Cytokines in Malignancy

Kim Margolin, MD

City of Hope Cancer Center

iSBTc 11/1/07

Immunobiology of Cytokines

For discussion

- Cytokine structure
- Stimuli leading to cytokine synthesis
- Cell(s) responsible for cytokine production
- Cytokine-responsive cell(s)/receptor structure
- Signaling induced by cytokine binding
- Preclinical/clinical status of cytokine

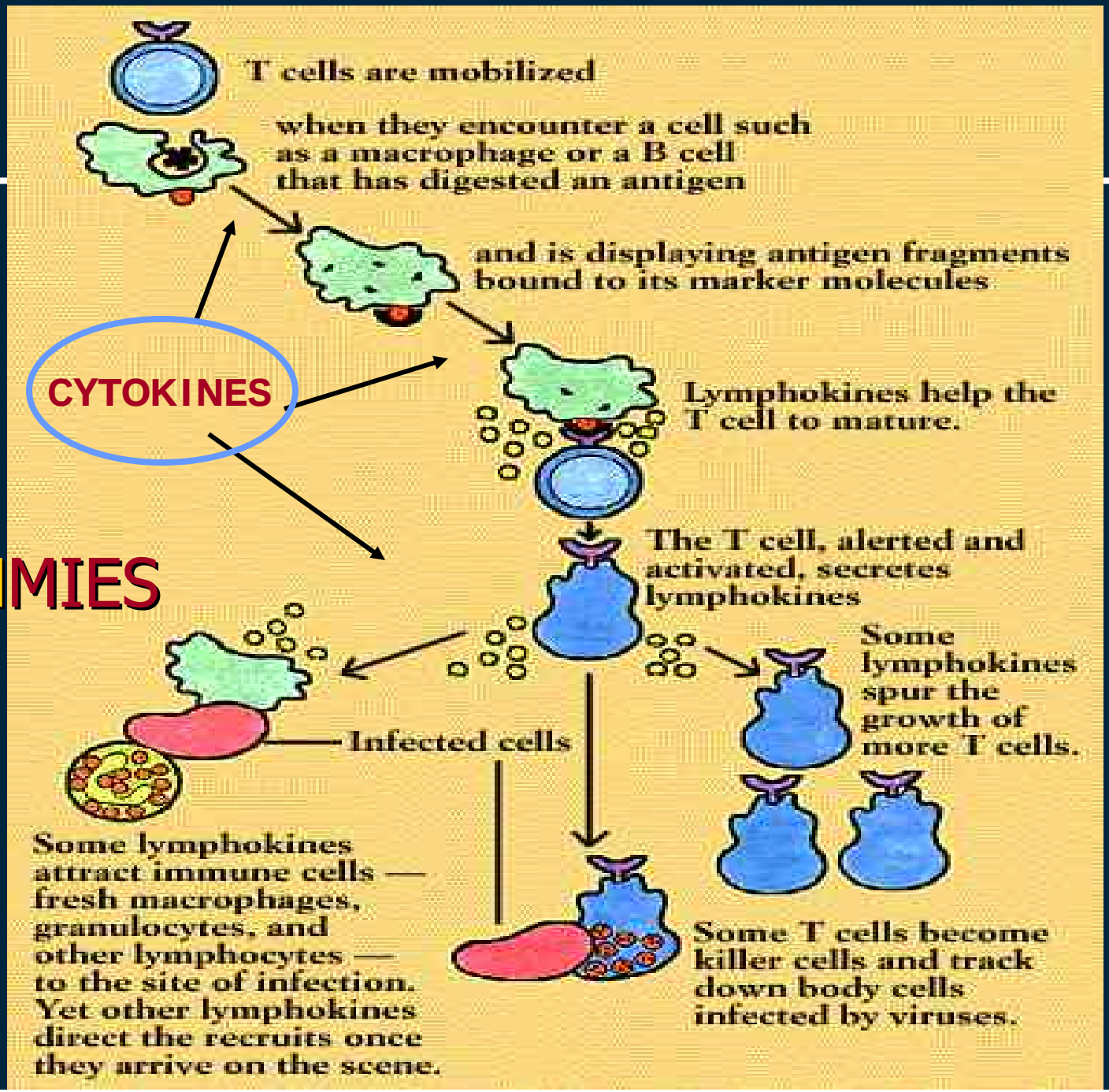
Not for discussion

- Cytokines in the pathogenesis of malignancy
- Other clinical aspects of cytokine manipulation
  - Autoimmune disease
  - Infectious disease
- Homeostatic cytokines

Cytokines during iSBTc 2007

- IL-2, many sessions, especially reconstitution and cellular therapies
- IL-7, Crystal Mackall, Friday Nov 2, p.m.
- IL-12, Mehmet Kilinc, Friday Nov 2, p.m.
- IL-24, Nancy Poindexter, Fri Nov 2, p.m.

