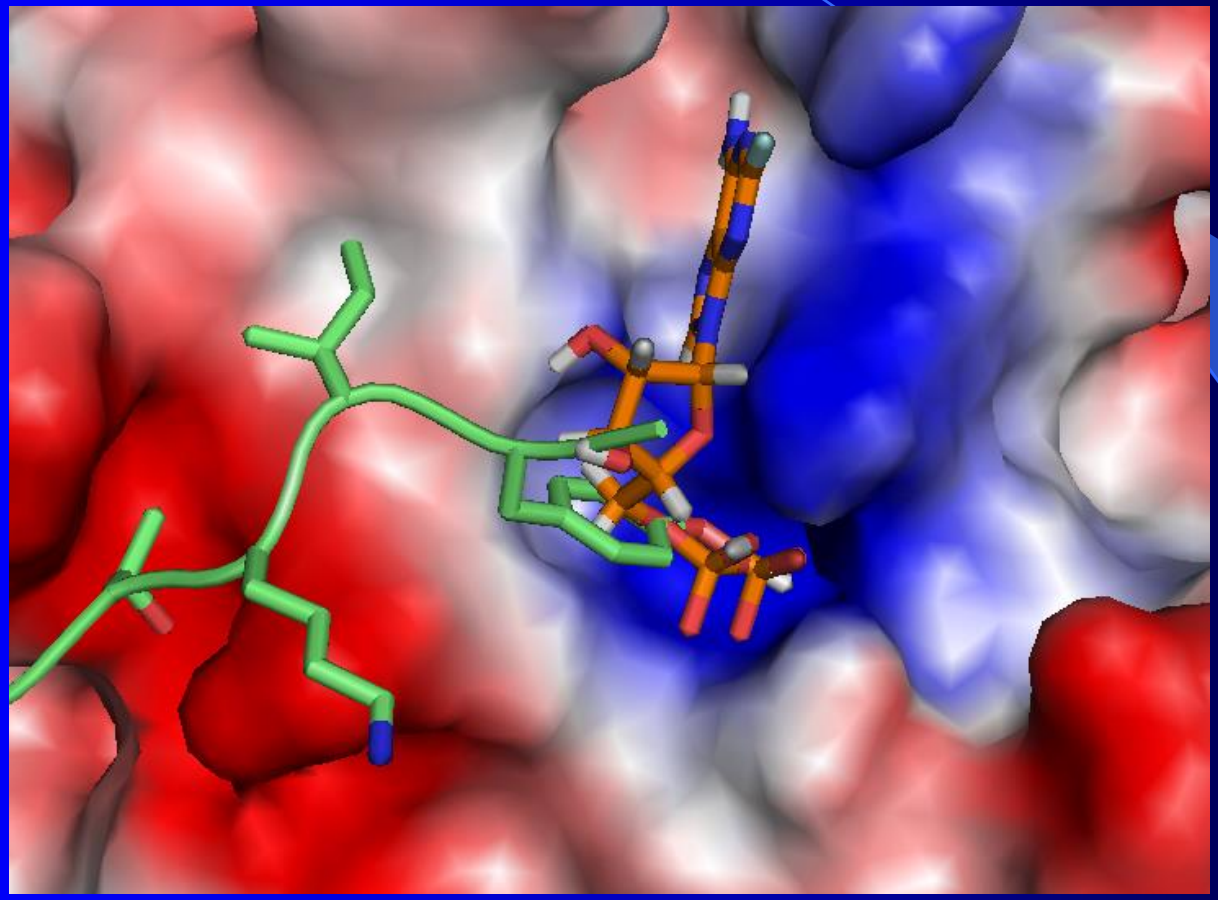
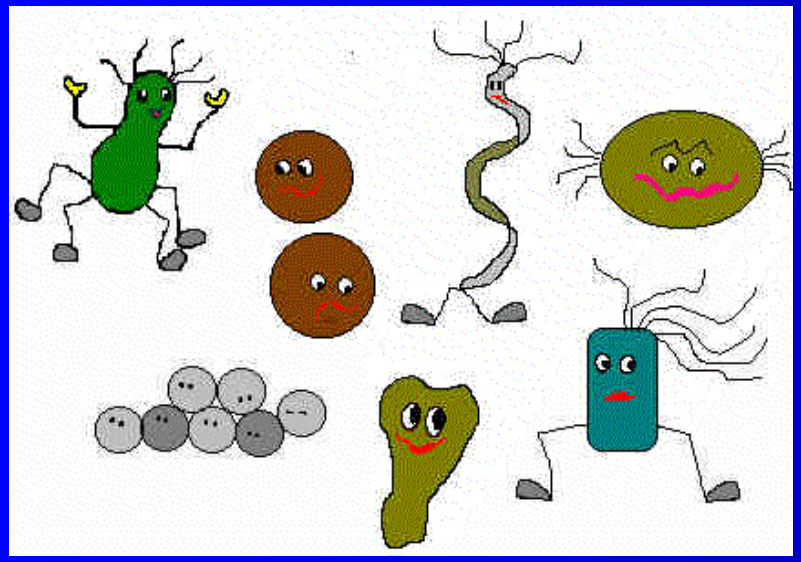
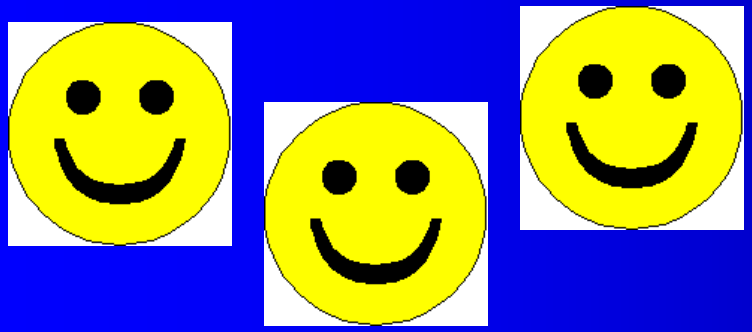
Immune Therapy



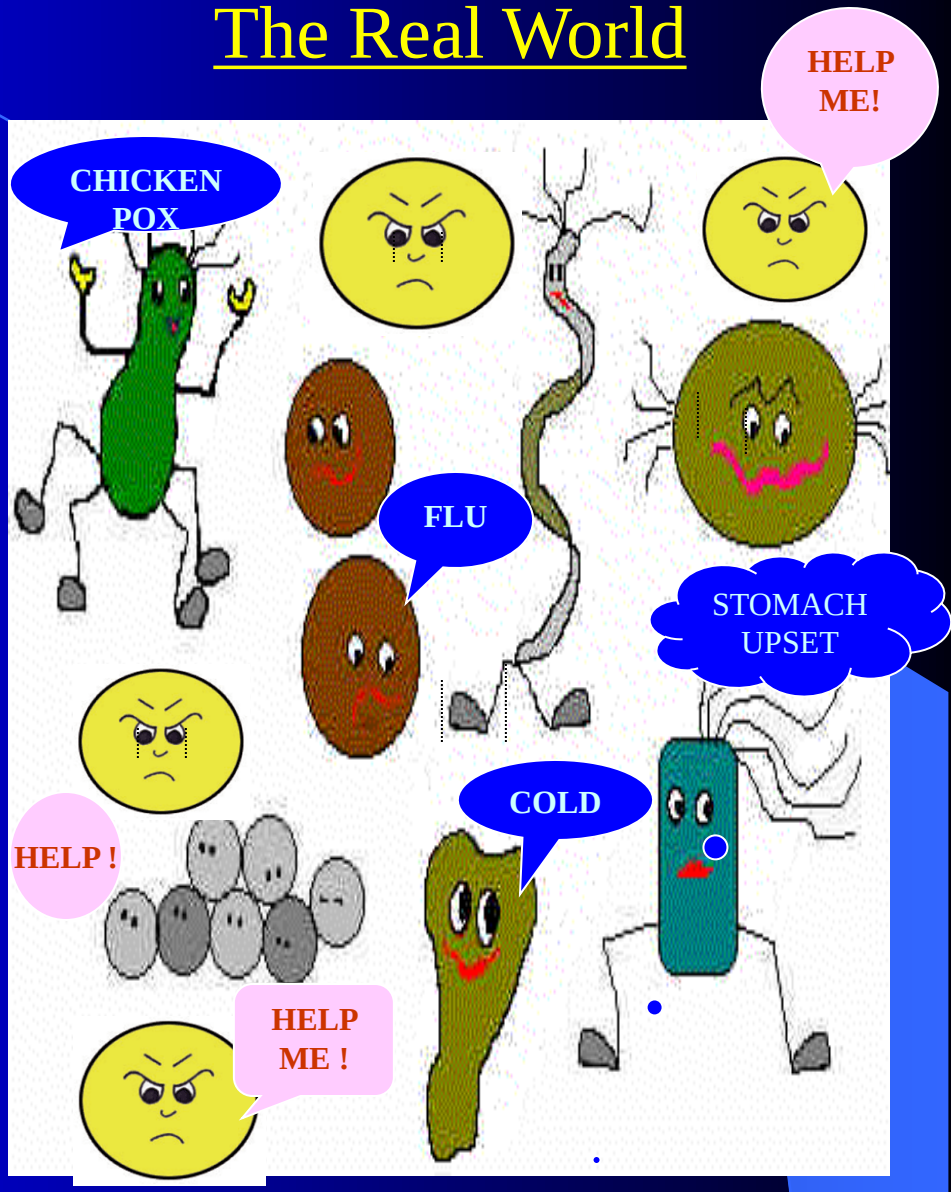
Hans Bluyssen, 20-10-2021

The Perfect World





The Real World



Immune System

The Latin term *"IMMUNIS"* means EXEMPT, referring to protection against foreign agents.

DEFINITION: - The integrated body system of organs, tissues, cells & cell products that differentiates self from non – self & neutralizes potentially pathogenic organisms.

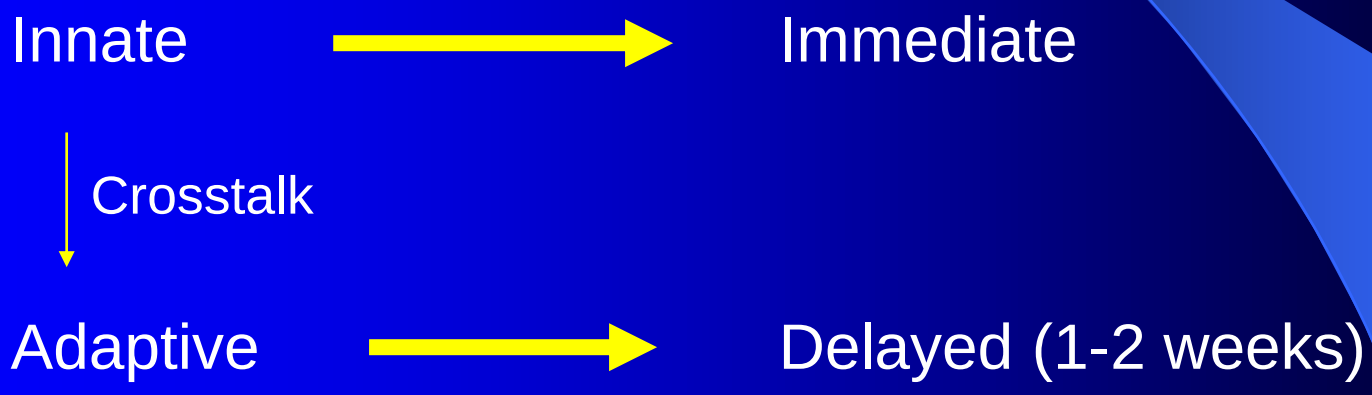
(The American Heritage Stedman's Medical Dictionary)

Errors in this recognition lead to autoimmune diseases, like type 1 diabetes, arthritis.

FLU

Timeframe?

What happens when a virus infects a host?



Time Course for Induction of Antiviral Response

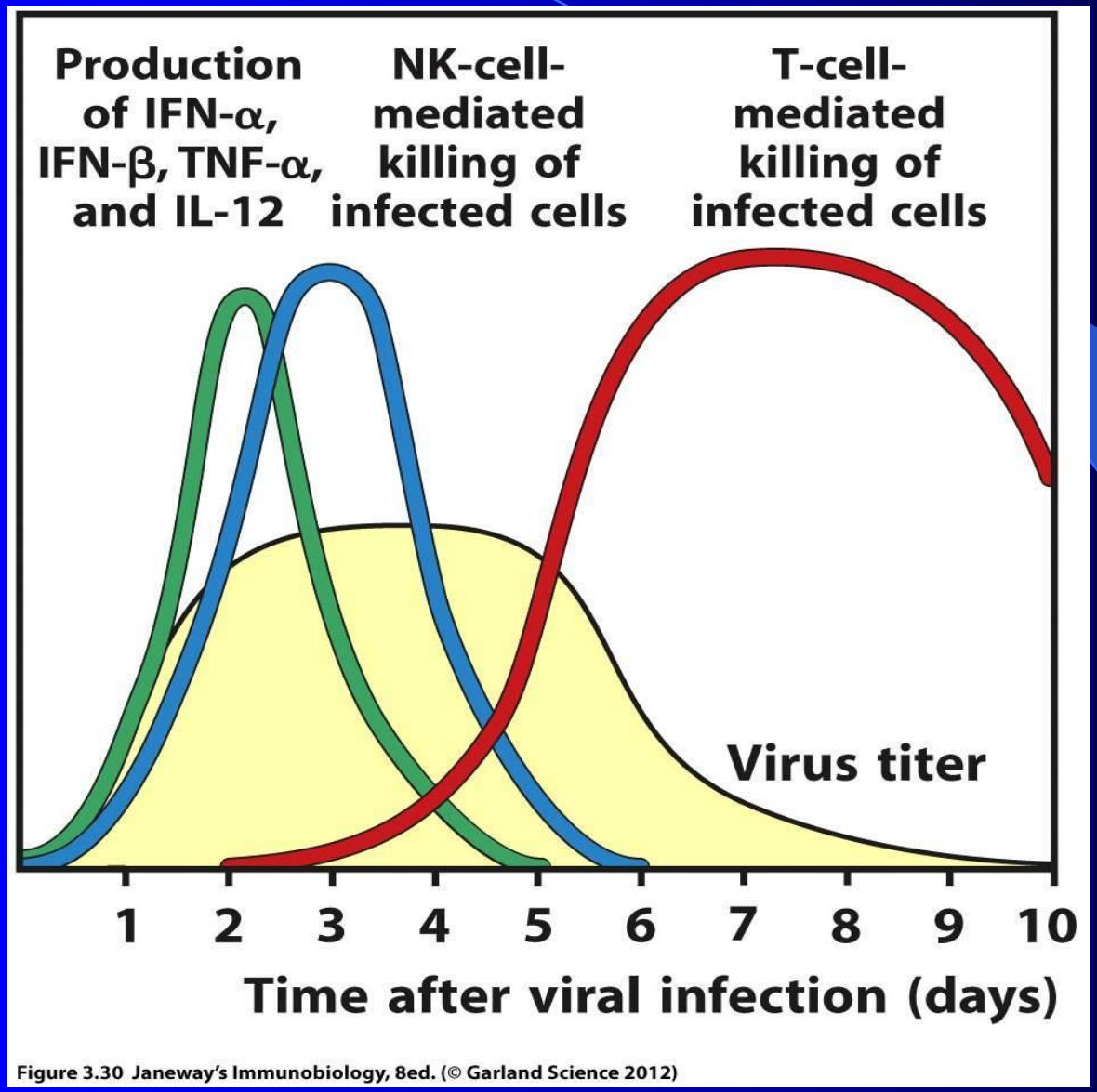


Figure 3.30 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Antiviral

Vaccine

Recognition of viral infection

How does a cell know it's infected
and what can it do about it?

Toll Is Required for Antifungal Response in Drosophila

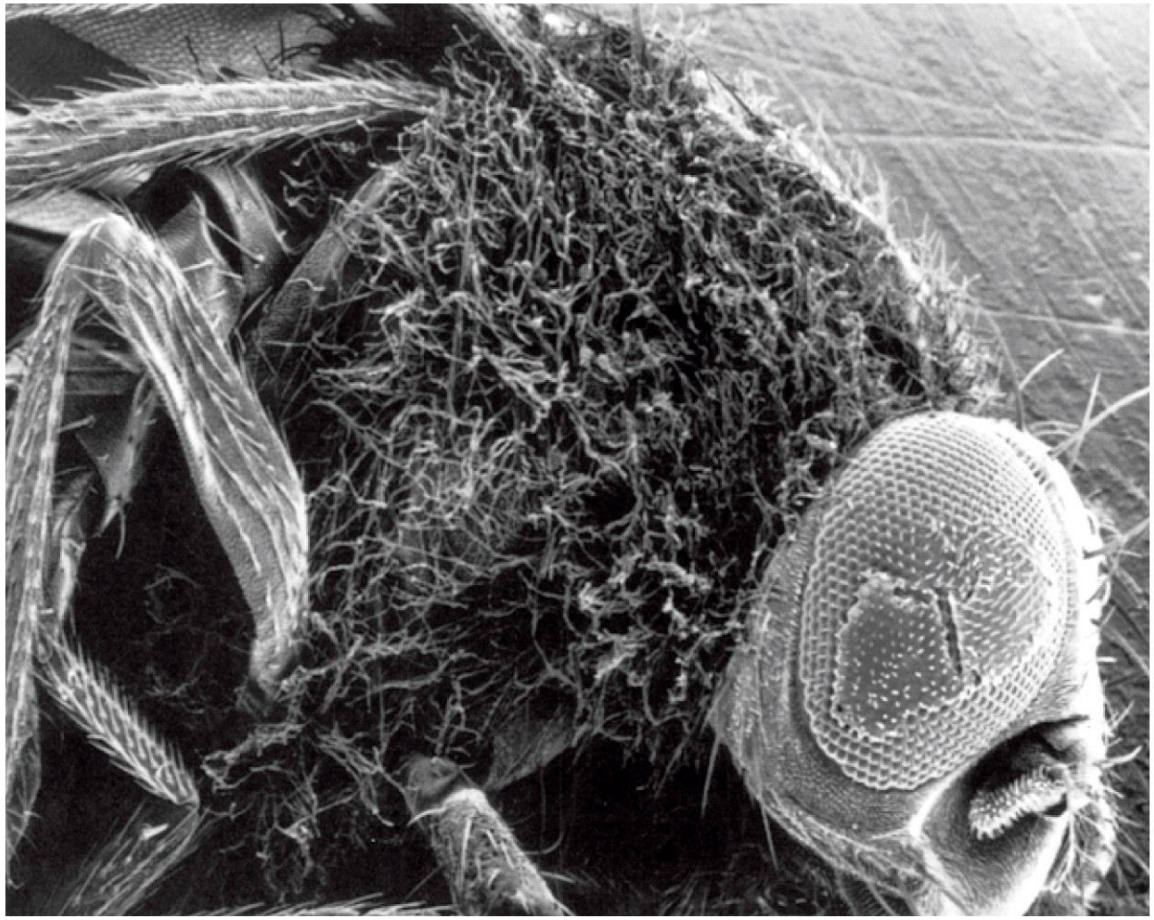


Figure 3.8 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Jules Hoffmann provided the first evidence that Toll receptors mediate immune defense and received Nobel Prize in 2011

How are pathogens recognized?

i.e. what turns on innate responses?

letters to nature

Nature **413**, 732 - 738 (2001) © Macmillan Publishers Ltd.

Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3

LENA ALEXOPOULOU*, AGNIESZKA CZOPIK HOLT*†, RUSLAN MEDZHITOV*‡§ & RICHARD A. FLAVELL*‡§

* Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut, 06520, USA
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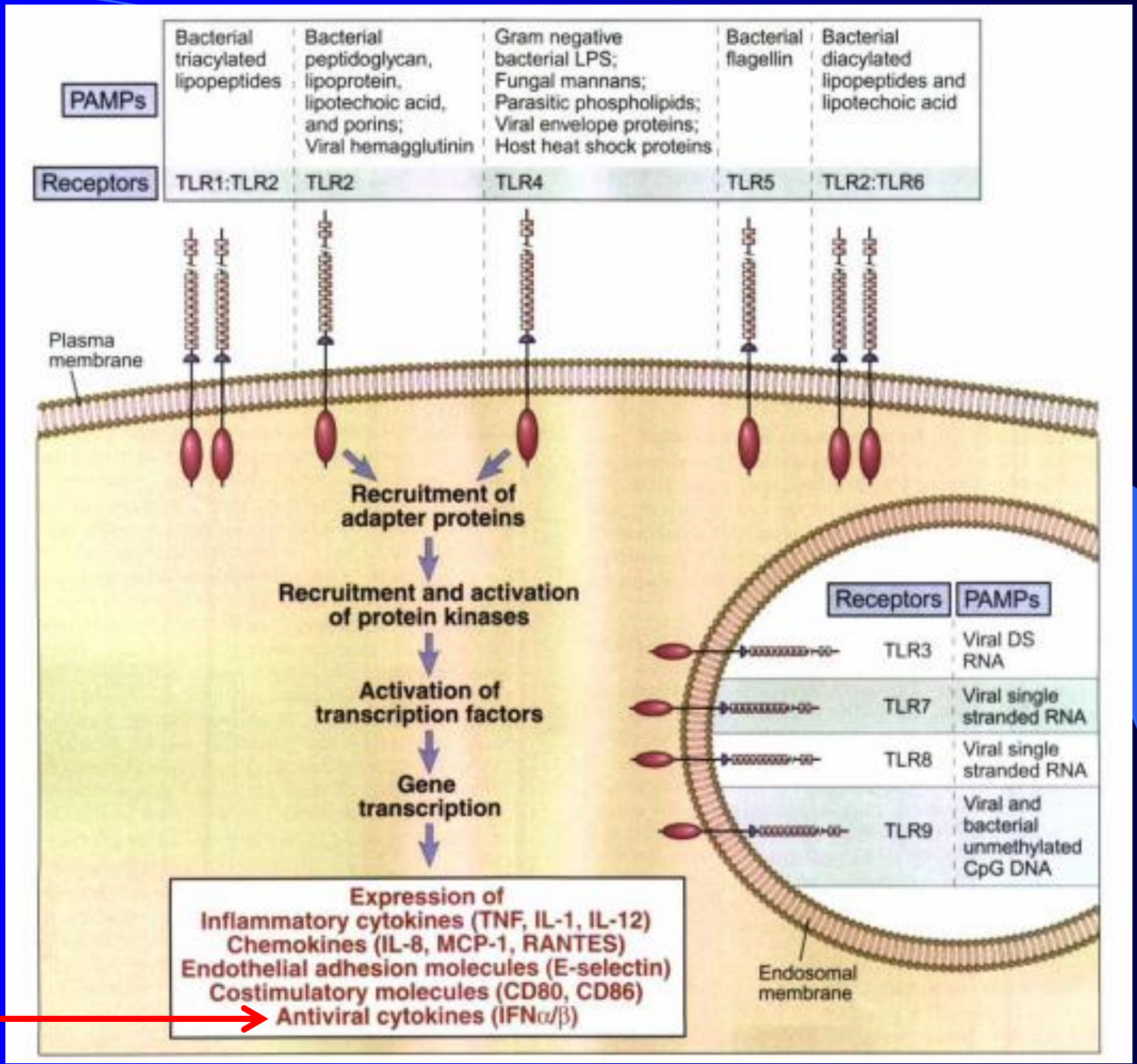
§ These authors contributed equally to the work

Correspondence and requests for material should be addressed to R.A.F. (e-mail: richard.flavell@yale.edu) or R.M. (e-mail: ruslan@yale.edu). The murine *TLR3* sequence has been deposited in GenBank under accession number AF420279.

