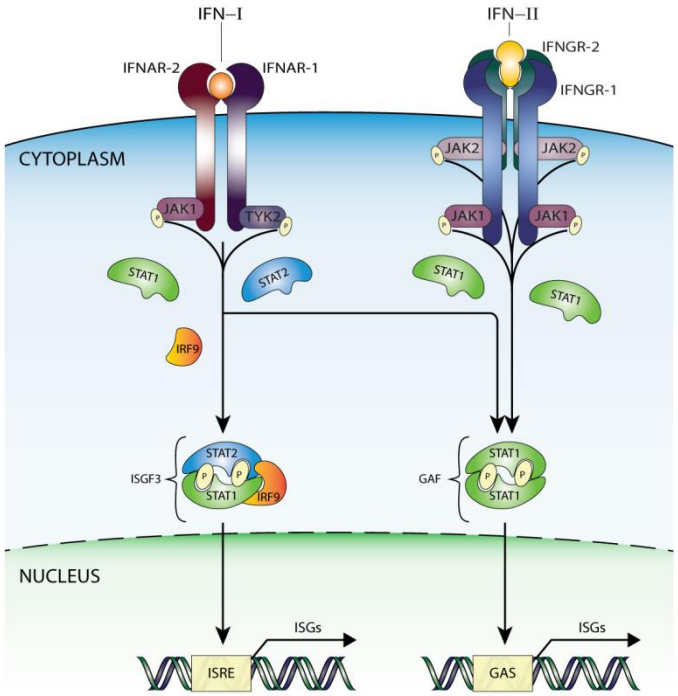
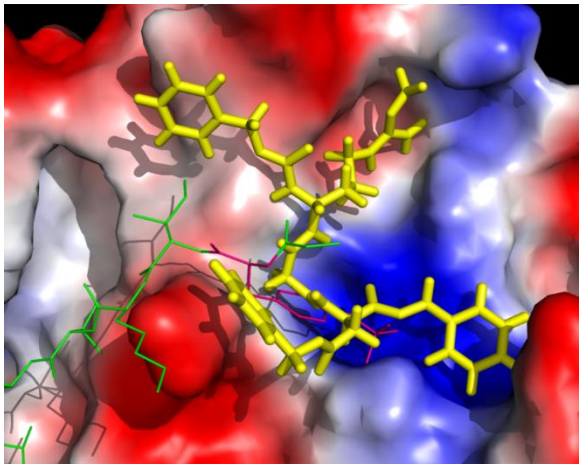
ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ
Faculty of Biology

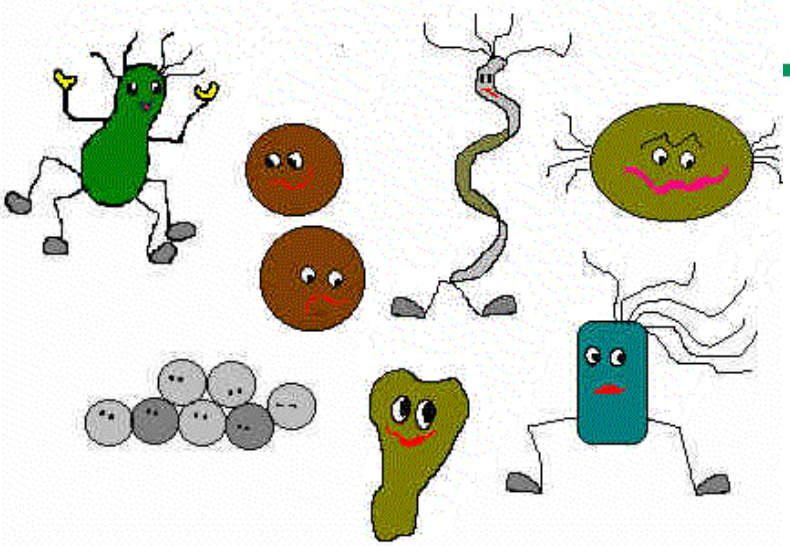
STATs in Infection & Cancer

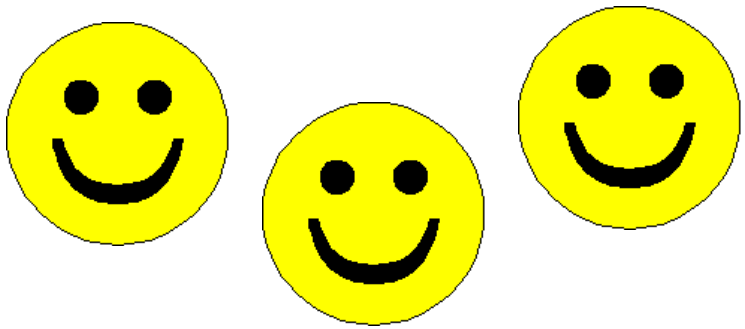




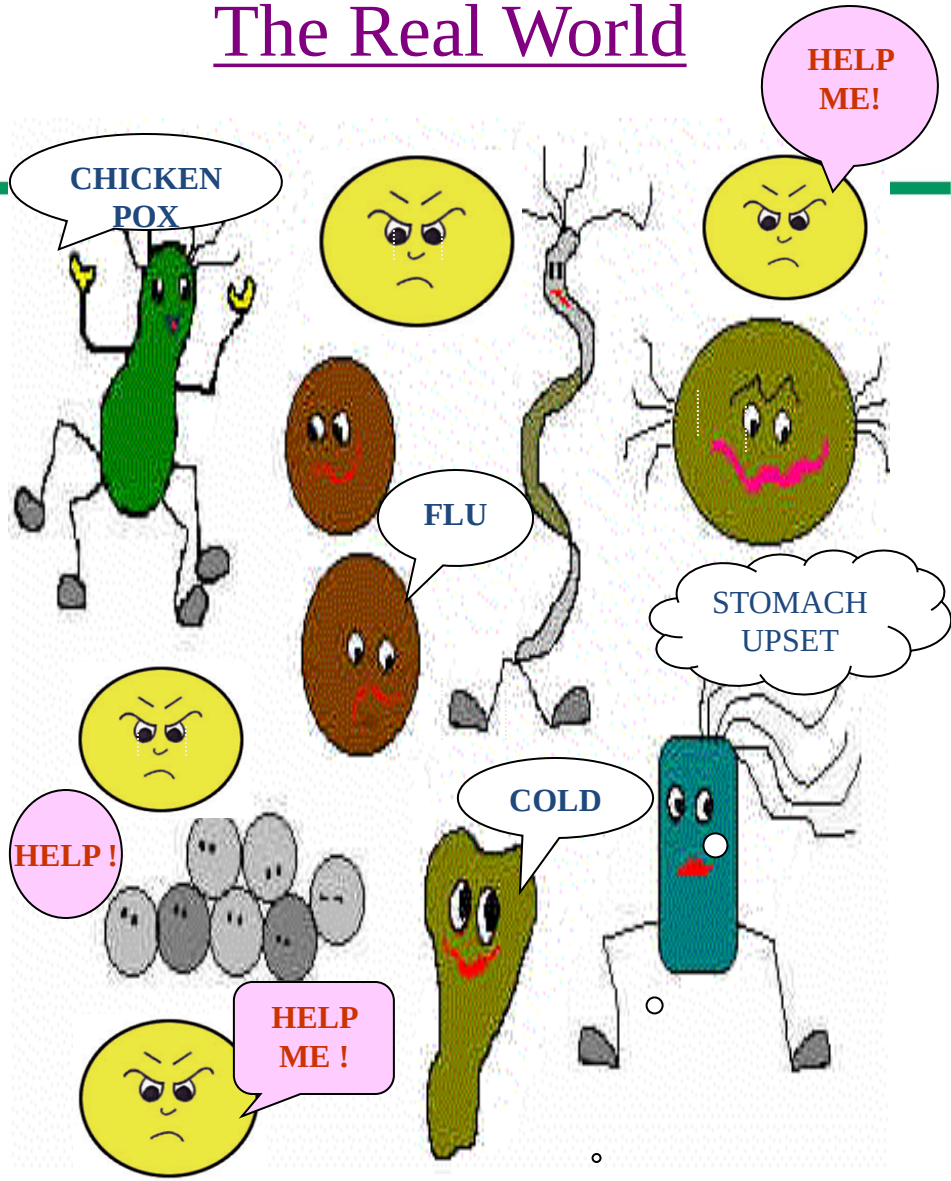
Prof. Hans Bluyssen
Lab Human Mol Genetics
28-04-2023

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.