IMMUNE SYSTEM FOR DUMMIES

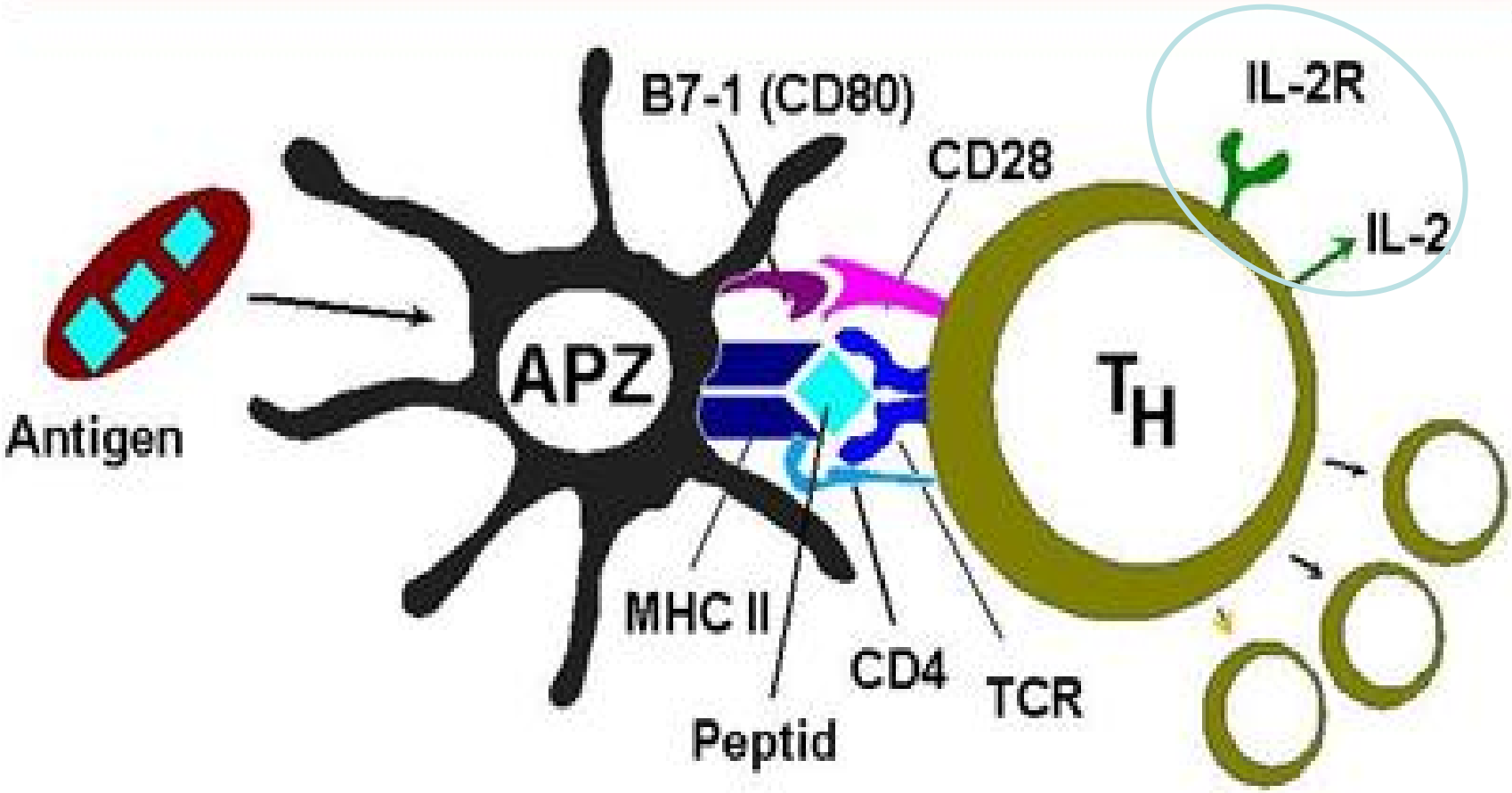


Induction of an immune response

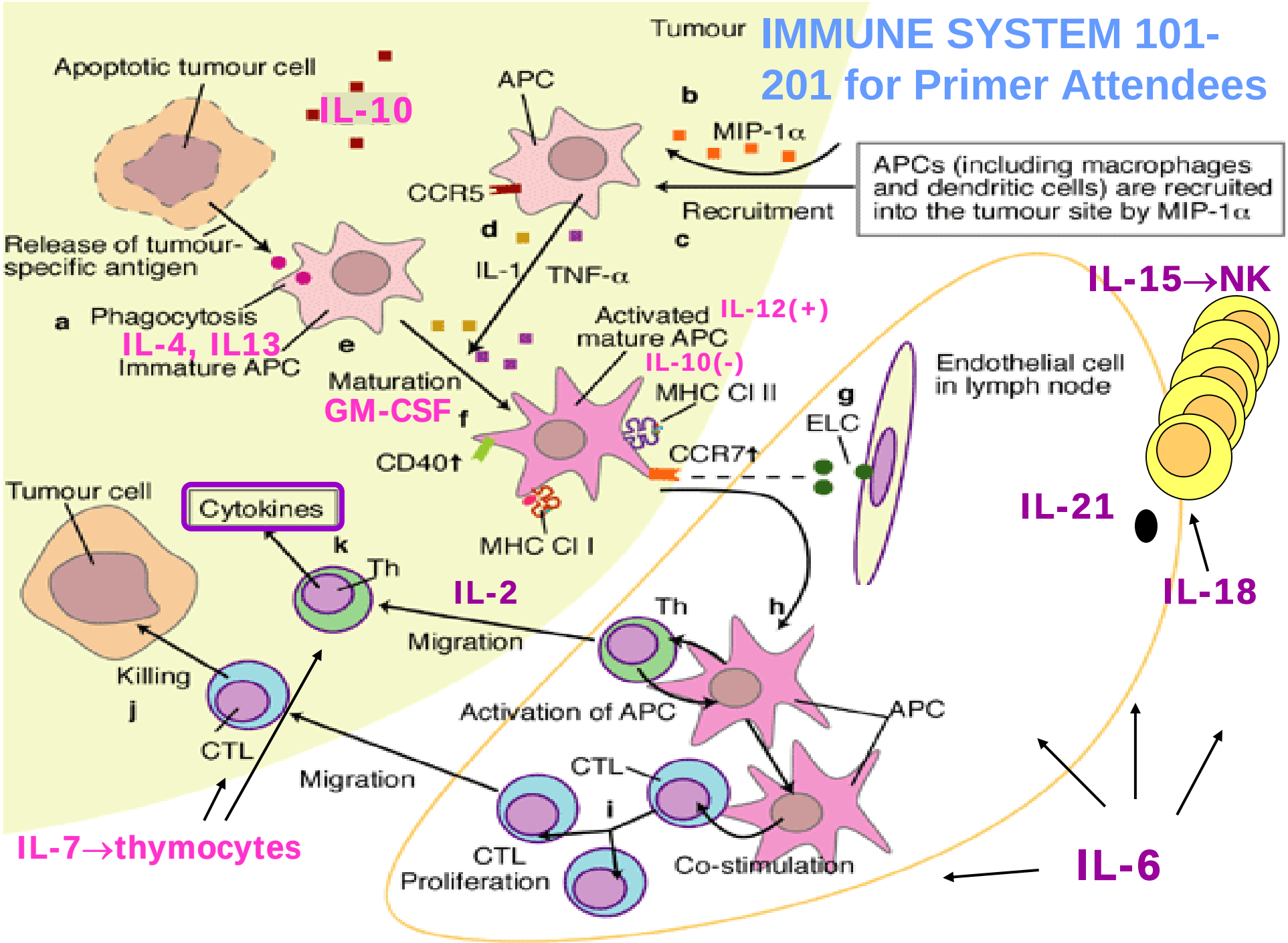
Induktion einer Immunantwort

Recognition Processing Presentation Activation

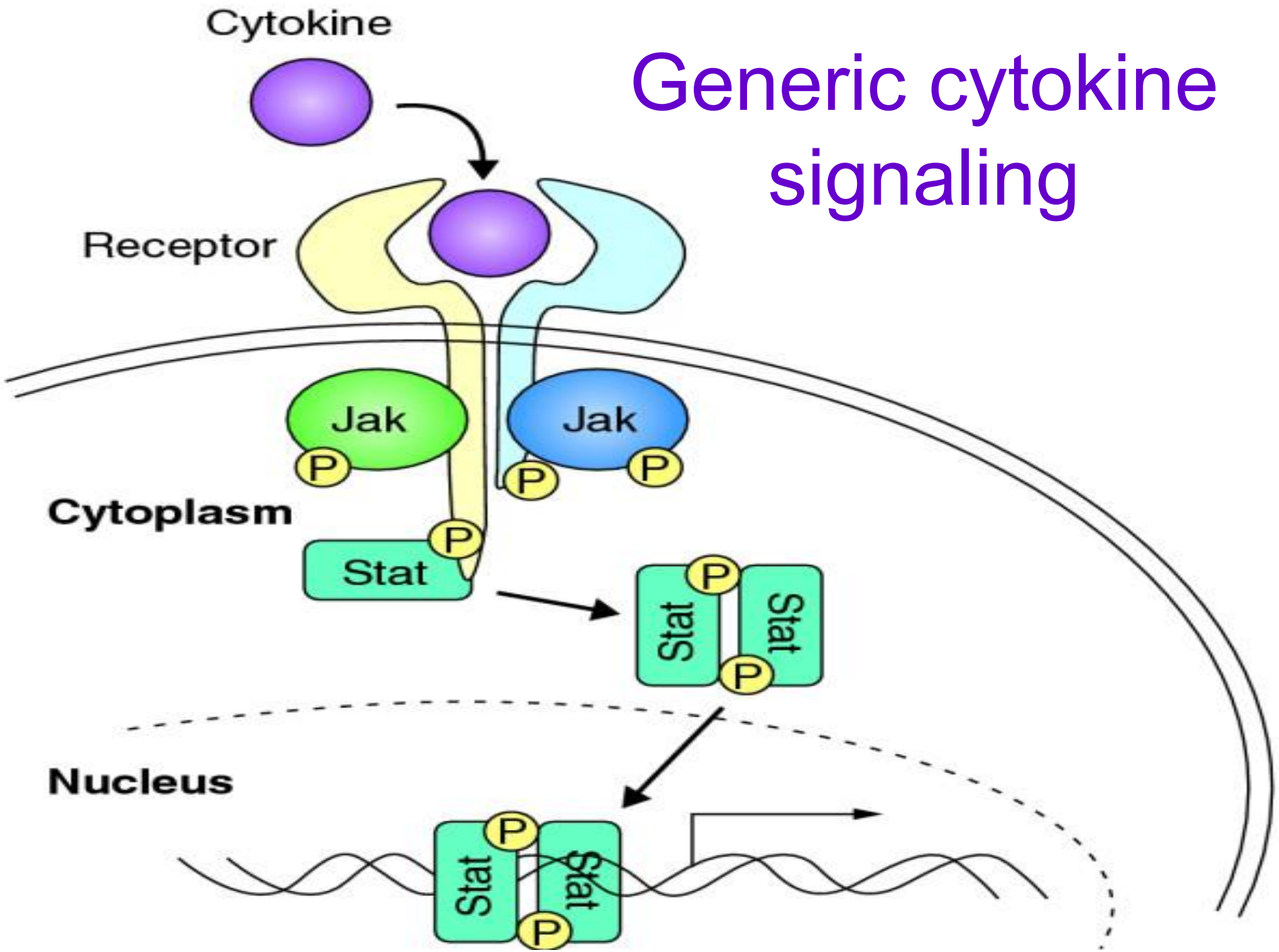
Erkennung Prozessierung Präsentation Aktivierung



IMMUNE SYSTEM 101-201 for Primer Attendees



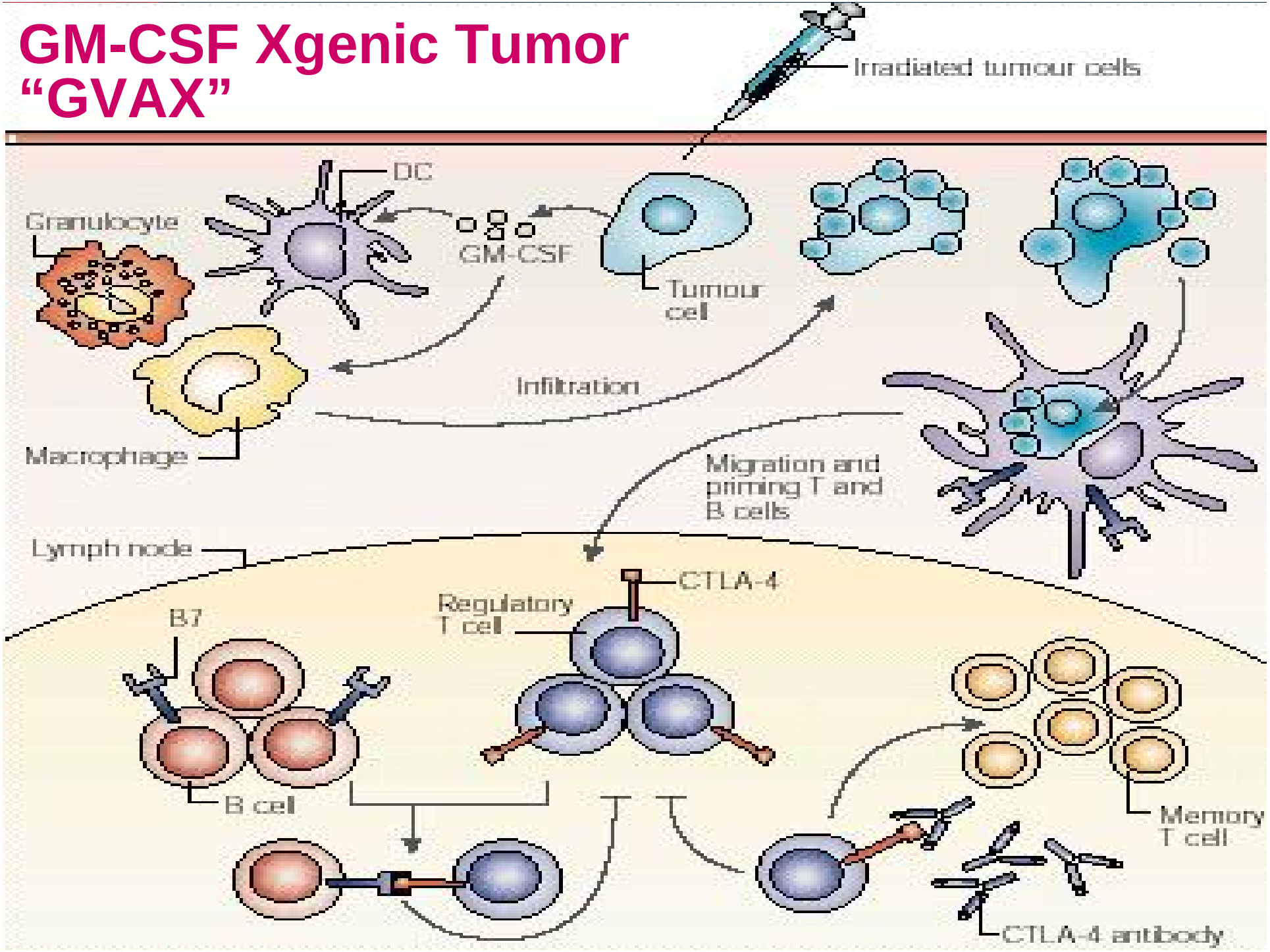
Generic cytokine signaling



GM-CSF as immunotherapy

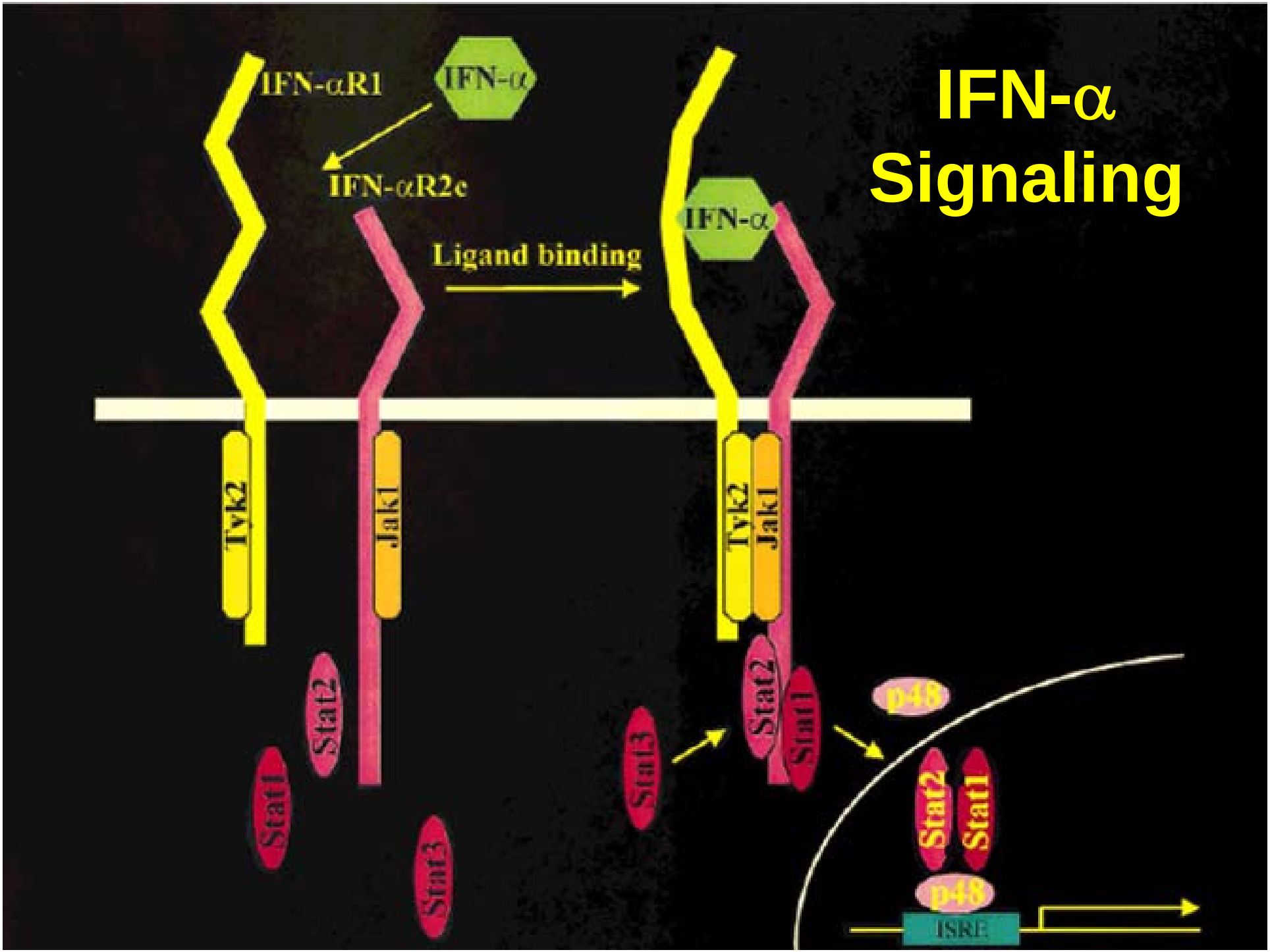
- Cells of origin
  - Th1, Th2
  - Others include epithelial, fibroblast, *tumor*
- Target cell: immature DC (& myeloid progenitor)
- Biological functions
  - Stimulation of T cell immunity via effect on APC
  - Myeloid cell proliferation, differentiation
- Clinical development
  - Hematopoietic support
  - Not a potent stand-alone cytokine in cancer
  - Transgenic expression (GVAX)
  - Adjunct to immunotherapy
    - Uncontrolled data for benefit in adjuvant Rx of melanoma
    - Phase III results pending +/- peptide

GM-CSF Xgenic Tumor "GVAX"