Toll-like receptors (TLRs)

- pattern recognition receptors recognize pathogen associated molecular patterns (PAMPs)
- Can identify a foreign invader (virus, bacterial, etc) via a conserved microbial product and initiate the innate response
- 13 identified in mammals


PAMPs
Receptors
Bacterial triacylated lipopeptides
Bacterial peptidoglycan, lipoprotein, lipotechoic acid, and porins; Viral hemagglutinin
Gram negative bacterial LPS; Fungal mannans; Parasitic phospholipids; Viral envelope proteins; Host heat shock proteins
Bacterial flagellin
Bacterial diacylated lipopeptides and lipotechoic acid
TLR1:TLR2
TLR2
TLR4
TLR5
TLR2:TLR6
Plasma membrane
Recruitment of adapter proteins
Recruitment and activation of protein kinases
Activation of transcription factors
Gene transcription
Expression of
Inflammatory cytokines (TNF, IL-1, IL-12)
Chemokines (IL-8, MCP-1, RANTES)
Endothelial adhesion molecules (E-selectin)
Costimulatory molecules (CD80, CD86)
Antiviral cytokines (IFNα/β)
Receptors
PAMPs
TLR3
Viral DS RNA
TLR7
Viral single stranded RNA
TLR8
Viral single stranded RNA
TLR9
Viral and bacterial unmethylated CpG DNA
Endosomal membrane

Interferon

"Interferons are protein components of animal cells which are synthesized and excreted under a variety of stimuli and make other cells of the same species incapable of replicating virus".

DeSomer and Cocito 1968

Interferons

Type I IFNs:

- IFN-α (12 sub-types) and IFN-β
- are induced by viral infection of any cell type

Type II IFN:

- IFN-γ
- is induced by NK cells and macrophages and when T helper lymphocytes are stimulated to replicate and divide after binding a foreign antigen

Type III IFN:

- IFN-λ1, IFN-λ2 and IFN-λ3

What induces Type I IFNs?

- Something in viruses
  - Influenza virus - heat and UV treated
  - DNA viruses inactivated
    normal

dsRNA is best activator of IFN genes

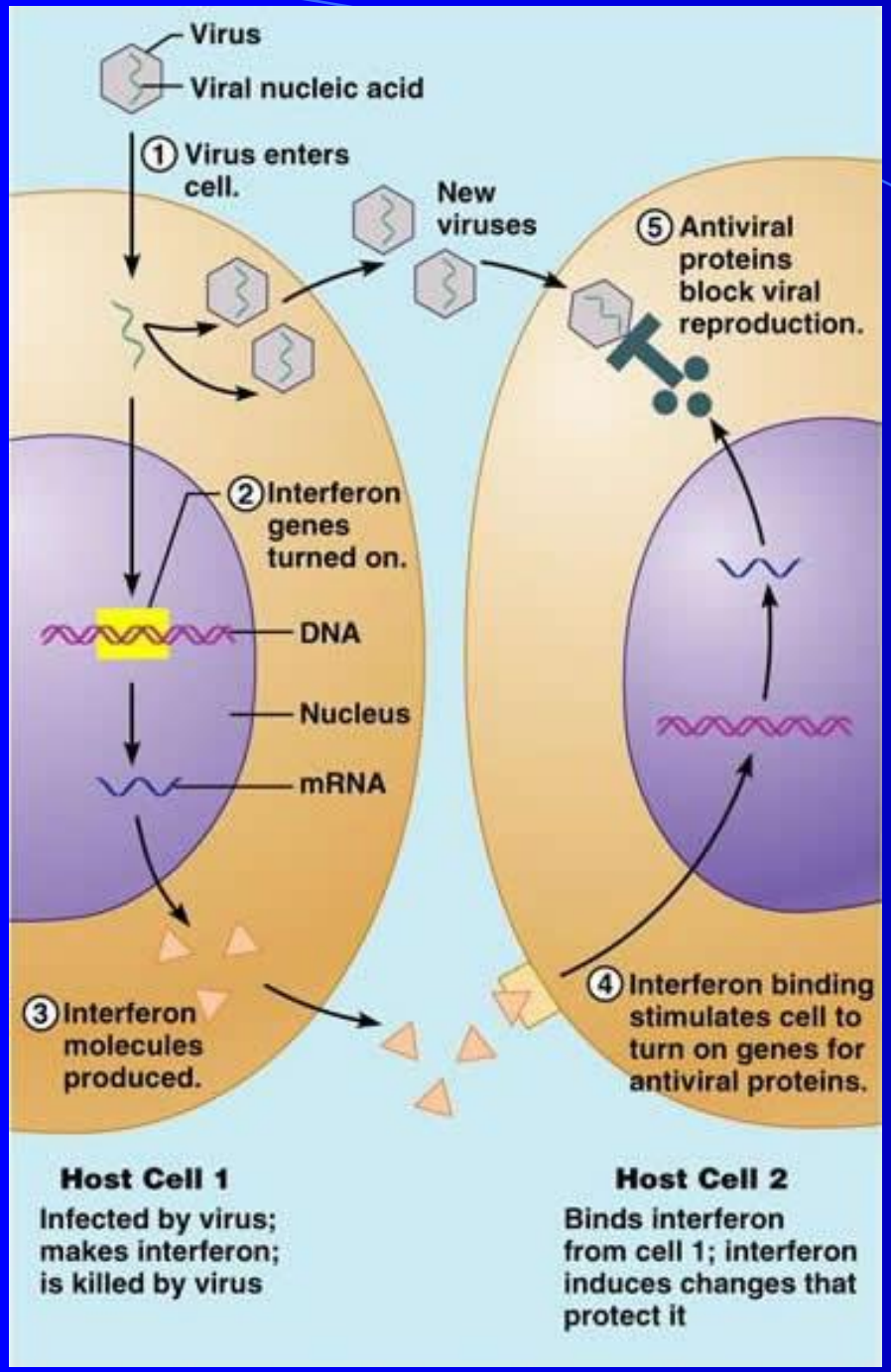
IFN is induced by many other substances

- viruses DNA (active) and RNA (active and inactive)
- bacteria (esp. gram-negative)
- live/killed mycoplasma
- protozoa
- nucleic acids esp. dsRNA

→ Toll like receptors

CPRR

Type I IFN Production & action

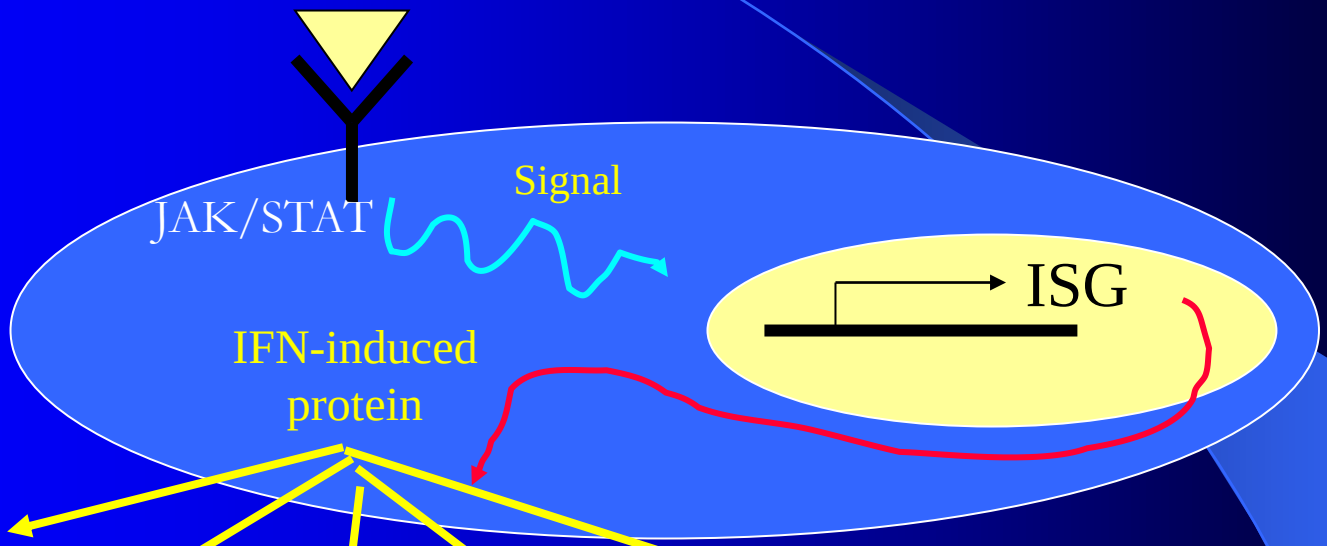


IFN

- Inhibition viral replication
- Inhibition cell growth
- Activation immune system

Anti-viral State
Adaptive immune response

Biological Activities of IFN



Apoptosis

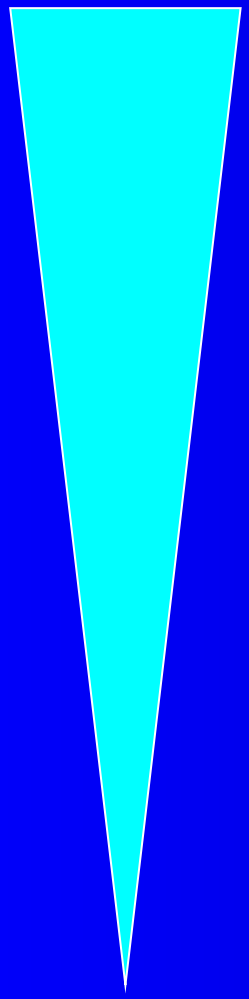
Inhibition of
viral replication

Inhibition of
Cell Growth

Regulation of
Cell differentiation

Activation of
Immune System

Virus Sensitivity to IFNs



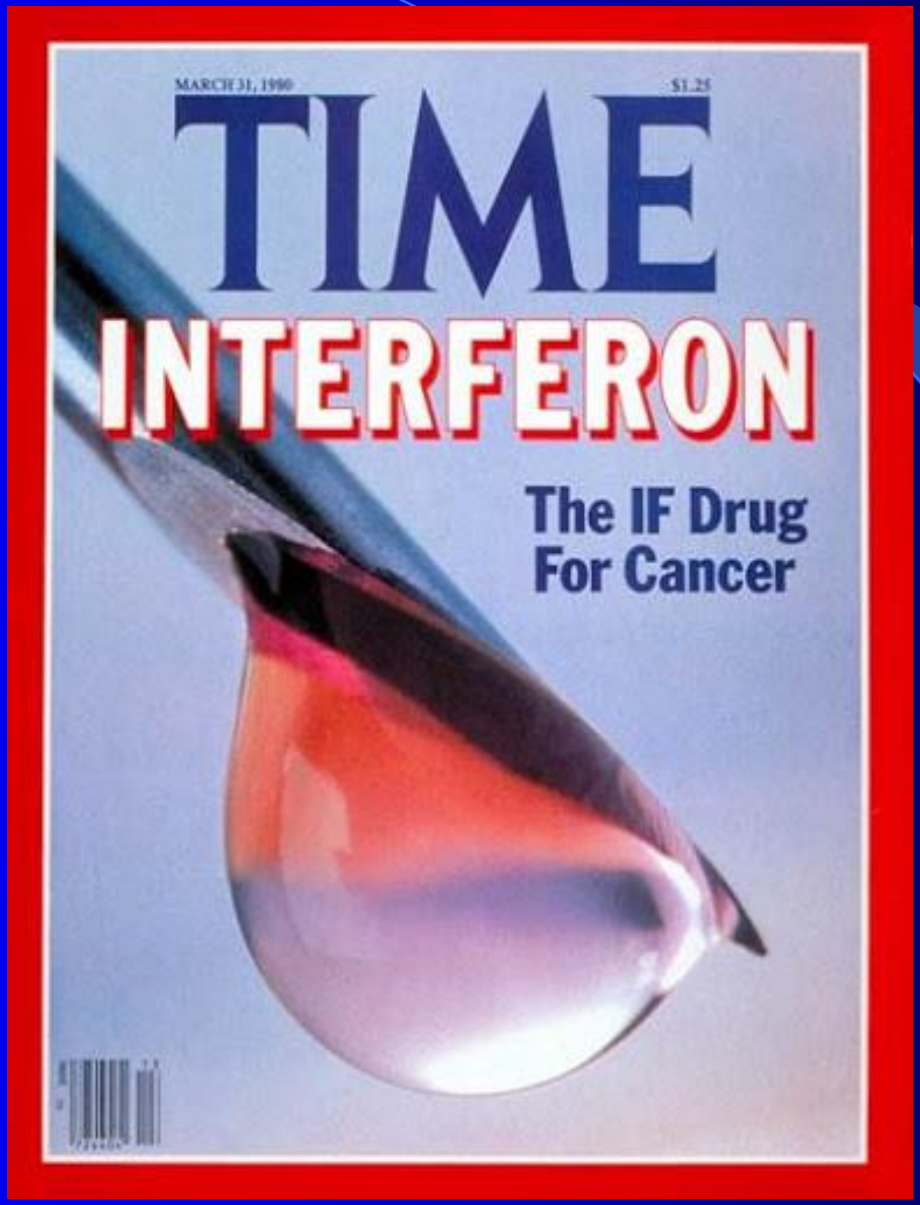
Small RNA viruses - picornaviruses

Large RNA viruses - Flu, rotovirus

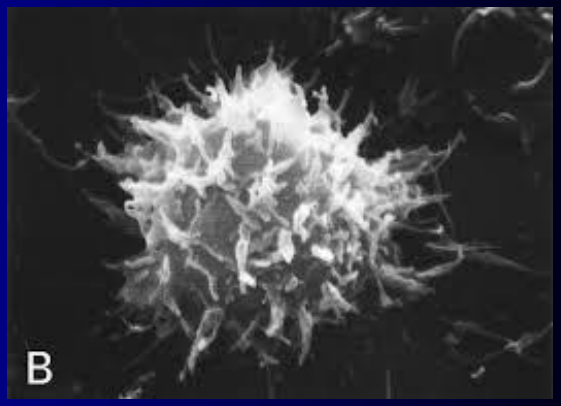
Small DNA viruses - papillomavirus

Large DNA viruses - Herpes, poxvirus

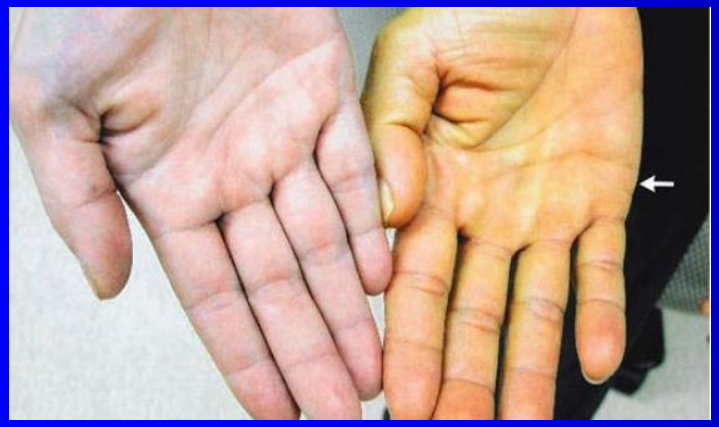
1980: IFN "Golden Bullet"



Clinical Use of IFNs



- Viral Infections
  - Hepatitis B and C
  - HPV warts
  - RSV



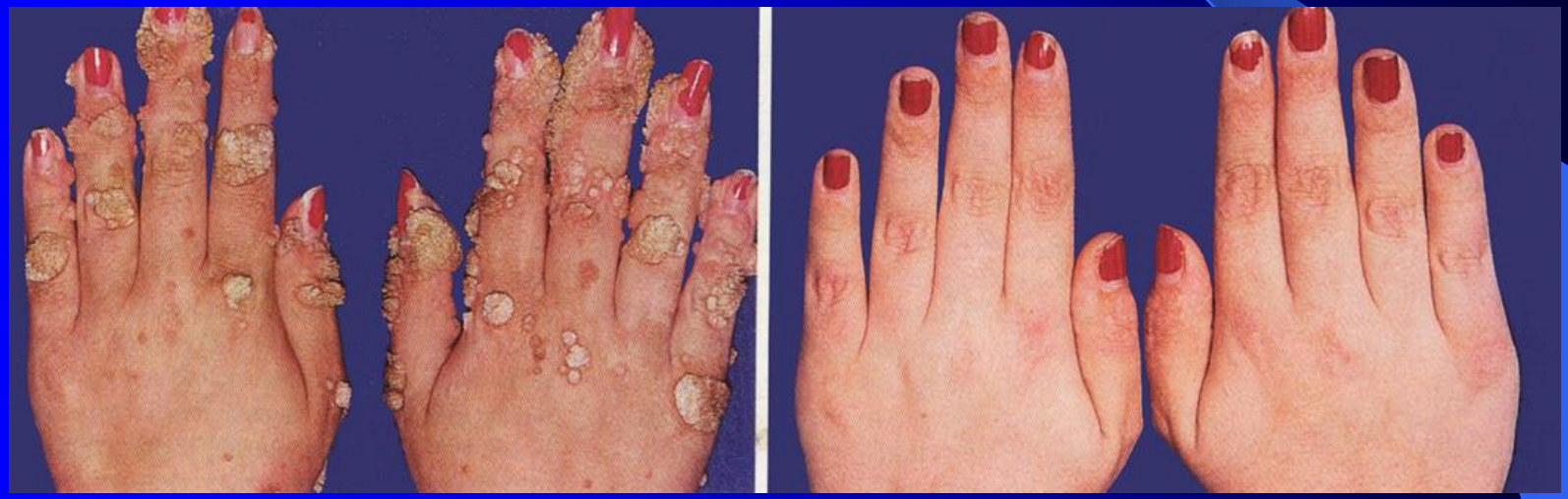
- Cancer
  - Hairy cell Leukemia (90% effective)
  - Follicular lymphoma
  - cervical (HPV)
  - basal cell cancer (80-90%)
  - Kaposi's sarcoma (HHV type 8)

- Other conditions
  - chronic granulomatous disease (IFN-γ)
  - multiple sclerosis
  - inflammatory bowel diease

IFN Therapy

Before

IFN therapy

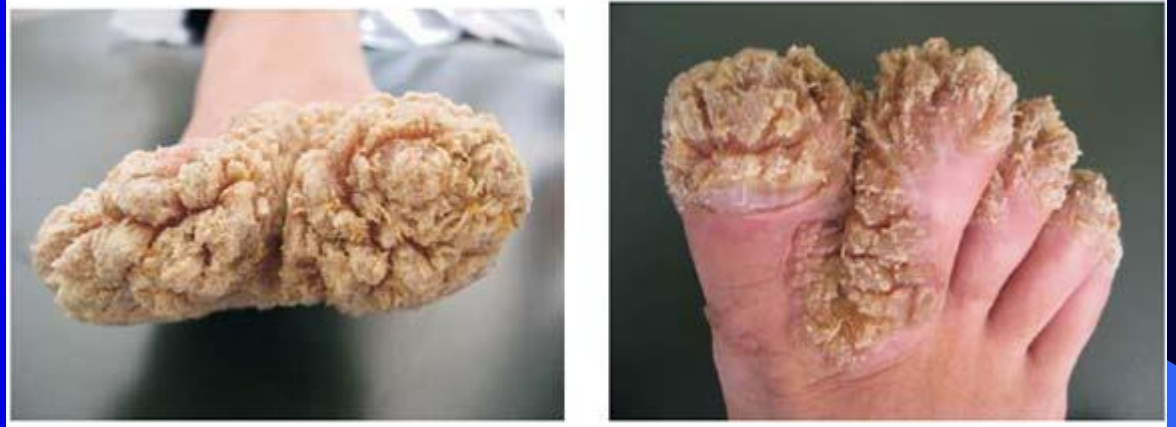


Human papillomavirus warts

Treatment of human papillomavirus with peg-interferon alfa-2b and ribavirin

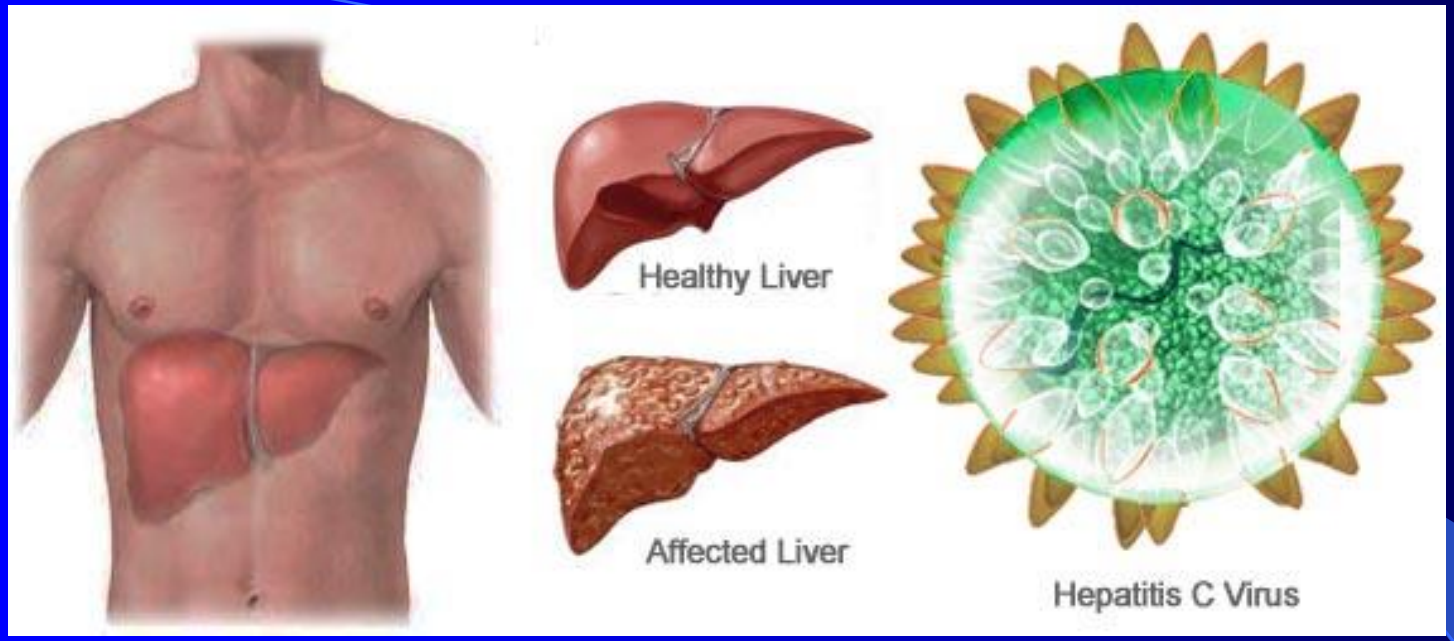
Before

Figure 1. Right foot lesions before treatment.



