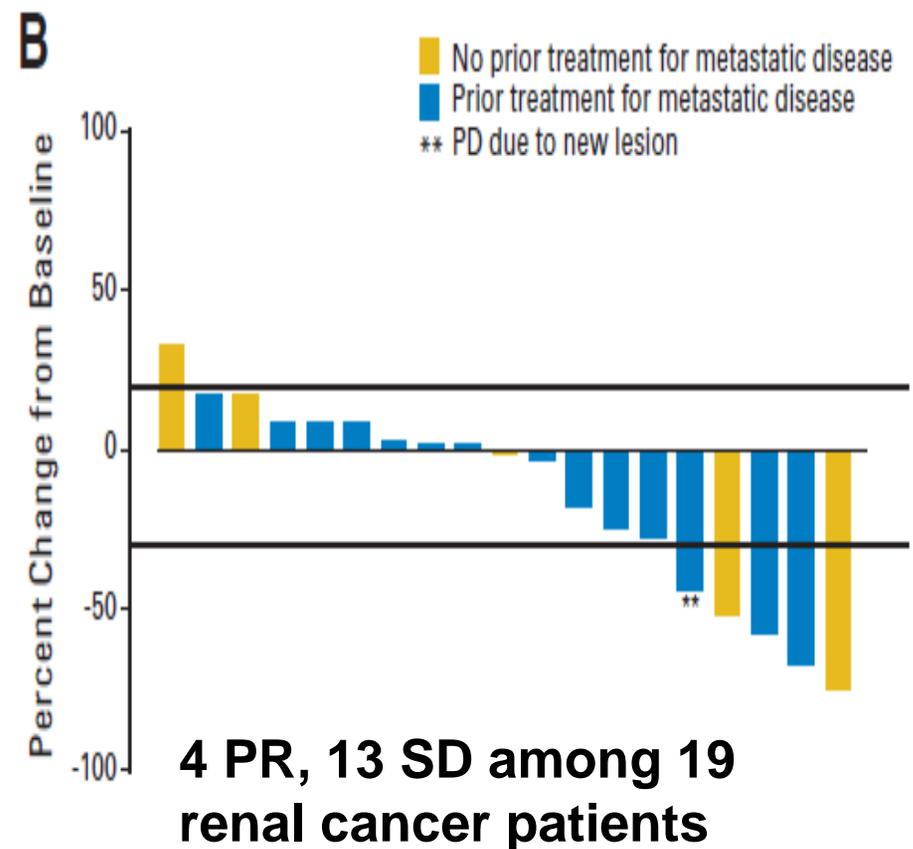
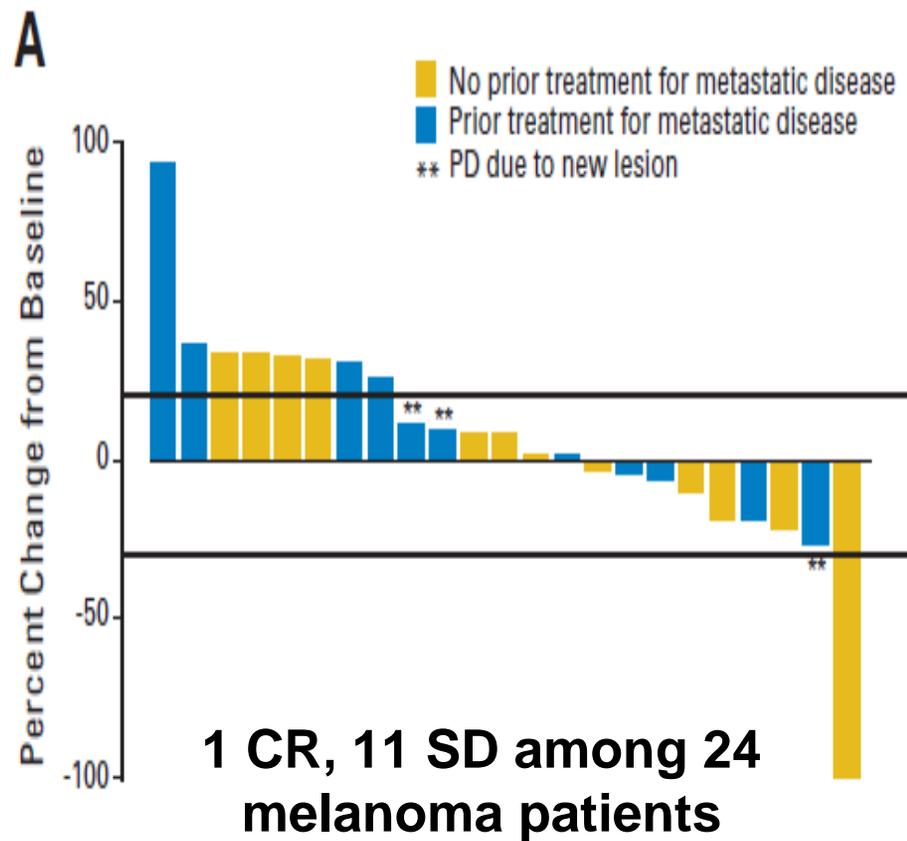
# IL-21 is a pleiotropic cytokine