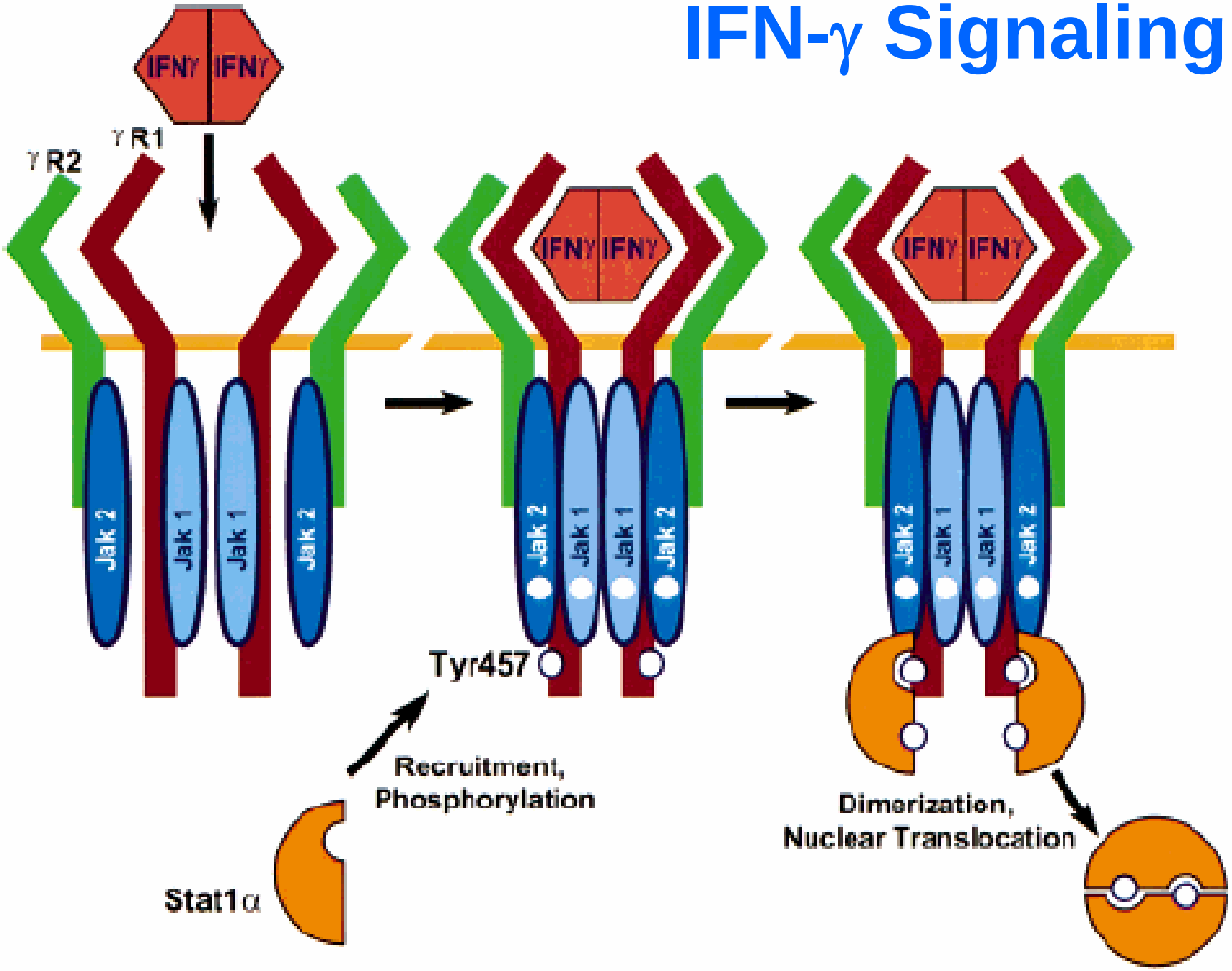
Interferons

- Type I
  - Alpha interferons: produced by WBC, mϕ
  - Beta interferon: produced by fibroblasts, epithelial cells
- Type II
  - IFN-γ: produced by T and NK cells
  - Extensive range of targets
- Immunomodulatory effects
  - MHC class I/II upregulation
  - Modulation of T/NK cell cytolytic activity
  - Modulation of macrophage/DC function
- Direct effects on tumor cells
  - MHC upregulation
  - Antiproliferative/pro-apoptotic effects
- Anti-angiogenic effects
  - IP-10
  - Thrombospondin

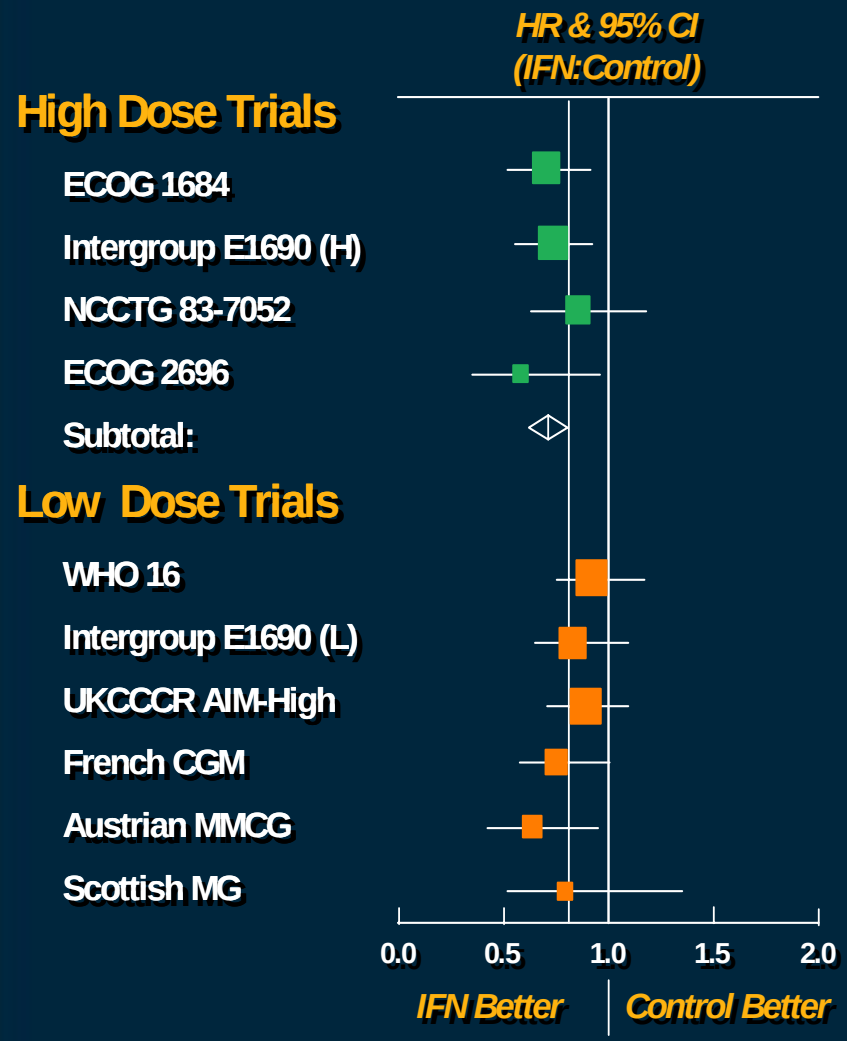


IFN-α Signaling

IFN-γ Signaling



Rates of Recurrent Melanoma: High-Dose and Low-Dose IFN



- HDI reduced risk of disease recurrence by 26%, $P_2 = 0.00009$
- Trend for increased benefit with high dose, $P = 0.02$

P_2=2-sided P

Cancer Treat Rev. 2003;29:241-252.

High-dose IFNa-2b toxicities

	Number (%) of Patients		
	Any Severity	Grade 3	Grade 4
Fatigue*	137 (96)	39 (27)	6 (4)
Neutropenia/Leukopenia	132 (92)	37 (26)	1 (1)
Fever*	116 (81)	26 (18)	0
Myalgia*	107 (75)	27 (19)	2 (1)
Anorexia*	99 (69)	14 (10)	0
Vomiting/Nausea	95 (66)	5 (3)	0
Increased SGOT	90 (63)	20 (14)†	0†
Headache*	89 (62)	25 (17)	1 (1)
Chills*	77 (54)	23 (16)	0
Depression	57 (40)	9 (6)	3 (2)
Diarrhea	50 (35)	0	0
Alopecia	42 (29)	2 (1)	0
Altered Taste Sensation	34 (24)	3 (2)	0
Dizziness/Vertigo*	33 (23)	3 (2)	0
Anemia	31 (22)	2 (1)	1 (1)

*Consistent with "flu-like illness."
†2/143 (1.4%)—grade 5.

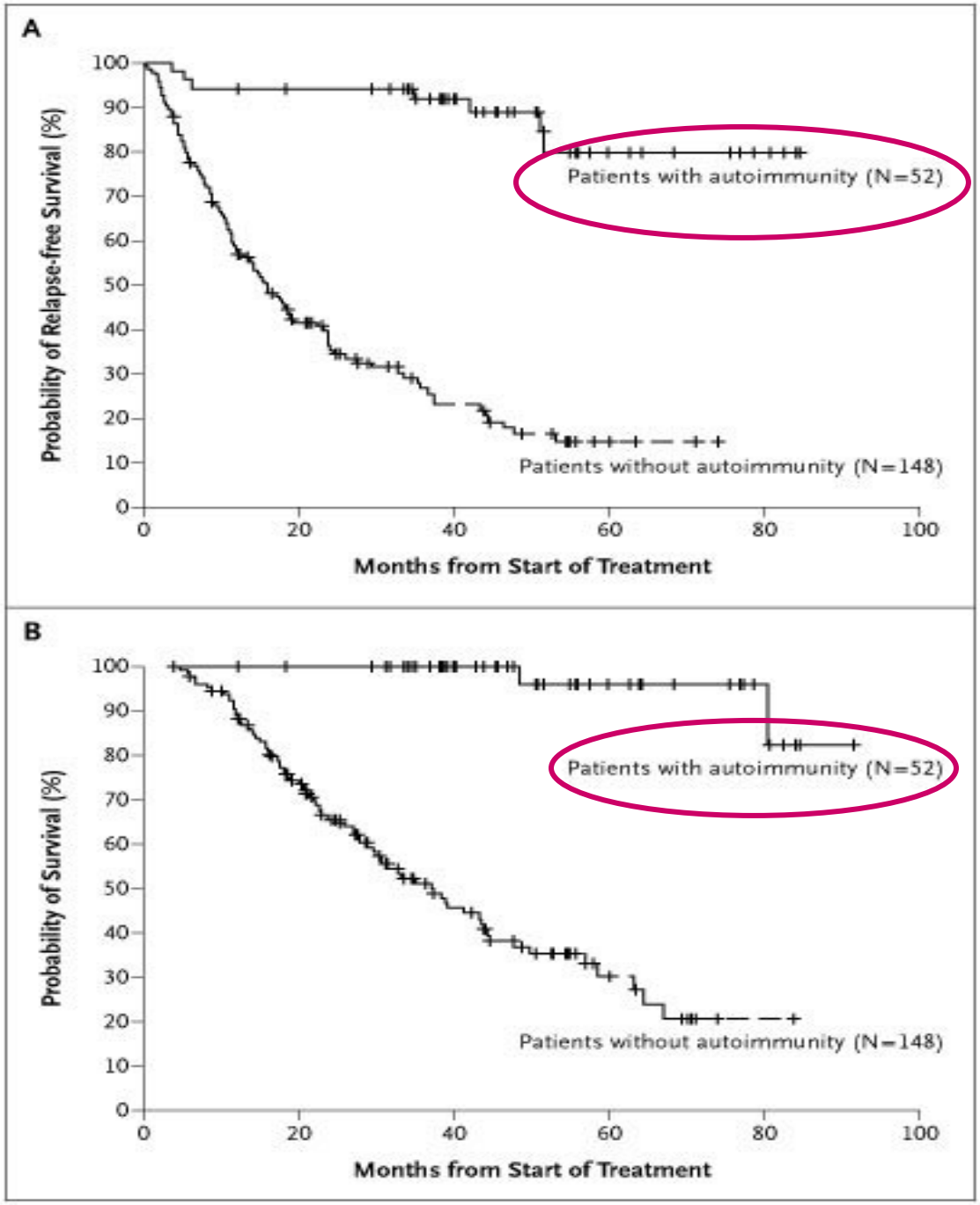
Autoimmunity-Adjuvant IFN-α2b-Melanoma

Autoantibodies or Manifestations of Autoimmunity	All Patients (N=200)	Induction-Therapy Group (N=96)	Extended-Therapy Group (N=104)
		no. of patients (%)	
Autoantibodies or autoimmune disorders	52 (26)	23 (24)	29 (28)
Antithyroid antibodies	43 (22)	16 (17)	27 (26)
Antinuclear antibodies	12 (6)	2 (2)	10 (10)
Anticardiolipin antibodies	10 (5)	2 (2)	8 (8)
Vitiligo	11 (6)	5 (5)	6 (6)
Clinical manifestations	19 (10)	2 (2)	17 (16)
With autoantibodies	16 (8)	2 (2)	14 (13)
Without autoantibodies (vitiligo)	3 (2)	1 (1)	2 (2)
Multiple manifestations of autoimmunity	16 (8)	1 (1)	15 (14)

* Patients in the induction-therapy group received interferon alfa-2b (15 million IU per square meter of body-surface area per day, intravenously, five days per week for four weeks) followed by observation. Patients in the extended-therapy group received the same induction dose for 4 weeks, followed by subcutaneous therapy (10 million IU per day thrice weekly) for an additional 48 weeks.

