Cytokines in the (Immuno)Therapy of Malignancy

Kim Margolin, M.D.

City of Hope Comprehensive Cancer Center

Cytokines-according to Google

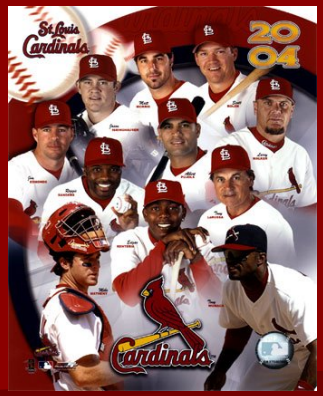
Biology of the cytokines

- Details to be discussed for IL-2>>other cytokines
  - Cytokine structure
  - Stimuli leading to induction of cytokine synthesis
  - Cell(s) responsible for cytokine production
  - Cytokine-responsive cell(s)/receptor structure
  - Signaling induced by cytokine binding
  - Overall result of cytokine function

- NOT for detailed discussion
  - Transgenic cytokine expression
  - Cytokines in adoptive cell therapies
  - Cytokines in tumor vaccine investigation

Clinical development

- Early trials
  - Dose-seeking, proof of principle
  - Toxicity, schedule considerations
- Disease-directed studies
  - Pilots
  - Phase II
  - Phase III
- Modulation of activity, toxicity
- Current status and future possibilities
  - Combination cytokines
  - With other modalities and classes of Rx, esp. “targeted”
  - Fusion molecules for focussed therapies



The players

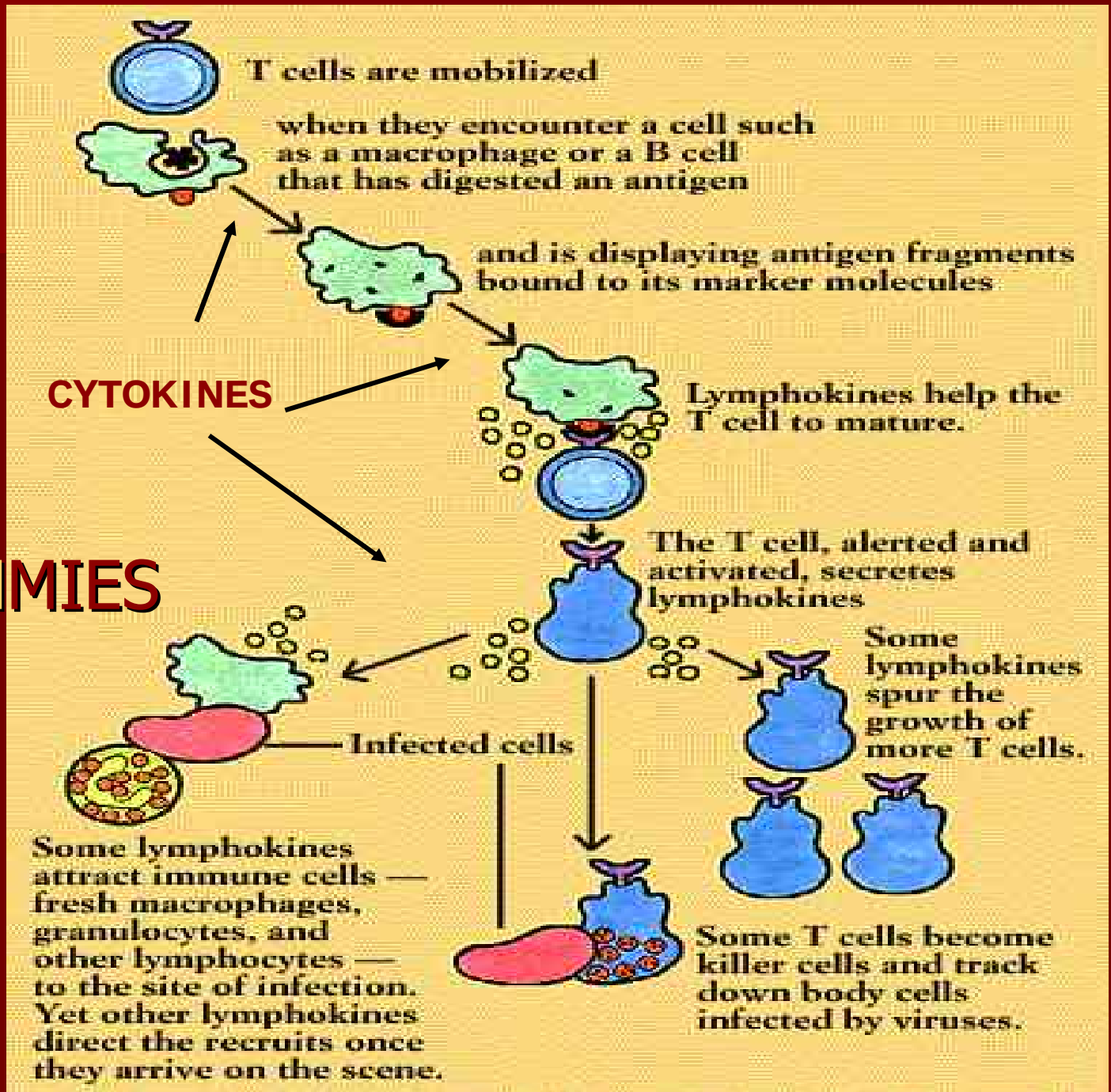
For discussion

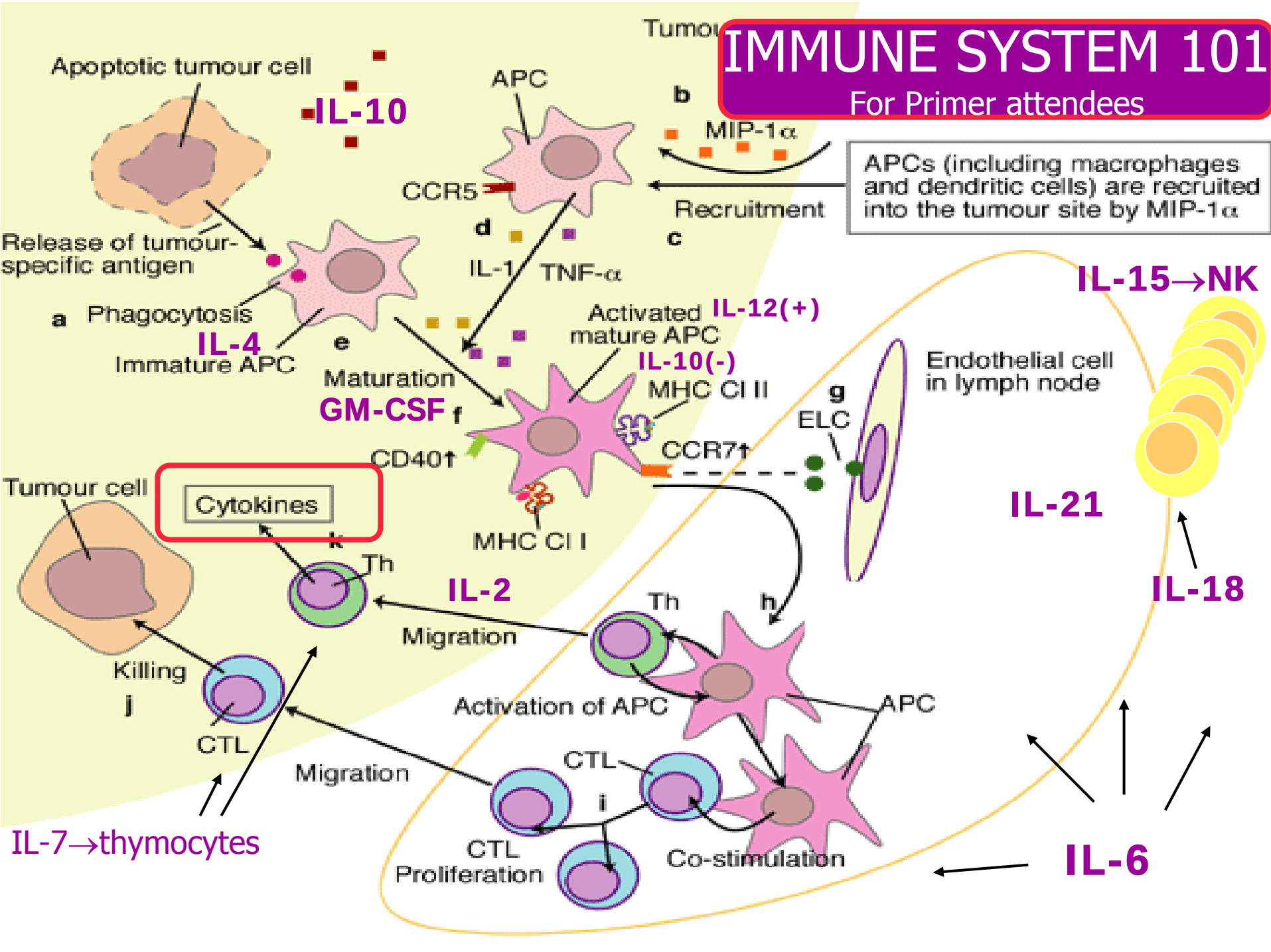
- GM-CSF
- Interleukin-2
- Interleukin-4/13
- Interleukin-6
- Interleukin-7/15
- Interleukin-12
- Interleukin-18
- Interleukin-21