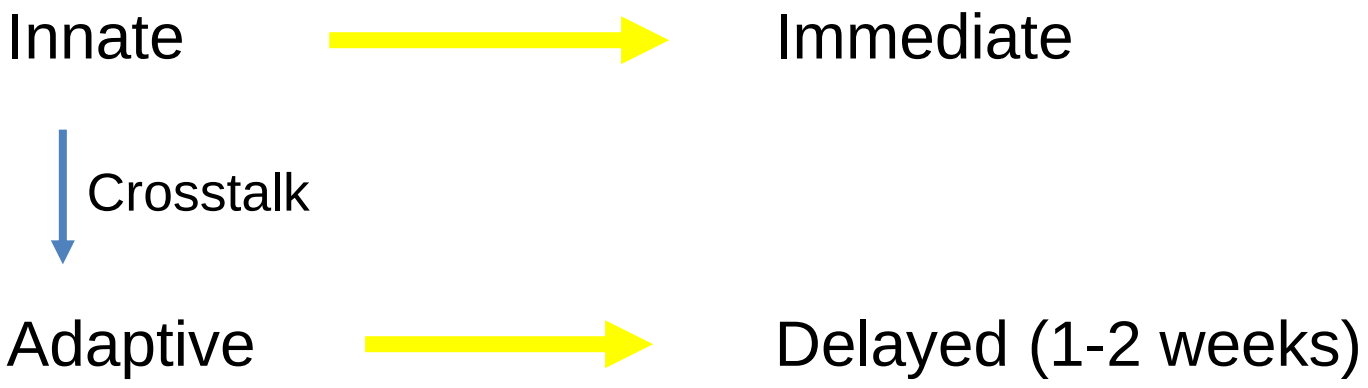
(The American Heritage Stedman's Medical Dictionary)