# Interleukin-21 and its receptor



# Clinical activity of IL-21



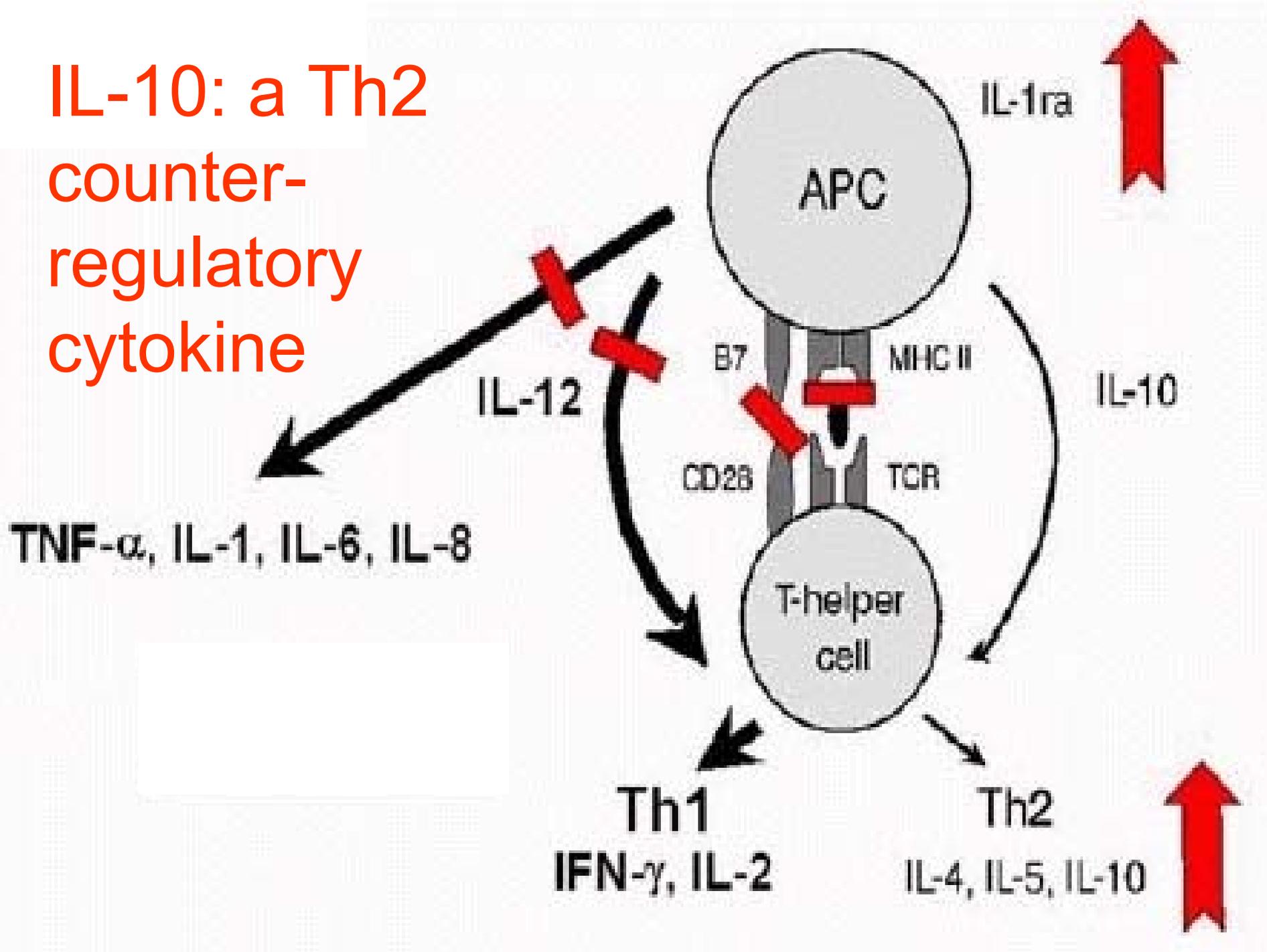
# Other Clinical Trials of IL-21

Disease Type	Trial Design
<b>Melanoma</b>	<sup>1</sup> Phase I: Alternative schedules 5+9 schedule x 3 and 3x/week
	<sup>2</sup> Phase IIa: 5+9 schedule x 3
	<sup>3</sup> NCIC PHASE II: 5+9 schedule x 3, q 8 weeks Treatment-naive; bulky disease excluded
<b>Renal cell Cancer</b>	IL-21 plus Sunitinib
<b>Lymphoma</b>	<sup>4</sup> IL-21 and Rituximab in Relapsed/Refractory Indolent Lymphoma

<sup>1</sup>Davis ID. Clin Cancer Res 2007  
<sup>3</sup>Petrella T et al. ASCO 2010

<sup>2</sup>Davis ID. Clin Cancer Res 2009  
<sup>4</sup>Timmerman JM et al. ASCO 2008

**IL-10: a Th2  
counter-  
regulatory  
cytokine**



# Thank you

- Any questions?