Not for detailed discussion

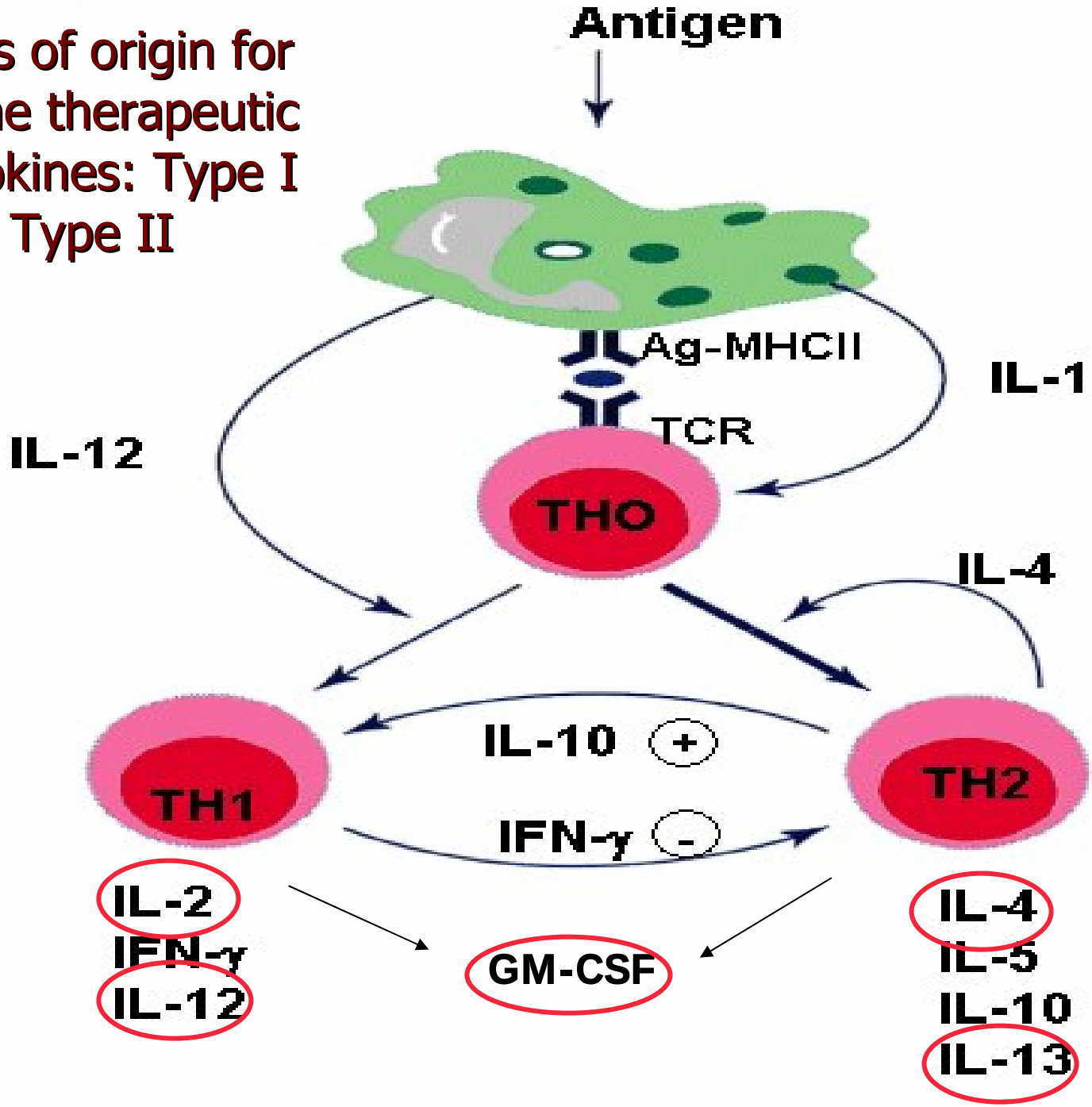
- Interferons
- Hematopoietic: 3, 11, flt-3L
- Complex innate: TNF, IL-1
- "Suppressive": IL-10, TGFβ
- Miscellaneous: TRAIL etc.

IMMUNE SYSTEM FOR DUMMIES




IMMUNE SYSTEM 101
For Primer attendees
Apoptotic tumour cell
IL-10
APC
b
MIP-1α
APCs (including macrophages and dendritic cells) are recruited into the tumour site by MIP-1α
CCR5
Recruitment
c
d
IL-1
TNF-α
Release of tumour-specific antigen
a
Phagocytosis
IL-4
Immature APC
e
Maturation
GM-CSF
f
Activated mature APC
IL-12(+)
IL-10(-)
MHC Cl II
CD40↑
CCR7↑
MHC Cl I
g
ELC
Endothelial cell in lymph node
IL-15→NK
IL-21
IL-18
Tumour cell
Cytokines
k
Th
IL-2
Migration
Th
h
Killing
j
CTL
Activation of APC
APC
Migration
CTL
i
CTL Proliferation
Co-stimulation
IL-7→thymocytes
IL-6

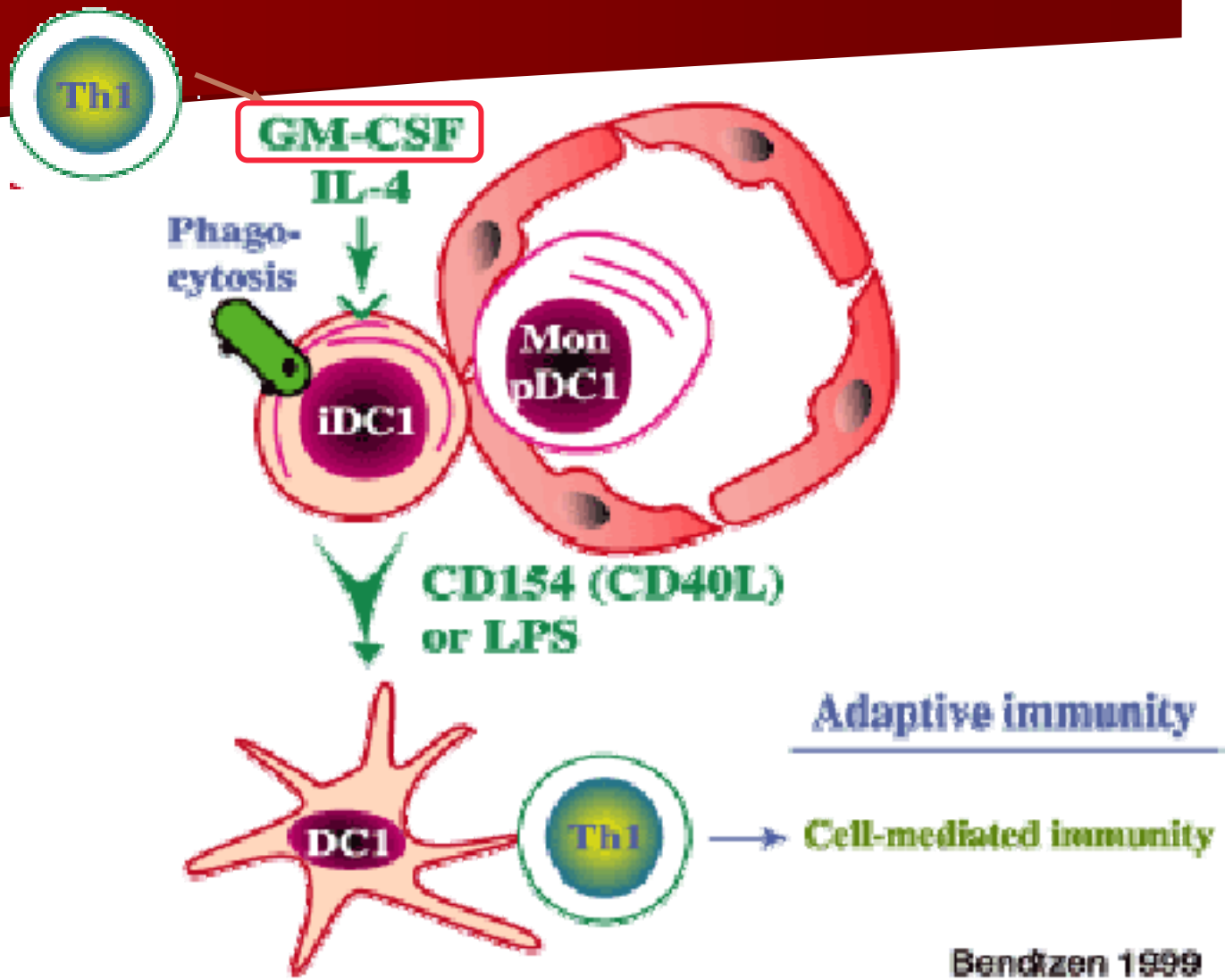
Cells of origin for some therapeutic cytokines: Type I and Type II