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

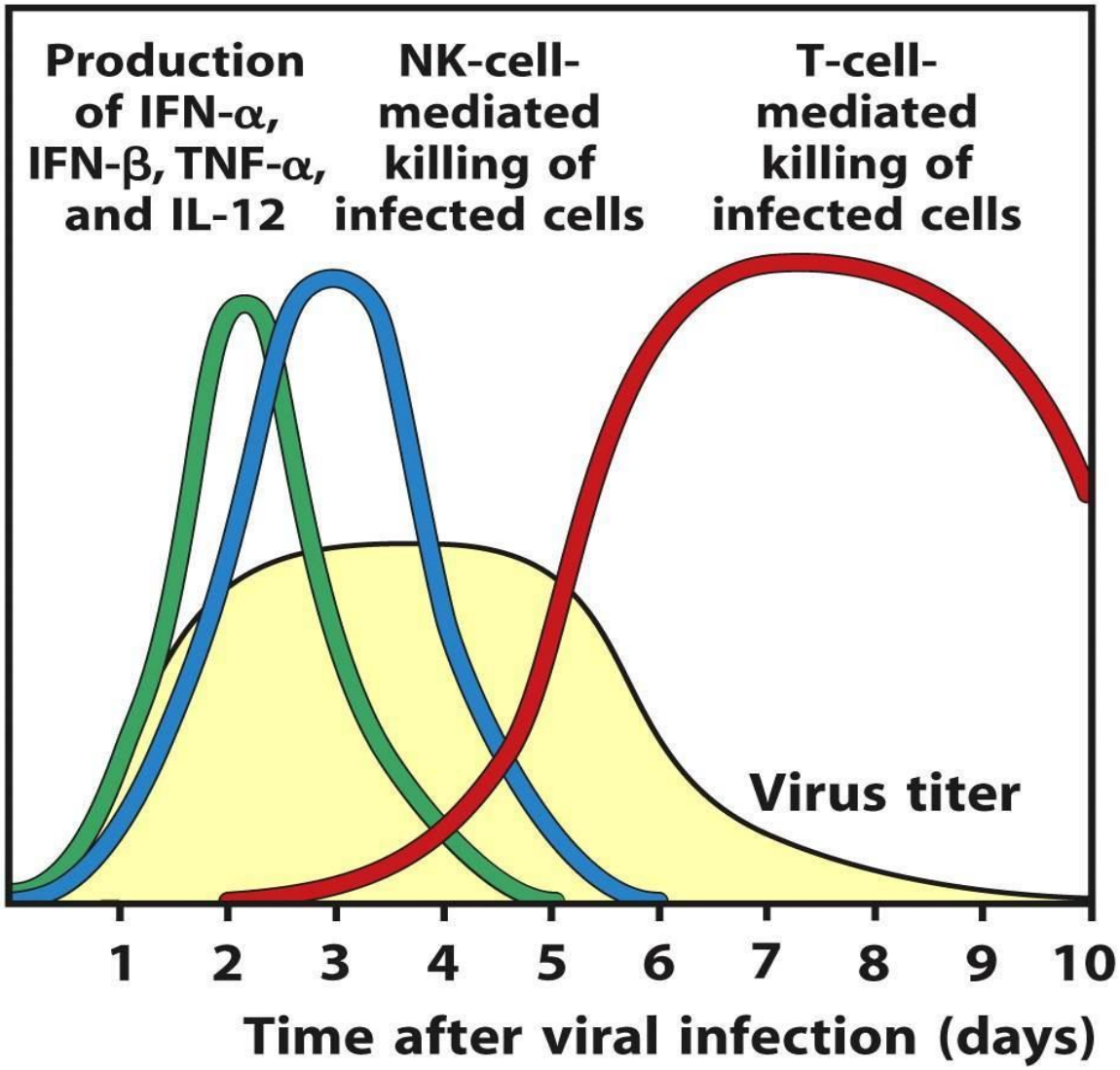


Figure 3.30 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

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

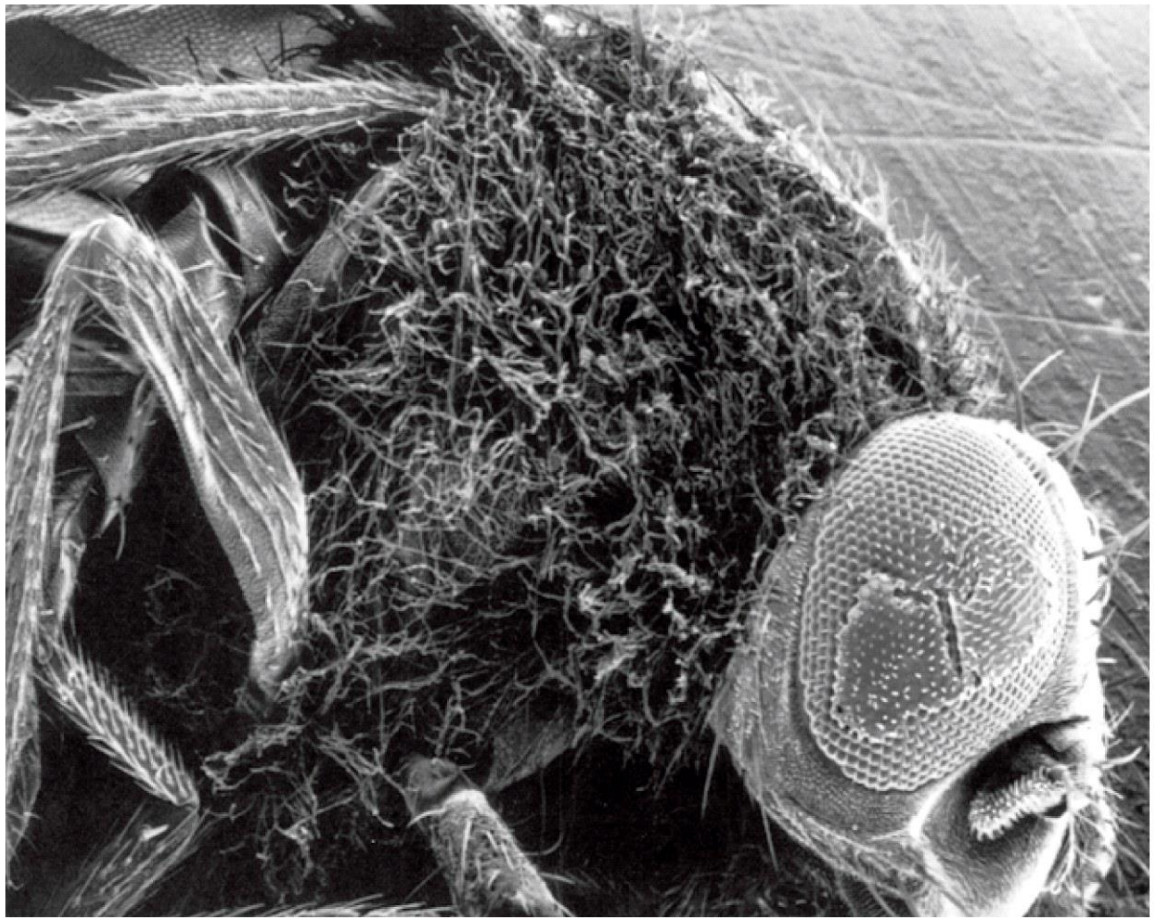


Figure 3.8 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

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

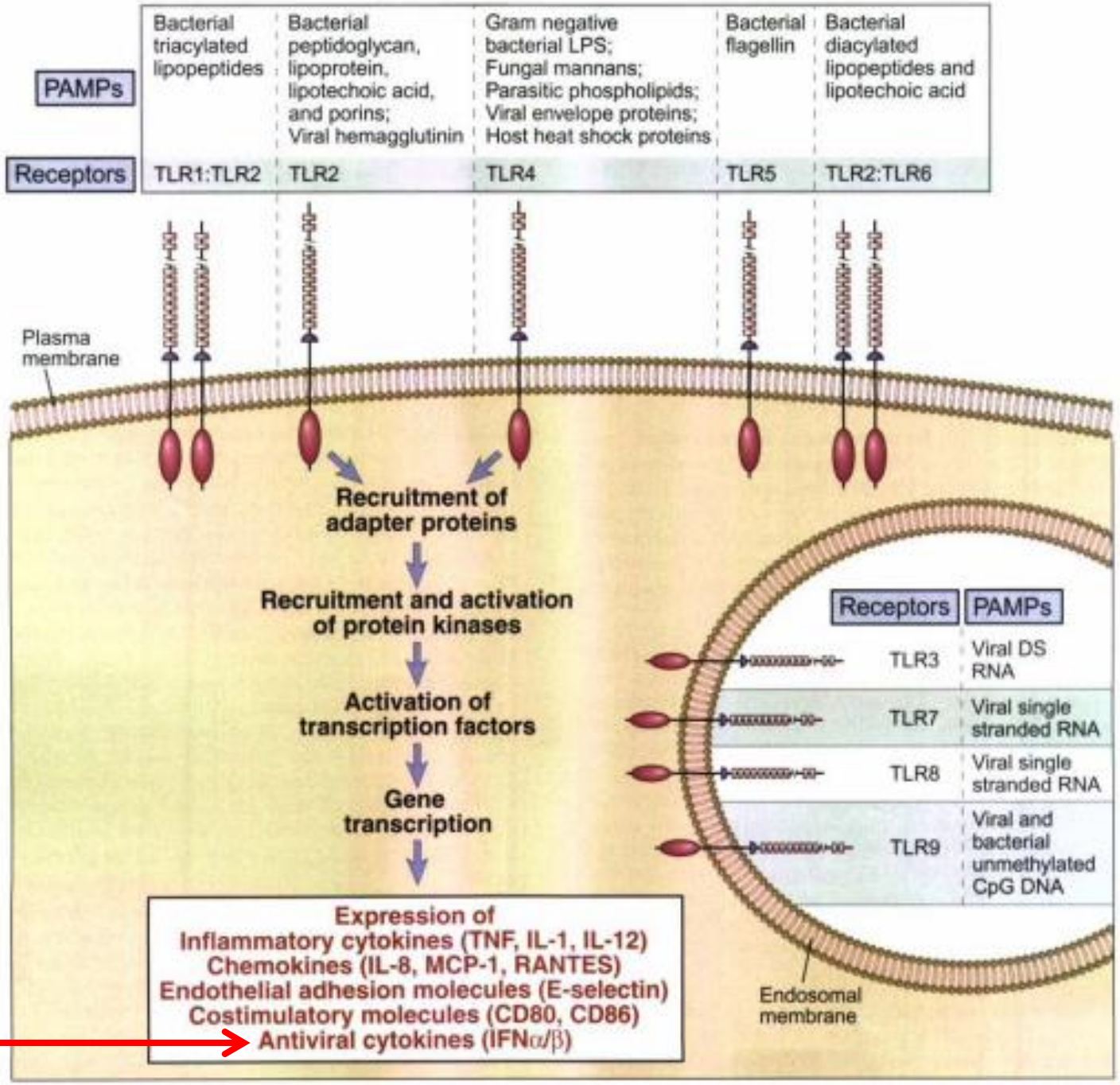
LENA ALEXOPOULOU*, AGNIESZKA CZOPIK HOLT*†, RUSLAN MEDZHITOV*‡§ & RICHARD A. FLAVELL*‡§

* Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut, 06520, USA
† Department of Molecular, Cellular and Developmental Biology, Yale University School of Medicine, New Haven, Connecticut, 06520, USA
‡ Howard Hughes Medical Institute, and Yale University School of Medicine, New Haven, Connecticut, 06520, USA
§ 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
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
Receptors
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