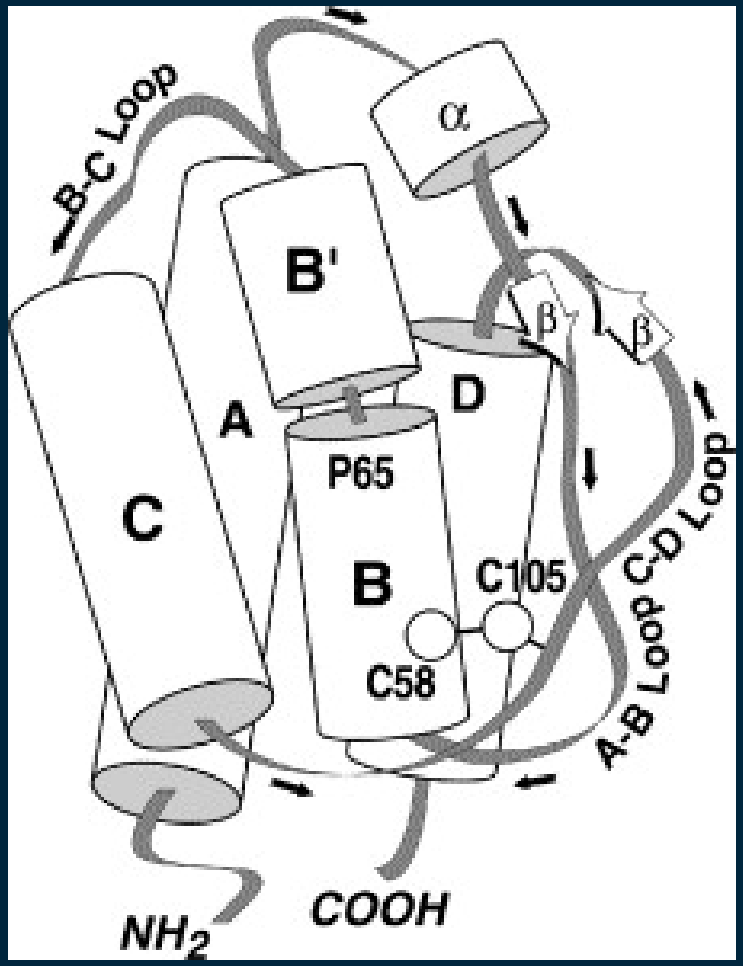
Gogas H, *et al. NEJM* 2006;354:709-18.

Autoimmunity and benefit from adjuvant IFN-a2b



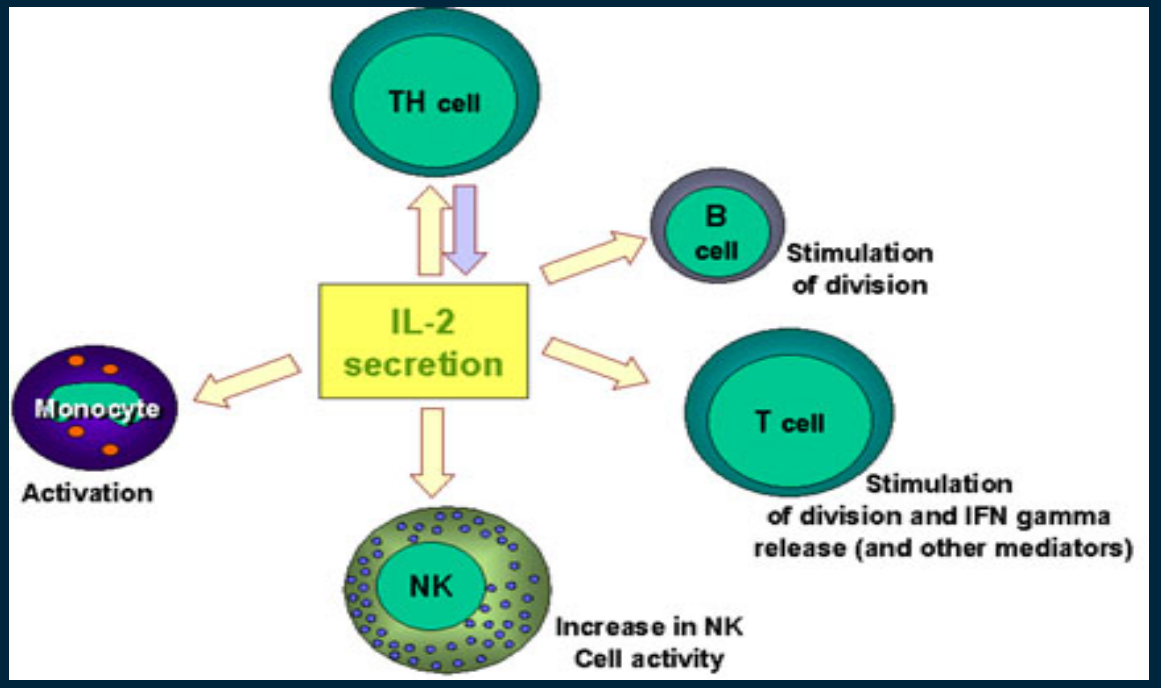
Interleukin-2

- Short chain type I cytokine
- Four α-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

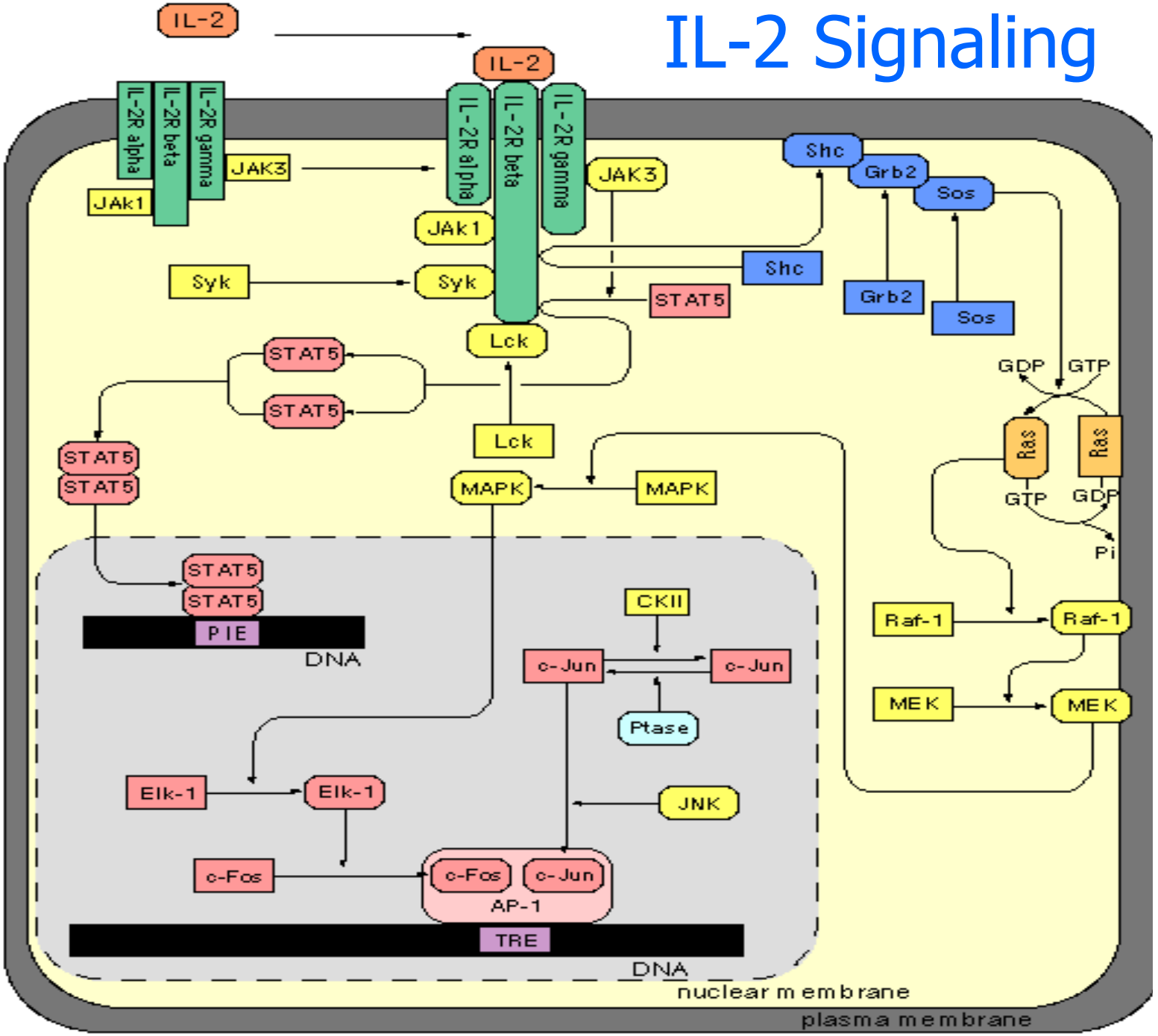


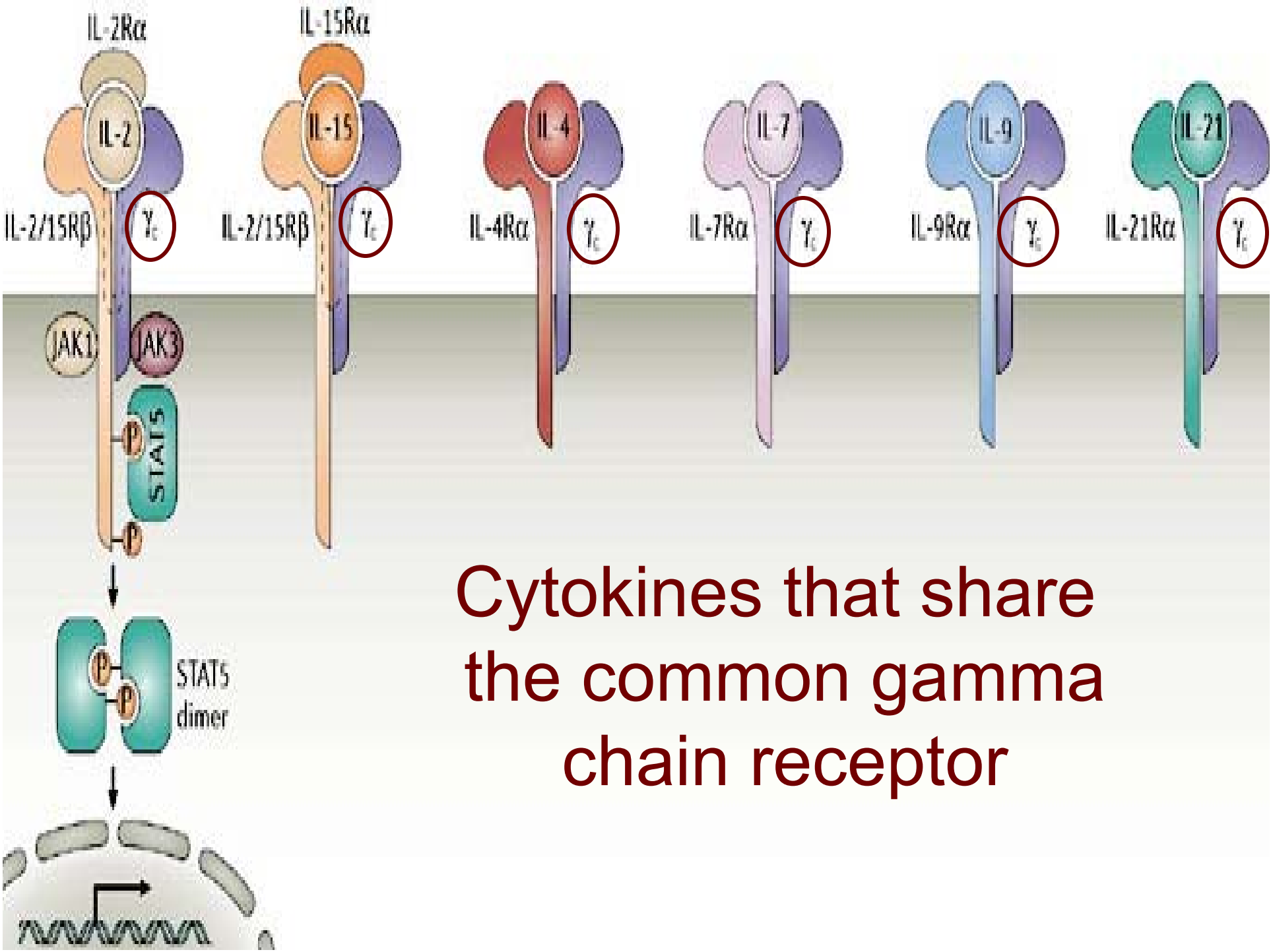
Interleukin-2

- "T cell growth factor"
- Produced *by* Th1 cells *for* T cells but…
- Many other cells express IL-2R
  - B, NK/NKT, monocytes
  - Variable affinity depending on subunit expression
  - Response to IL-2 depends on cell type, receptor, milieu
- Signaling
  - JAK-STAT>
  - MAPK
  - PI3K
- Proliferation, cytotoxicity