GM-CSF as immunotherapy

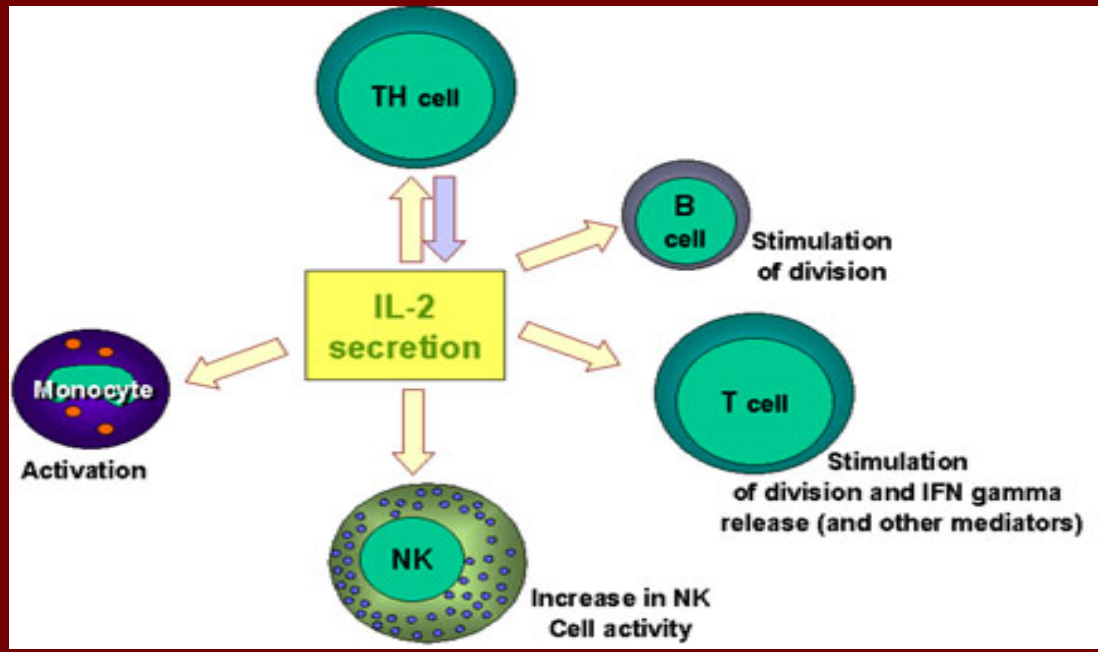
- Cells of origin
  - Th1, Th2
  - Others include epithelial, fibroblast, *tumor*
- Target cell: immature DC (& myeloid progenitor)
- Biological functions
  - Stimulation of T cell immunity via effect on APC
  - Myeloid cell proliferation, differentiation
- Clinical development
  - Hematopoietic support
  - Not a potent stand-alone cytokine in cancer
  - Transgenic expression (GVAX)
  - Adjunct to immunotherapy

GM-CSF function in immunity

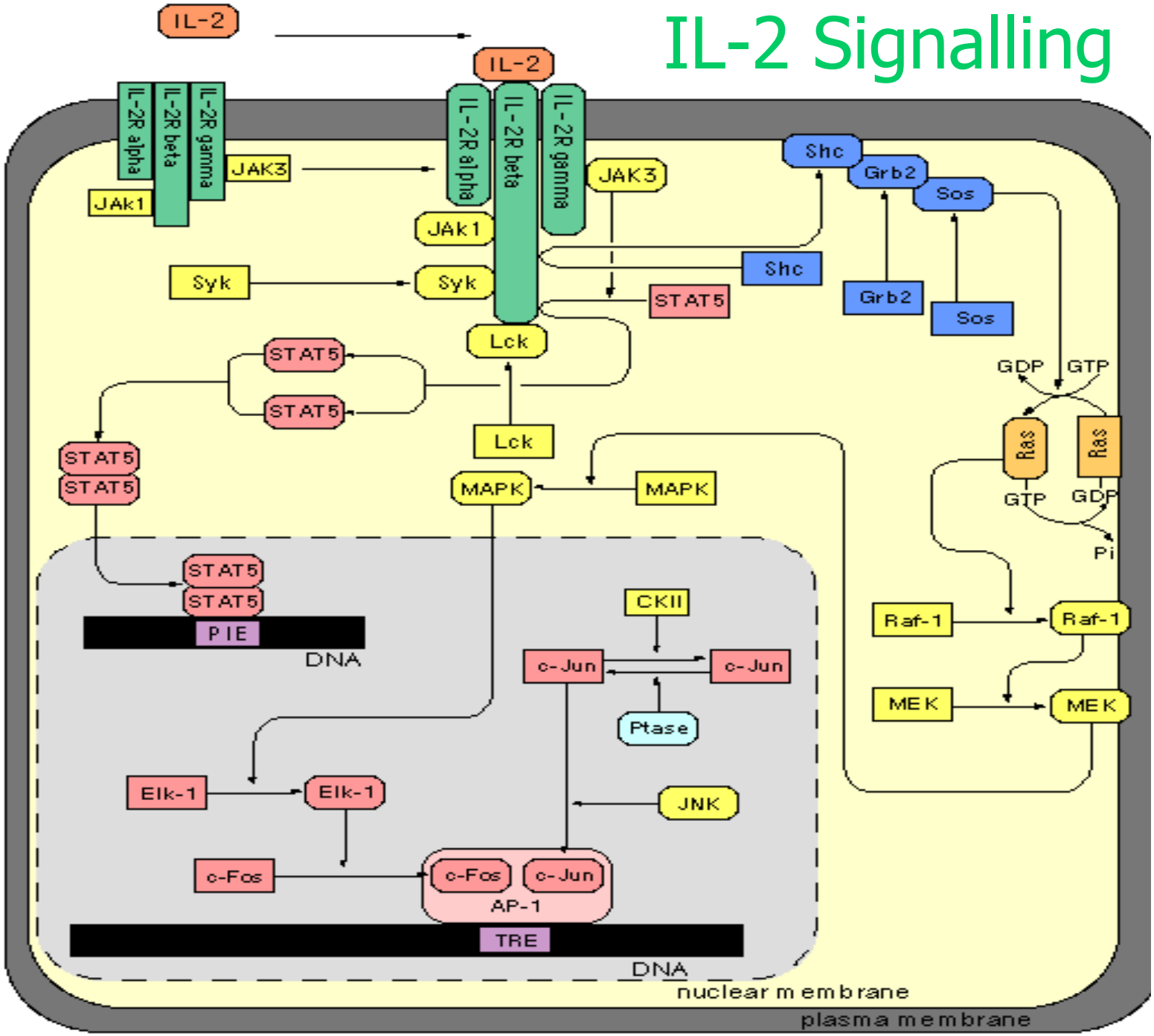


Interleukin-2

- The mother of all therapeutic cytokines
- 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 Signalling