After

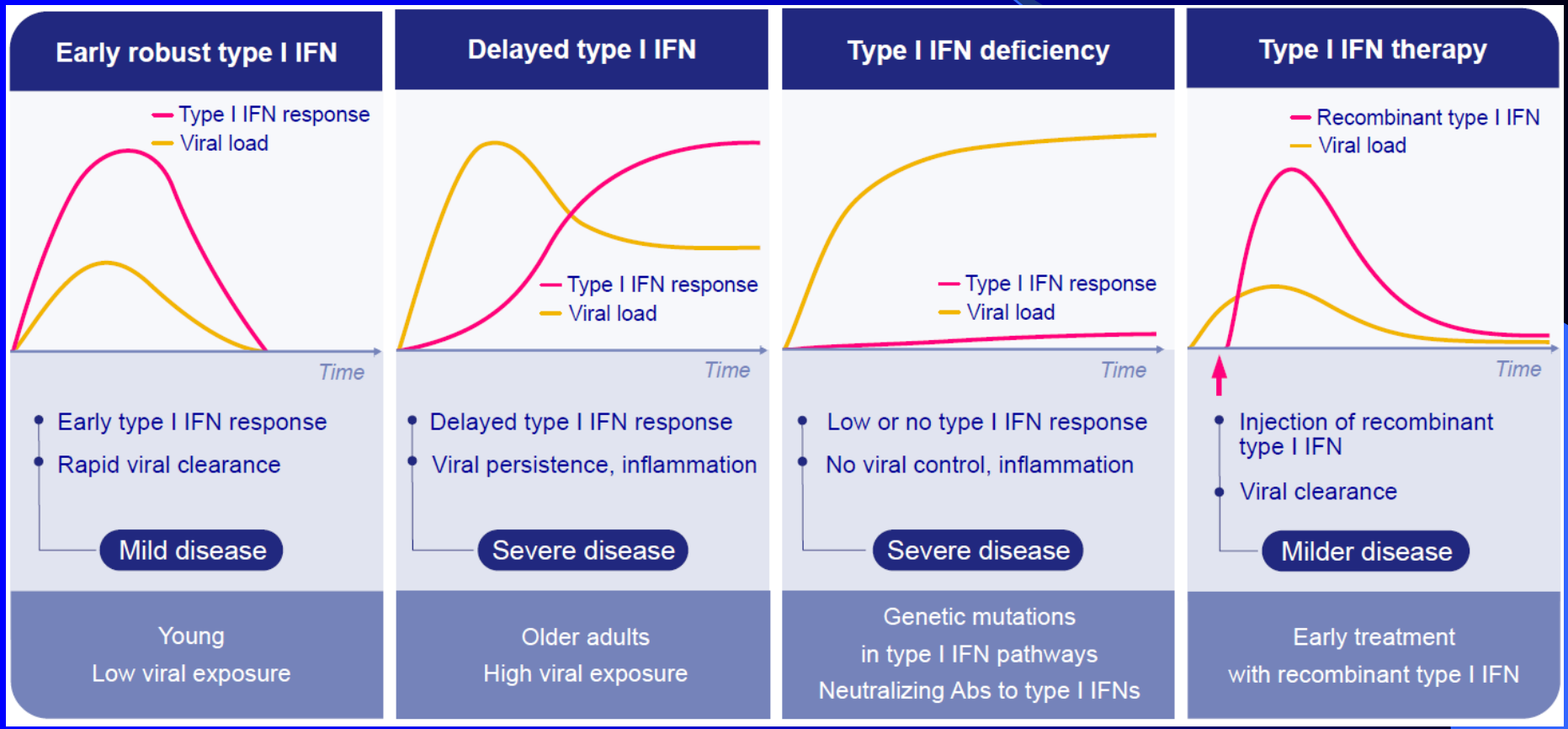
Figure 2. Right foot without lesions seven months after treatment with peg-interferon alfa-2b and ribavirin.



HCV infection Treatment

Therapy	Trade name (manufacturer)
•Interferon alfa-2b	•Intron A (Schering-Plough)
•Interferon alfa-2a	•Roferon (Roche)
•Interferon alfacon-1	•Infergen (?Amgen)
•Interferon alfa-2b plus Ribavirin	•Rebetron (Schering-Plough)
•Pegylated Interferon alfa-2a	•Pegasys (Roche)
•Pegylated Interferon alfa-2b	•PEG-Intron (Schering-Plough)

Covid-19:
IFN-I & Clinical Symptoms



NEWS

Inhaled interferon beta therapy shows promise in COVID-19 trial

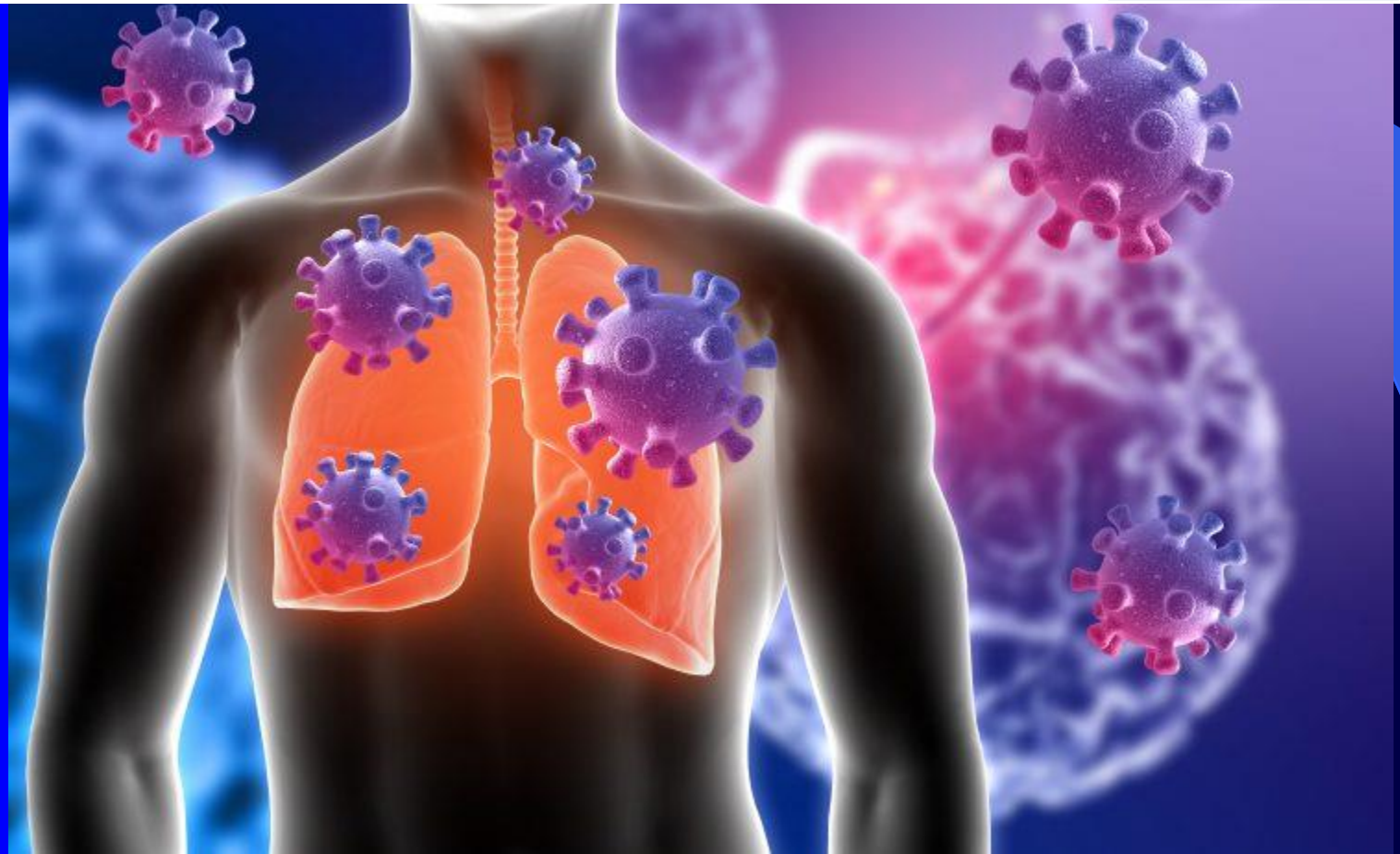
SNG001 diminished the risk of COVID-19 patients developing severe symptoms, reduced breathlessness and improved recovery rates.

By Hannah Balfour (European Pharmaceutical Review)

20 July 2020

No comments yet

SHARES



NEWS

Inhaled interferon beta therapy shows promise in COVID-19 trial

Synairgen, a company based in Southampton, UK, has announced positive results from a clinical trial of SNG001 in hospitalised COVID-19 patients. SNG001 is an inhaled formulation of interferon beta.

According to the study, the risk of developing severe COVID-19 symptoms that required ventilation or caused death during the treatment period of 16 days was reduced by 79 percent for patients receiving SNG001 compared to those who received placebo.

The company also reported that patients who received SNG001 were more than twice as likely to recover within the course of the treatment period compared to those receiving placebo. The measure of breathlessness was “markedly reduced” in those treated with the drug compared to those in the control arm.

IMMUNOTHERAPY

Treatment of the disease by Inducing, Enhancing or Suppressing the Immune System.

Active Immunotherapy:

-It stimulates the body's own immune system to fight the disease.

Passive Immunotherapy:

-It does not rely on the body to attack the disease, instead it uses the immune system components (such as antibodies) created outside the body.

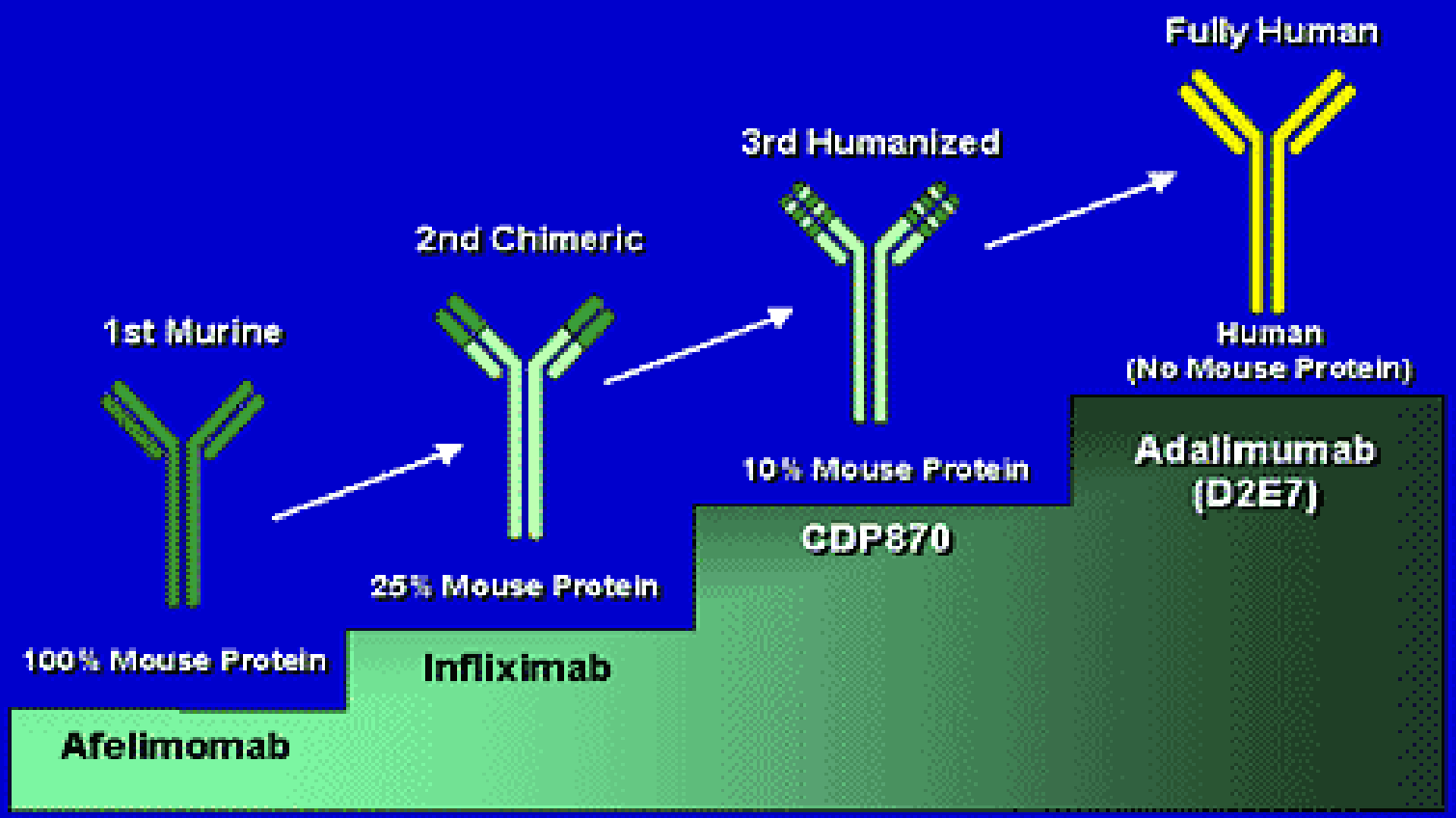
mAb designed for Immunotherapy

A. Murine source mAbs with excellent affinities and specificities. Clinical efficacy compromised by HAMA (human anti murine antibody) response, which lead to allergic or immune complex hypersensitivities.

B. Chimeric mAbs: chimers combine the human constant regions with the intact rodent variable regions. Affinity and specificity unchanged. Also cause human anti-chimeric antibody response.

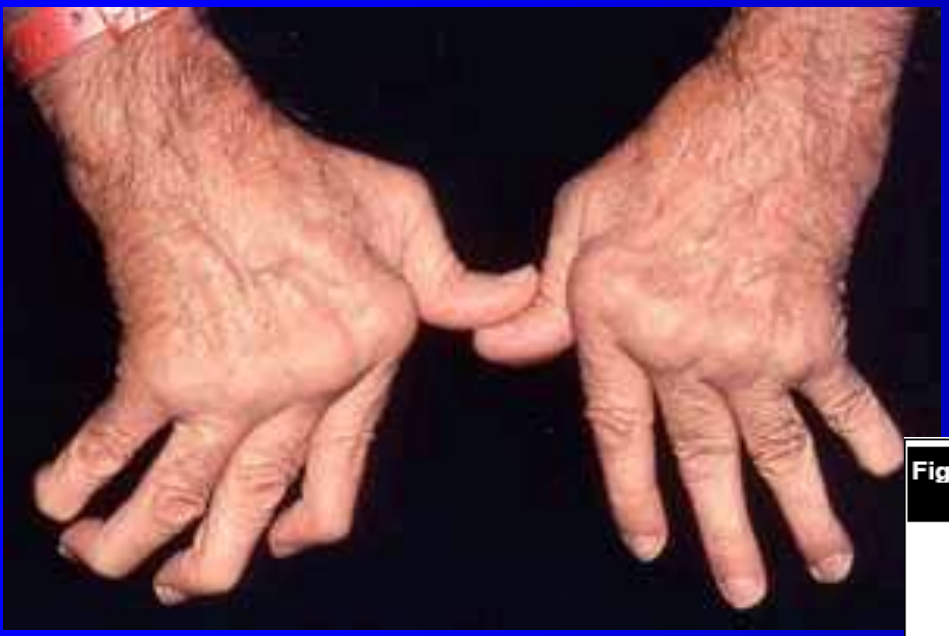
C. Humanized mAbs: contain only the complementarity determining regions (CDRs) of the rodent variable region grafted onto human variable region framework.

EVOLUTION OF MONOCLONAL ANTIBODY

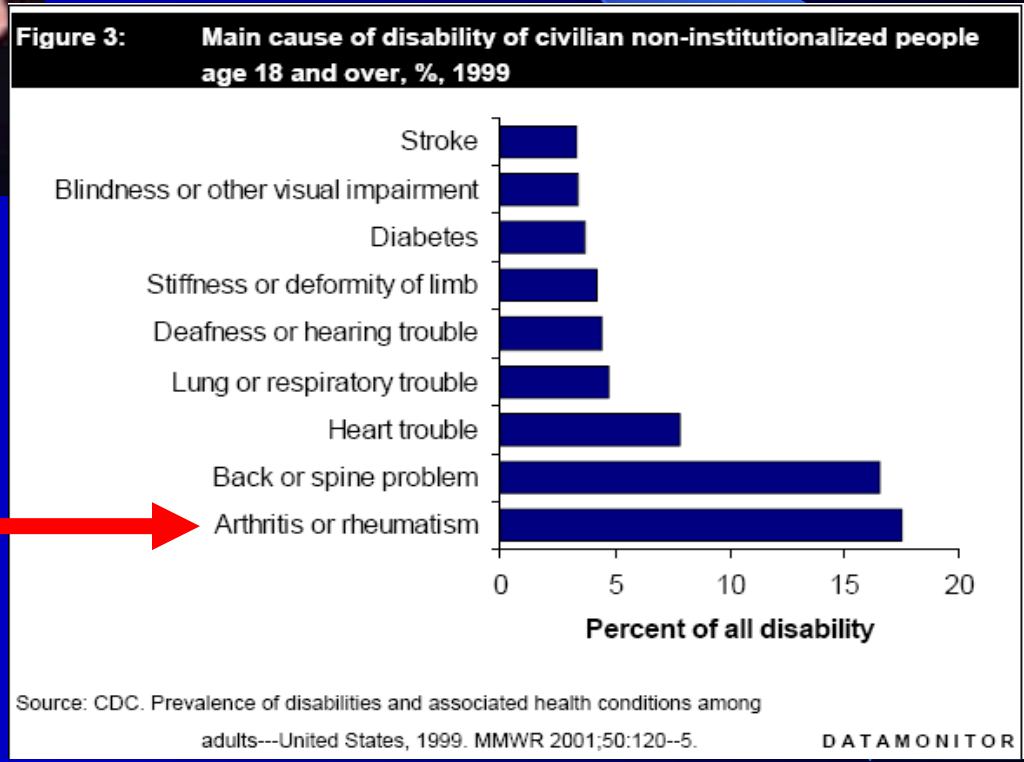


Company Name	Name of Product[1]	Indications	Date of FDA Approval	Antibody Type [2]
Ortho Biotech	**Orthoclone-OKT®**	Organ Transplant Rejection	1986	M
J&J/Eli Lilly	**ReoPro®**	Acute Cardiac Conditions	1994	C
BiogenIdec/Genentech/Roche	**Rituxan®**	Non-Hodgkin's Lymphoma	1997	C
BiogenIdec	**Zevalin™**	Non-Hodgkin's Lymphoma	2002	M
PDLI	**Zenapax®**	Acute Transplant Rejection	1997	H
MedImmune/Abbott	**Synagis®**	Viral Respiratory Disease	1998	H
Genentech/Roche	**Herceptin®** **Avastin ®**	Breast Cancer Colorectal Cancer	1998 2004	H H
J & J	**Remicade®**	Crohn's, Rheumatoid Arthritis	1998	C
Novartis	**Simulect®**	Acute Transplant Rejection	1998	C
Wyeth	**Mylotarg™**	Acute Myleoid Leukemia	2000	H
Schering /ILEX Oncology	**Campath®**	Chronic Lymphocytic Leukemia	2001	H
Abbott/CAT	**Humira™**	Rheumatoid Arthritis	2002	PD
Novartis/Genentech/Tanox	**Xolair®**	Asthma	2003	H
Genentech/Xoma	**Raptiva™**	Psoriasis	2003	H
Corixa/GlaxoSmithKline	**Bexxar®**	Non-Hodgkin's Lymphoma	2003	M
BMS/ImClone Systems	**Erbitux ™**	Colorectal Cancer	2004	C

Rheumatoid Arthritis



Rheumatic diseases are the leading cause of disability among adults age 65 and older



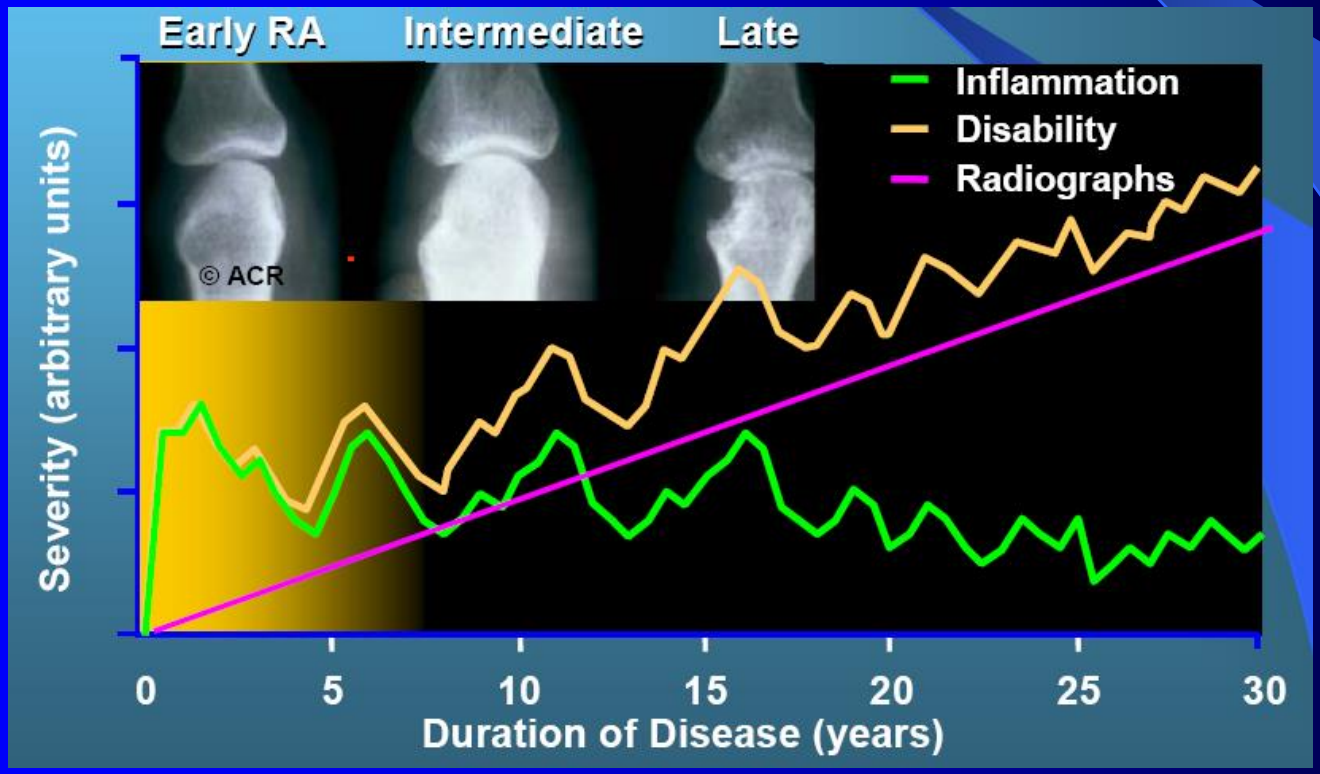
Rheumatoid Arthritis (RA)

- ✓ Common human autoimmune disease
- ✓ Chronic inflammation of the joints and infiltration by blood-derived cells
- ✓ Progressive destruction of cartilage and bone
  - invasion by cellular synovial tissue
  - cytokine induction of destructive enzymes, matrix metalloproteinases (MMP)

Prognosis of RA

- ✓ Long-term prognosis: poor
  - 80% of patients are disabled after 20 years
  - life expectancy is reduced by 3-18 years
- ✓ Disease modifying anti-rheumatic drug (DMARD) like methotrexate or steroids
  - limited efficacy and many side effects
  - do not improve long-term prognosis
- ✓ Efforts to develop safer and more effective treatments