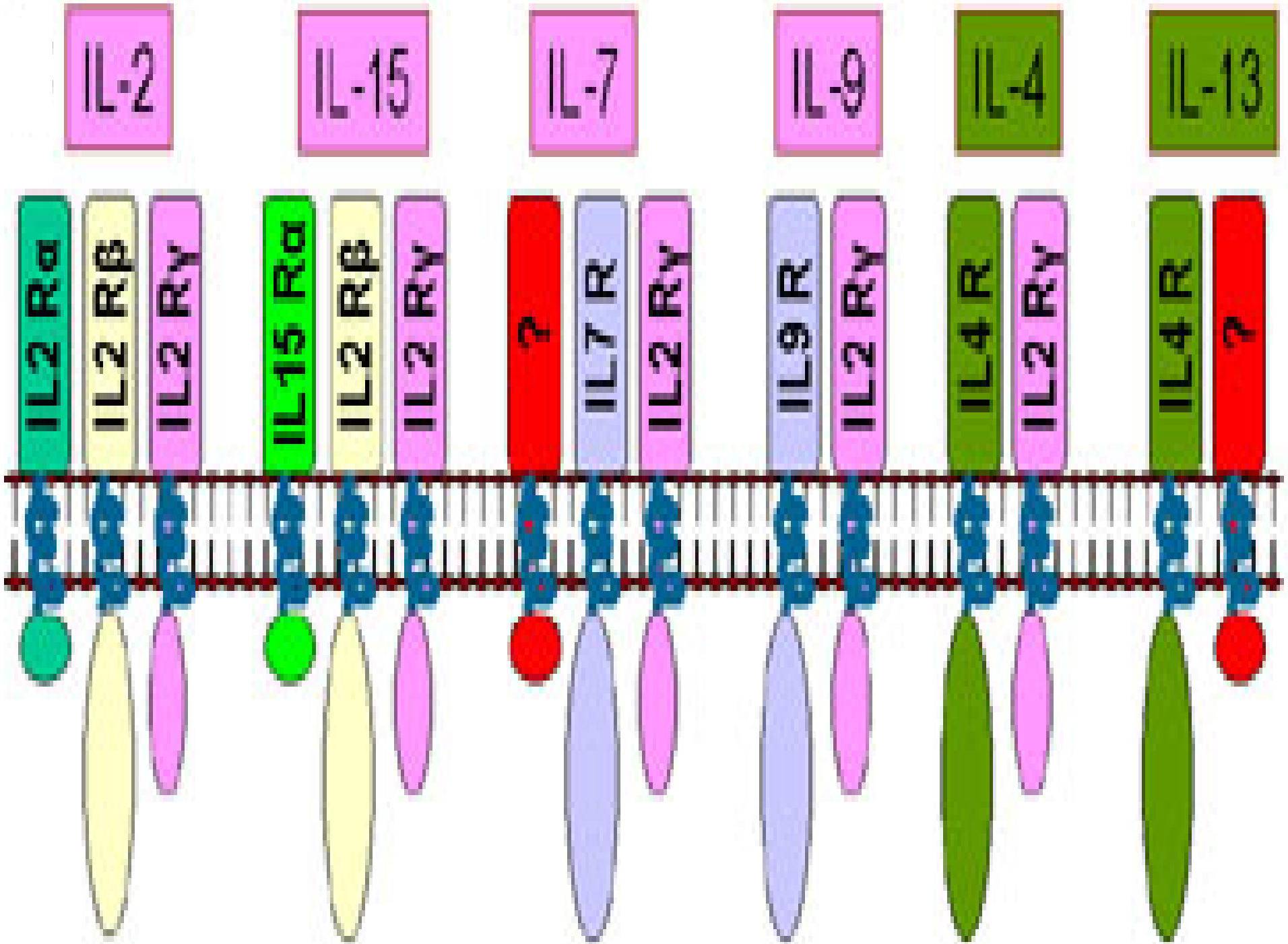


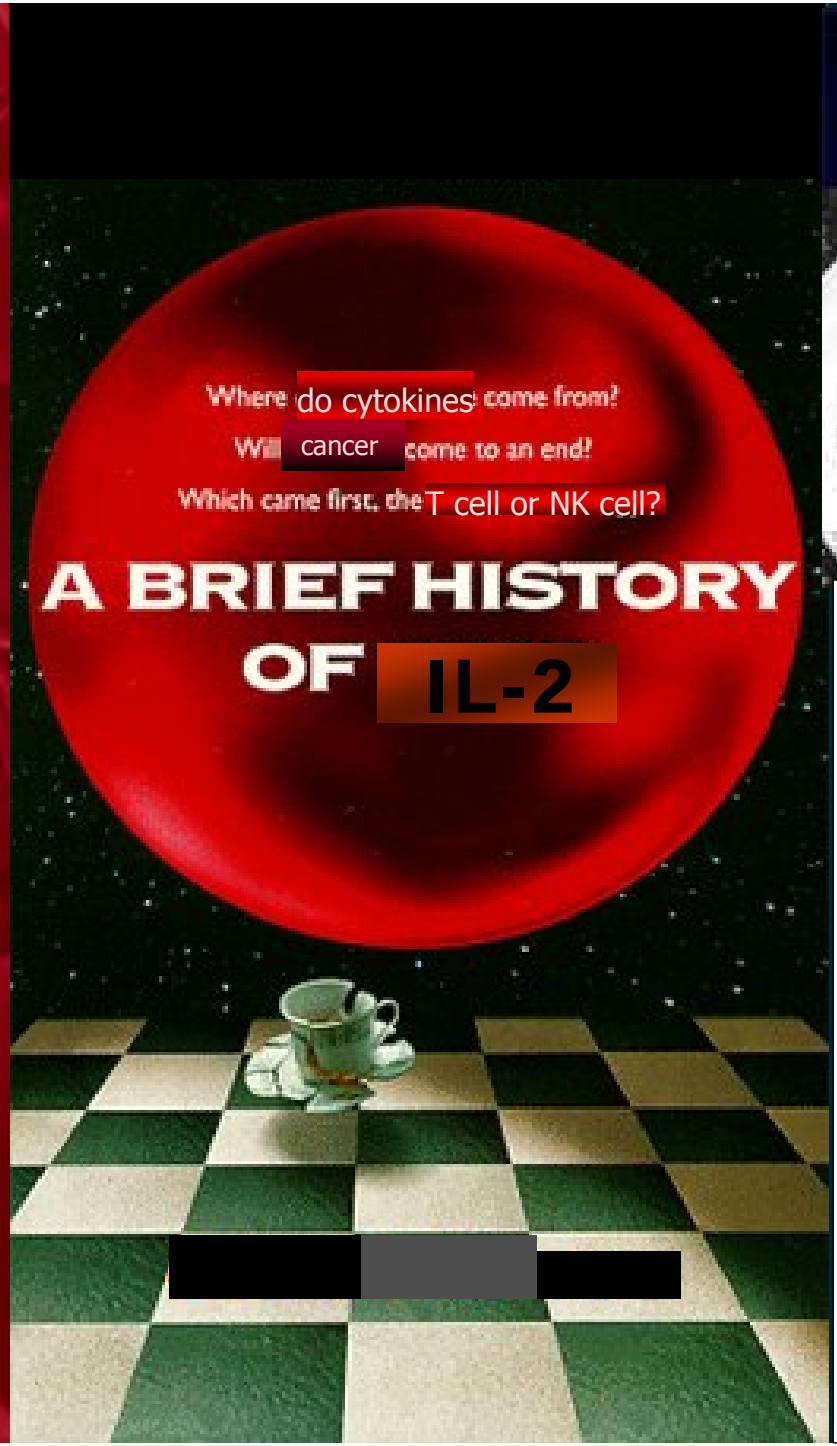
IL-2 Signaling

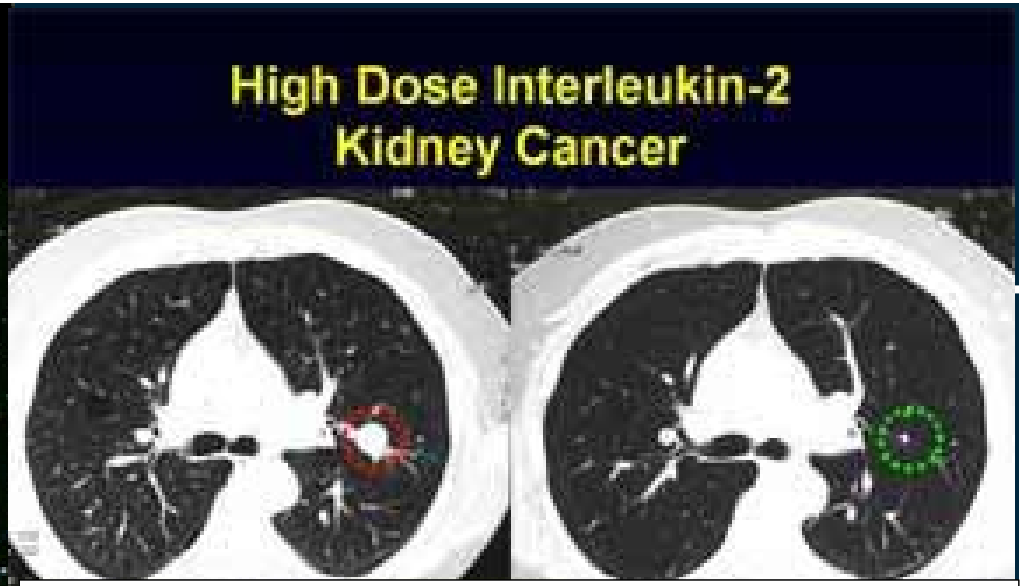


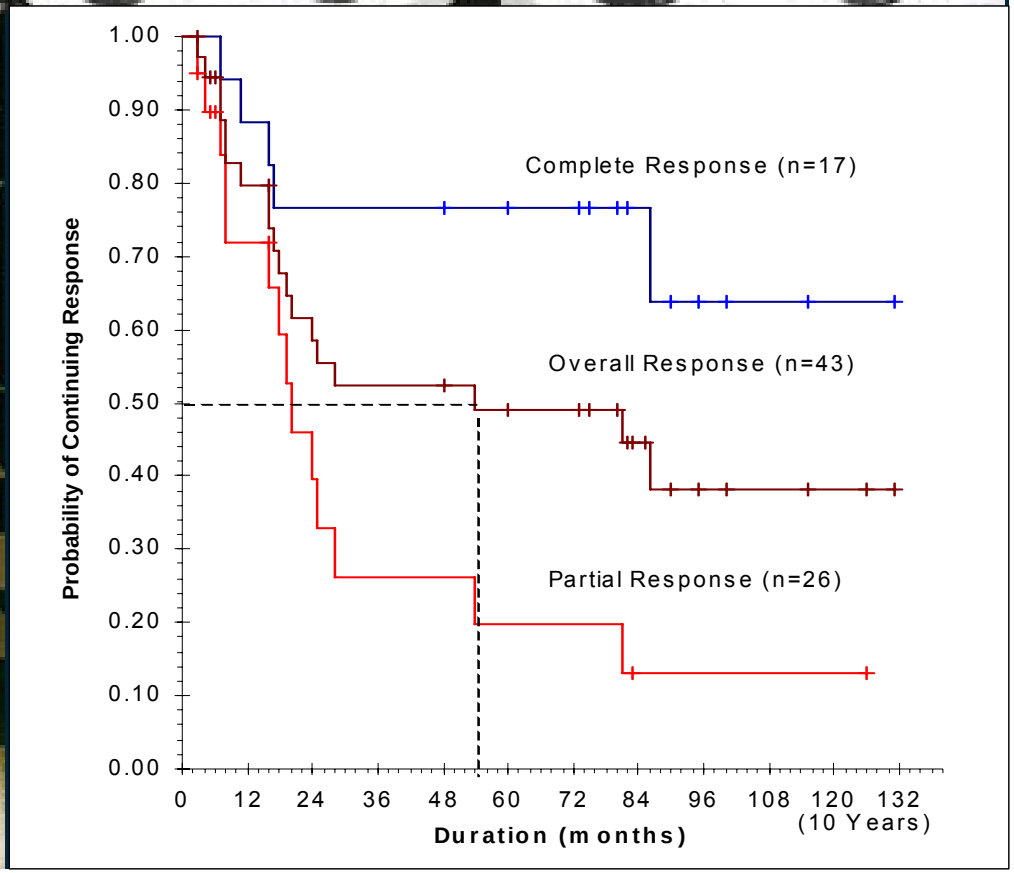


Cytokines that share the common gamma chain receptor


IL-2
IL-15
IL-7
IL-9
IL-4
IL-13
IL2 Rα
IL2 Rβ
IL2 Rγ
IL15 Rα
IL2 Rβ
IL2 Rγ
?
IL7 R
IL2 Rγ
IL9 R
IL2 Rγ
IL4 R
IL2 Rγ
IL4 R
?