Interferons

“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

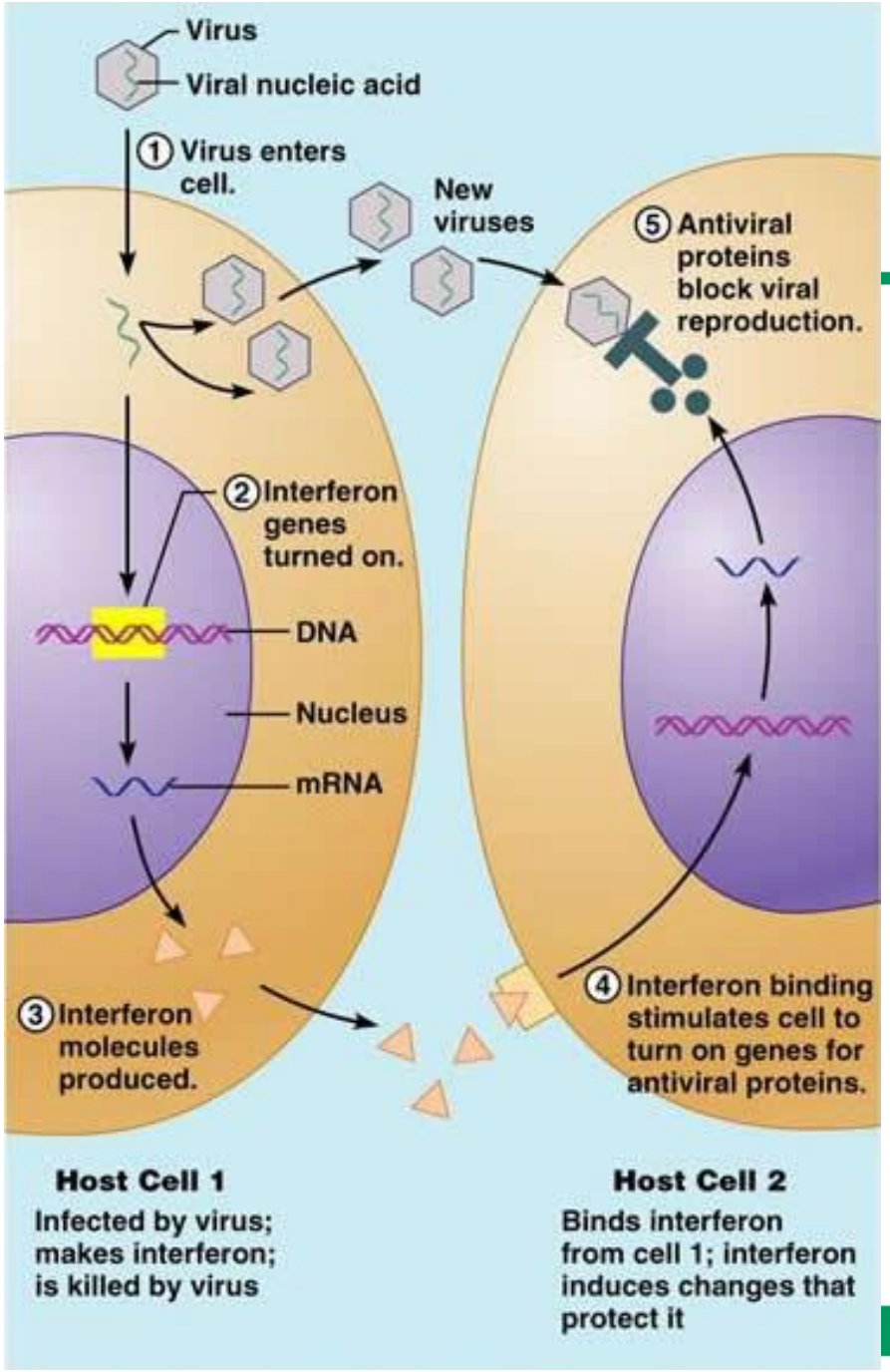
dsRNA is best activator of IFN genes

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

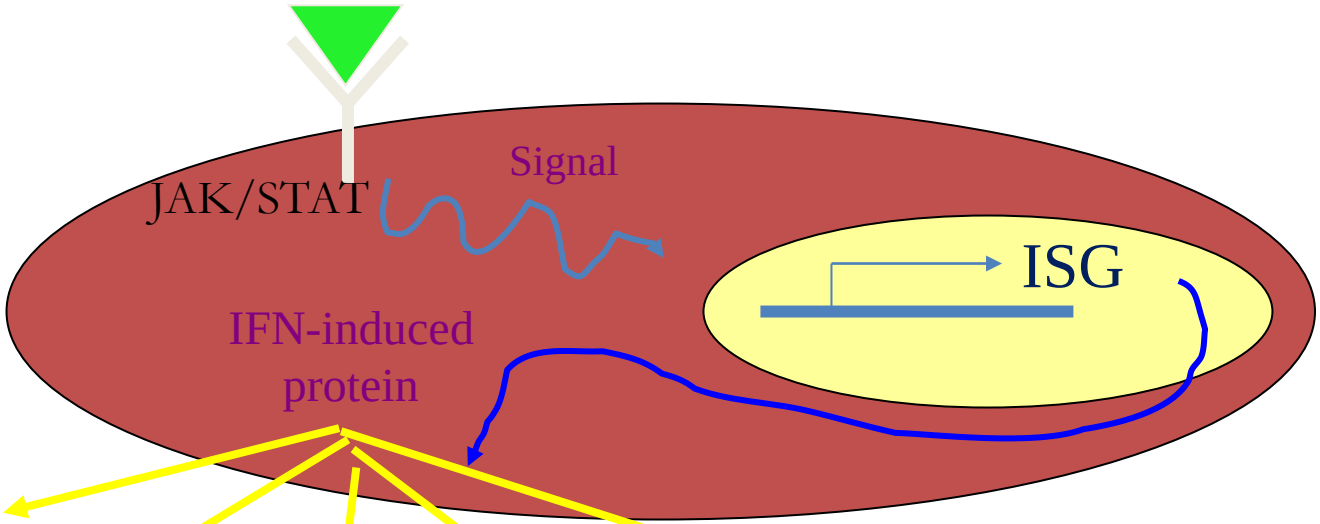
Inhibition cell growth

Activation immune system

Anti-viral State

Adaptive immune response

Biological Activities of IFN



Apoptosis

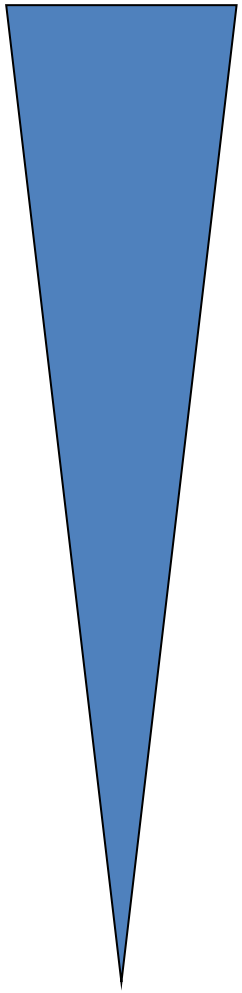
Inhibition of
viral replication

Activation of
Immune System

Inhibition of
Cell Growth

Regulation of
Cell differentiation

Virus Sensitivity to IFNs



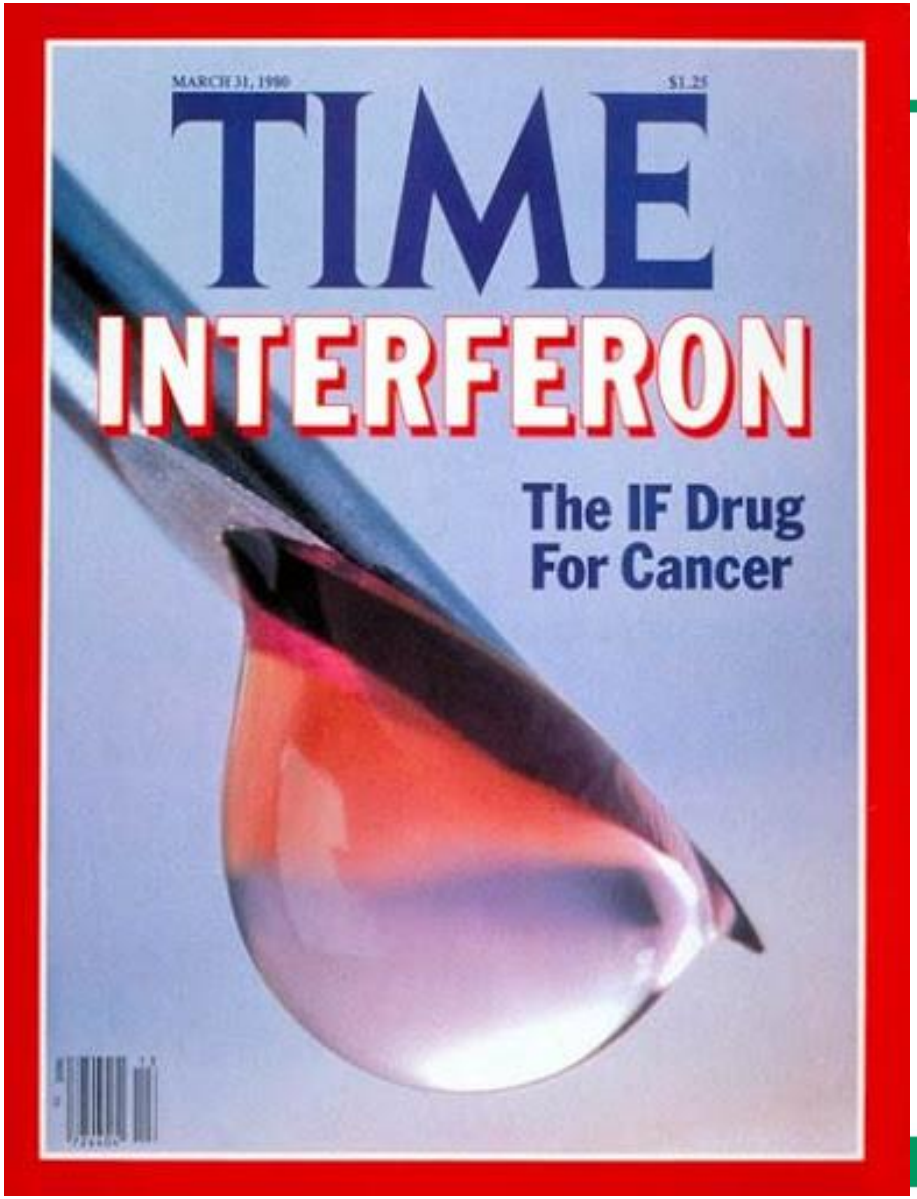
Small RNA viruses - picornaviruses

Large RNA viruses - Flu, rotovirus

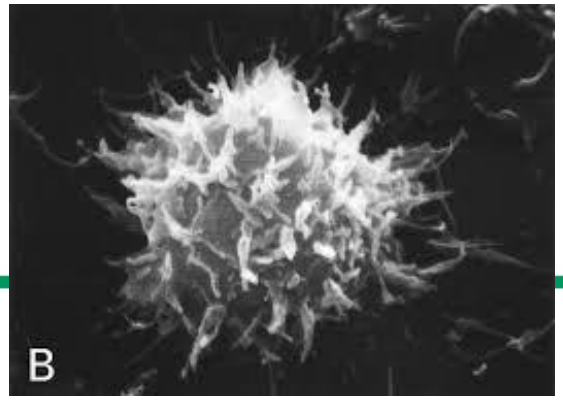
Small DNA viruses - papillomavirus