RA Progression



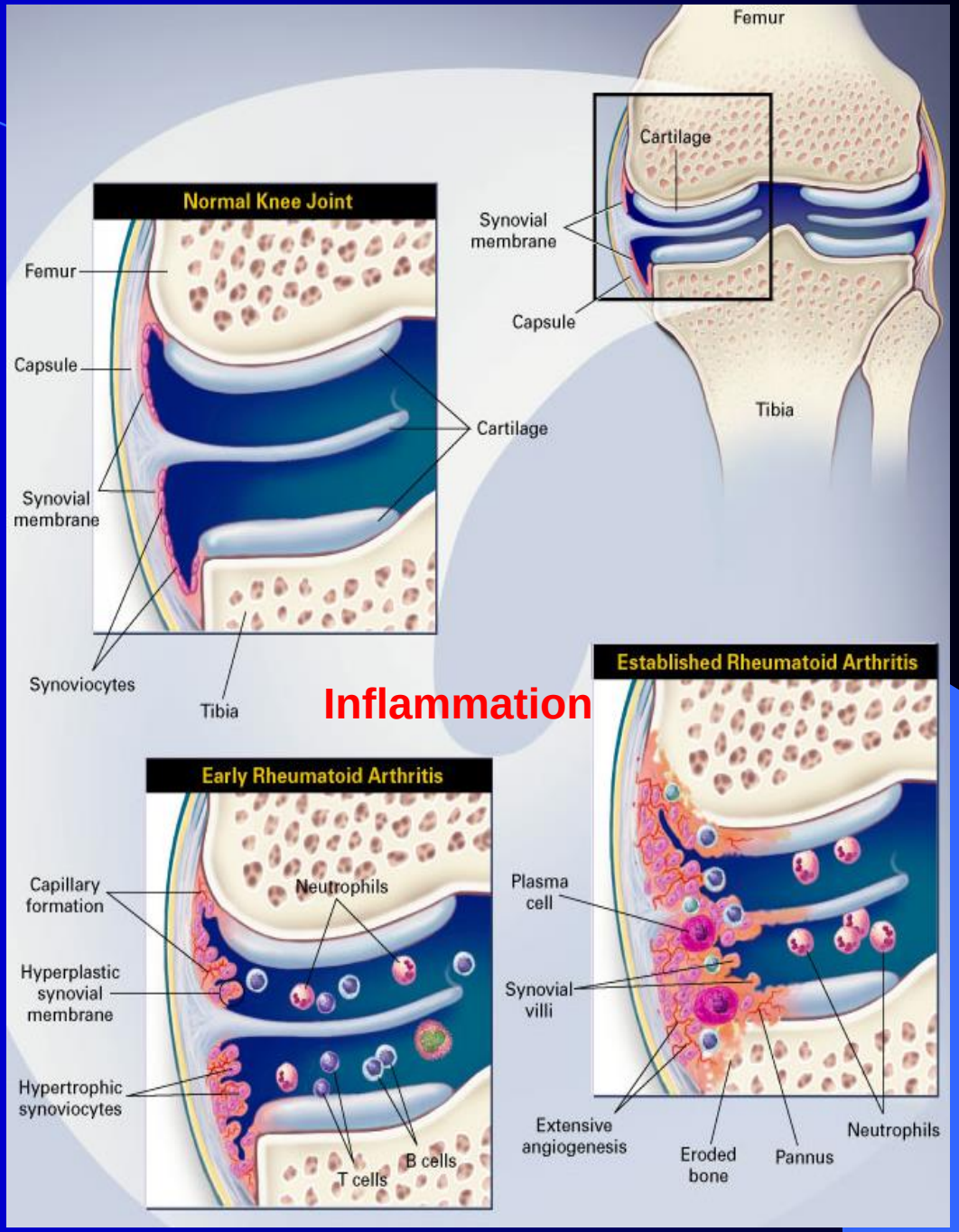
Radiographic Monitoring

Rheumatoid Arthritis

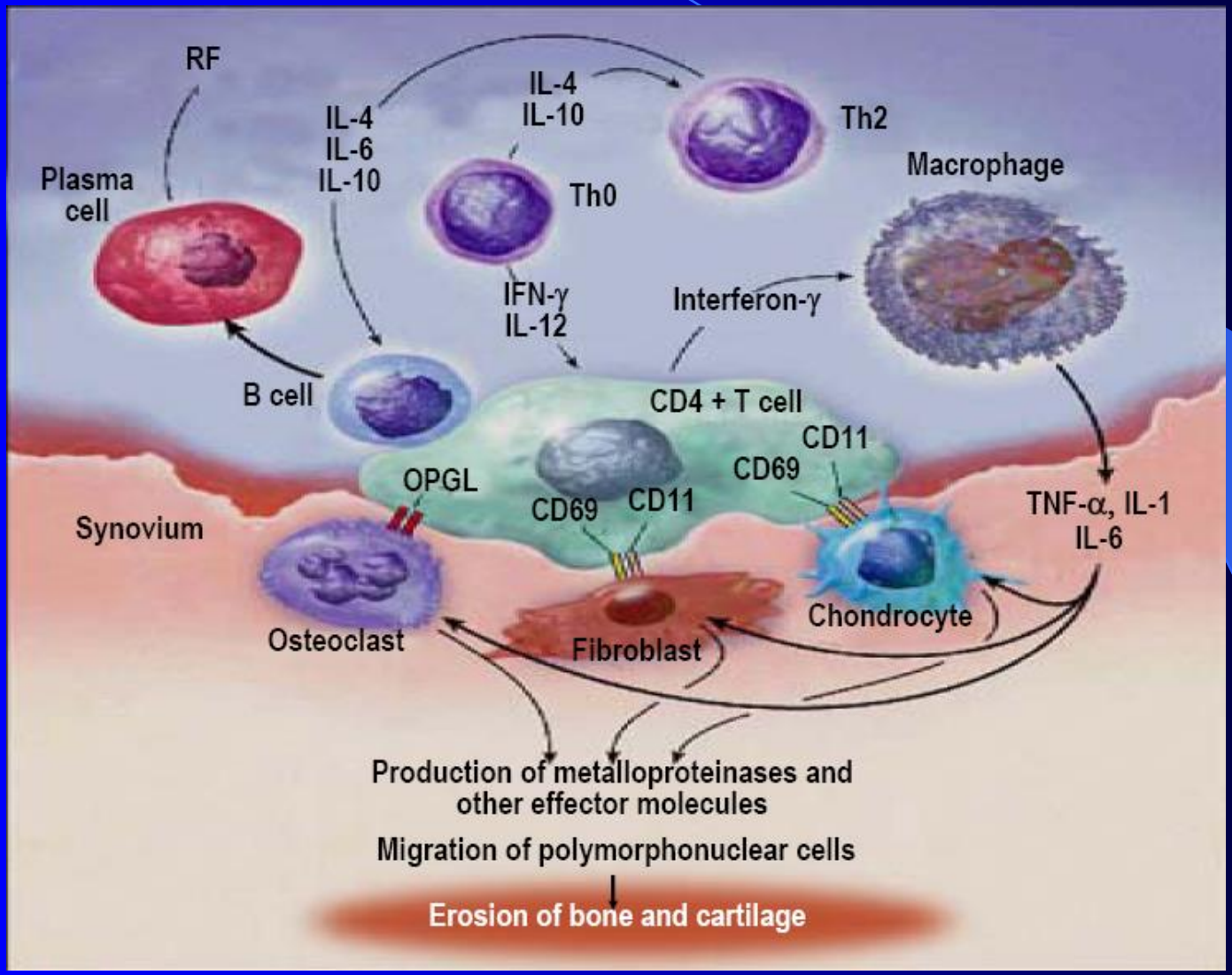
- ✓ hyperplasia of synovial

increase of the cellularity of the synovial membrane and leads to synovial thickening

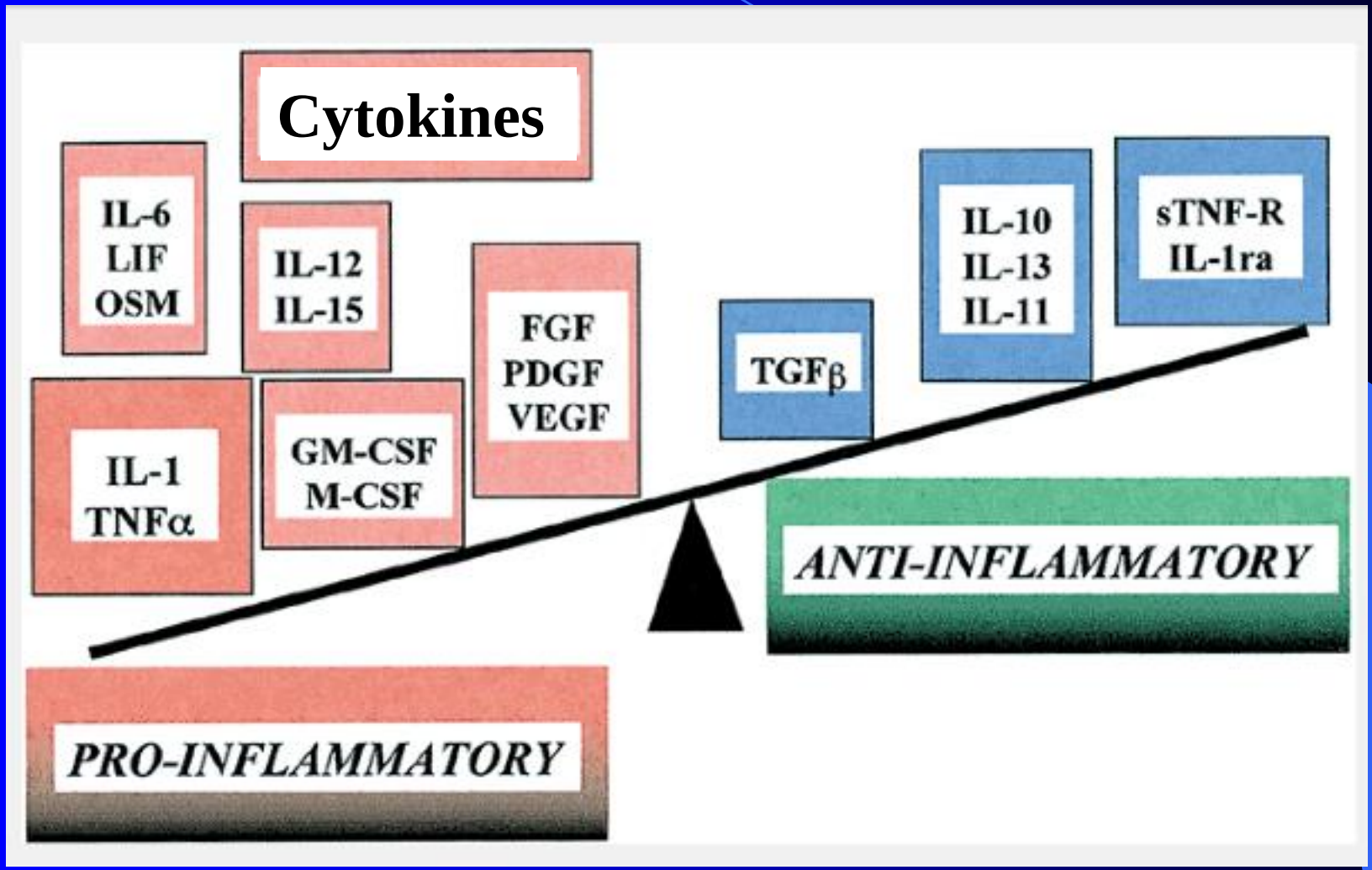
- ✓ Neovascularization
- ✓ chronic inflammatory disease of the joints
- ✓ accumulation of large numbers of leukocytes within the inflamed synovium
- ✓ cartilage and bone damage



Cytokine Signaling Pathways in RA



Role of Cytokines in RA

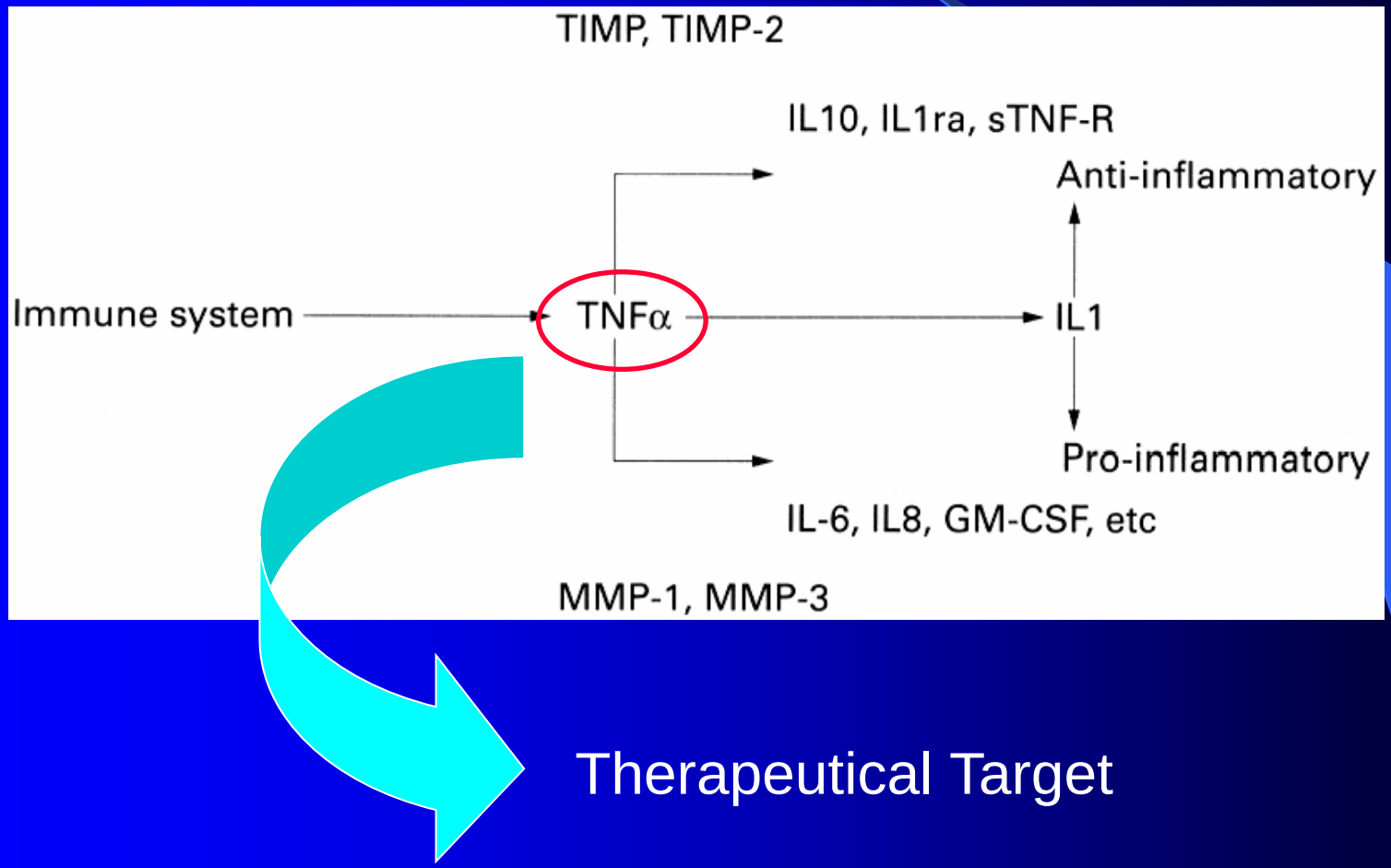


Cytokine Sources, Targets, Levels and Damage

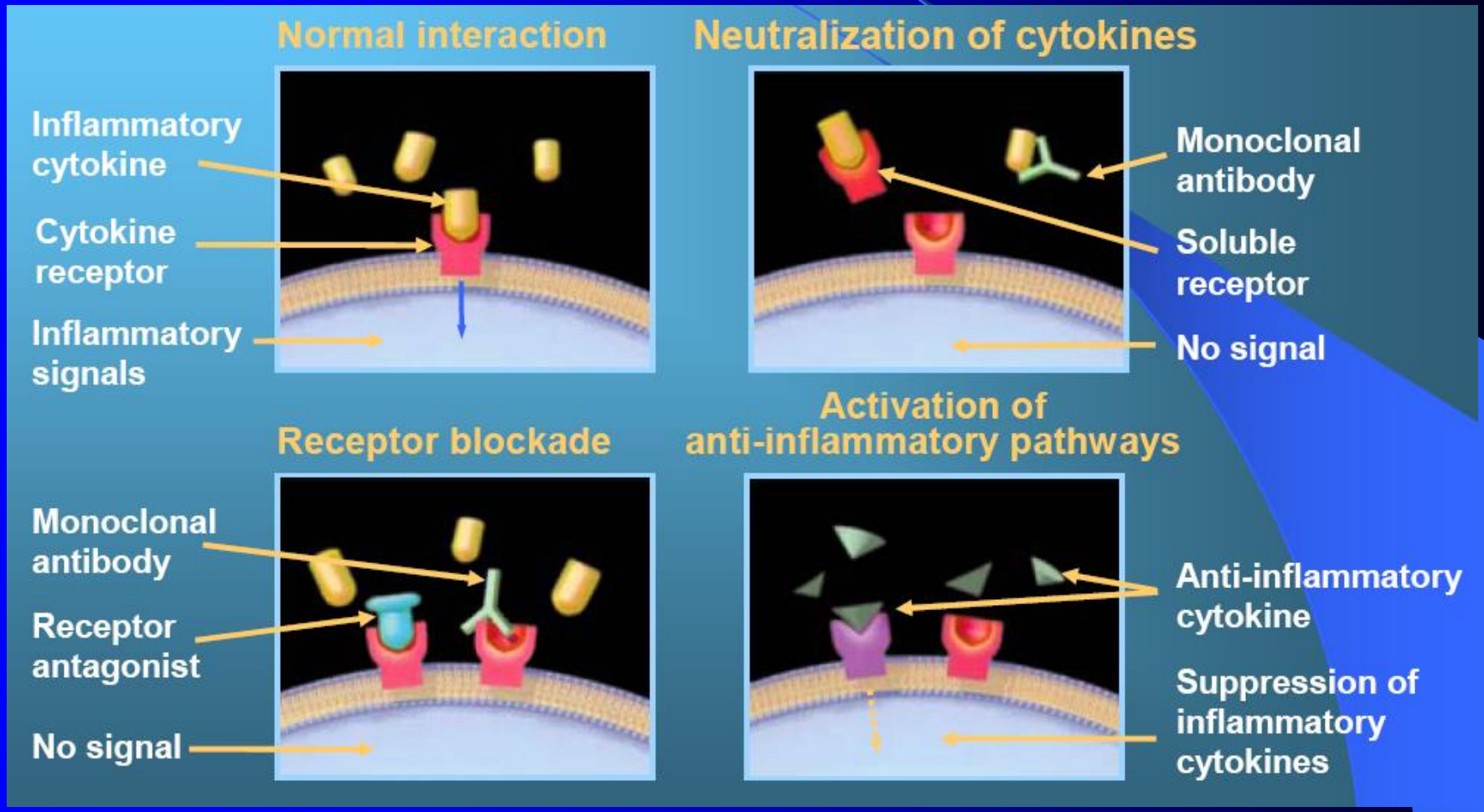
Cytokine	Source	Target	Abundance	Effect on Inflammation or Tissue Damage
TNF	M	Multiple	+++	+++
IL-1β	M	Multiple	+++	+++
IL-6	M, F	Multiple	++	++
IL-8	Multiple	Neutrophils	++	++
IL-10	M, T	T	++	–
IL-12	M	T	+	++
IL-15	F, M	T	+	++
IL-2	T	T	+/–	+
IL-17	T	F	+	++
IFN-γ	T	Multiple	+	++
TGF-β	Multiple	T	++	–
GM-CSF	M, T	Multiple	++	++

**TNF indicates tumor necrosis factor; IL, interleukin; IFN, interferon; TGF, transforming growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; M, monocyte/macrophage; F, fibroblast; T, T lymphocyte; –, inhibitory effect; +, low abundance/mild effect; ++, moderate abundance/moderate effect; and +++, high abundance/high effect.*

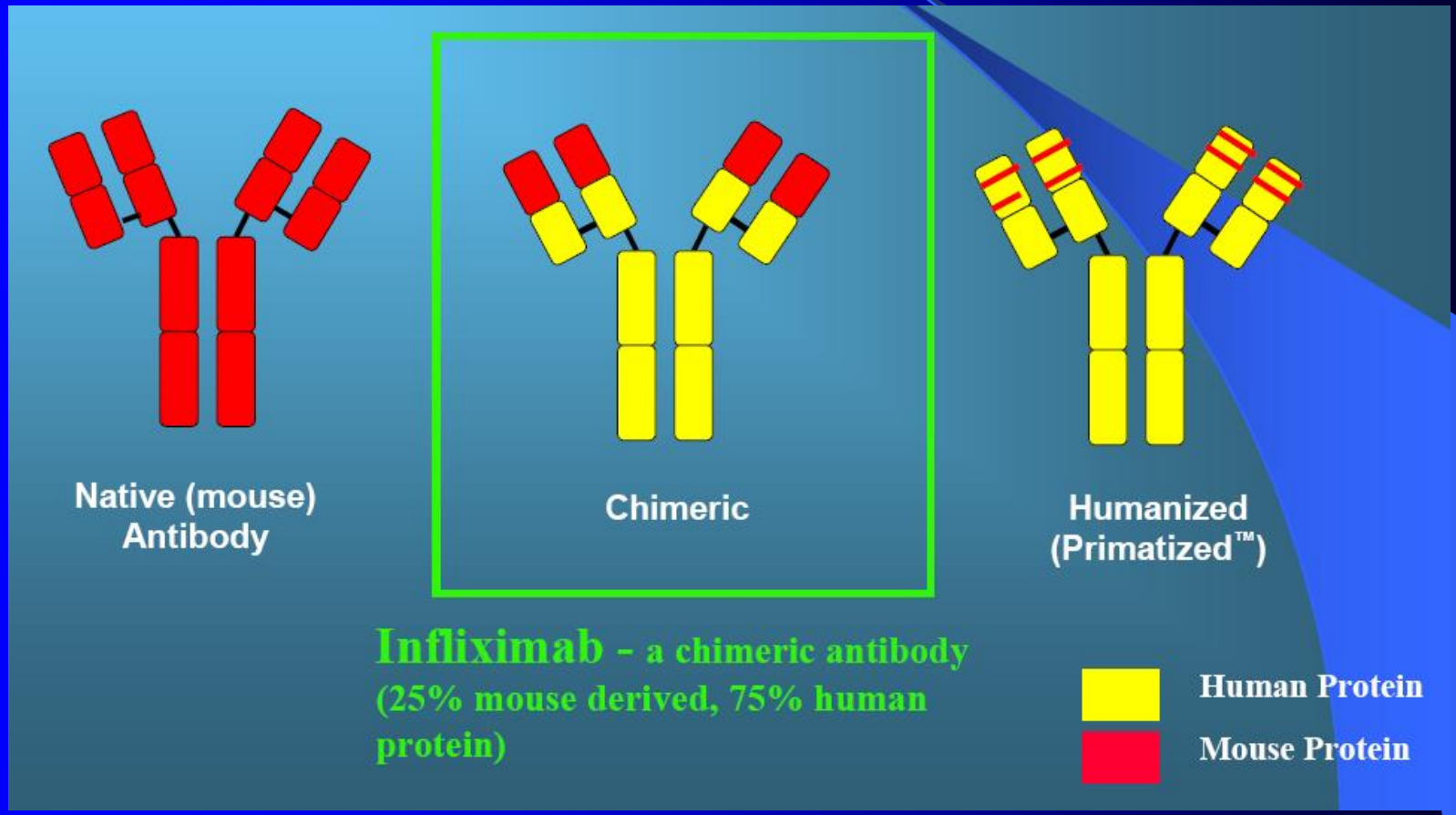
Role of Cytokines in RA (continued)