Where do cytokines come from?
Will cancer come to an end!
Which came first, the T cell or NK cell?
A BRIEF HISTORY
OF IL-2


High Dose Interleukin-2
Kidney Cancer


Probability of Continuing Response
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Complete Response (n=17)
Overall Response (n=43)
Partial Response (n=26)
0
12
24
36
48
60
72
84
96
108
120
132
(10 Years)
Duration (months)

Pioneering NCI studies

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

Preclinical models
- DLTs due to CLS
- Toxicities vary by species
- Dose-dependent activity

Early clinical studies±LAK

Role of IL-2 in adoptive cell-Rx strategies

Extramural IL-2 studies

In solid tumors
- With LAK cells
- Single agent
- With α-IFN
- With other cytokines
- With chemotherapy
- Toxicity modulation

- In heme malignancies
  - Trial methodology challenging
  - Phase II data promising
  - Phase III data disappointing

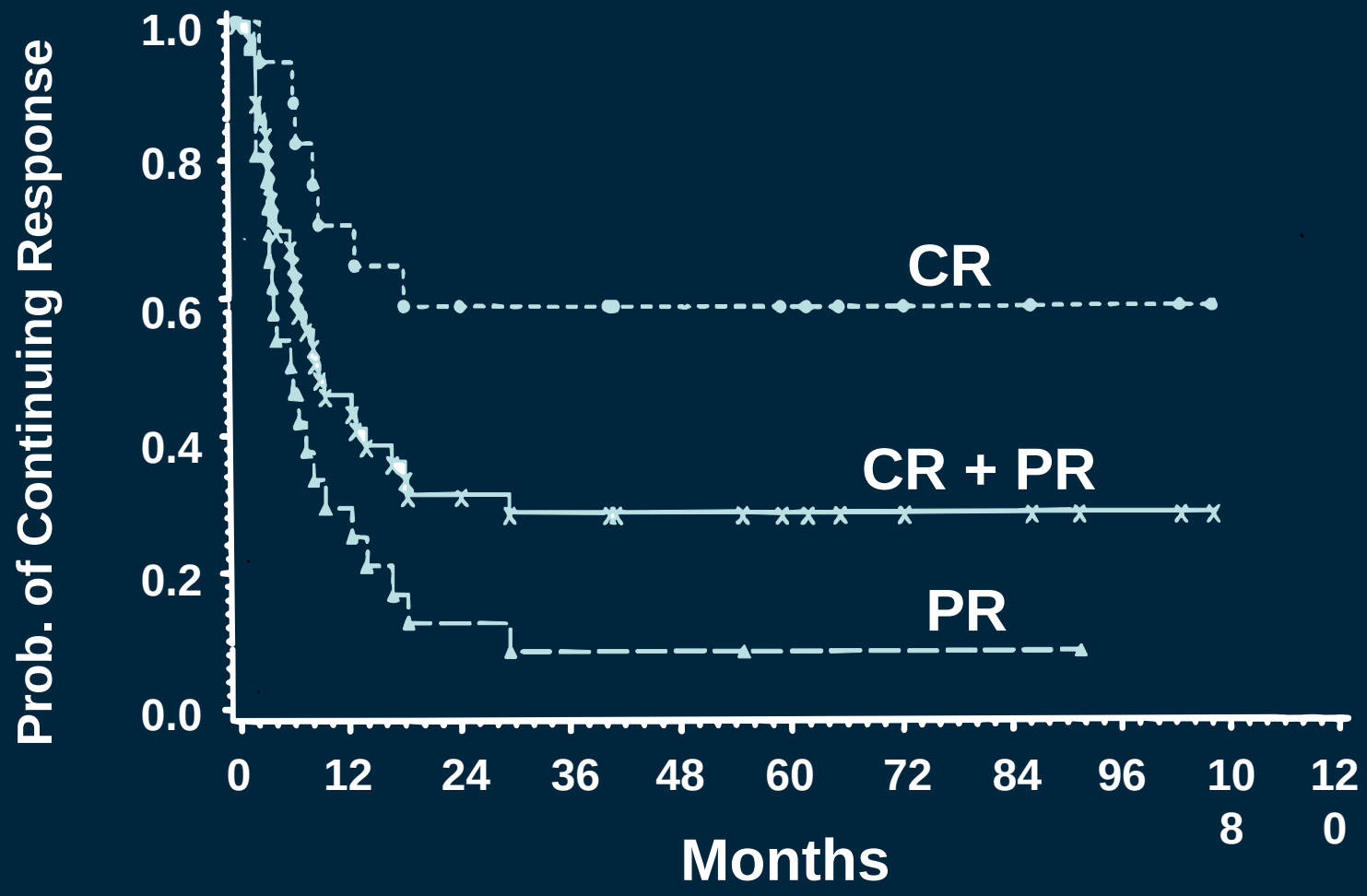
High-dose IL-2 in advanced melanoma-270 patients

Response	No.	%	Response Duration (mo) Median	Range
CR	17	6	Not yet reached	3-158+
PR	26	10	5.9	1.5-91.5
CR + PR	43	16	8.9	1.5-158+

- Median survival for all responders: 62+ mo
- 29% of responders progression-free at 9 years

Atkins MB, et al. *J Clin Oncol* 1999;17:2105.

Durability of responses to high-dose IL-2 in melanoma



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

IL-2 Conclusions ~1985-2000

- 15-20% pts w/RCC, melanoma benefit
- Rx ratio not improved by
  - IL-1 receptor agonist (decoy)
  - TNF blockade (Ab or decoy)
  - Lysophylline (lipid mediators)
  - Histamine (inhibit mϕ ROS)
  - iNOS blockade (inhibit CLS)
- Dose-response inconclusive
- Not effective in biochemo
  - RCC w/pyrimidines, vincas
  - Melanoma w/DTIC, CDDP

- Novel strategies did not improve therapeutic index
  - With IFN α or γ
  - With tumor-directed Ab
  - With agonistic OKT3 Ab
  - Structure-function alterations
    - PEG-IL-2
    - Liposomal IL-2
    - IL-2 “specific agonist”
    - Albuleukin

Worth pursuing in RCC, melanoma, ?heme

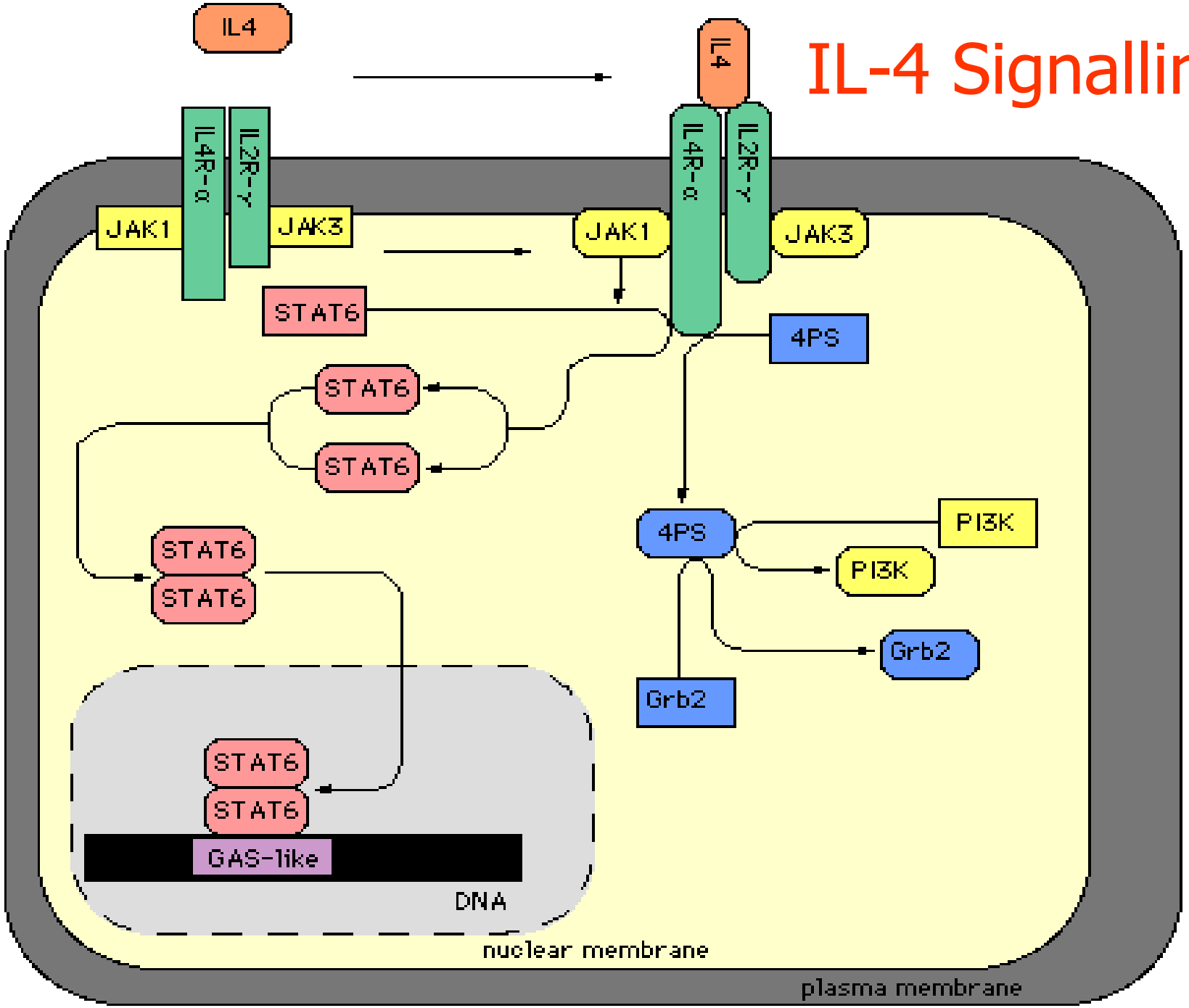
IL-2: 2001-2007 and beyond

- Structural alterations
- Toxicity modulating agents
- Rational combinations hold promise for improving therapeutic ratio
  - Anti-vascular Rxs, small molecules, cytotoxics
  - Unique toxicities need further understanding
- ↑ insight into mechanisms
  - Host-immunogenetics, pharmacodynamics
  - Tumor (“Select” trial to validate CA-IX in RCC)

Interleukin-4

- Pleomorphic cytokine signals through STAT 6
- Th_2 cytokine mediates T-B, other interactions
- Net effects depend on cytokine and cell milieu
  - Mainly a B cell-stimulatory cytokine
  - Inhibits non-specific NK activity
  - Enhances other adaptive immune functions
    - Growth factor for Th2
    - Promotes proliferation and cytotoxicity of CTL
    - Stimulates MHC class II expression
    - Contributes to DC maturation
    - Enhances mΦ tumorcidal activity

IL-4 Signalling



Interleukin-4

- Promising data/proof of principle in Xgenic tumor (prototype for the GVAX strategy)
- Clinical experience limited
  - Studied like IL-2
  - Unfavorable therapeutic index
- Most promise as Rx to "elicit" moDC from PBMC
  - Used ex vivo w/GM-CSF
  - ? Comparison w/IL-13