Large DNA viruses - Herpes, poxvirus

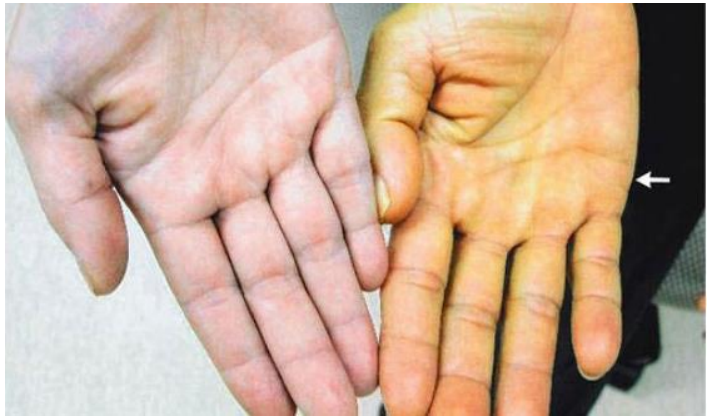
1980: IFN “Golden Bullet”



Clinical Use of IFNs



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



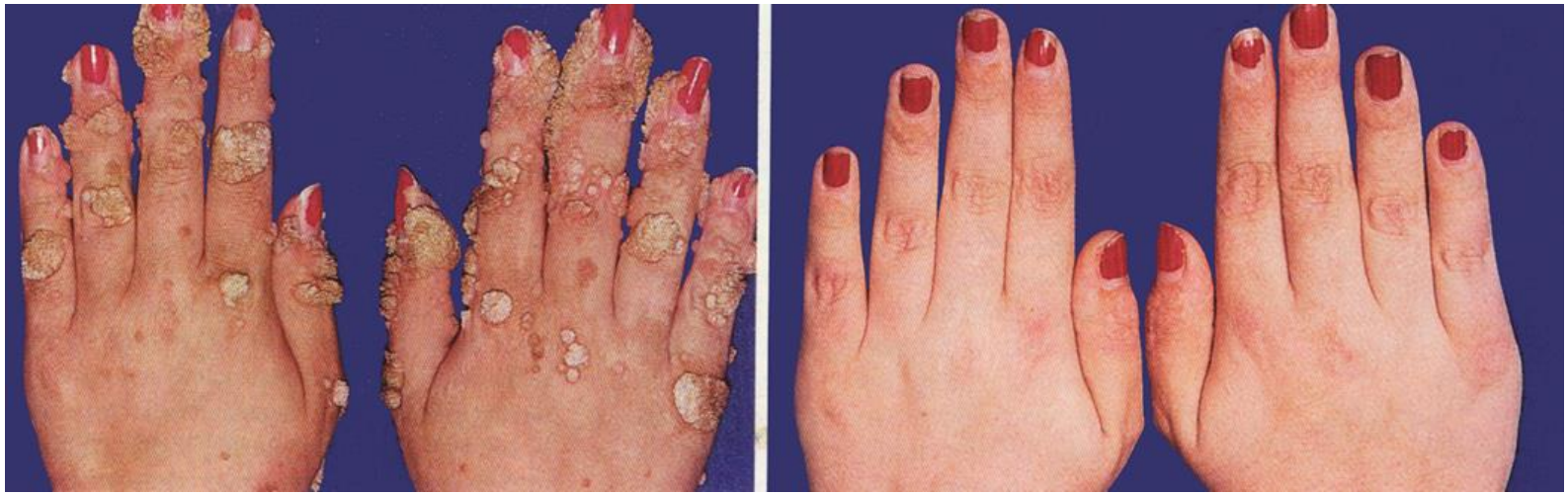
- Cancer
  - Hairy cell Leukemia (90% effective)
  - Follicular lymphoma
  - cervical (HPV)
  - basal cell cancer (80-90%)
  - Kaposi's sarcoma (HHV type 8)

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

IFN Therapy

Before

IFN therapy

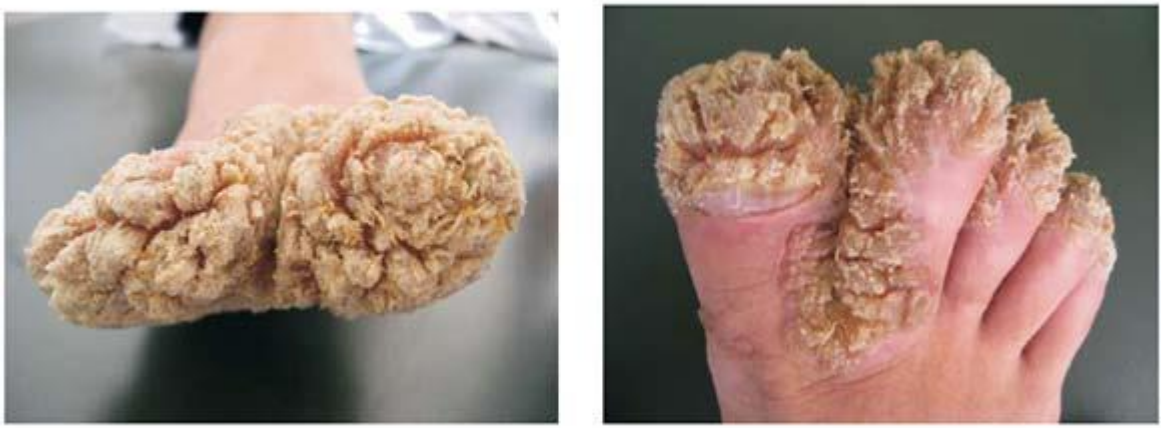


Human papillomavirus warts

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

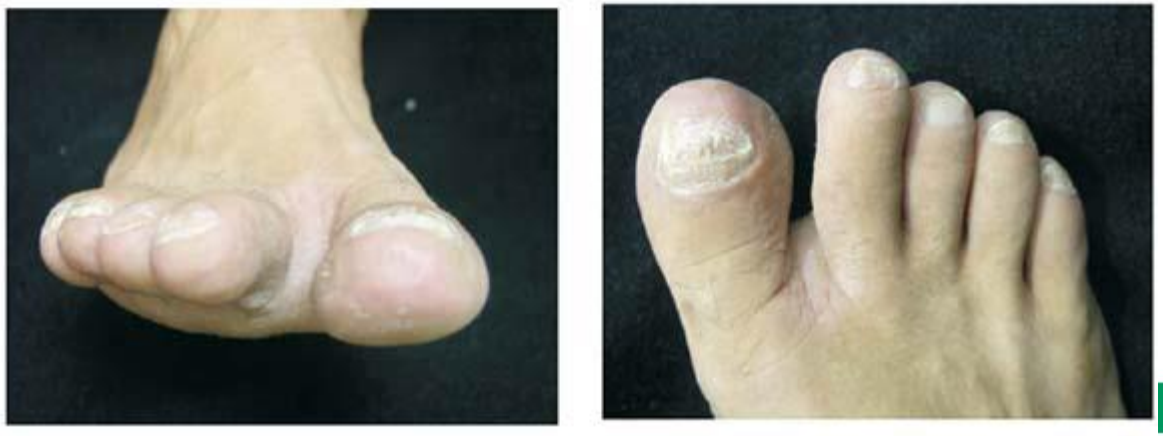
Before

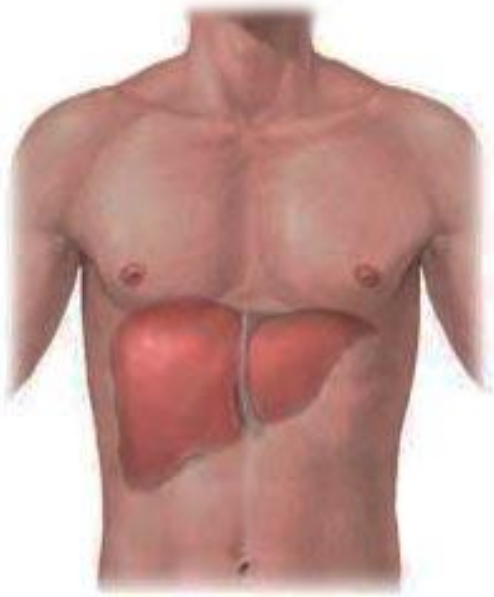
Figure 1. Right foot lesions before treatment.