Strategies for Inhibition of Cytokine Action (Current Drug Strategies)

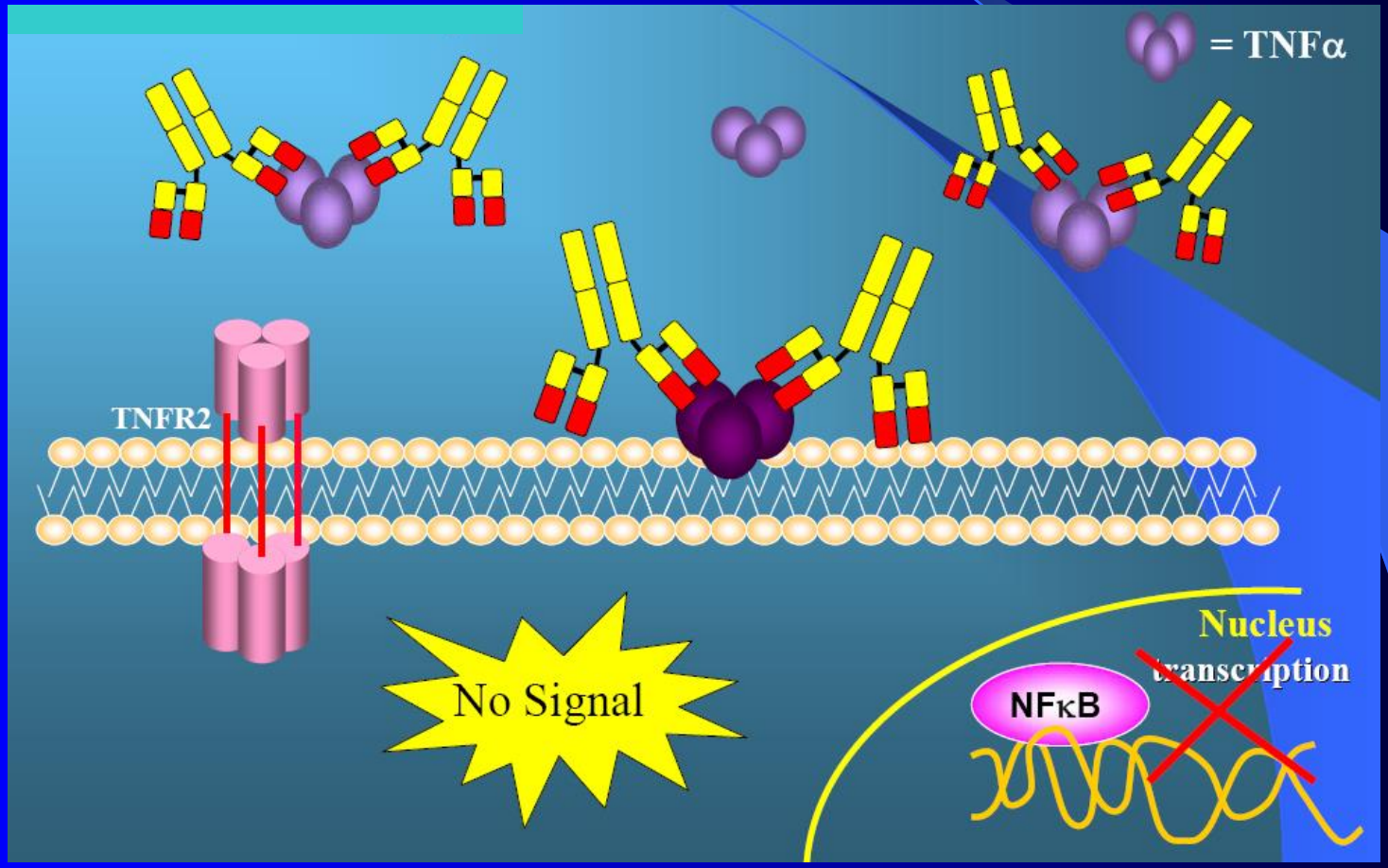


Structure of Infliximab (Remicade®)



Infliximab: Mechanism of Action

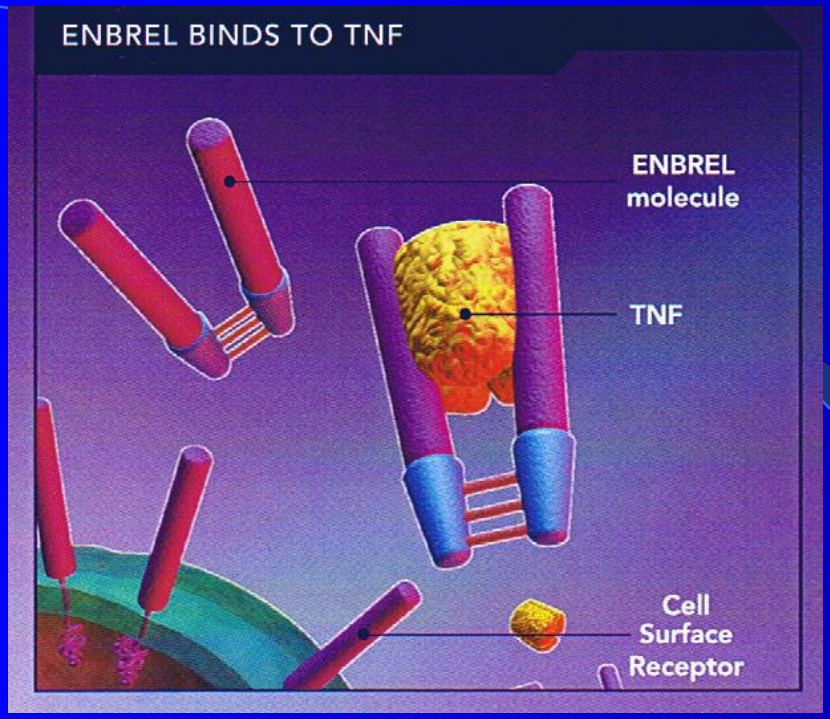
Binds and neutralizes both soluble and membrane bound TNFα -inhibits further activity



Safety and Side Effects of Infliximab Use

- ✓ Most common: infusion reactions (itching, nausea), headache & abdominal pain.
- ✓ Increased risk of serious infection due to immuno-suppression
- ✓ Upper respiratory tract infections (tuberculosis)
- ✓ Increased risk of non-Hodgkins lymphoma
- ✓ Lupus
- ✓ Immunogenicity: patient develops HAMA (human anti-mouse antibodies) towards Infliximab

Currently Available TNF Inhibitors



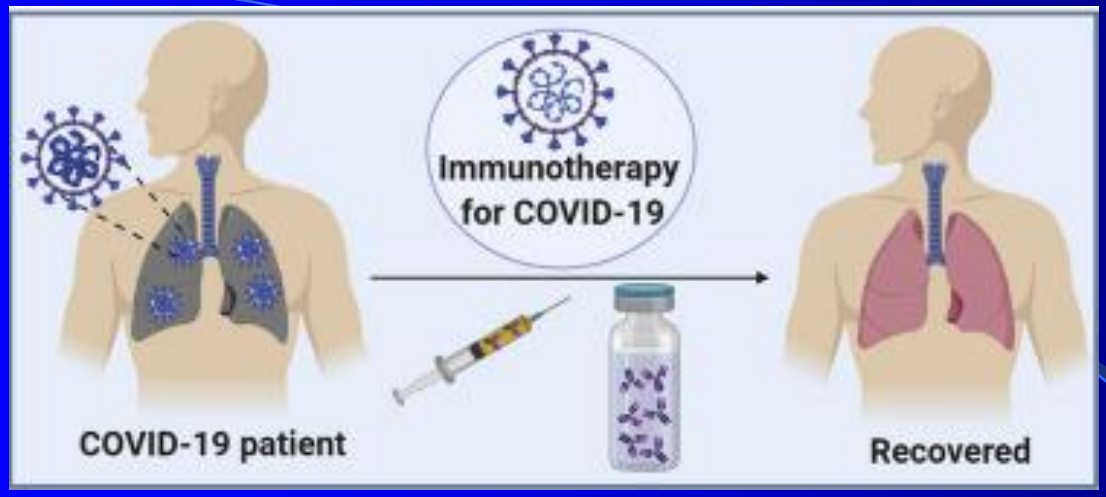
- Etanercept: Soluble receptor
- Adalimumab: Human MAb
- Infliximab: Murine/human MAb

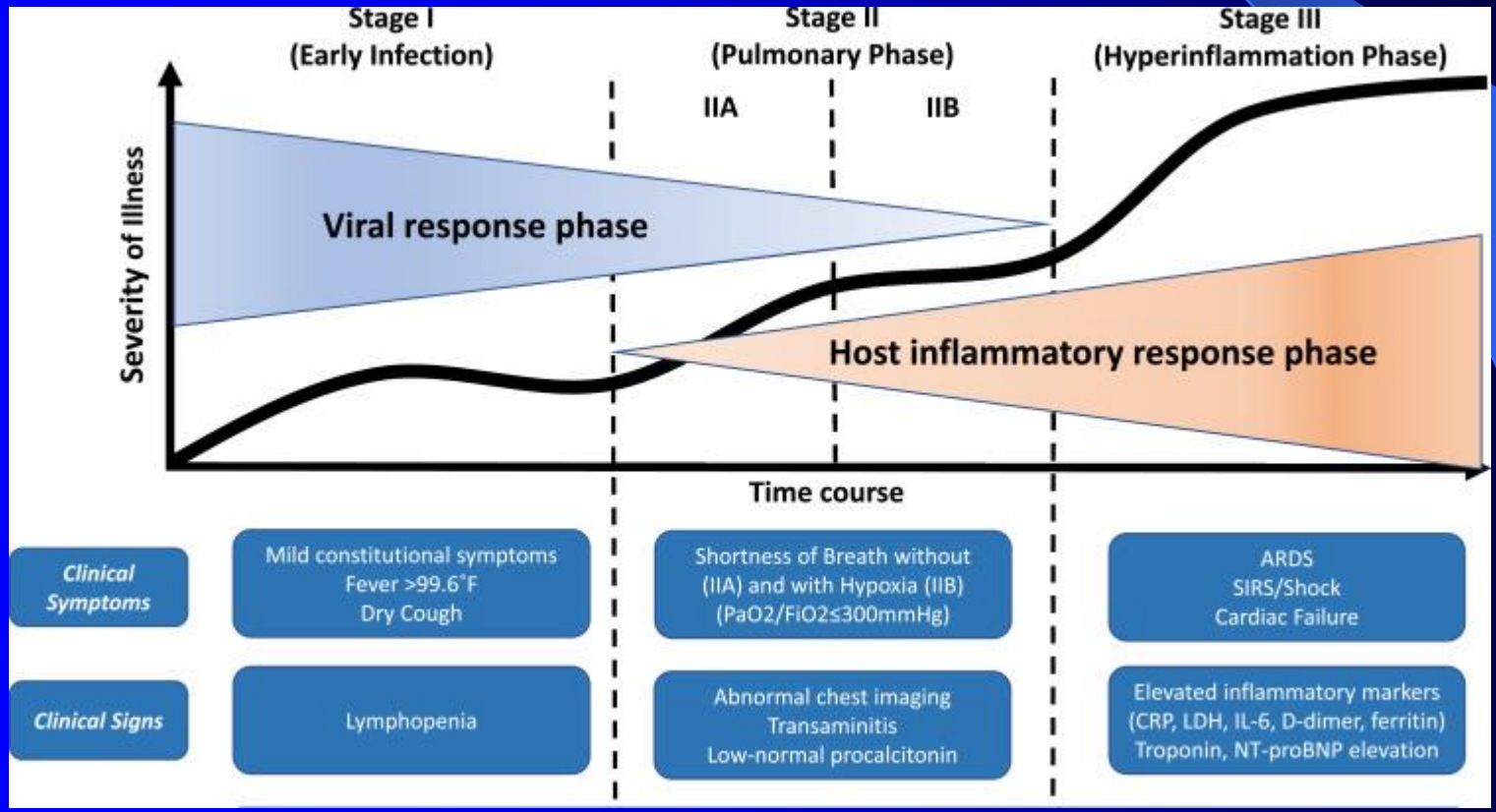
Other Uses for Anti-TNFα

Autoimmunity	Infectious Agents	Tumours	Other
Crohn's Disease	HIV infection	Angiogenesis	Asthma
Insulin-dependent diabetes mellitus	Septic shock	Ovarian cancer	Graft Versus Host Disease (GVHD)
Multiple Schlerosis	Hepatitis C	Lymphoma	Glomerulonephritis
Rheumatoid Arthritis			Pancreatitis

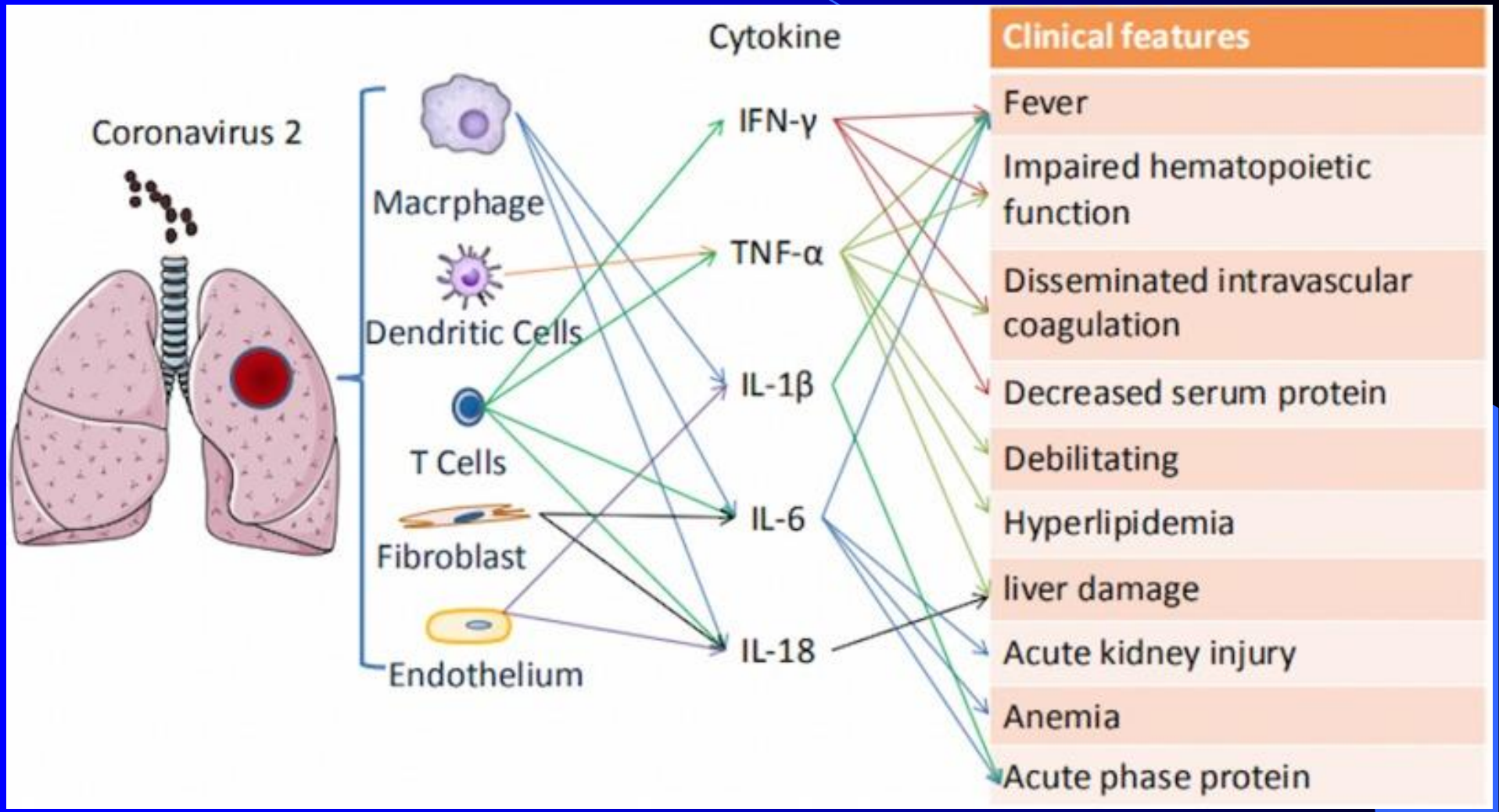
*** Conditions associated with overproduction of TNFα***

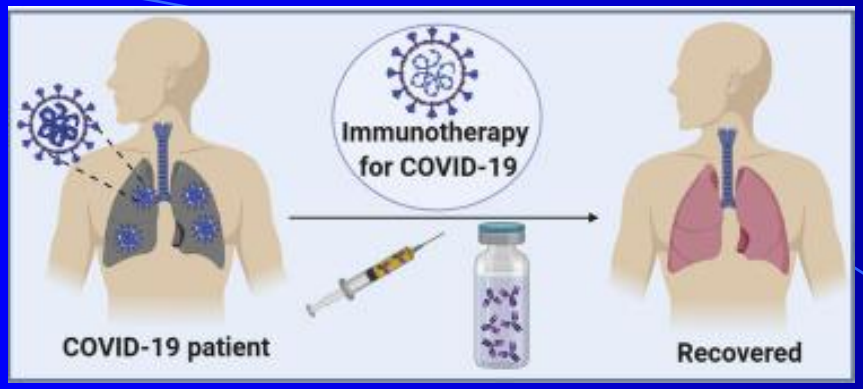
Covid-19: Clinical Symptoms



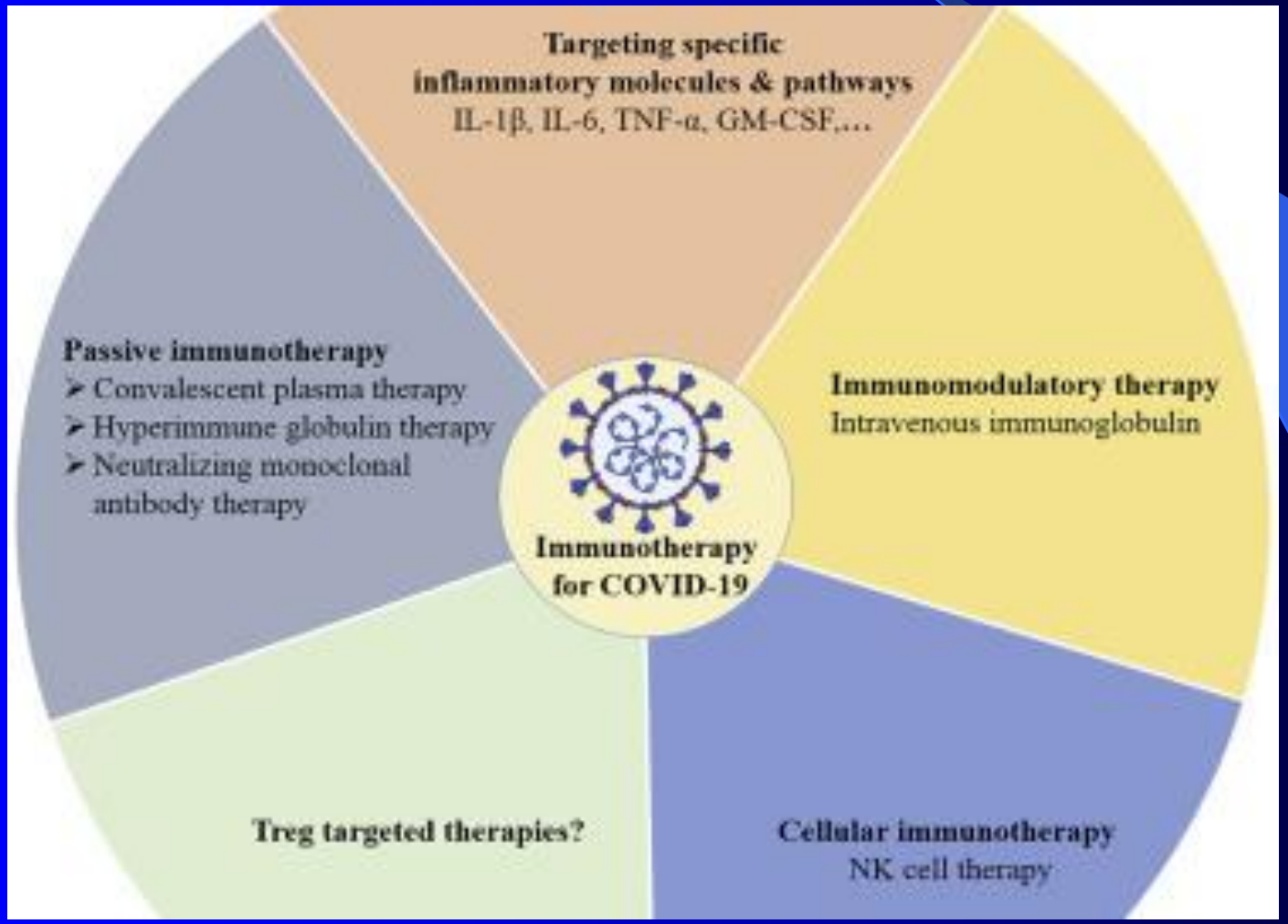


Covid-19: Inflammatory Cytokines





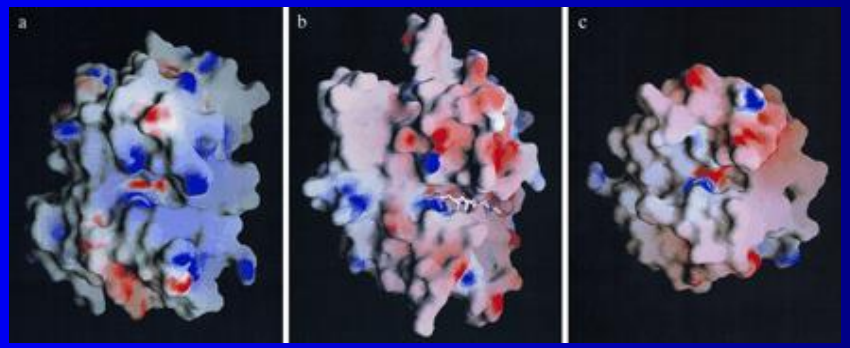
Covid-19: Immune Therapy

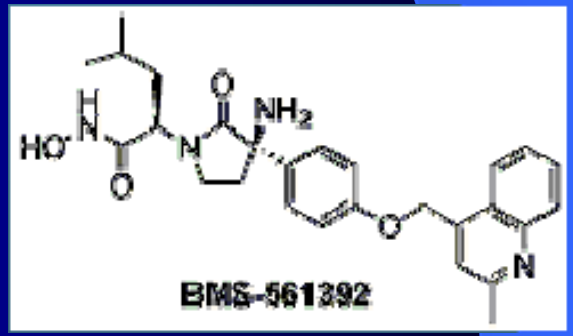


Small Molecule Anti-TNFα Agents in Development

Class of Inhibitor	Product	Company	Clinical Status
p38 Kinase	BIRB796	Boehringer Ingelheim	Phase 2
TACE	BMS-561392	Bristol Myers	Phase 2
Thalomid	Thalidomide	Celgene	Phase 3
Rationally Designed L-amino acid peptide	RDP58	Sangstat Medical	Phase 2