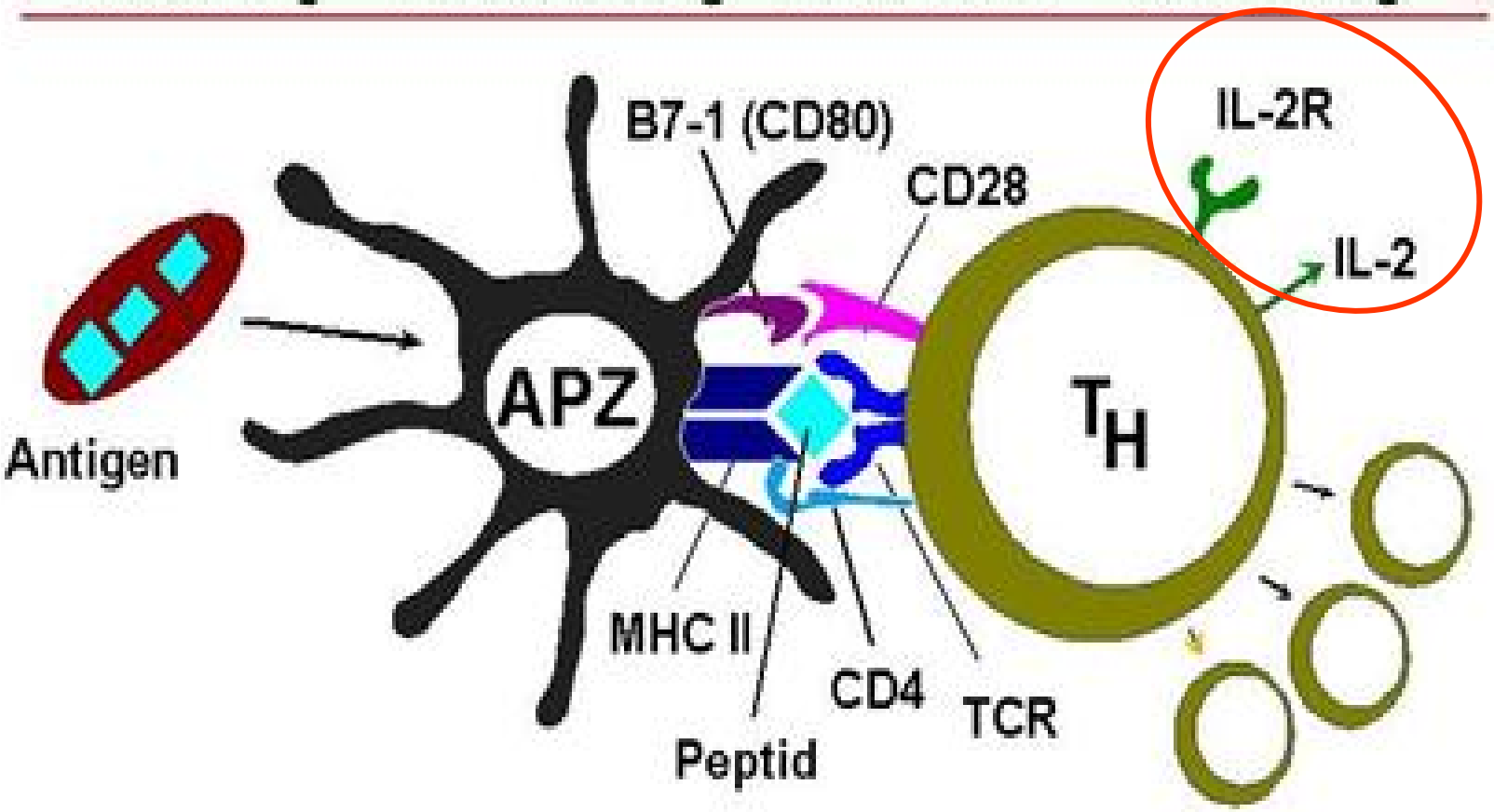


Induction of an immune response

Induktion einer Immunantwort

Recognition Processing Presentation Activation

Erkennung Prozessierung Präsentation Aktivierung



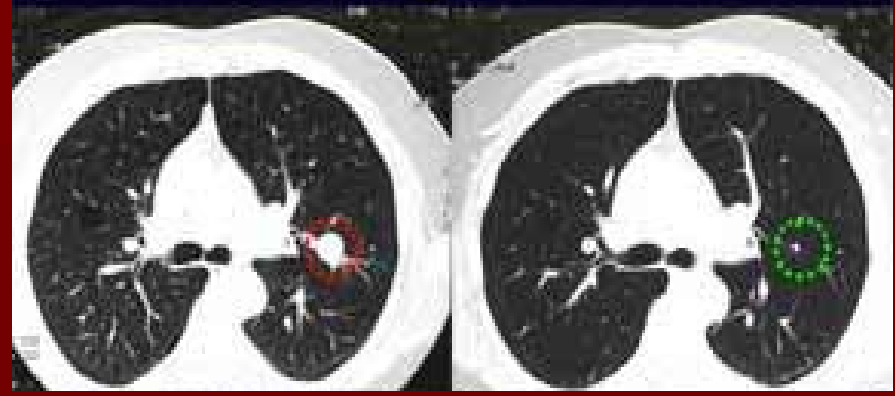
Where do cytokines come from?

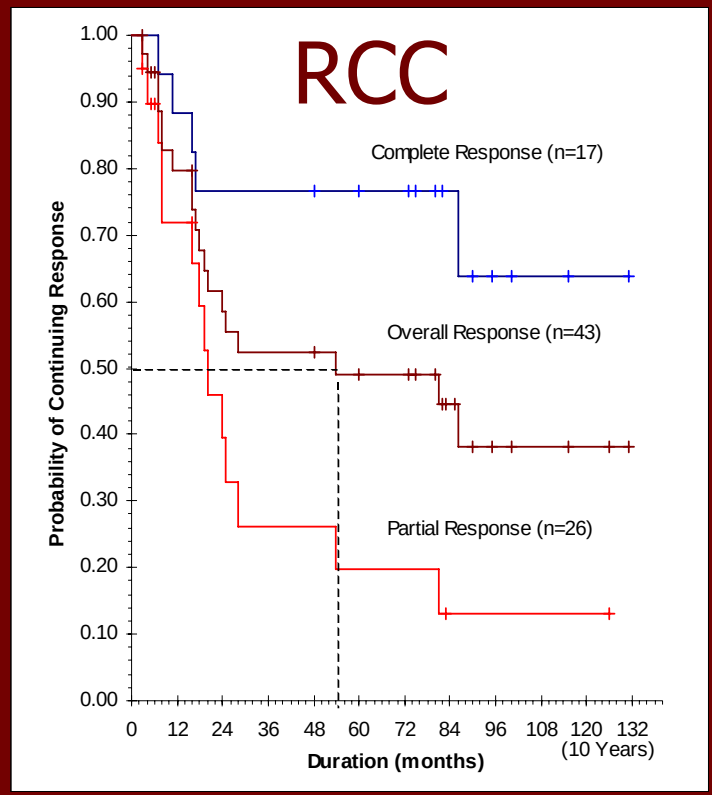
Will cancer come to an end?

Which came first, the T cell or NK cell?

A BRIEF HISTORY OF IL-2

High Dose Interleukin-2 Kidney Cancer





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

IL-2 in hematologic malignancy

- Preclinical: IL-2 exposure of BM/PB induced cytotoxic lymphocytes vs. chemo-S/R leukemia, NHL, cancer
- Early clinical
  - Ex vivo Rx of HCT product w/IL-2 feasible, may promote h'poiesis
  - IL-2/LAK cell Rx had slight activity in HD, NHL
  - Autologous GvHD endpoint promising
- Post-transplant IL-2 had dose-dependent toxicities
  - Less technically demanding than treating cells w/IL-2
  - Encouraging pilot data from Seattle, other centers
  - Auto-HCT feasible, allo-HCT too complex
- Phase III designs included HCT and non-HCT regimens
  - Acute leukemia: feasibility problems, better alternatives
  - NHL: Negative result of post-aHCT IL-2 (J Thompson/SWOGl)

Overall conclusions: Clinical IL-2 studies ~1985-2000

- 15-20% pts w/RCC, Mel 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 2001-2006: General considerations

Investigations continuing on structural alterations to reduce capillary leak

Toxicity modulation approaches have been overtaken by investigation of mechanisms and patient selection factors: different paths for different diseases

Rational combinations hold promise for improving therapeutic ratio

RCC: Ag-specific strategies elusive

Defining correlates of benefit

- Target organs
  - Lymphocytes, ?other cells
  - Blood count changes
  - Cytokines
  - Autoimmune events
- Prognostic
  - Sites of mets
  - Pace of mets
  - Nephrectomy state
- Predictive
  - Histology
  - Hypoxia-related, other cell pathways

New directions for IL-2-based Rx in RCC

- "Select" trial to validate predictors, correlates
  - CA-IX
  - Histology
    - Favorable:  clear, alveolar, <50% granular,
    - Unfavorable:  papillary, >50% granular
  - New exploratory endpoints
    - VHL gene mutations
    - Other pathways: glut-1, PTEN/AKT, CXCR4
    - Immuno"suppressive" factors-preRx analyses
      - CD11b immature myeloid cells
      - arginase, ξ-chain function
- Rational new combos
    - With targeted agents
    - With angiogenesis inhibitors

} ongoing

Melanoma: Ag-specific strategies remain at forefront

- Multicomponent Rx
  - Lymphodepletion/reconstitution
  - Vaccine (many options)
  - Regulatory blockade (IL-2 effects on Treg need further elucidation)
    - CTLA-4Ab
    - Ontak
    - Other resistance modulators

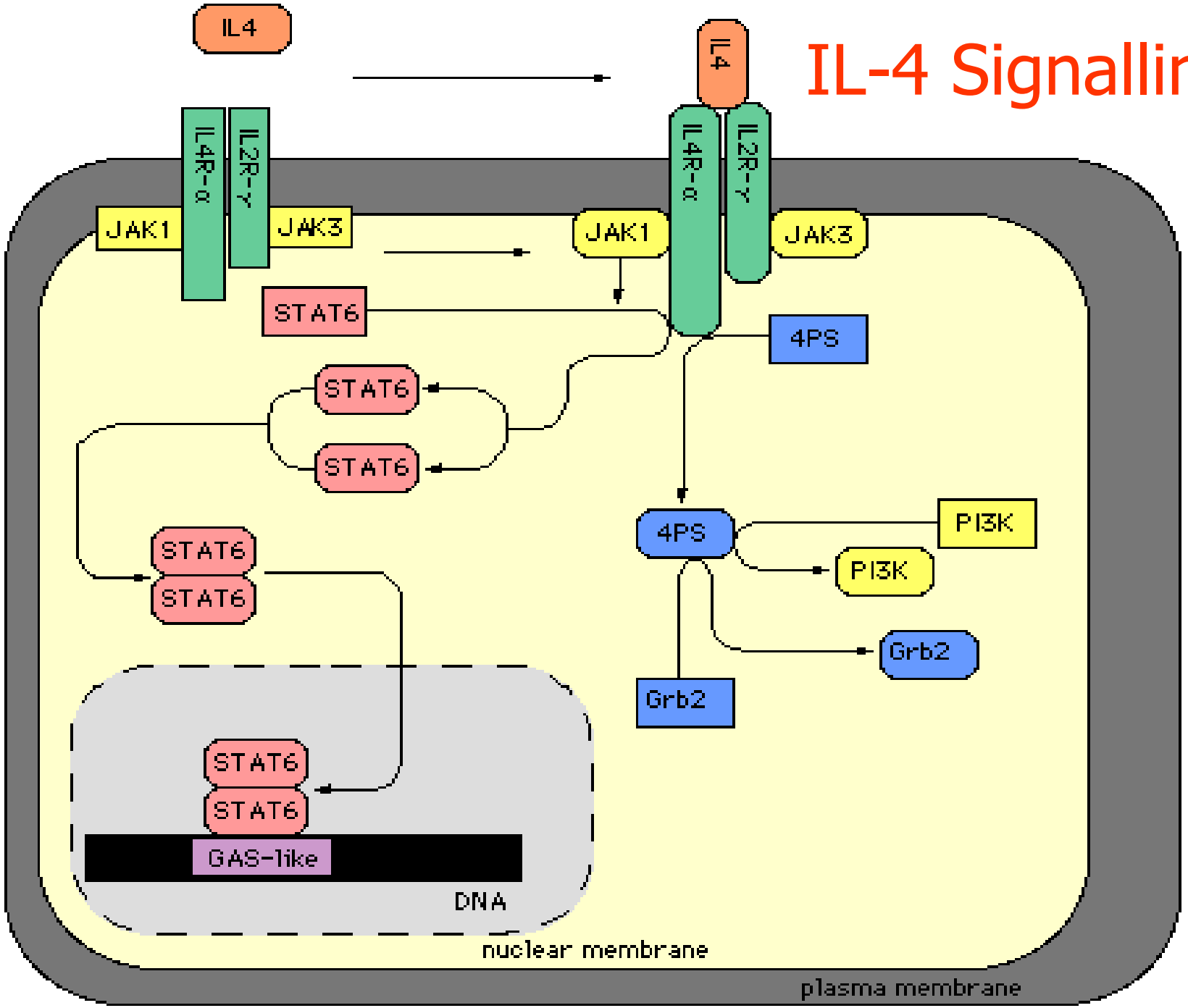
    } under investigation
  - Passive/active
    - Cloned/expanded Ag-specific effector lymphocytes
    - Chimeric receptor-expressing T cells
    - Immunocytokine to redirect effector cells
  - Methylation inhibitors to ↑ expression of immune response genes