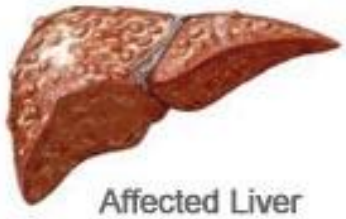


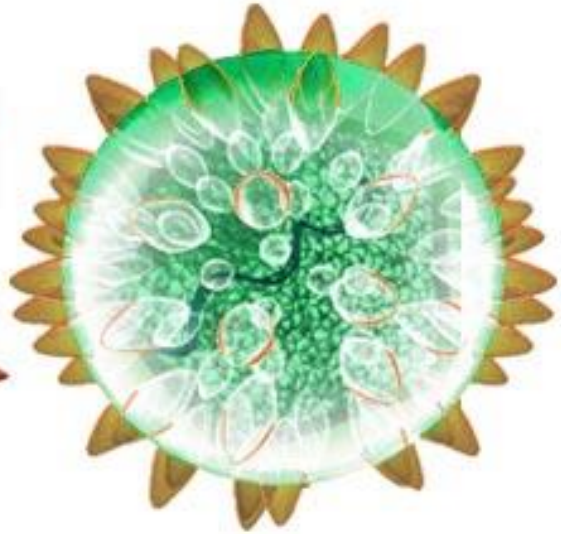
After

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





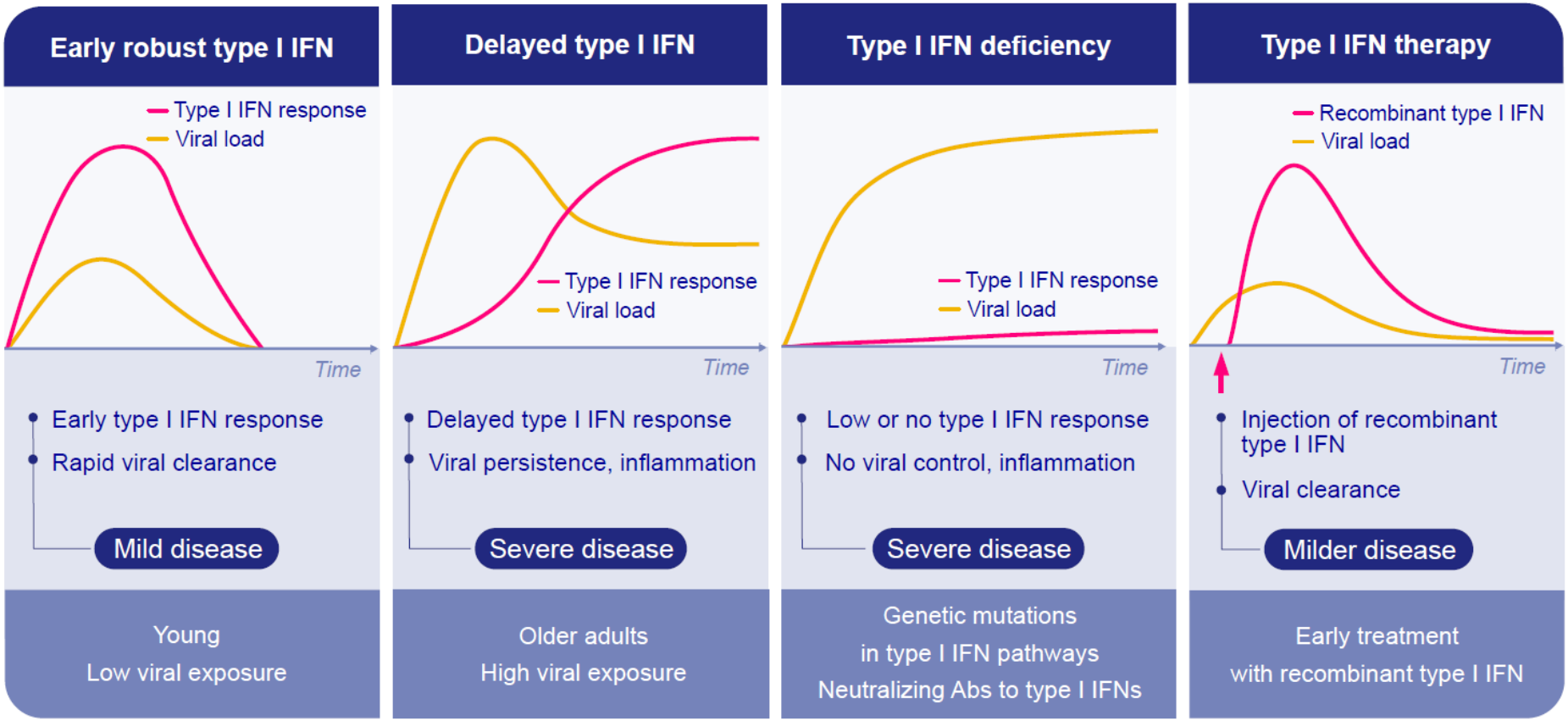




HCV infection Treatment

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



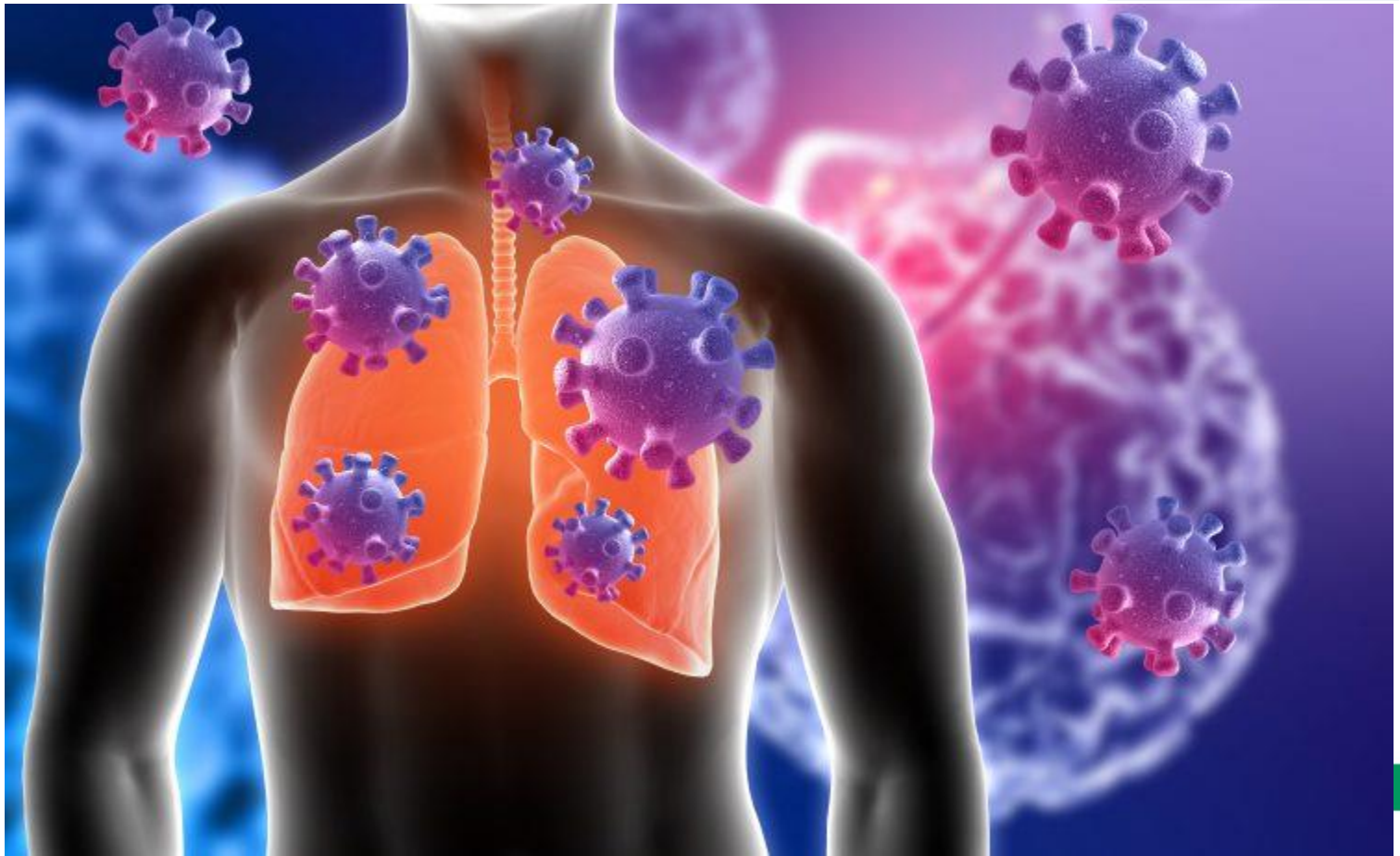
NEWS