Crystal structure of the catalytic domain of human tumor necrosis factor- -converting enzyme



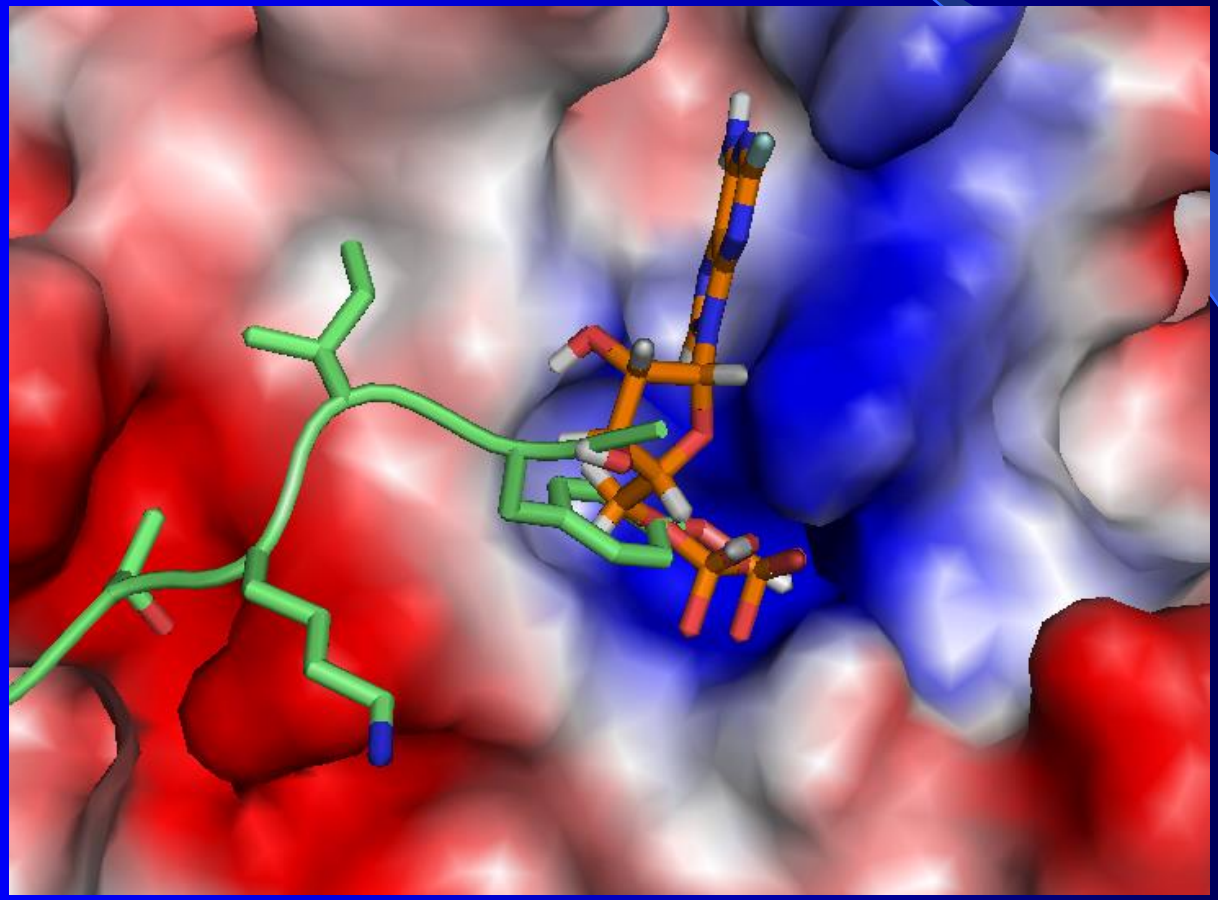


Small Molecule Approaches to Anti-TNF-α Therapy

Potential Advantages of Small-Molecule, Oral TNF-α Inhibitors:

- ✓ Convenient, non-injectable with greater patient compliance
- ✓ Small molecule might facilitate tissue penetration
- ✓ Possibility for once a day dosing
- ✓ Non-immunogenic
- ✓ Easier manufacturing and lower cost
- ✓ Potential use in combination with other anti-inflammatory therapies.

Targeting STAT3 as a novel strategy to treat cancer



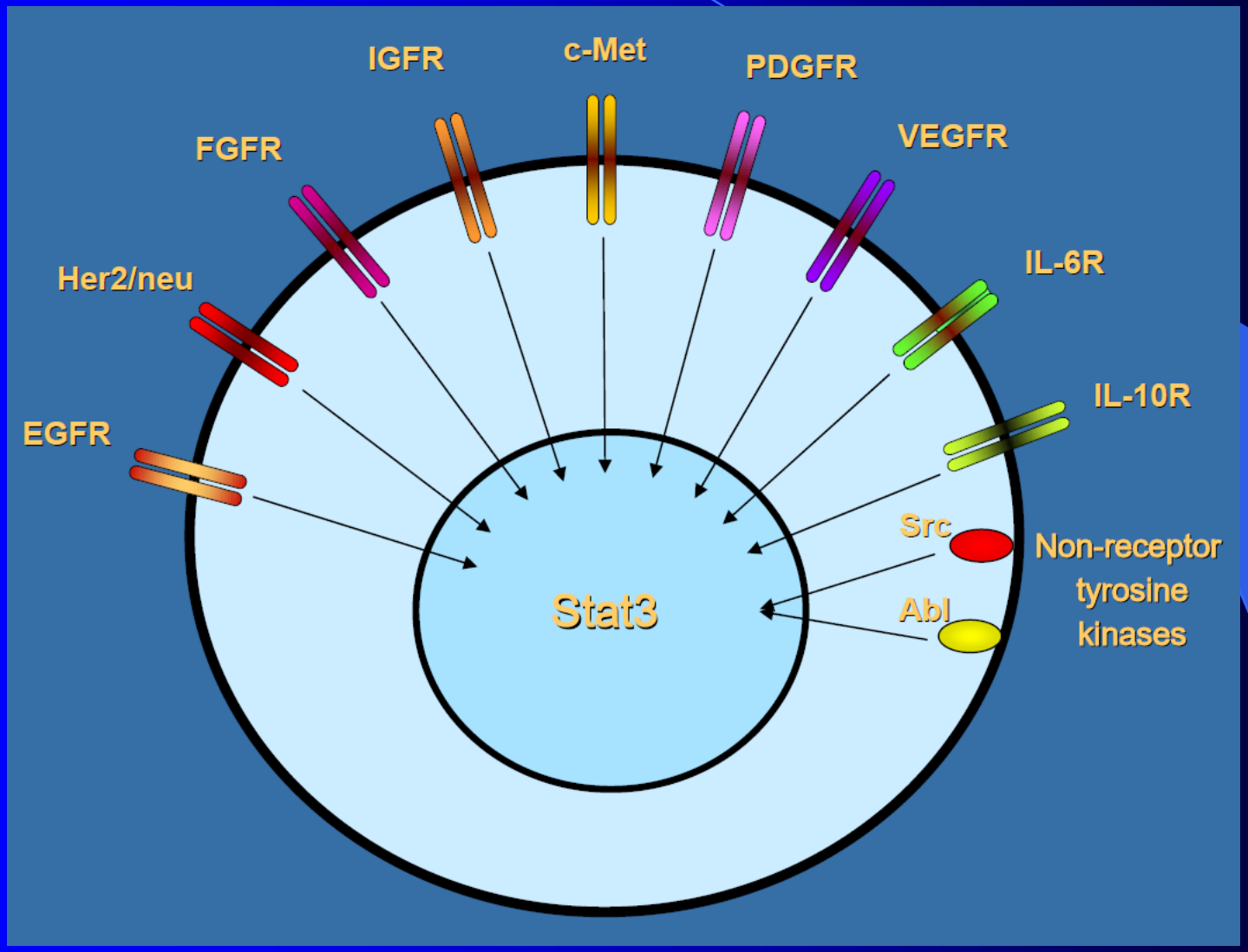
STAT3: A target for many human cancers

50-90% STAT3 activation in:

Solid Tumors	
Prostate cancer	STAT3
Non-small Cell Lung cancer	STAT3
Breast cancer	STAT3, STAT5
Head and Neck cancer	STAT3
Melanoma	STAT3
Ovarian cancer	STAT3
Pancreatic cancer	STAT3
Glioma	STAT3
Stomach Cancer	STAT3
Cervical Cancer	STAT3
Blood Tumors	
Multiple Myeloma	STAT3
Acute Myelogenous Leukemia (AML)	STAT3, STAT5
Chronic Myelogenous Leukemia (CML)	STAT5
Burkitt's Lymphoma	STAT3
Non-Hodgkins Lymphoma	STAT3
Cutaneous T cell Lymphoma	STAT3

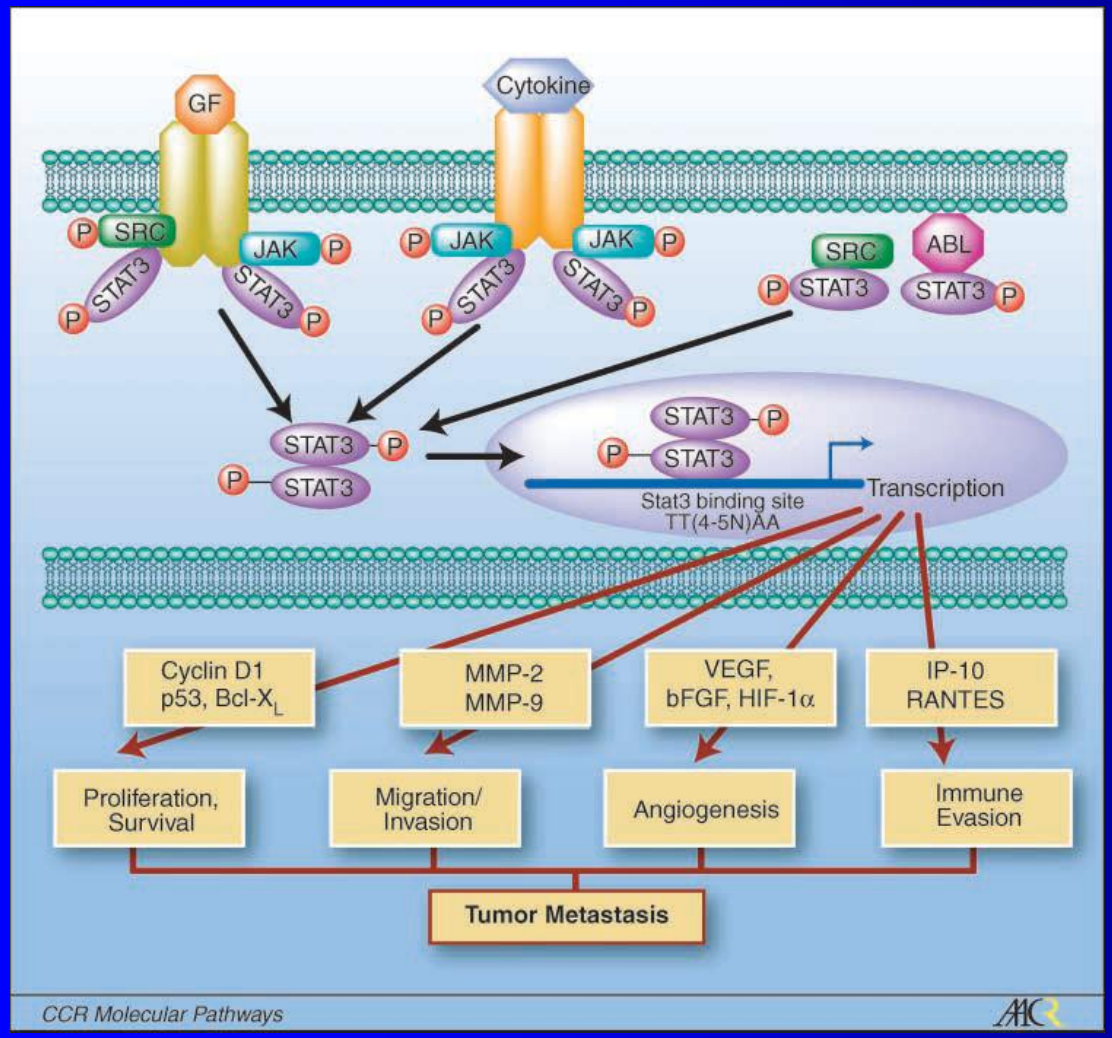
Hua Yu - Marcin Kortylewski et al., 2007

STAT3: Point of convergence in oncogenic signaling



Yu et al., 2007

Role of STAT3 in Oncogenesis & Tumor Metastasis



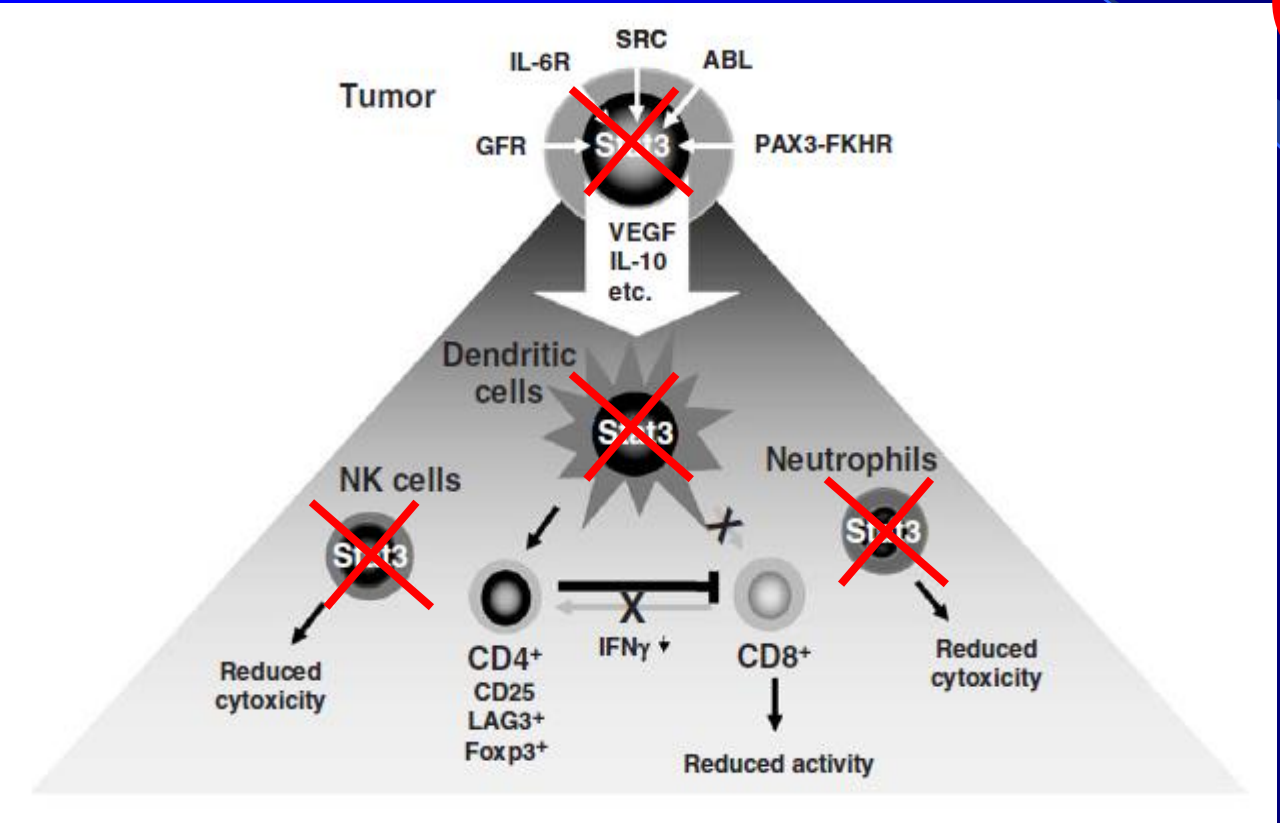
STAT3:
a novel multi-functional protein involved in

tumor development
tumor progression
tumor-induced immuno suppression
metastasis

in different types of cancer.

STAT3 in Cancer

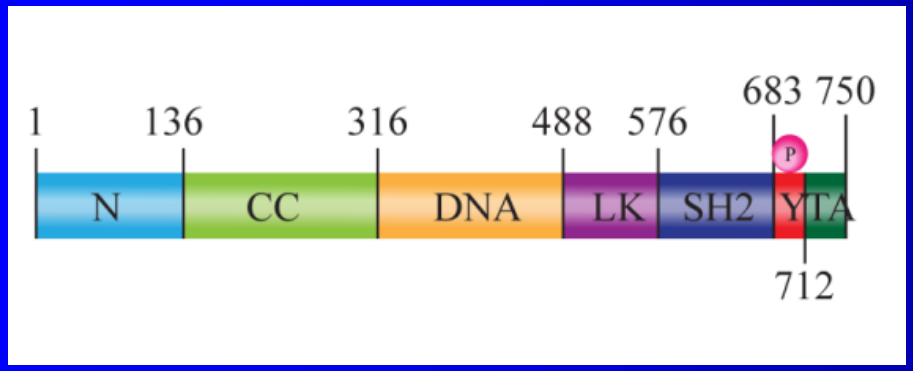
Solid Tumors

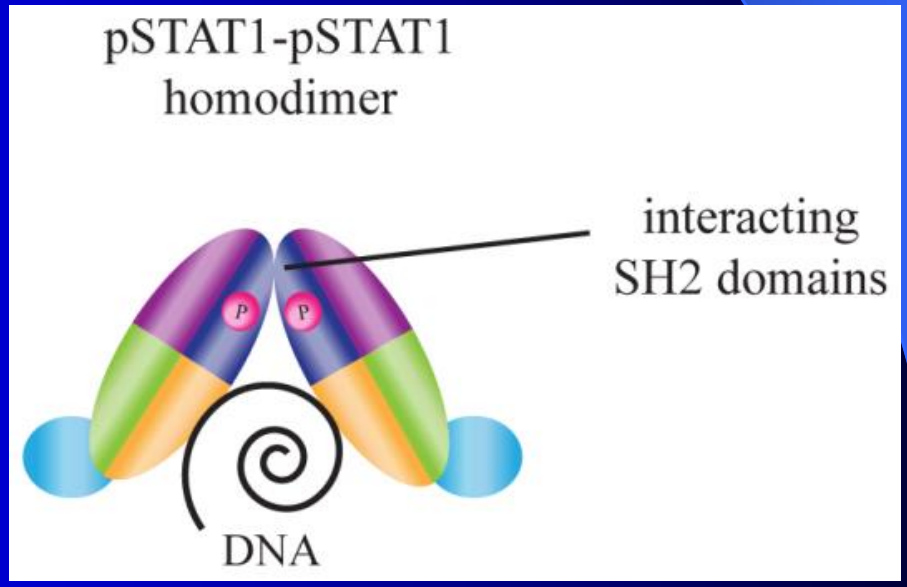


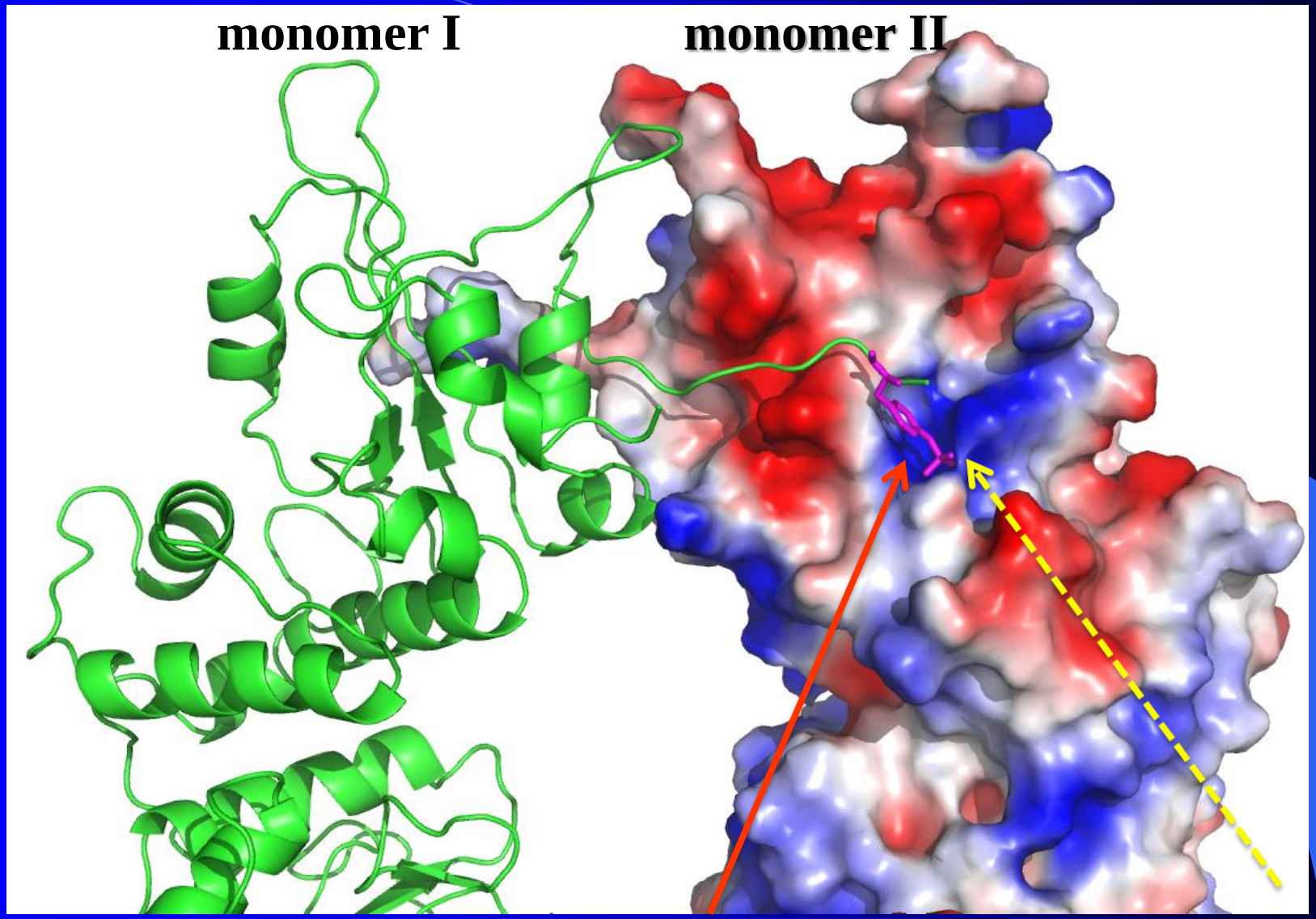
- Small molecule inhibitors
  - compounds
  - phosphopeptides
  - peptidomimetics
- Gene therapy
  - DN-STAT3
  - SOCS3
- RNAi + targeting vectors
- Combination therapy
  - Immune therapy