- Immunological insights plentiful, but useful predictors remain under investigation

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 but
  - 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

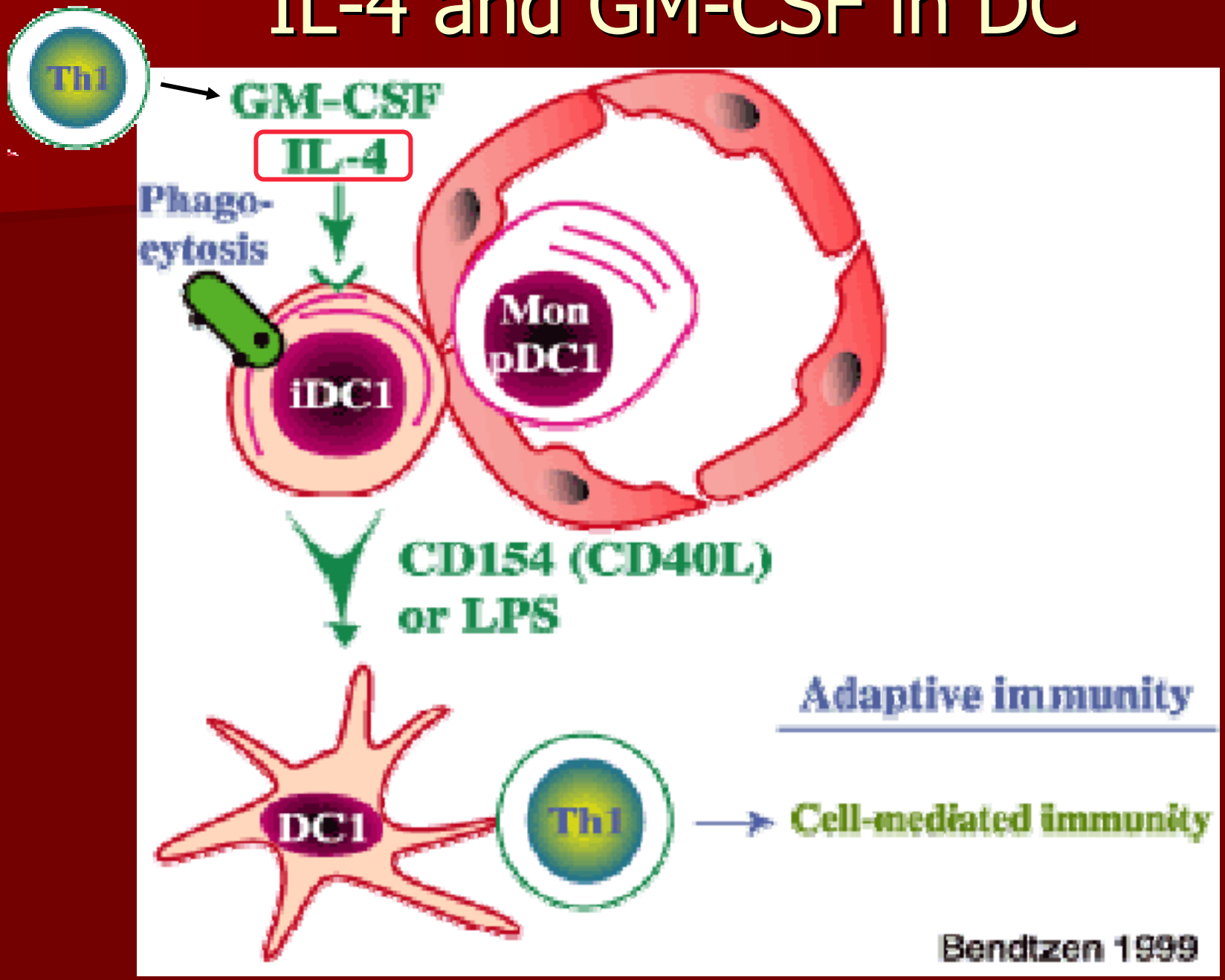
IL-4 Signalling



Interleukin-4

- Promising original data
  - One of the first transgenically expressed cytokines
  - Tumor-associated immune infiltrate was prototype
- Clinical experience limited
  - Studied like IL-2
  - Minimal activity, much toxicity (mucocutaneous, cardiac)
- Most promise as Rx to "elicit" moDC from PBMC
  - Phase I was directed at *in vivo* DC expansion (Gitlitz JIT '03)
  - How does this compare w/flt-3L?
  - IL-13 may be superior in vitro to IL-4
  - What is the current status of *in vivo* DC work?

IL-4 and GM-CSF in DC



IL-4 and IL-13

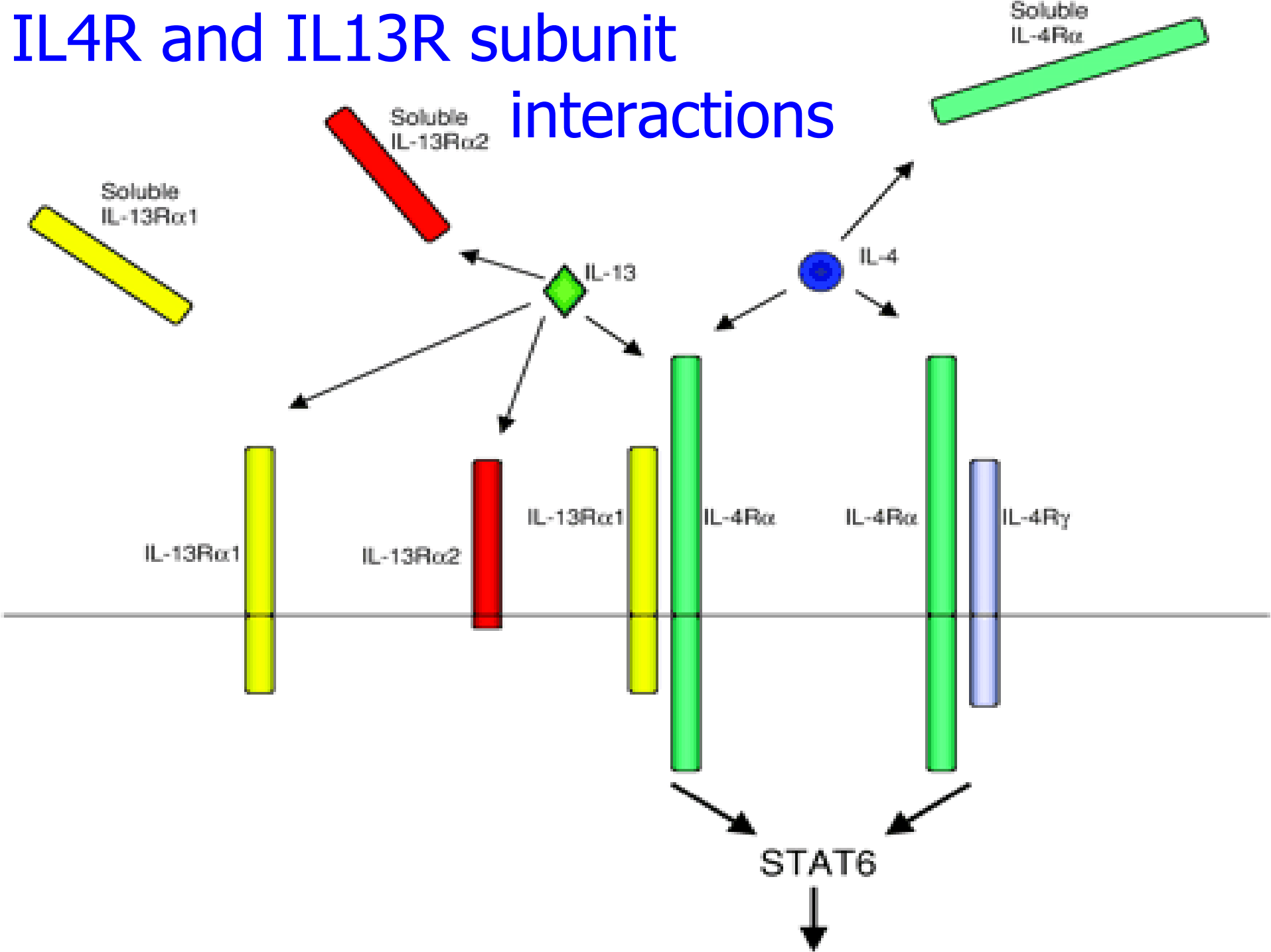
- Similarities
  - Predominantly anti-inflammatory effects
  - Favor Th_2 responses
  - Partially common receptor
  - Promotes Ig class switch
  - Used w/ GM-CSF to elicit moDCs

Shared receptor subunits depend on cell type

- Differences
  - IL-13 activity predominantly on monocyte/mΦ cells
  - IL-13 lacks B, T cell effects