Inhaled interferon beta therapy shows promise in COVID-19 trial

By Hannah Balfour (European Pharmaceutical Review)

20 July 2020

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



NEWS

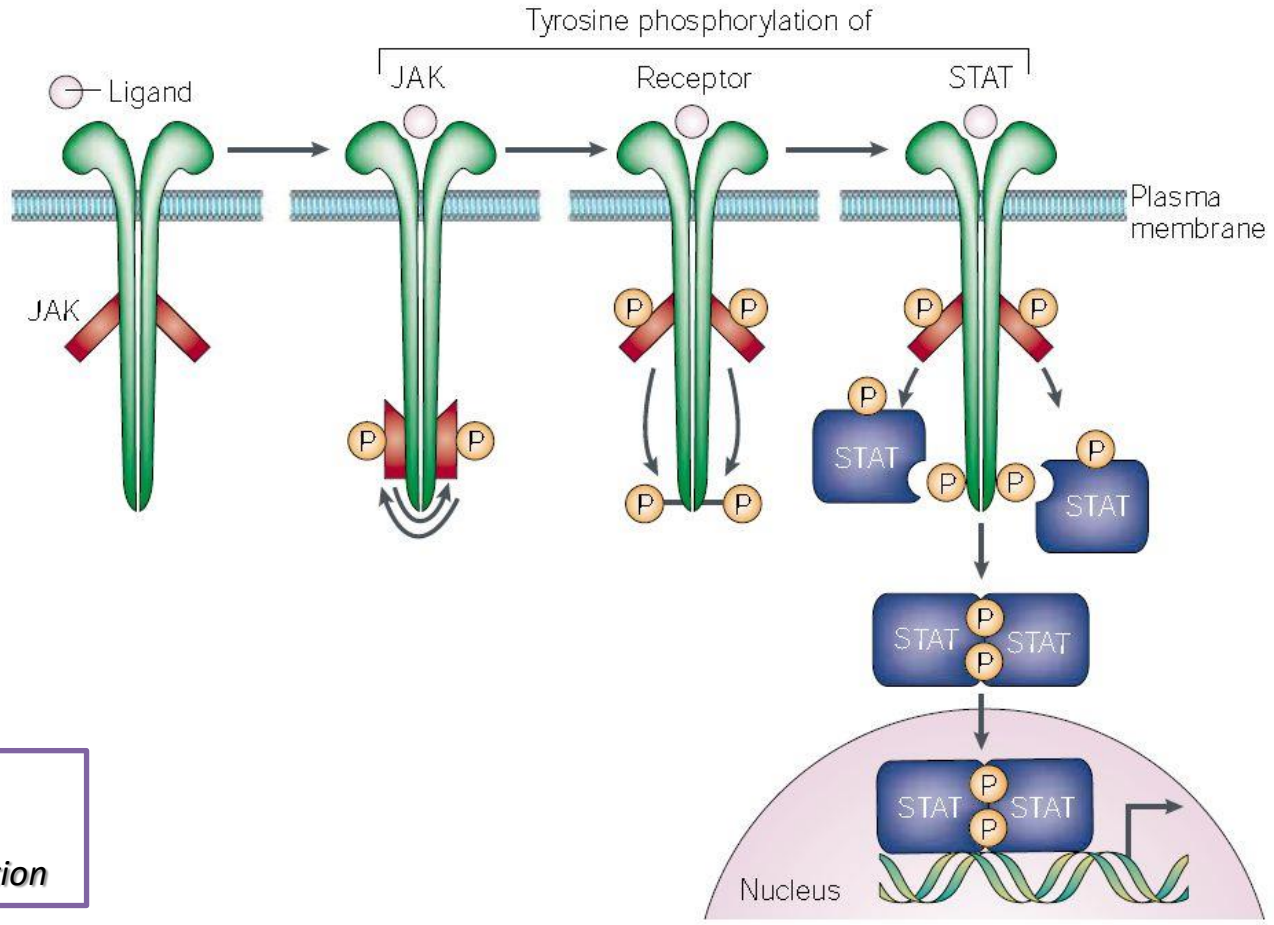
Inhaled interferon beta therapy shows promise in COVID-19 trial

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.