Inhibition of tumor development + progression

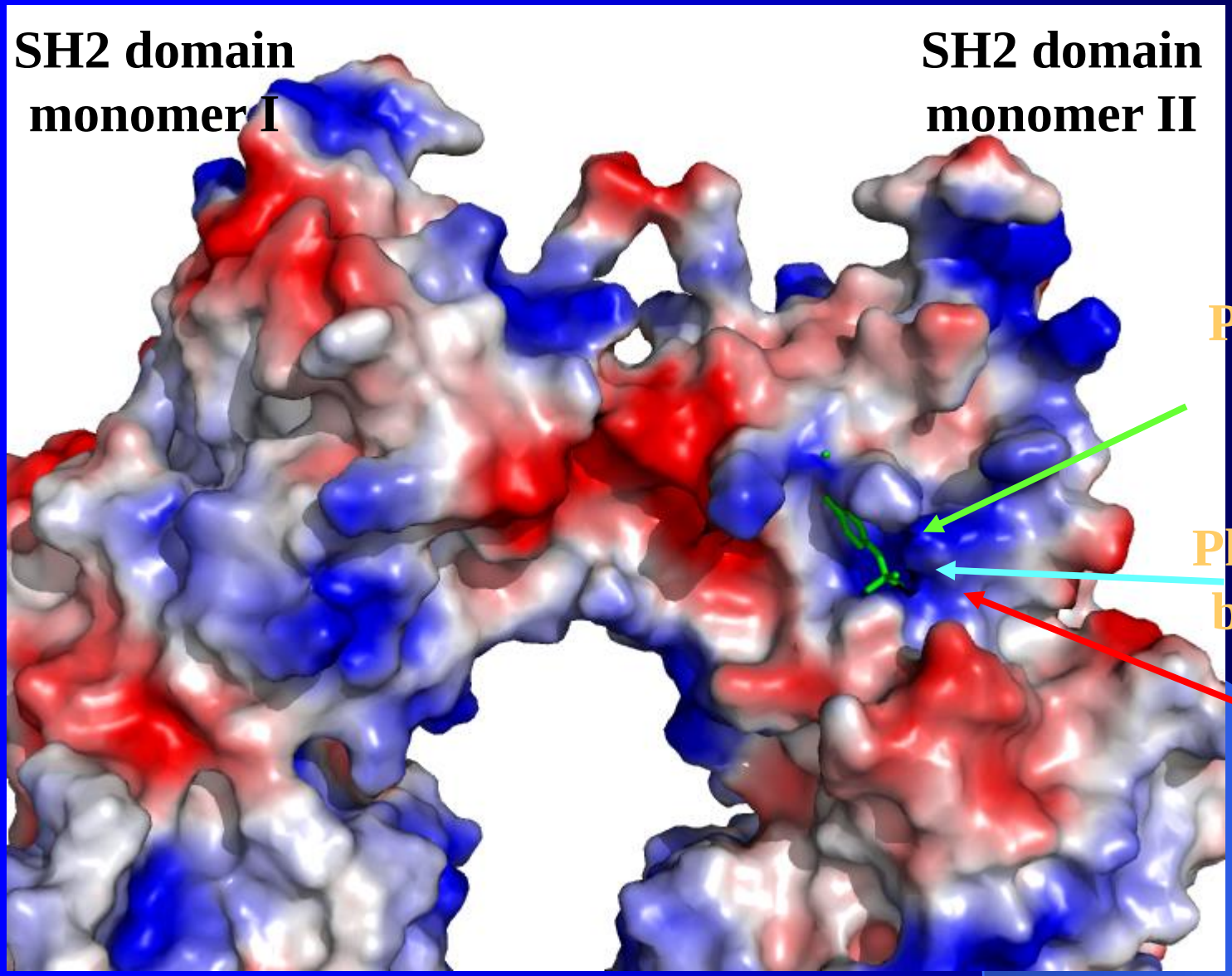
STAT activation and dimerization






monomer I
monomer II
pTyr binding pocket
in SH2 domain
Phosphorylated
tyrosine

Structural information STAT3



Chemical
compounds
to block
STAT3 activity

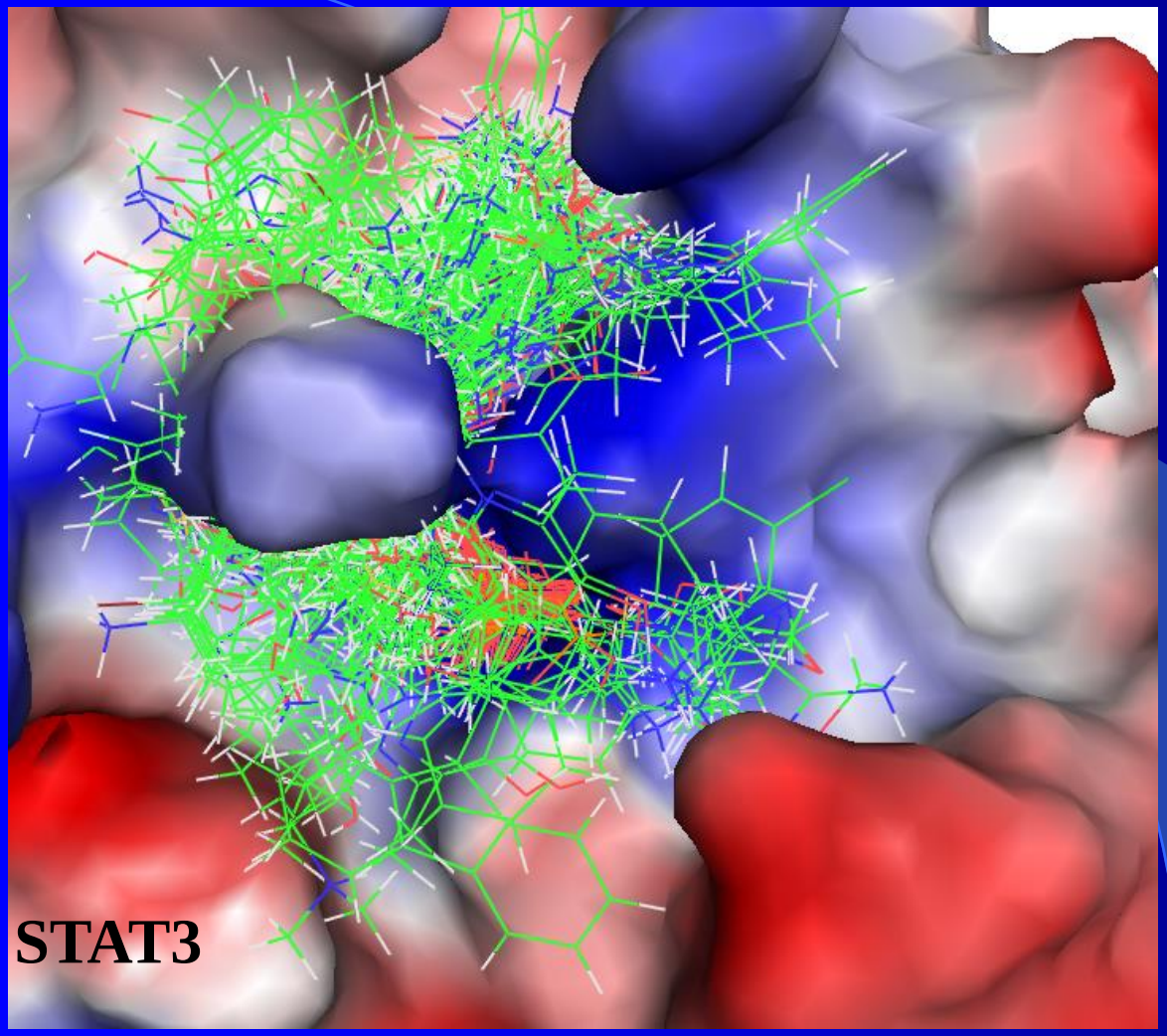
Screening Compound Libraries

- Synthesize chemical compound library
  biological selection screening

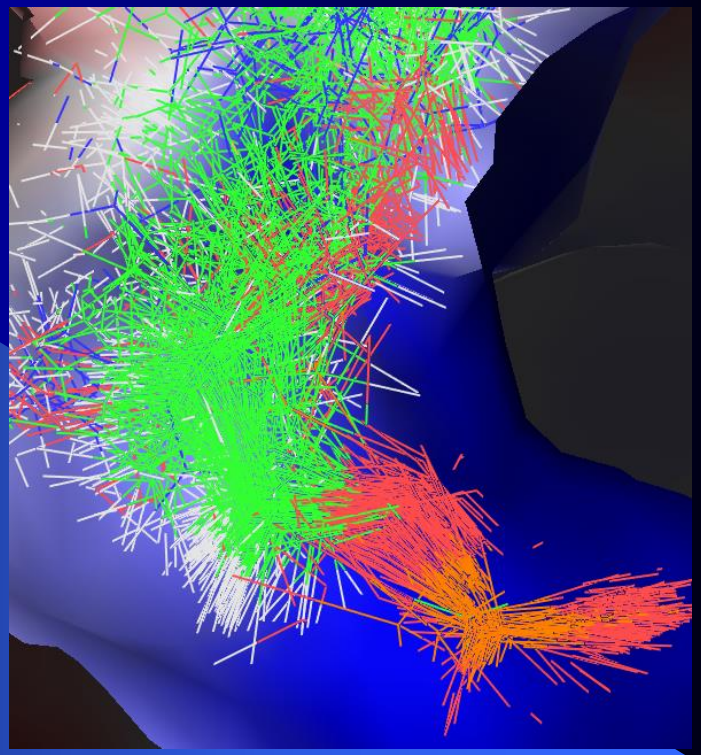
- Structural modeling + Virtual Screening
  virtual selection screening
  biological selection subscreening

⟶ Combination of Both

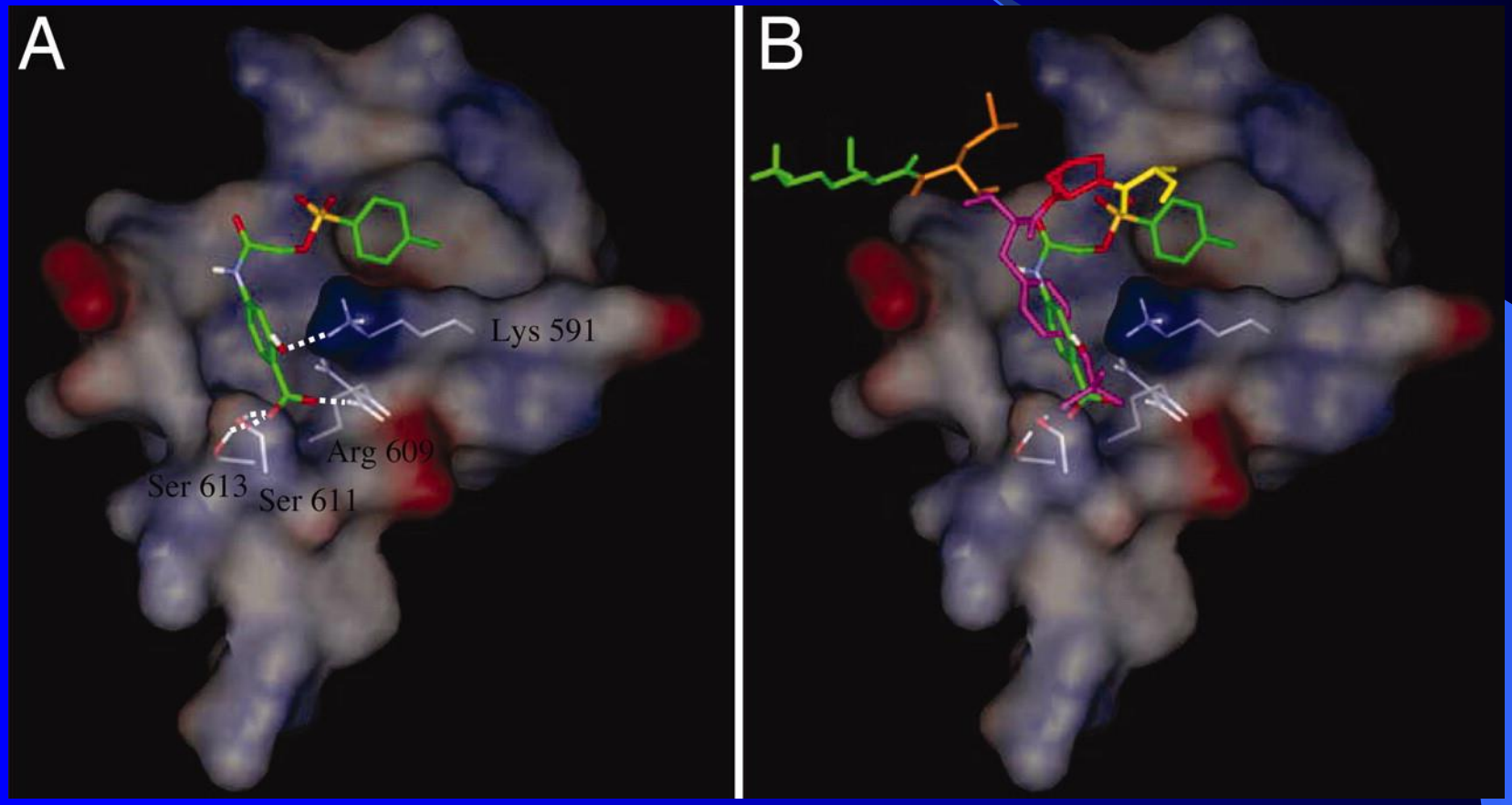
Application of computational modelling in virtual screening to identify STAT3 inhibitory compounds from a chemical database



Docking

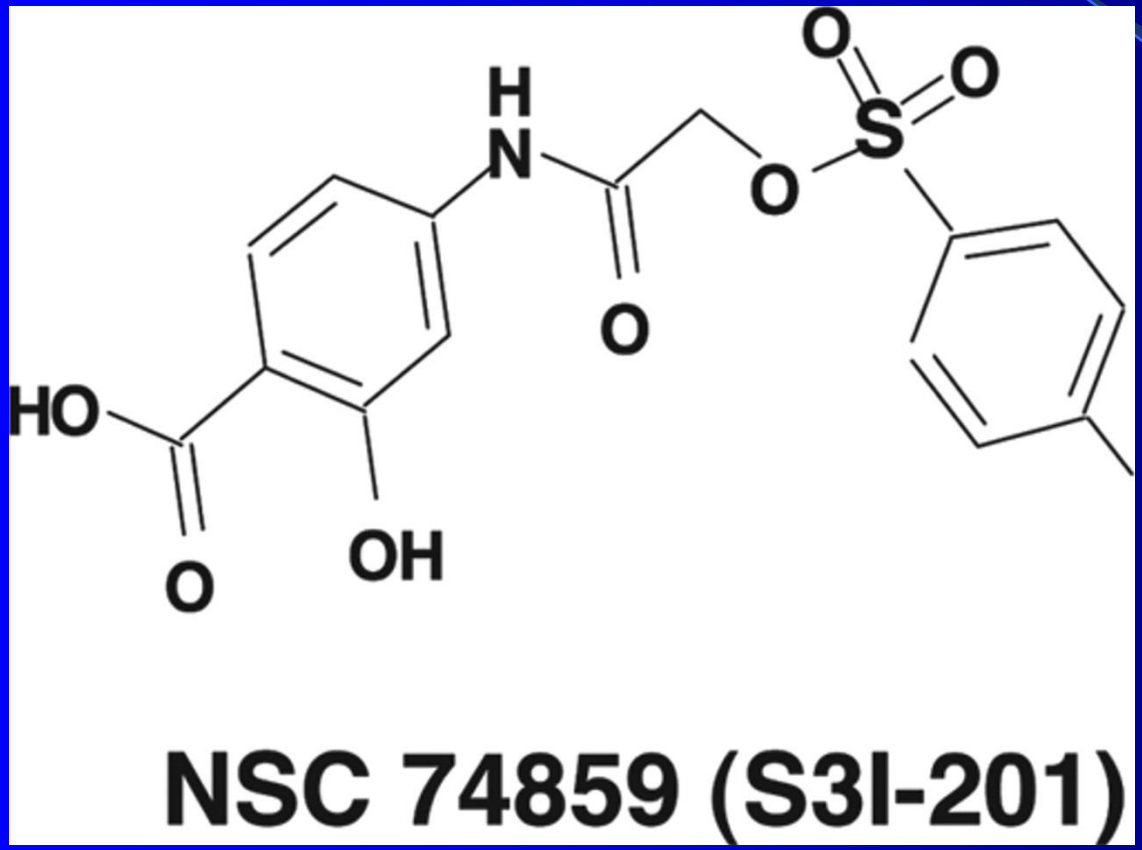


Application of computational modeling in virtual screening to identify the compound S3I-201 from a chemical database



Siddiquee K. et.al. PNAS 2007;104:7391-7396

STAT3 inhibitory compound



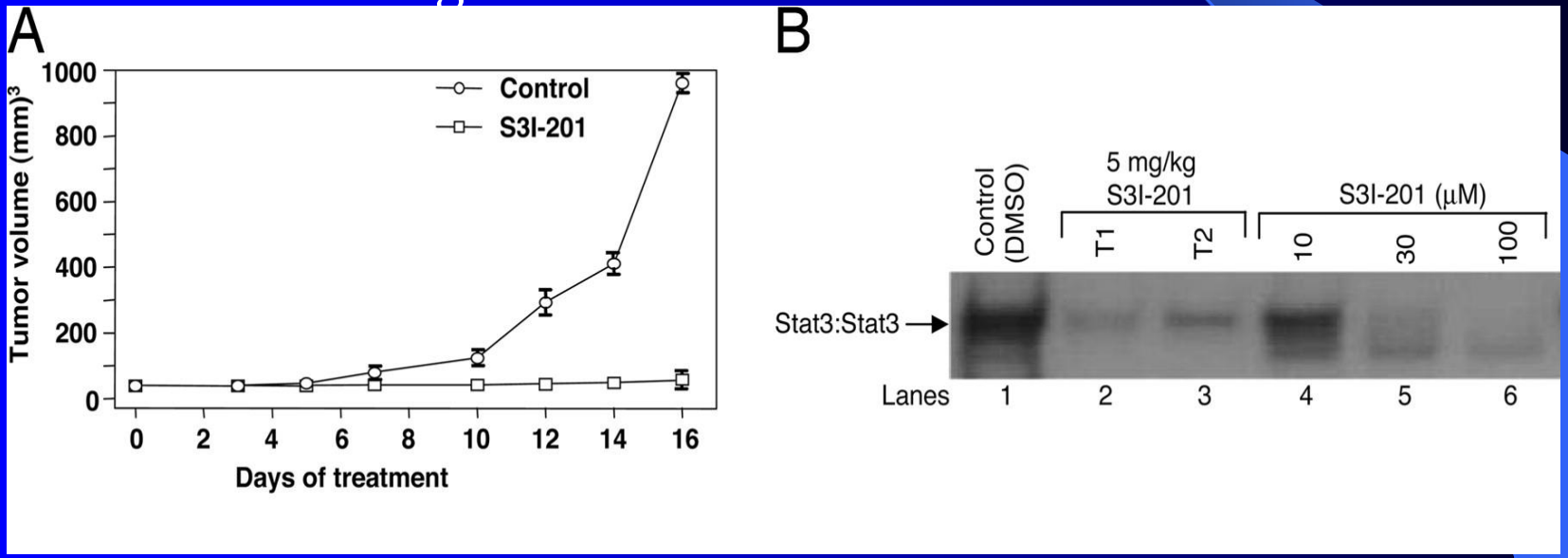
Dimerization

DNA binding

Siddiquee K. et.al. PNAS 2007;104:7391-7396

In vivo Tumor growth inhibition by S3I-201

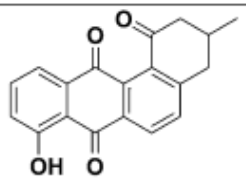
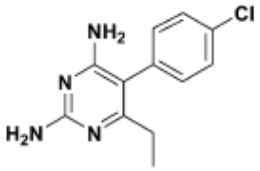
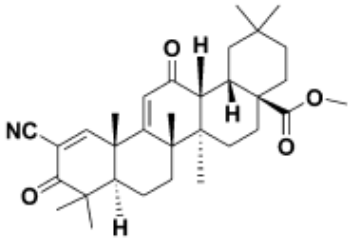
Human breast (MDA-MB-231) tumor-bearing mice



Siddiquee K. et.al. PNAS 2007;104:7391-7396

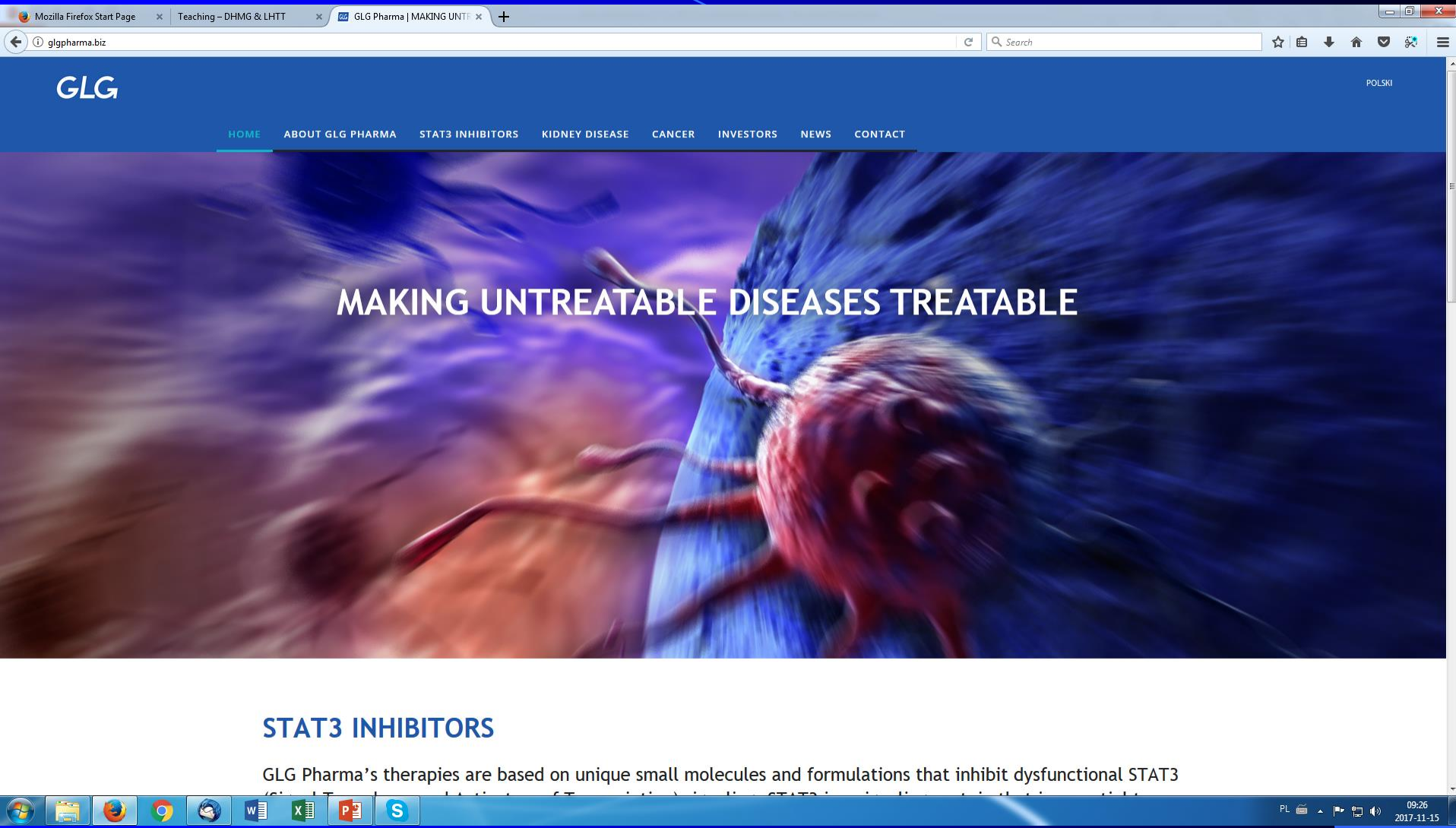
STAT3 inhibitors in clinical trials

Table 9. Stat3 Inhibitors in Clinical Trials

Agent	Structure	Trial phase	Indication	References
1		Phase I/II	Psoriasis	142
Pyrimethamine		Phase I/II	Chronic lymphocytic leukemia / Small lymphocytic lymphoma	143
OPB-31121	Structure not disclosed	Phase I	Advanced solid tumor	144
53		Phase I/II	Pancreatic cancer	145
		Phase II	Solid tumors and lymphoid malignancies	146