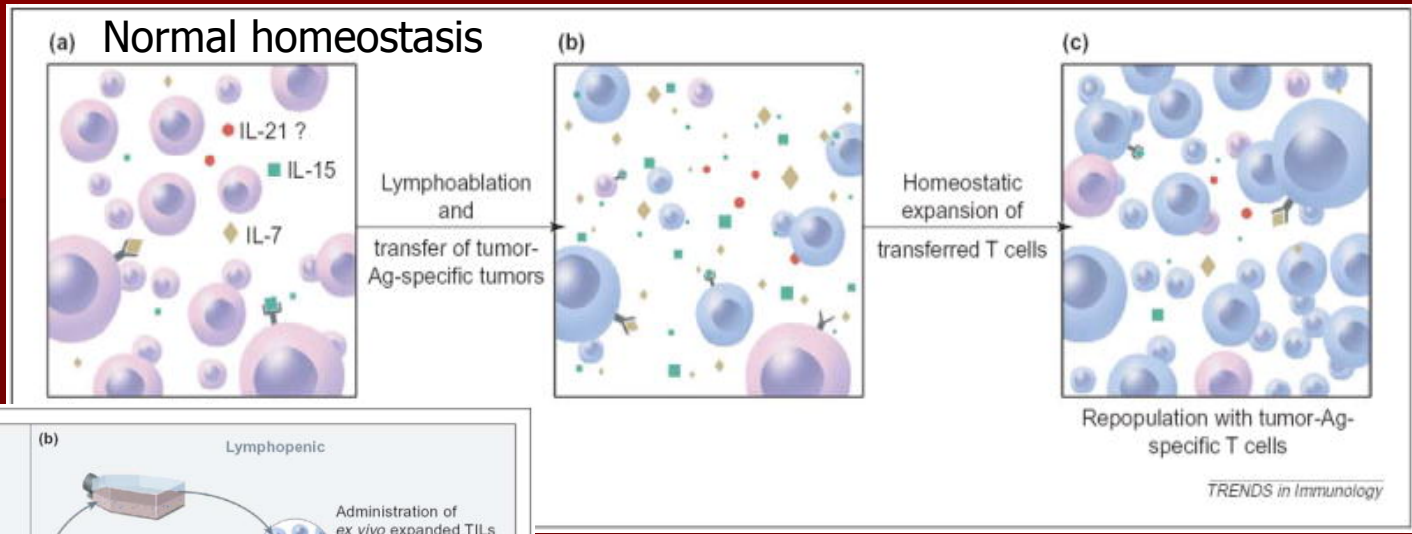
- Most important: IL-13 receptors on tumor cells, especially glioma
  - Immunotoxins under evaluation
  - Chimeric T cell Ag receptor in clinical trials

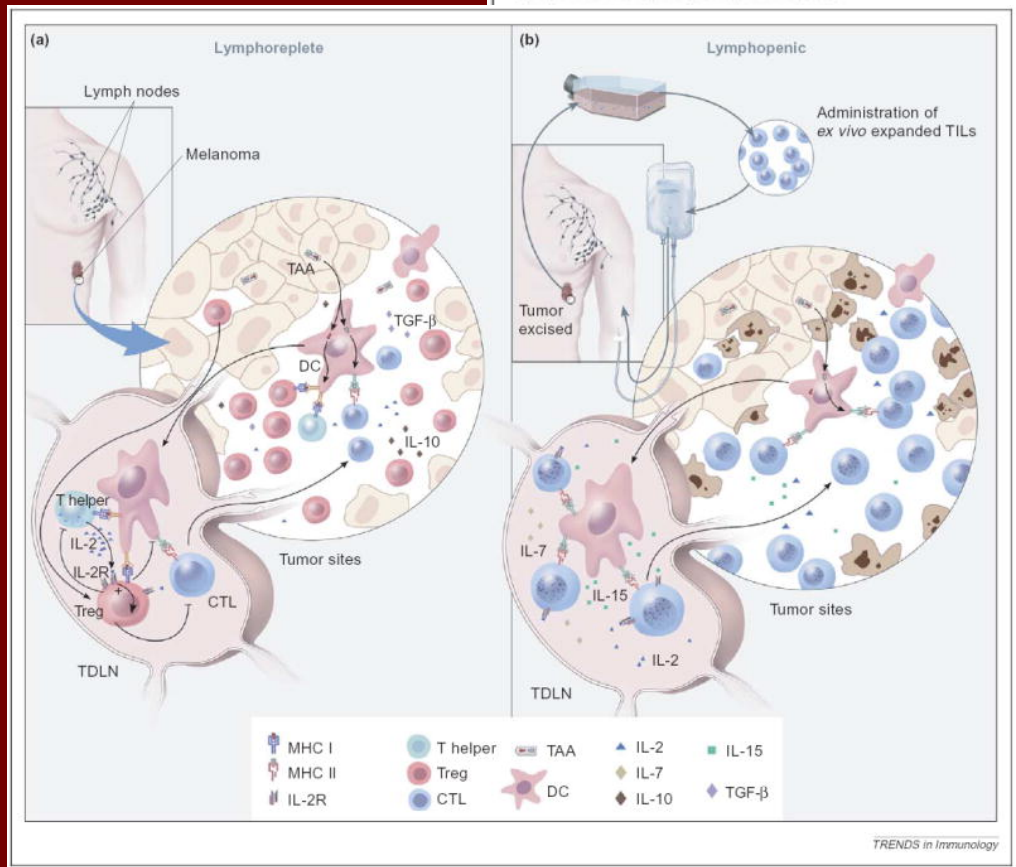
IL4R and IL13R subunit interactions

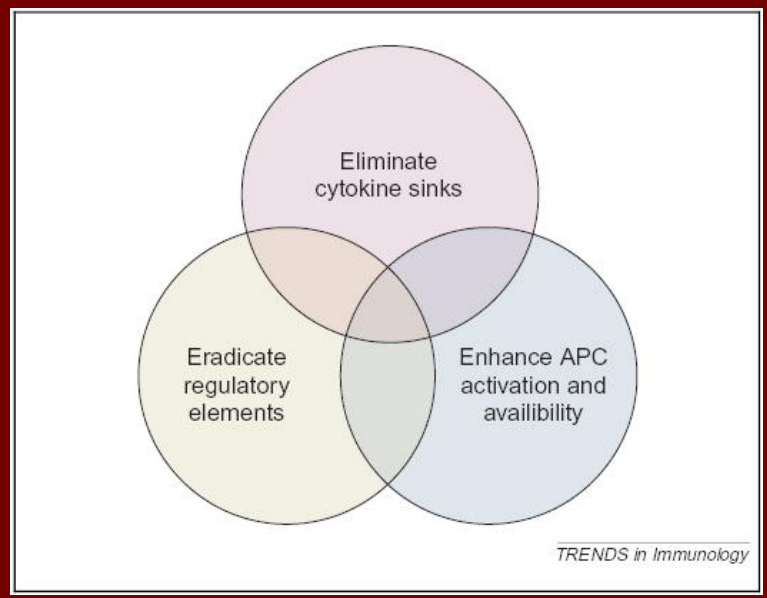


Sinks, suppressors and antigen presenters: how lympho-depletion enhances T cell-mediated tumor immunoRx

Klebanoff, Trends in Immunol (2005)







IL-6

A very pleiotropic cytokine

IL-6

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

Induction of T-Cell Differentiation → T Cell → Cytotoxic T Lymphocyte

Induction of Acute-Phase Reactants → Hepatocytes → C-Reactive Protein

Induction of Nerve Cell Differentiation → Astrocytes → NGF–Like Activity

Induction of B-Cell Differentiation → B Cell → Plasma Cell

Induction of Myeloma-Plasmacytoma Growth → Myeloma Cell → Malignant Transformation