IL-4 and IL-13

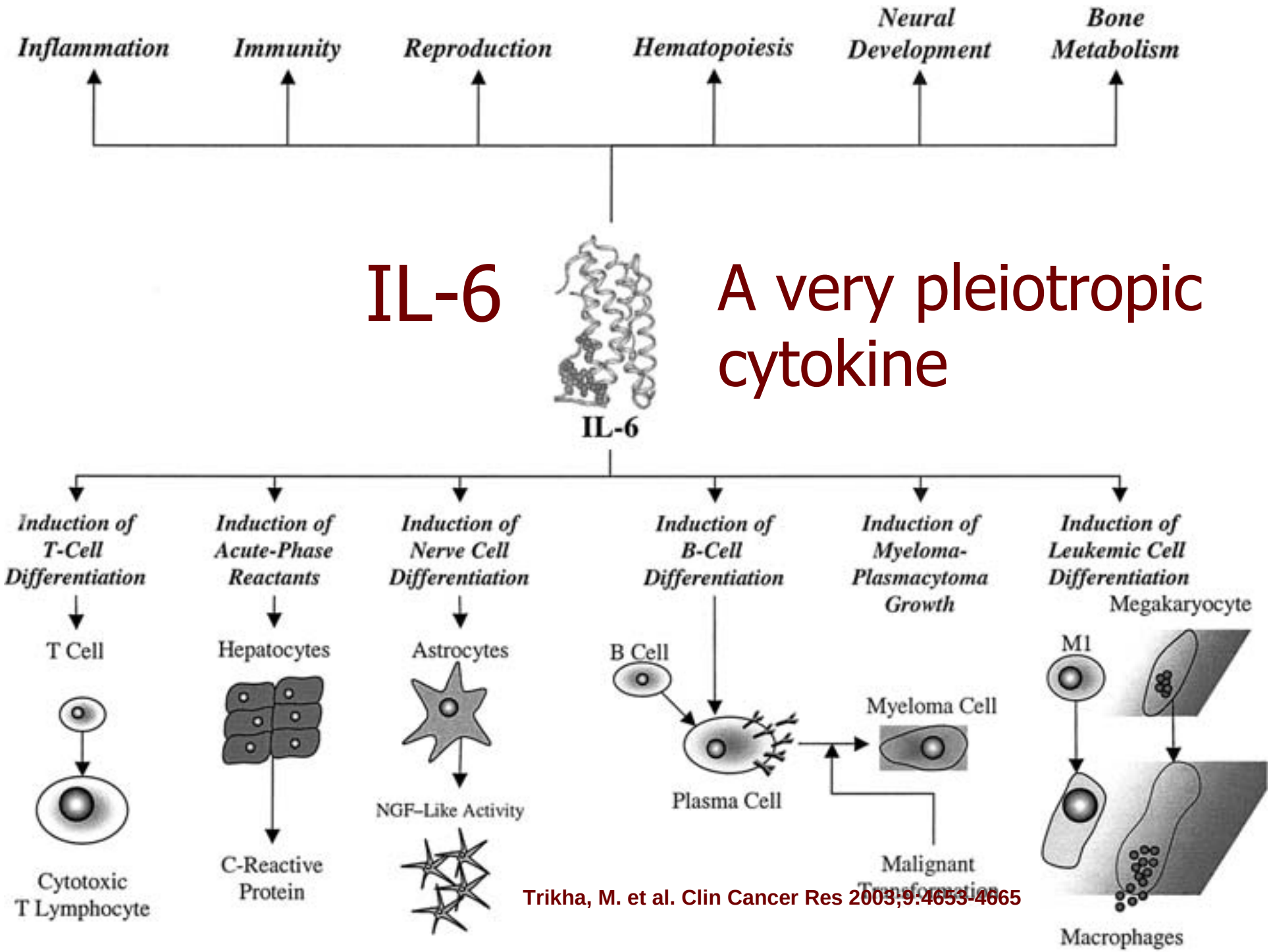
- Similarities
  - Predominantly anti-inflammatory effects
  - Favor Th_2 responses
  - Partially common receptor
  - Promotes Ig class switch
  - Used w/ GM-CSF→moDCs

Assortment of receptor subunits depend on cell type

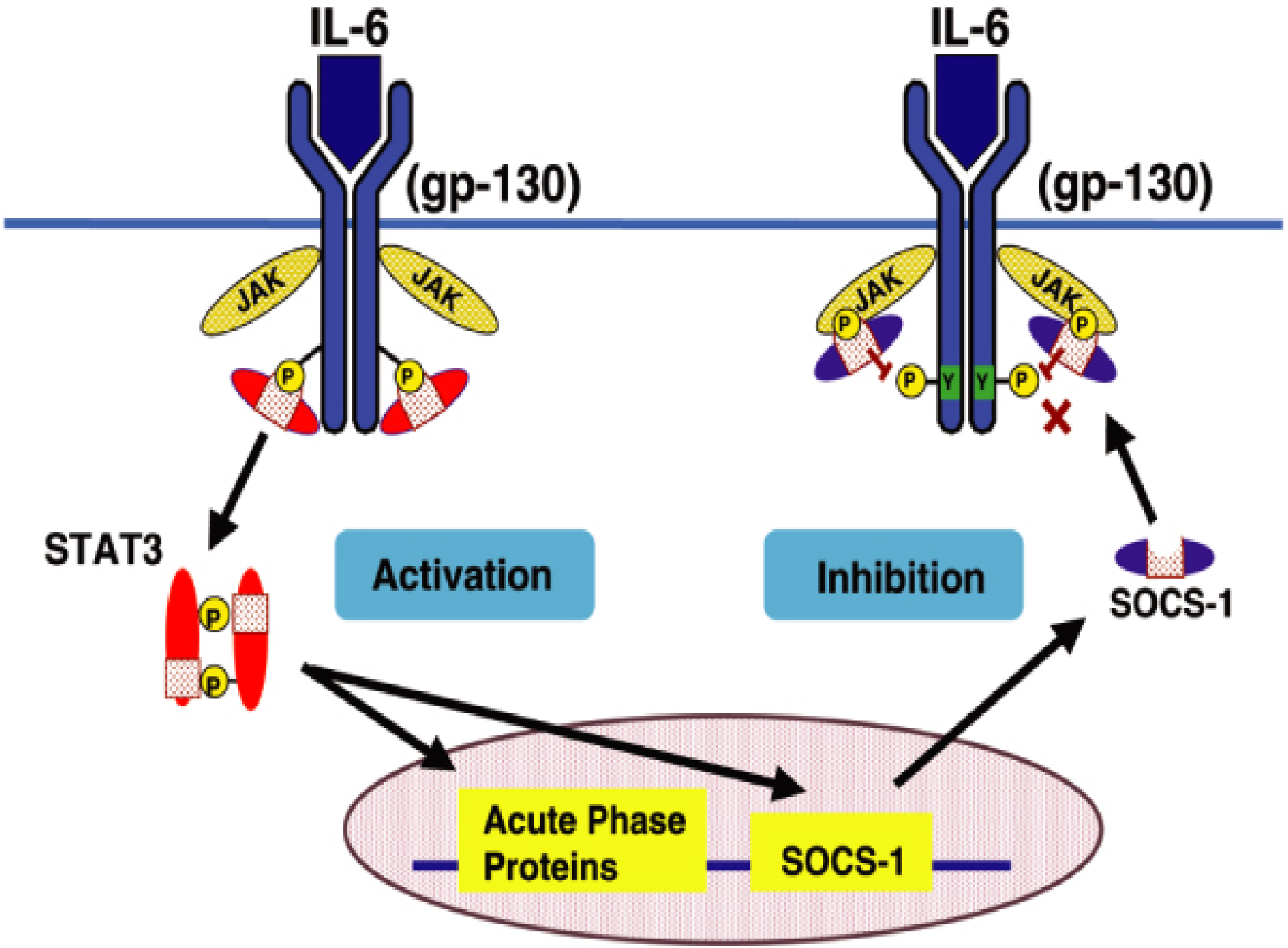
- Differences
  - IL-13 activity on monocyte/mΦ cells
  - IL-13 lacks B, T cell effects
- IL-13 receptors on tumor cells, especially glioma
  - Immunotoxins
  - Chimeric T cell Ag receptor

IL-6

A very pleiotropic cytokine



Trikha, M. et al. Clin Cancer Res 2003;9:4653-4665


IL-6
(gp-130)
JAK
JAK
P
P
IL-6
(gp-130)
JAK
JAK
P
P
Y
Y
P
STAT3
P
P
Activation
Inhibition
SOCS-1
Acute Phase
Proteins
SOCS-1

IL-6

- Tumor source
  - Unfavorable prognostic factor in renal CA, melanoma
  - An important growth factor for myeloma
  - Major effector of paraneoplastic thrombocytosis
- Adaptive system
  - B cell growth/differentiation
  - CTL differentiation
  - Type 2 responses
- Preclinical data showed activity in selected tumor models
- Phase I and II clinical data
  - Hematologic (thrombocytosis, anemia), arrhythmias, neurotox
  - Insufficient clinical activity
  - Concern about potential tumor-promoting effects
- Paradox: IL-6 *Ab* now in clinical trials

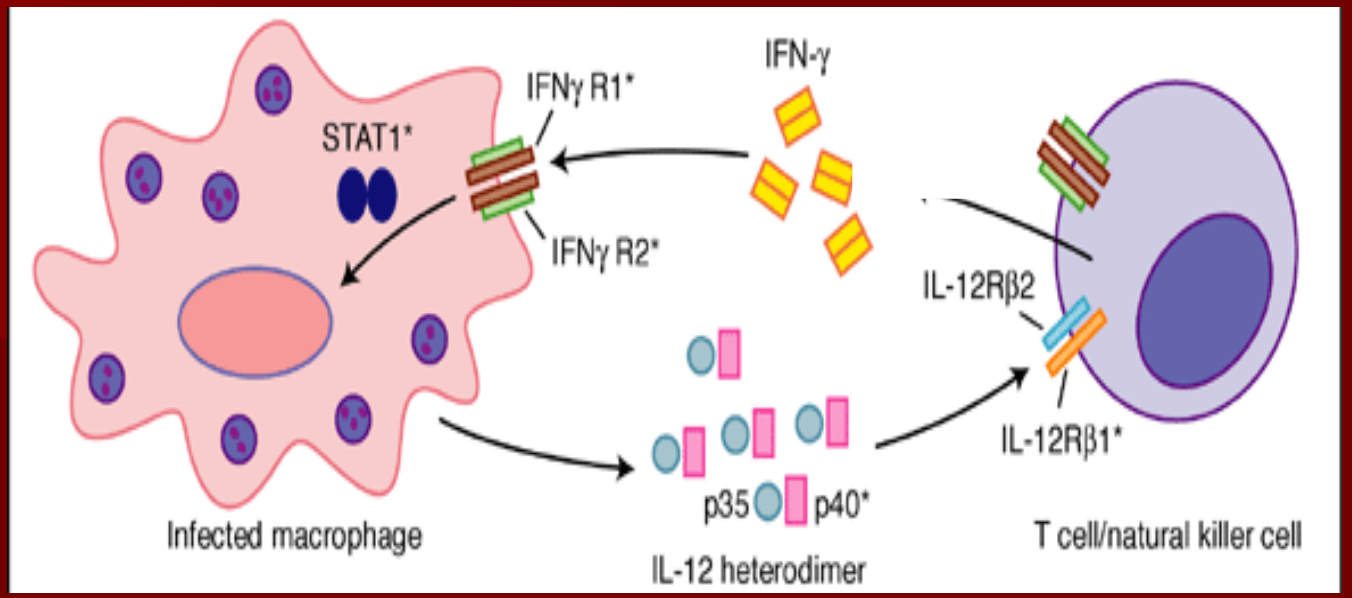
IL-7

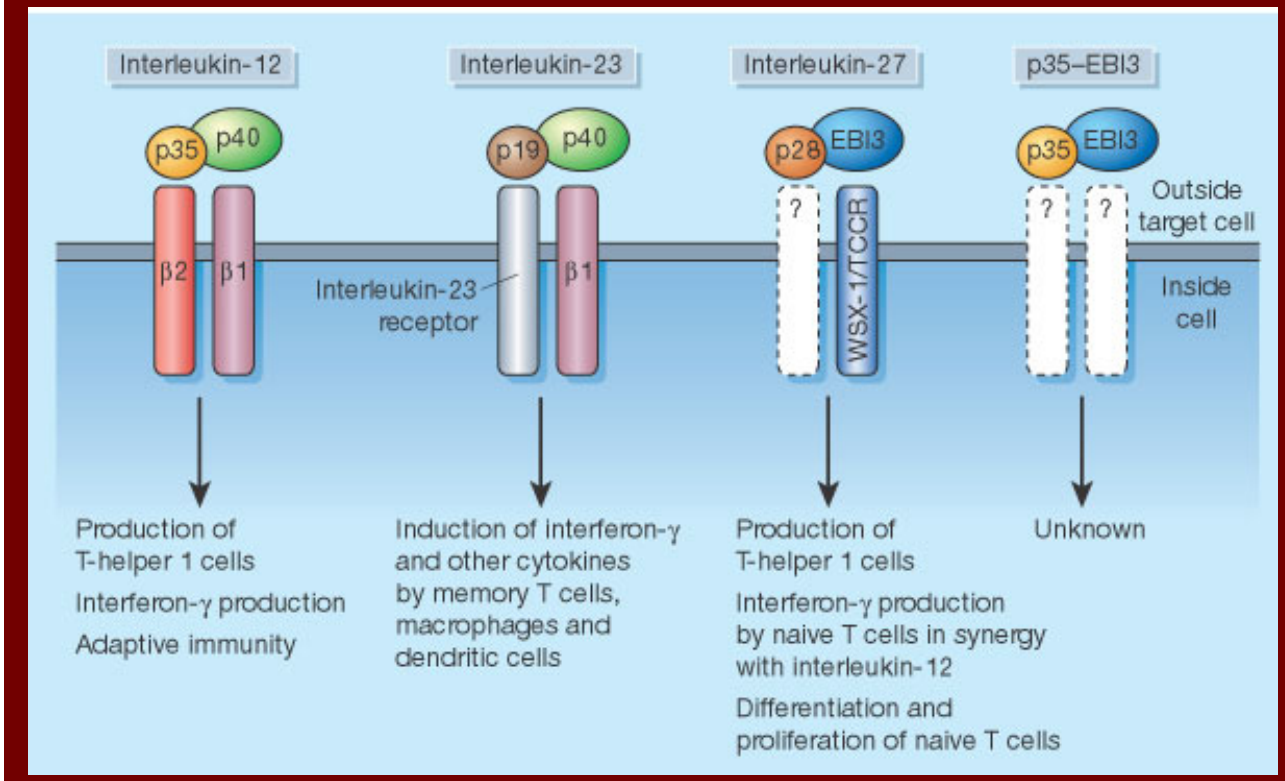
- Signaling
  - Jak1, Jak3/STAT5
  - PI3K activation, mTOR activation
  - IL-7 signaling and/or T-cell activation→receptor downregulation
- Opposite regulation compared to IL-2, IL-15
  - IL-7 accumulates during lymphopenia as a result of diminished utilization
  - Homeostatic expansion of naïve cells during lymphopenia
  - Can substitute for IL-15 for homeostatic expansion of memory cells during lymphopenia
- IL-7Rα expression marks cells destined to become memory during the evolution of the immune response
  - (Kaech, Nat Imm 2003)

IL-12

- Link between innate, adaptive immune response
  - Receptors on variety of immune cells
  - Prototypical type I cytokine, induces IFN-γ
- 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/α-IFN, IL-2?