Debnath et al., 2012

GLG Pharma


Mozilla Firefox Start Page
Teaching – DHMG & LHTT
GLG Pharma | MAKING UNTR
glgpharma.biz
Search
GLG
POLSKI
HOME
ABOUT GLG PHARMA
STAT3 INHIBITORS
KIDNEY DISEASE
CANCER
INVESTORS
NEWS
CONTACT
MAKING UNTREATABLE DISEASES TREATABLE
STAT3 INHIBITORS
GLG Pharma's therapies are based on unique small molecules and formulations that inhibit dysfunctional STAT3
PL
09:26
2017-11-15

GLG PHARMA

STAT3 Mediated Diseases Result from Uncontrolled STAT3 Activation

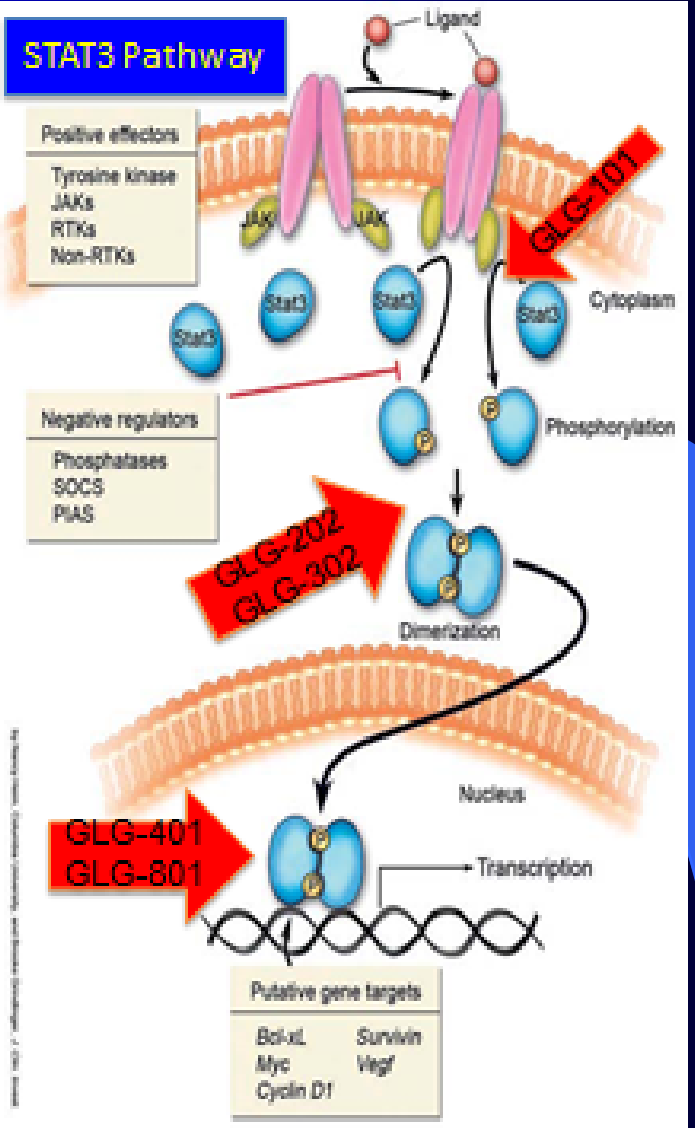
Activation of STAT3 PROTEIN is essential for

- cell growth
- division
- apoptosis

Normal cell: activation is switched on/off by positive effectors & negative regulators

Diseased cell: **switch stays on,** process occurs constantly at high levels, keeping cells growing & dividing uncontrollably

Diseases: Kidney disease, cancer, psoriasis



GLG Pharma: Pipeline

There are currently 12 STAT3 inhibitors in the GLG Pharma pipeline.

Phase II clinical trials are currently underway with GLG-801 for chronic lymphatic leukemia (CLL). It is anticipated that Phase II studies will be completed in 2016 and Phase III clinical trials will begin in 2017 in the United States, Poland, Germany and France.

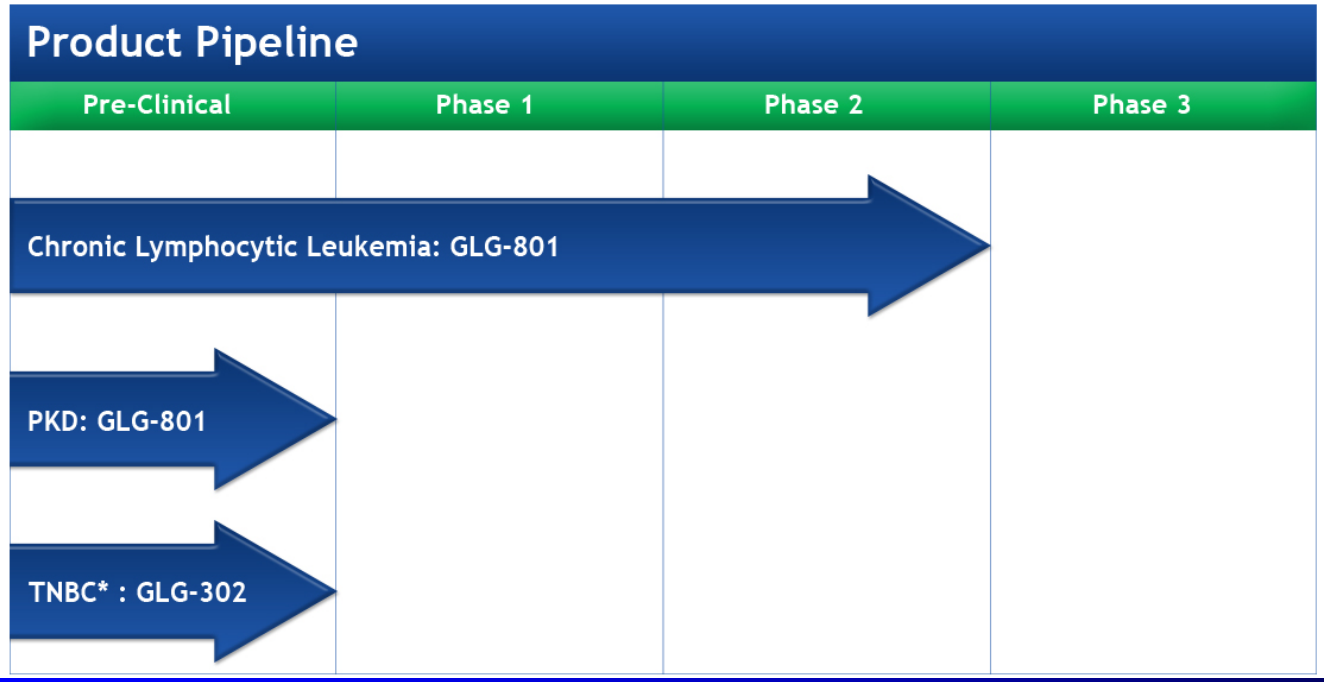
Pre-clinical work on GLG-801 has been completed for polycystic kidney disease and Phase I clinical trials are planned for 2016.

Pre-clinical work on GLG-302 for the treatment of triple negative breast cancer (TNBC} has been completed. Phase I clinical trials are planned for. 2016.

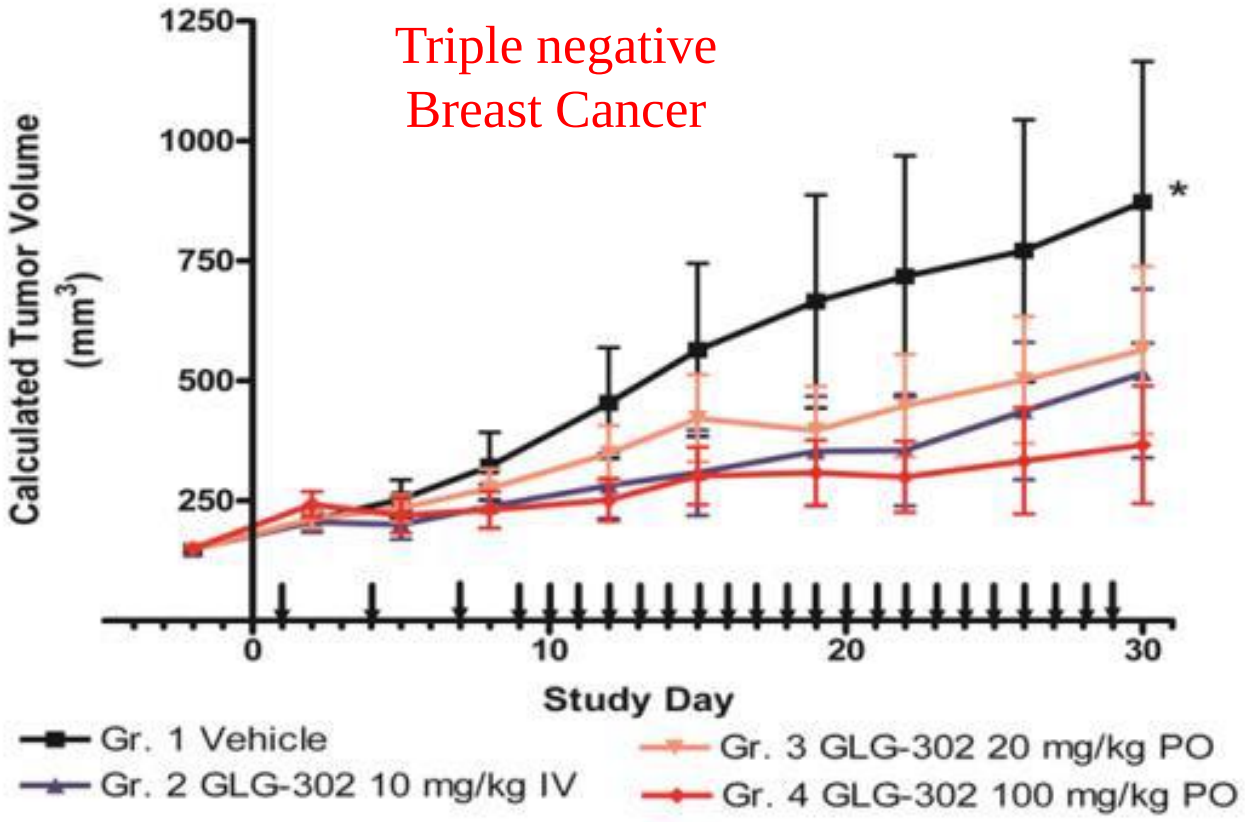
A number of toxicity studies are being completed for several indications

A unique diagnostic tool has been developed to identify promising candidates for STAT3 inhibitor therapy and monitor patient's progress.

Because there is no current effective treatment for these diseases, regulatory approval processes should be accelerated

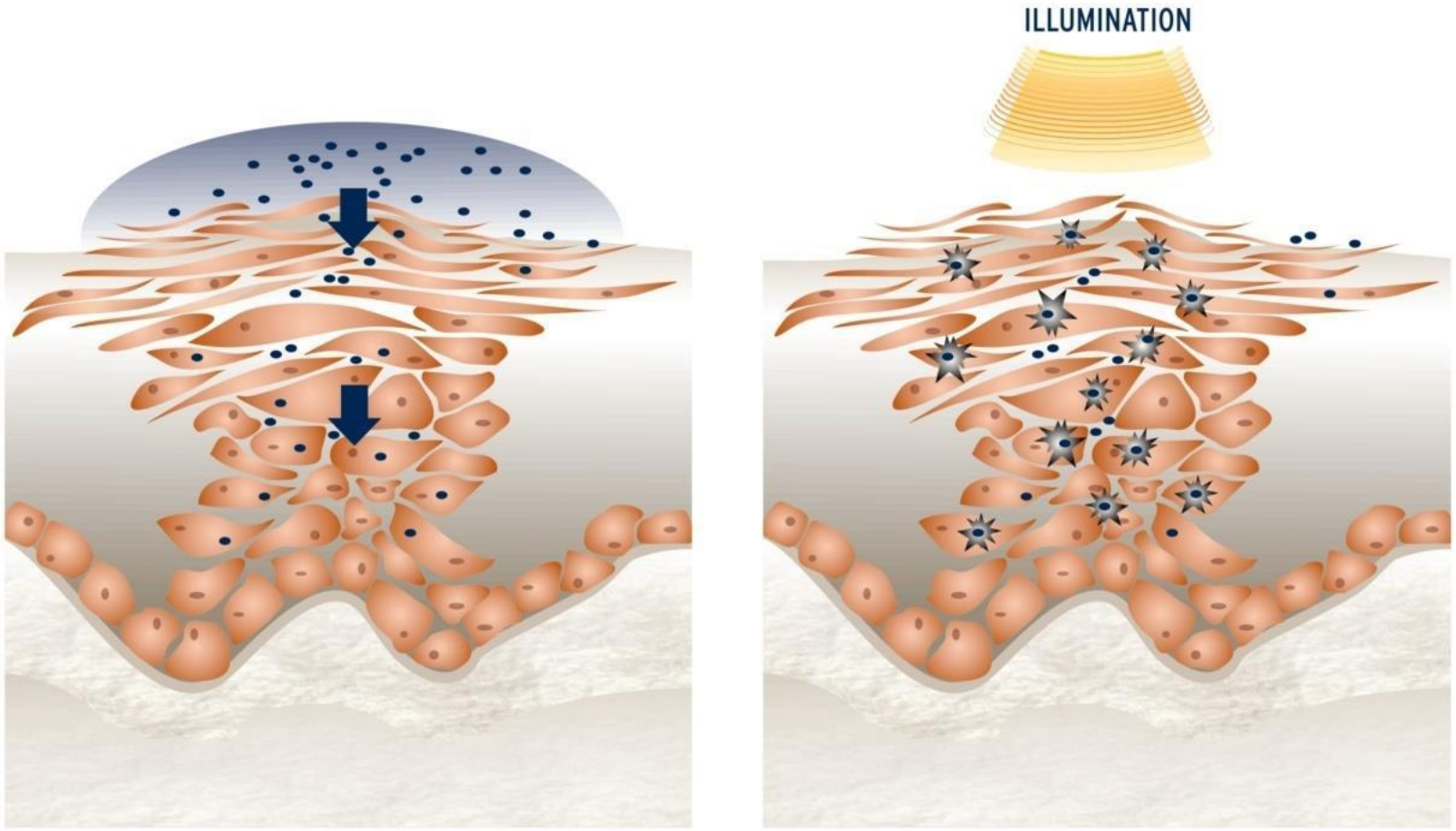


0711-GLG-011 (BTS 11175-02)
MDA-MB-231 Xenograft Study Examining GLG-302, IV or PO vs Vehicle
Group Average of Individual Calculated Tumor Volume



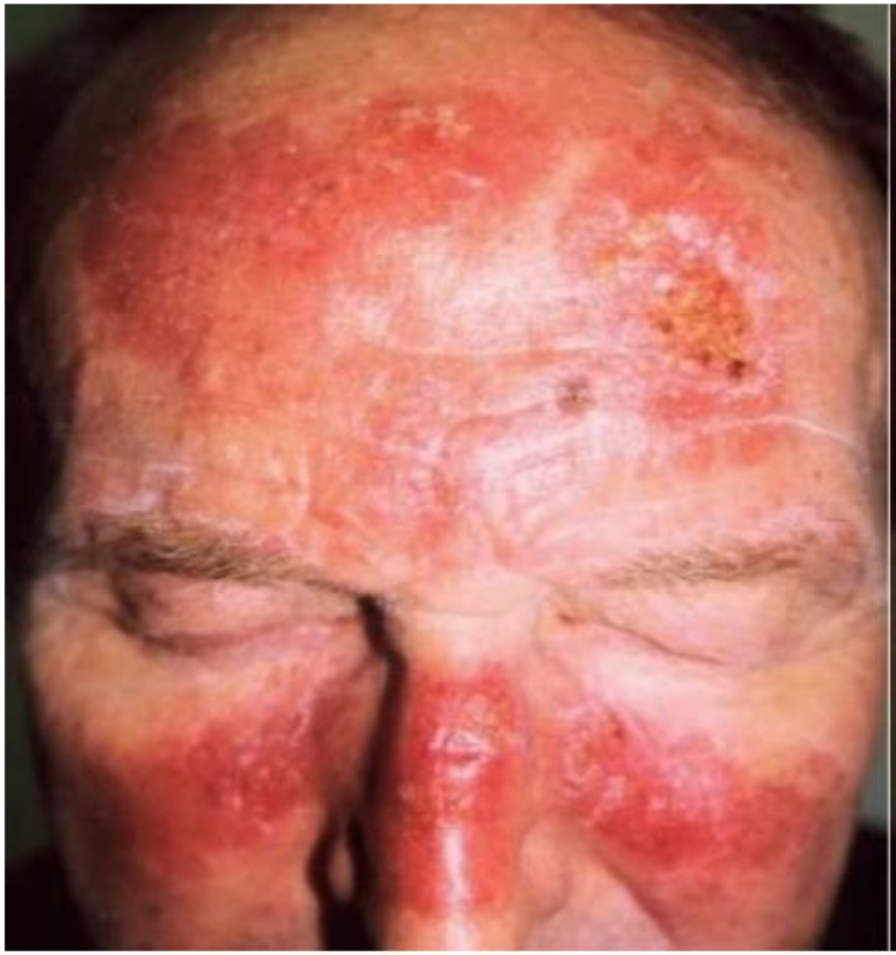
= Day of TA Administration

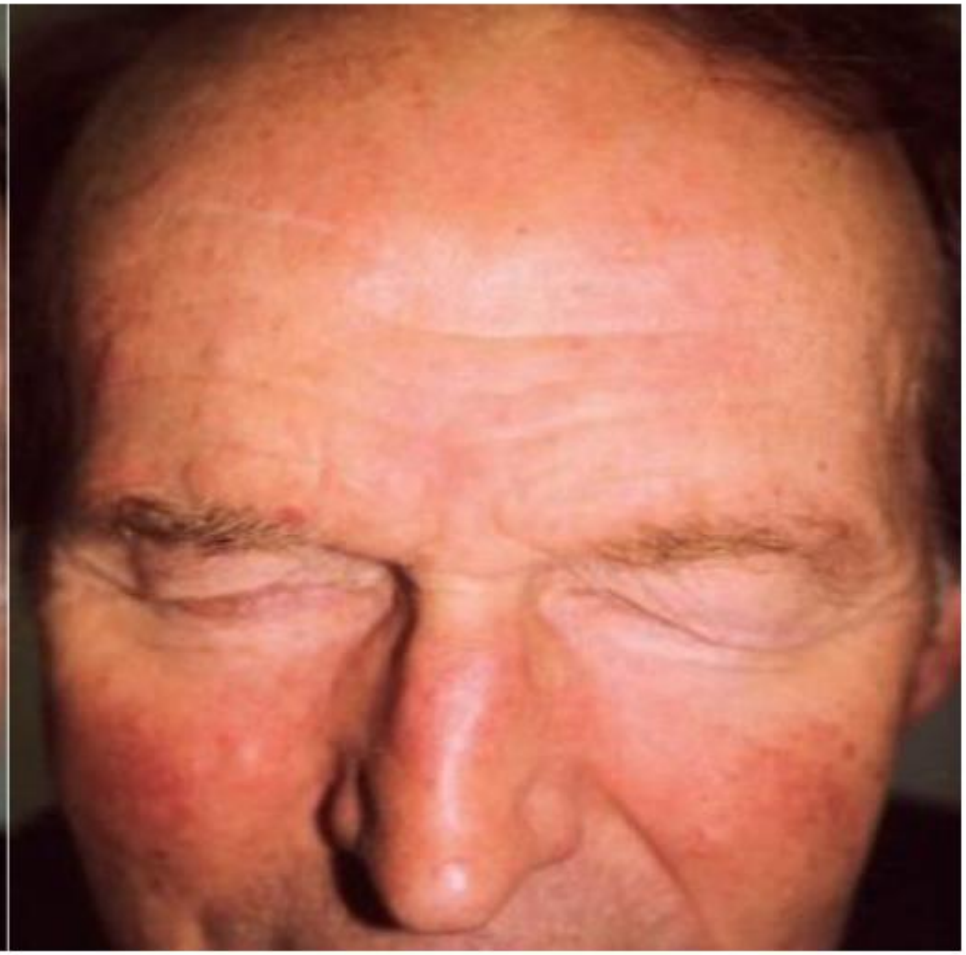
* n = 9, single animal euthanized on Day 26 (post tumor measurement), per protocol and IACUC, due to excessive tumor size (> 2000 mm3; actual 2577 mm3), animal data was carried forward for analysis


ILLUMINATION

Actinic keratosis Squamous cancer Treatment

7 day follow-up visit





Application/Publication Patent Number	Application/ Publication/ Issued Date	STATUS	Title
US 2007/0191490 Al 11/701,722	Feb. 2007	Filed	Withacnistin Compounds for the Treatment of Cancer **GLG-101**
WO 2008/070697 A2 12/517,453	Jun. 2008	Filed	STAT3 Inhibitor Having Anti-Cancer Activity and Associated Methods **- GLG-202**
European Patent No. 2120958	Mar. 2013	Issued	
Patent No. 7,960,434	Jun. 2011	Issued	Small Molecule Inhibitors of STAT3 with Anti-tumor – **GLG-302 and analogs**
61/551,737	Oct. 2011	Filed	A Novel Platinum Compound That Inhibits Constitutive STAT3 Signaling and Induces Cell Cycle Arrest and Apoptosis of Malignant Cells – **GLG-401**
Patent No. 8,445,517	Mar. 2013	Issued	STAT Modulators - **GLG-801 and others**
61/533,379	Sept. 2011	Filed	Method and Compositions for Reducing Ischemic Stroke-Induced Damage to Neural Cells **- GLG-302**
Patent No. 8,133,692	Mar. 2012	Issued	Methods of predicting responsiveness to chemotherapeutic agents and selecting treatments **- Diagnostic**

CURRENT CORPORATE (see disclaimer) REVENUE PROJECTIONS

- GLG-801 + Diagnostic for CLL and ADPKD - $728MM 3-5 years
- GLG-302 + Diagnostic for CLL and ADPKD - $4.5 BB 4-8 Years

Inhibiting
Signaling Pathways
as a novel strategy
to treat

Cancer
&
Inflammatory Diseases