Induction of Leukemic Cell Differentiation → Megakaryocyte → M1 → Macrophages

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

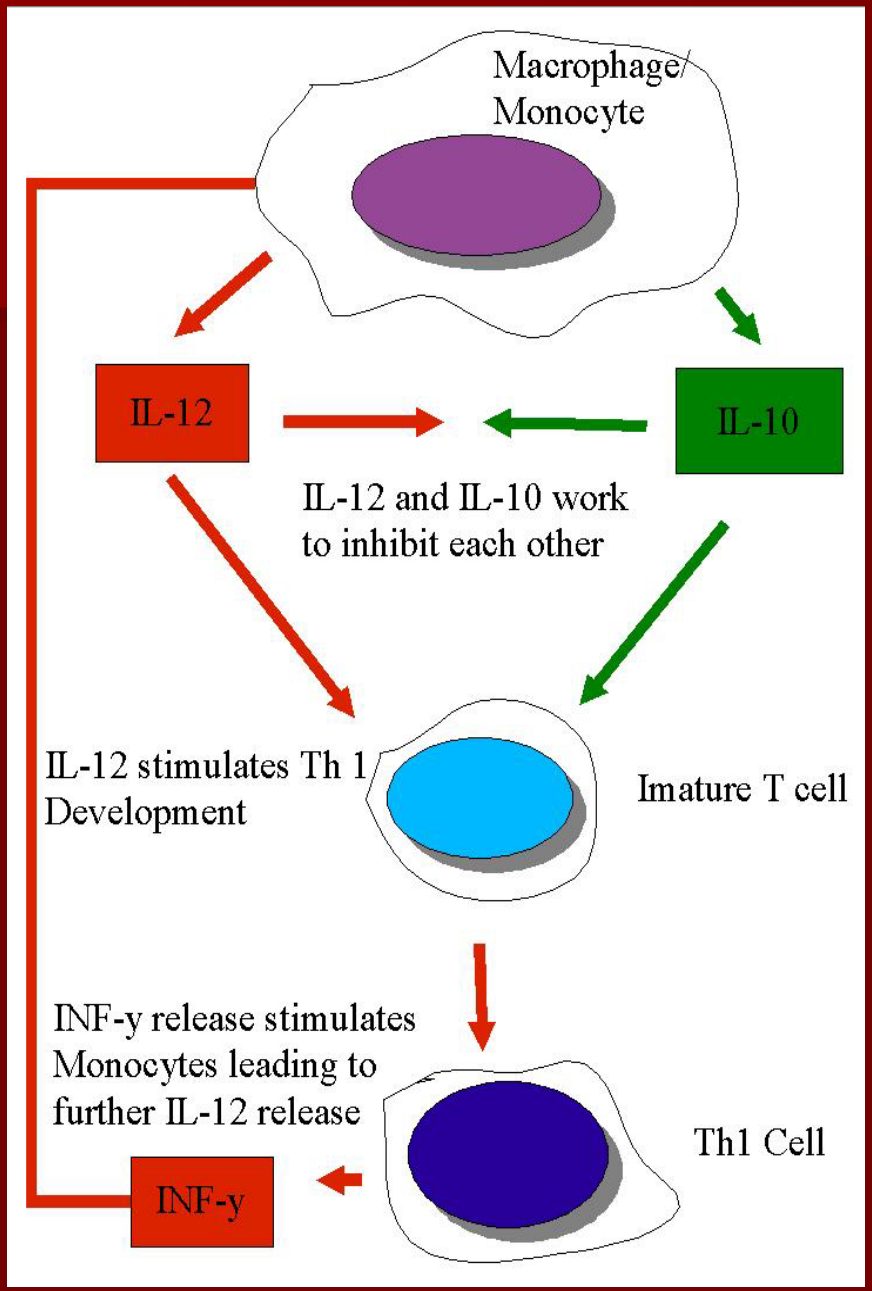
IL-6

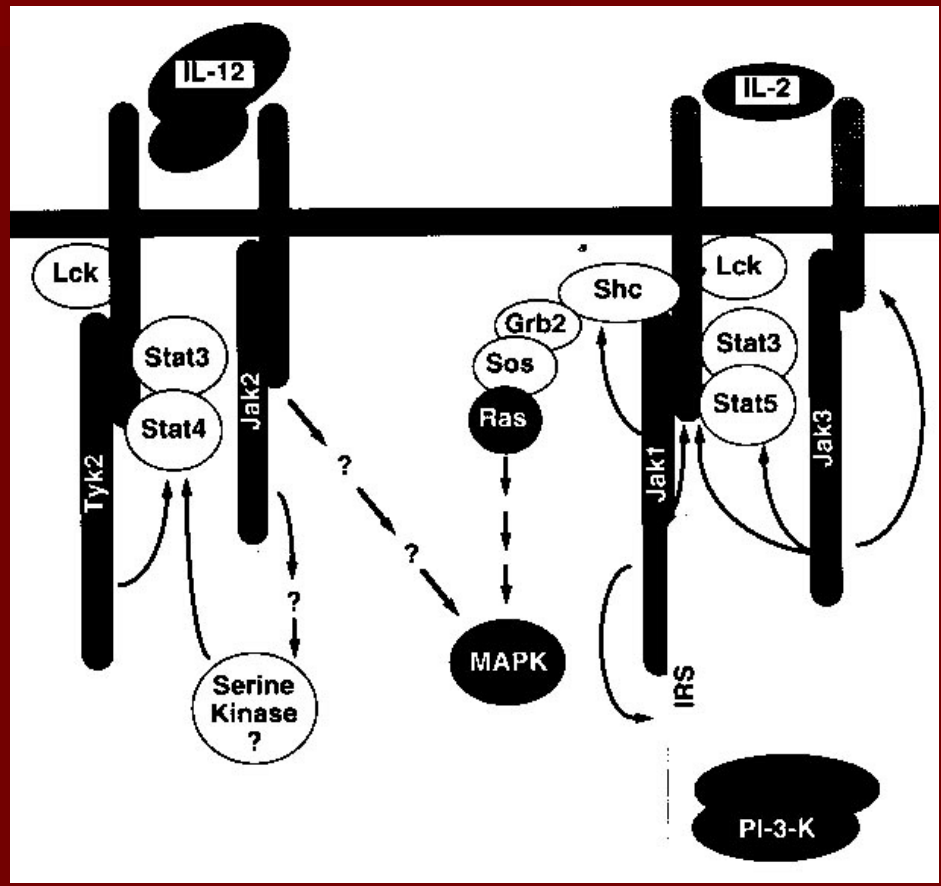
- Tumor source
  - Associated with unfavorable outcome renal CA, melanoma
  - An important growth factor for myeloma
  - Major effector of paraneoplastic thrombocytosis
- Adaptive system
  - B cell growth/differentiation
  - CTL differentiation
  - Type 2 responses
- Preclinical data showed activity 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 trials alone or w/IL-2 based on preclinical, clinical leads

IL-12

- Prototypical type I cytokine, induces IFN-γ
- Link between innate, adaptive immune response
- DC production triggered by variety of stimuli
- Receptors mainly on activated T and NK cells
- Anti-angiogenic activity via IFN induction
- In animals, $\uparrow$ antitumor effects in combo w/other type I cytokine (IL-2)
- Probable role in vaccine development, ? tumor vaccine

IL-12 and its receptor





IL-12 Clinical trial experience

- Subcutaneous dosing in initial trials
  - Lymphopenia, hepatoxicity dose-limiting
  - Fever, headache, fatigue, nausea common
  - Attenuation of toxicities with re-exposure
- i.v. dosing featured test dose
  - Markedly reduced toxicity at similar doses
  - Type II cytokines increased, especially IL-10
  - Type I cytokines decreased, especially IFN-γ
- Phase II i.v. IL-12 without test dose had excess toxicity
- b.i.w. schedule for i.v. IL-12
  - Less attenuation of IFN-γ
  - Clinical activity associated with maintenance of IFN-γ induction
- Combinations w/IFN-α
  - Feasible
  - Therapeutic ratio not favorable

IL-18

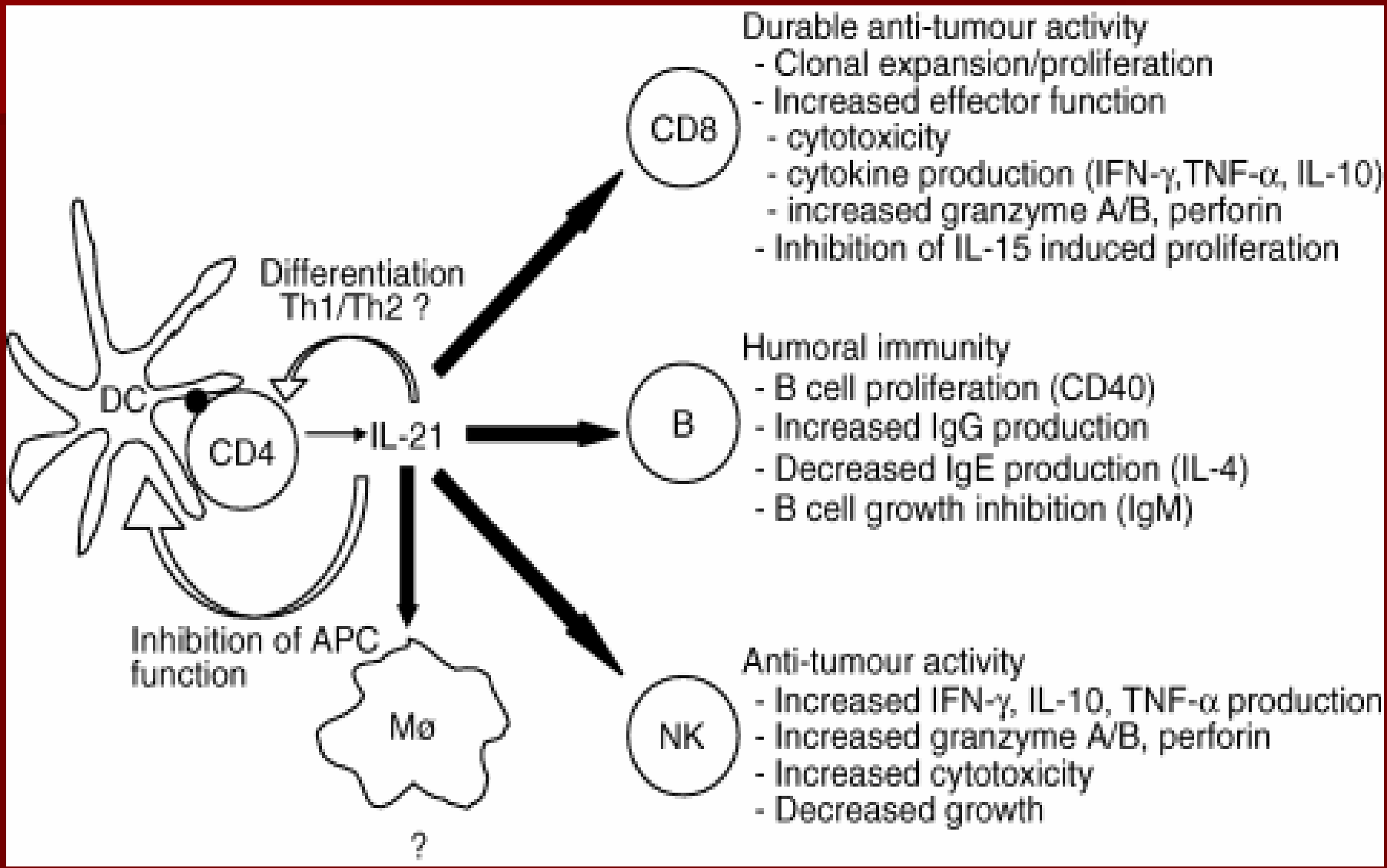
- Member of IL-1 family of cytokines
- Activates NK cells and induces type I cytokines
- Promotes Th1 and memory CD8 T cells
- Upregulates FasL on effector lymphocytes
- Cytotoxicity MOA "complementary" to that of IL-12
  - Not IFN-γ-dependent
  - 18 uses Fas/FasL while 12 uses perforin/granzyme