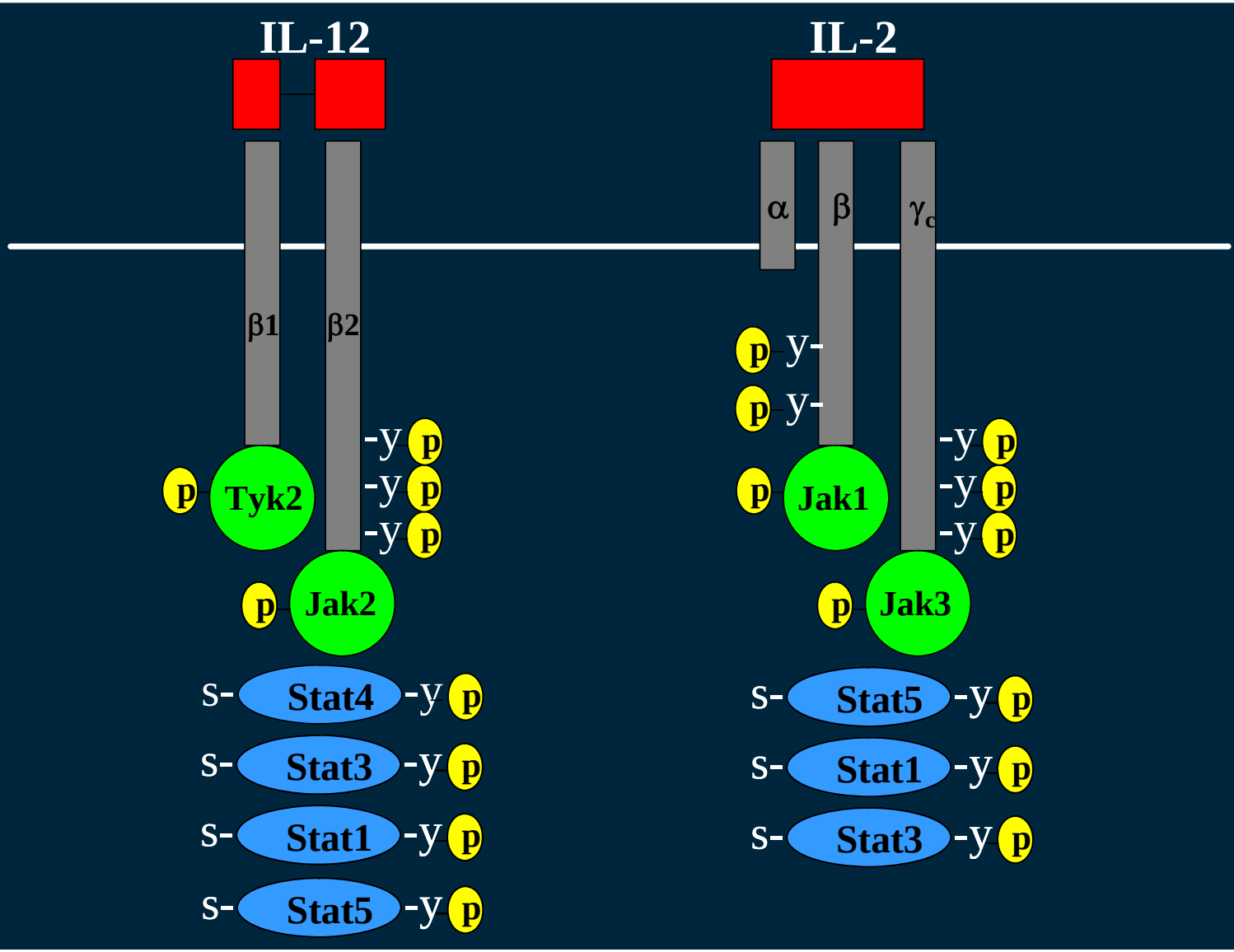
IL-12 and innate/ adaptive→ immune system





IL-12 family

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

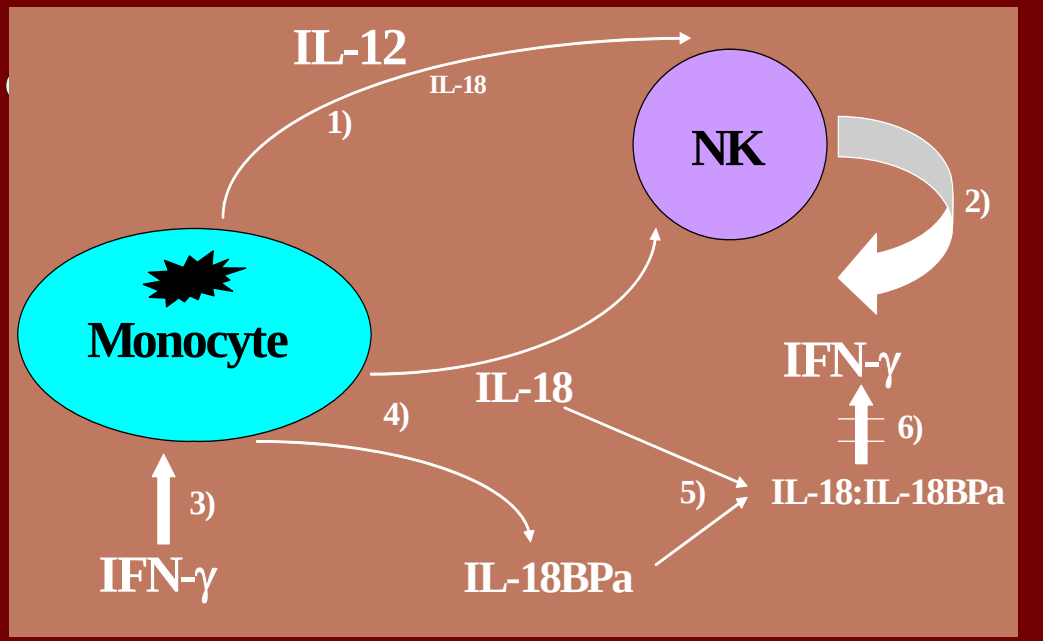


IL-15 and IL-2 compared

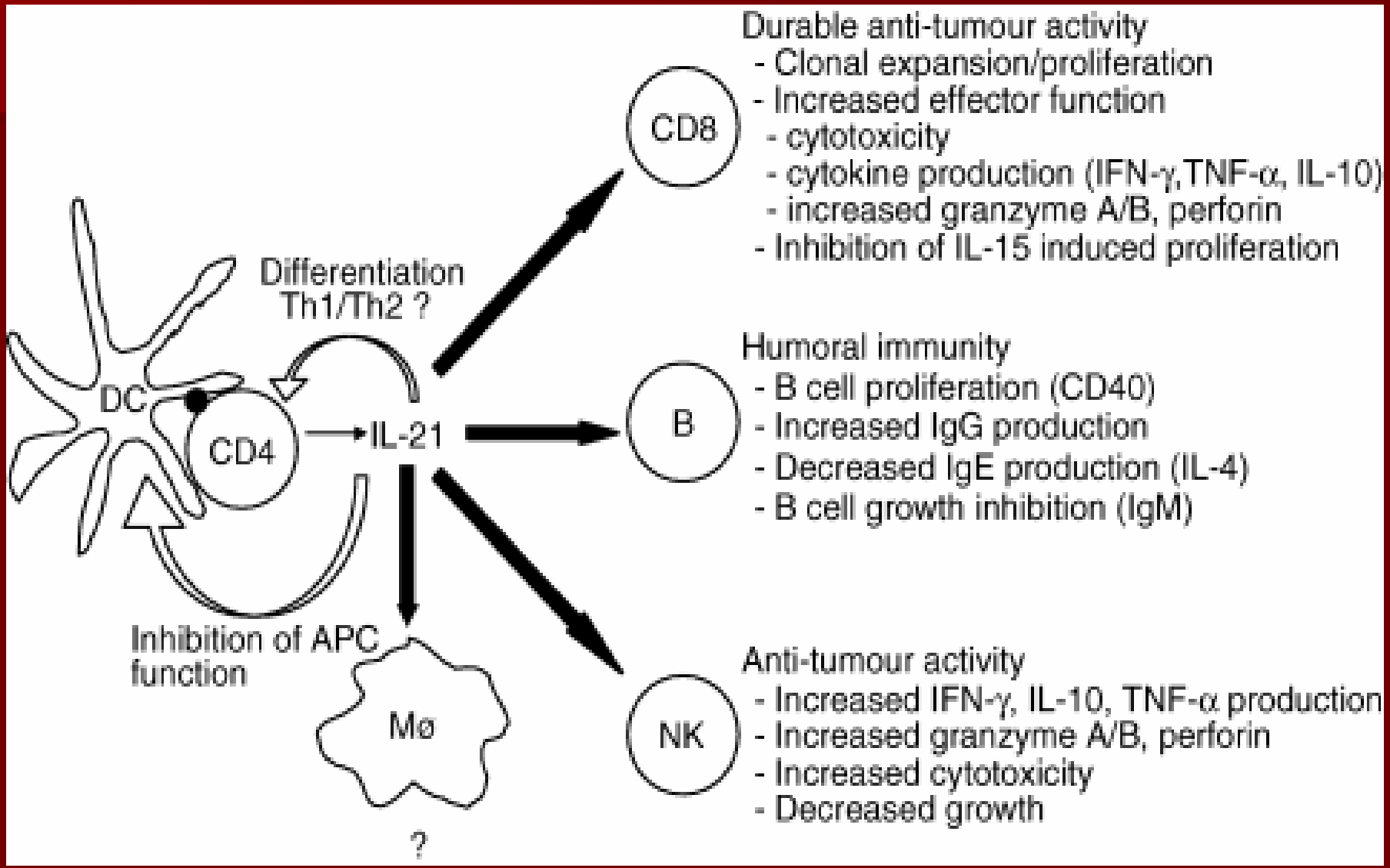
- IL-2
  - Activated T, B express high-affinity $\alpha\beta\gamma$
  - Prolif/differentiation NK, T, B
  - Elimination self-reactive T (AICD)
  - Maintenance Treg
  - -/- has autoimmune phenotype
- IL-15
  - Produced by DC, mono
  - Surface-bound on DC/mono $\leftrightarrow$ receptors on NK, CD8a1 cells
  - Promotes prolif NK, T, B, memory CD8
  - Inhibits AICD
  - -/- is lymphopenic for same populations

IL-18

- Member IL-1 family with IL-18BP counterregulation
- Activates NK cells and induces type I cytokines
- Promotes Th1 and memory CD8 T cells
- Upregulates FasL on effector lymphocytes
- Antitumor activity in
  - Alone
  - W/IL-2, IL-12
- Phase I DLTs
  - Leukopenia



IL-21: another pleiotropic γ_c cytokine



Phase I i.v. outpatient IL-21

J. Thompson et al, ASCO 2006

IL-21 Treatment Schedule

(outpatient administration of two 5-day cycles)

Dosing cycle = 5 consecutive daily doses of IL-21 delivered by IV push

- Tolerable outpatient regimen identified
- Multiple dosing cycles feasible
- IL-21 pharmacodynamic activity
  - Direct effect on lymphocyte count
  - Increase in sCD25
- Four responses observed at different dose levels (Mel, RCC)
  - One patient with Complete Response
  - Three patients with Partial Response

Phase II studies planned
RCC w/TKI (Phase I/II); Melanoma as SA

Thank you

[and to Jared Gollob]

Any questions?