- Antitumor activity in animals
  - Alone
  - W/IL-2, IL-12

- Phase I DLTs
  - Leukopenia
  - Fever/chills
  - Hepatoxicity

Phase II in melanoma ongoing [caveat: like IL-6, may be GF for selected CAs]

IL-21: another pleiotropic 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

The Corrections: Some of the Lessons Learned

Biological insights→potential new targets

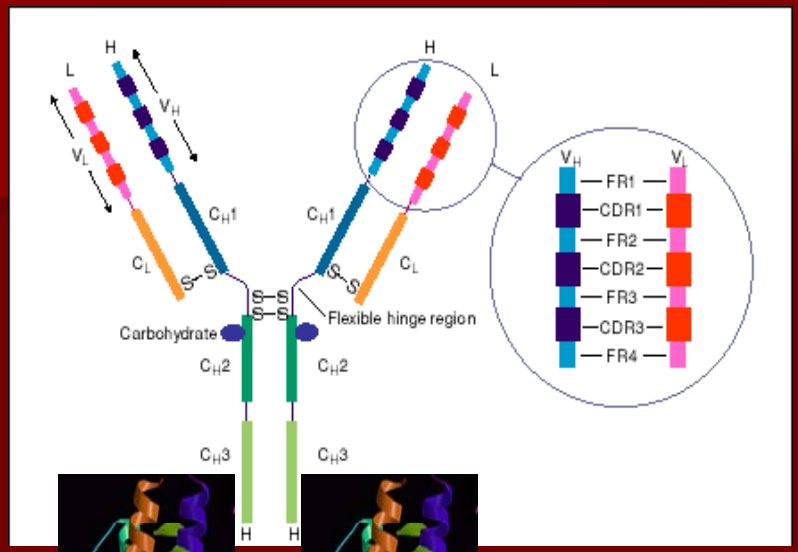
- Activation-induced death of effector T, NK cells
- Intrinsic, acquired tumor resistance mechanisms
- Counterregulatory cytokines, other substances in tumor-effector cell milieu
- CD4+CD25+FoxP3+ regulatory T cells
- Enhanced understanding of effector cell gene expression, polymorphisms

Other strategies based on cytokine structure and biology

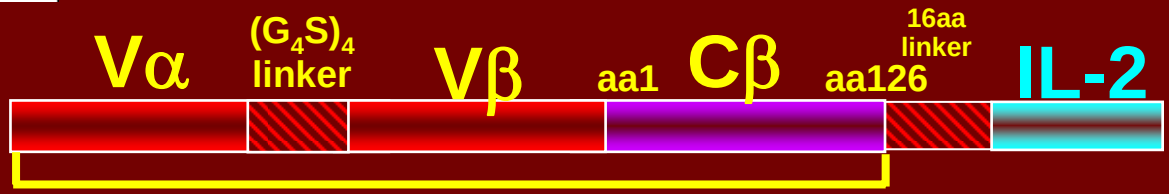
- Immunocytokines: Ab chain fused to cytokine
- Immunotoxins
  - Localized toxin delivery similar to Ab-based Rxs
  - Cytokine-receptor targeting e.g. Ontak (IL-2-diptheria)
- Novel T cell-receptor engineering
  - Xgenic TCR$\rightarrow$IL-13 recognizing IL13R on glioma
  - Immunofusions: Ag-specific TCR fused to cytokine

Immunocytokine structure

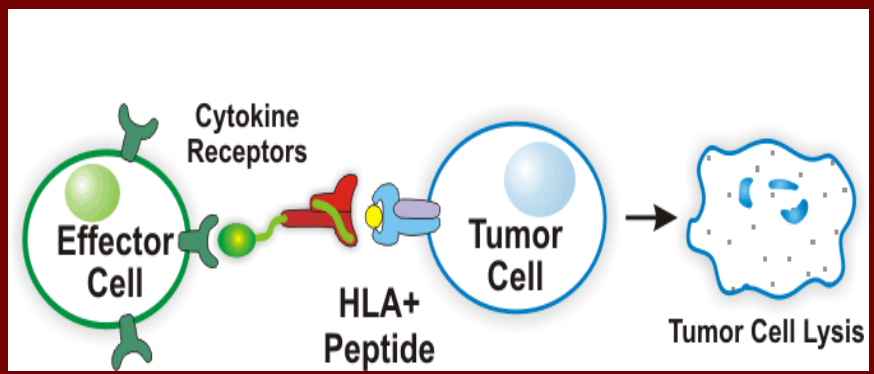
well, sort of…

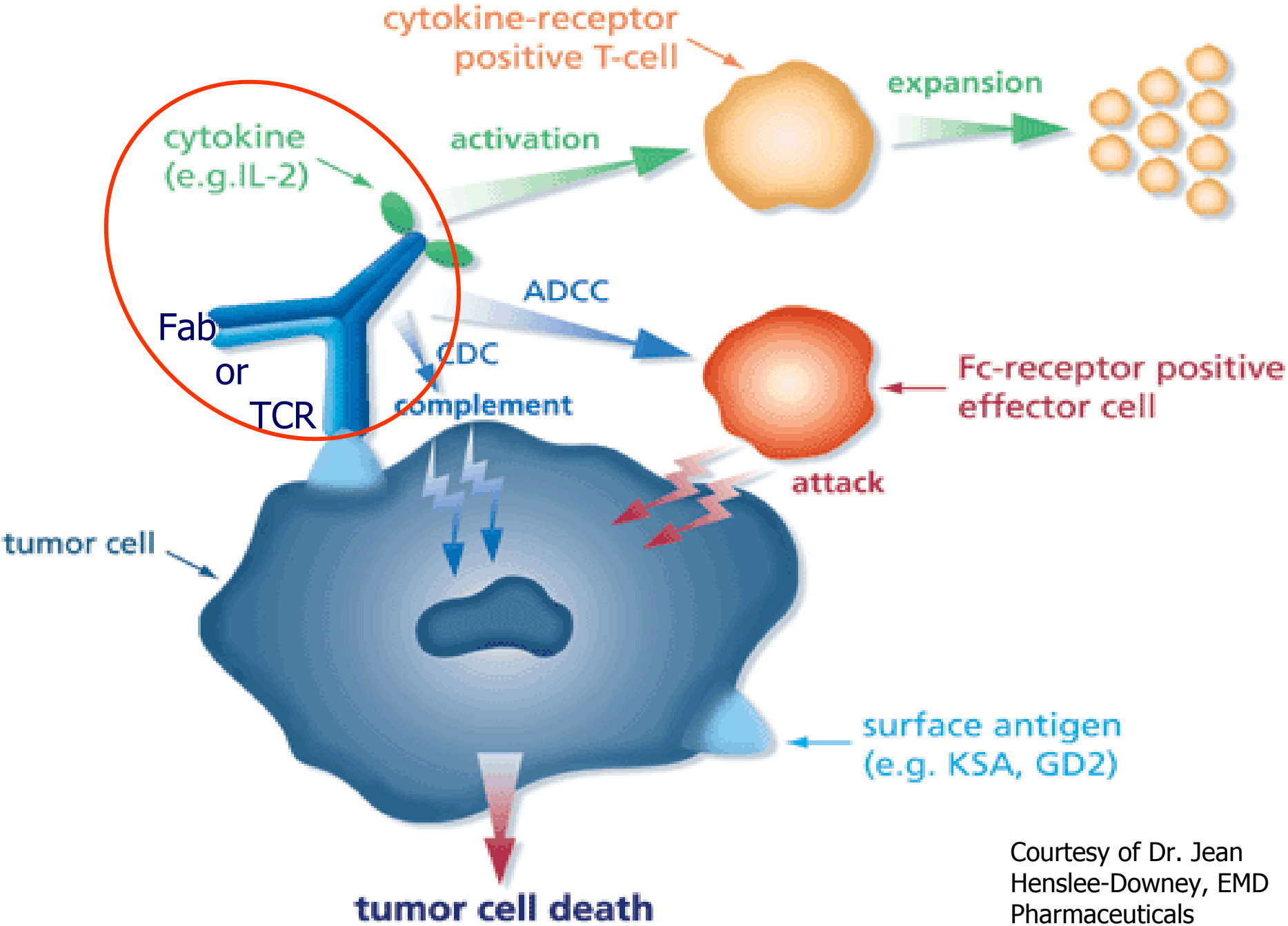


Immunofusion TCR-IL-2



Single chain TCR (264scTCR)





Courtesy of Dr. Jean Henslee-Downey, EMD Pharmaceuticals

If only it were so easy…

Cytokine Therapy report card

- Points taken off for
  - Empiric approach
  - "Me-too" research and development designs
- Extra credit given for
  - Incorporation of cytokine biology into novel structure, strategies
  - Recent exploration of mechanisms of resistance, predictive factors and selection strategies

- Overall grade B+

Thank you

Any questions?