Canonical JAK-STAT pathway



JAK - Janus Kinase
STAT - Signal Transducer and Activator of Transcription

Levy & Darnell (2002)
Nature Reviews Molecular Cell Biology 3, 651-662

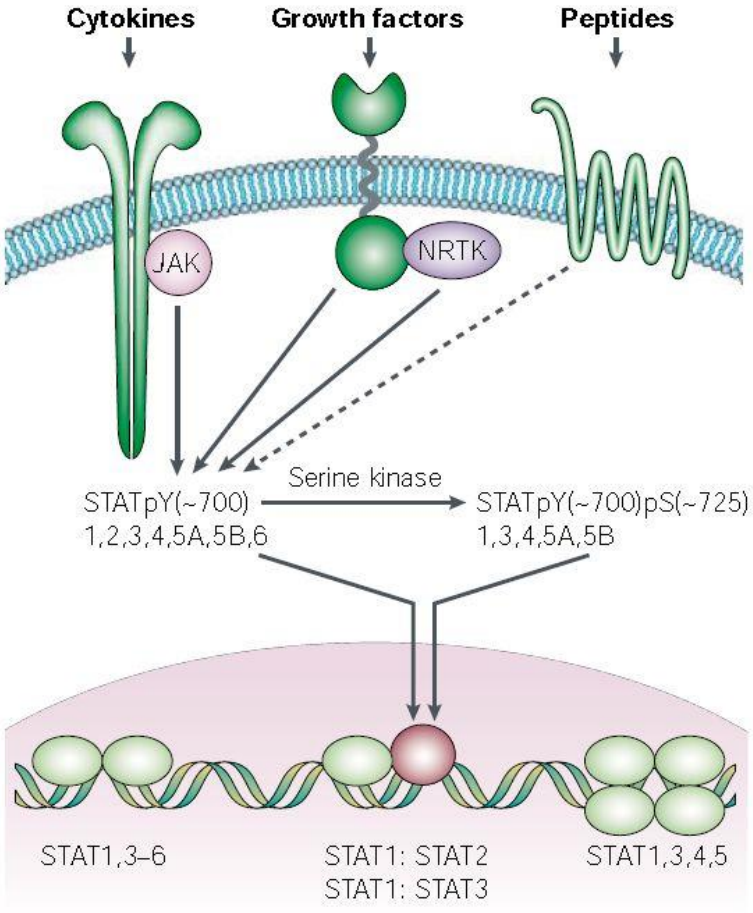
General triggers of JAK-STAT pathway

Cytokines
interferons
interleukines

Growth factors
EGF
PDGF

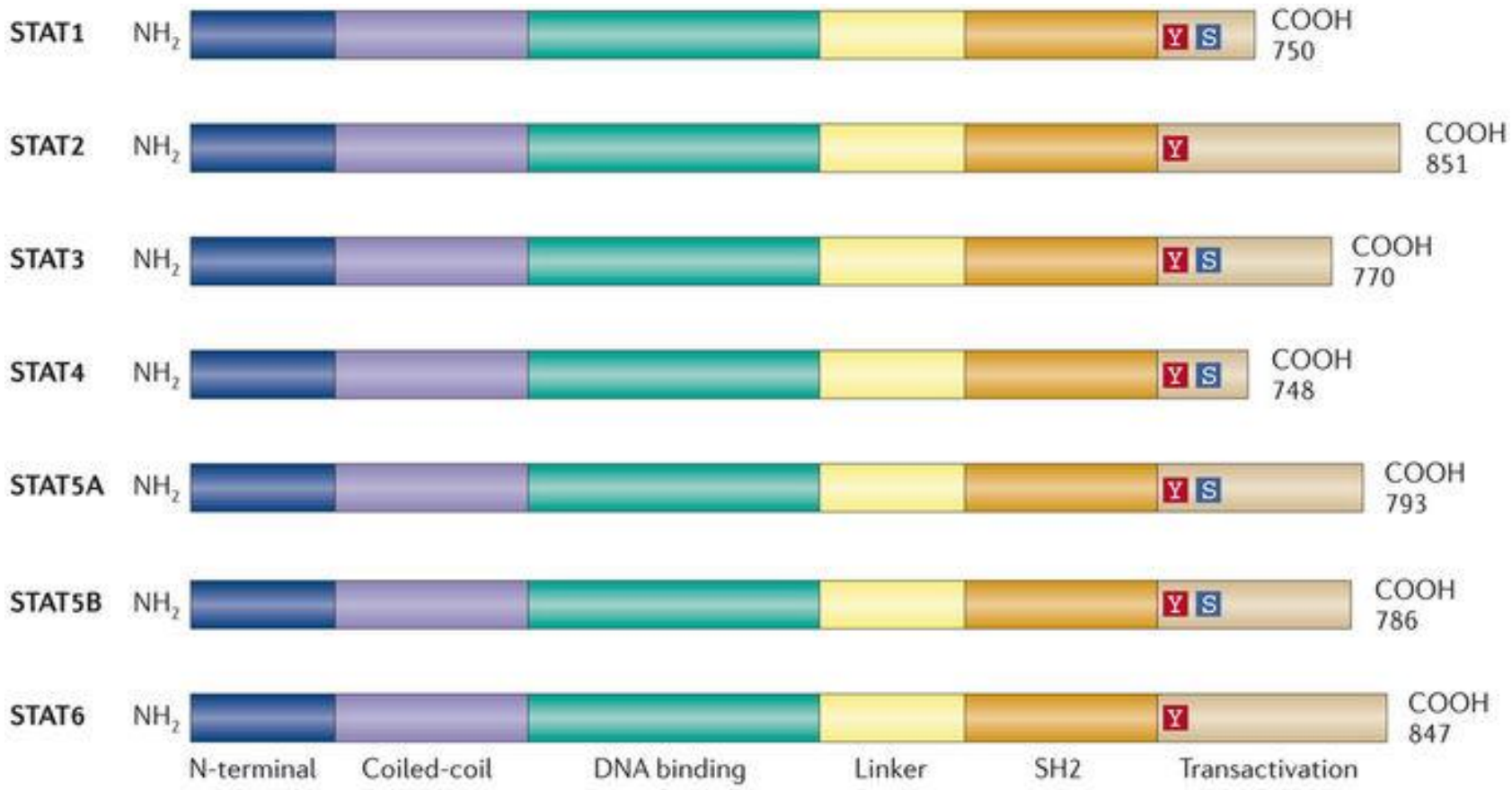
Bacterial molecules
LPS
LTA
dsRNA

Src and Abl kinases
G-coupled receptors
AngII



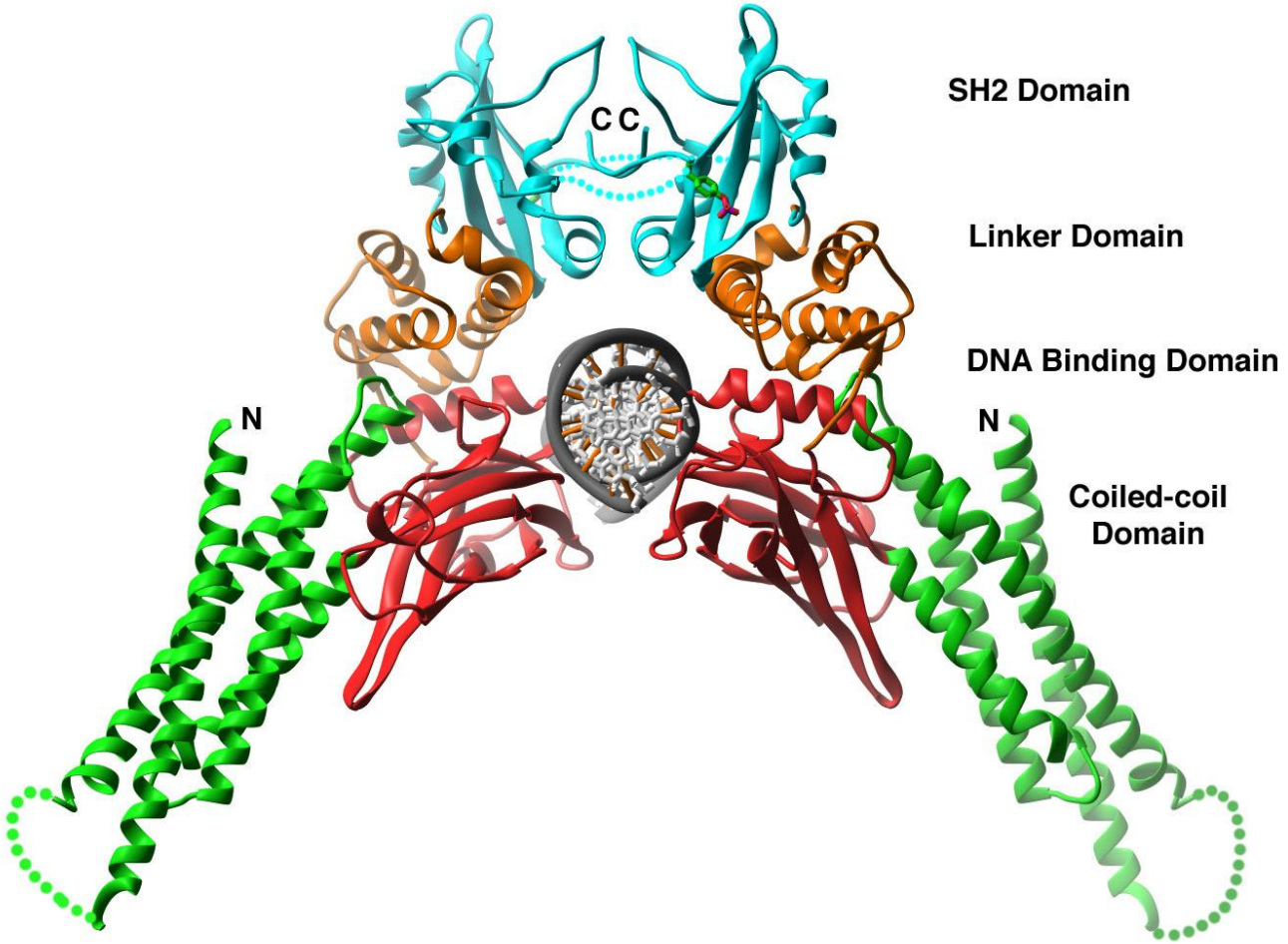
Levy & Darnell (2002)
Nature Reviews Molecular Cell Biology 3, 651-662

STAT Family: Structure



Nature Reviews | Drug Discovery

STAT-DNA Binding

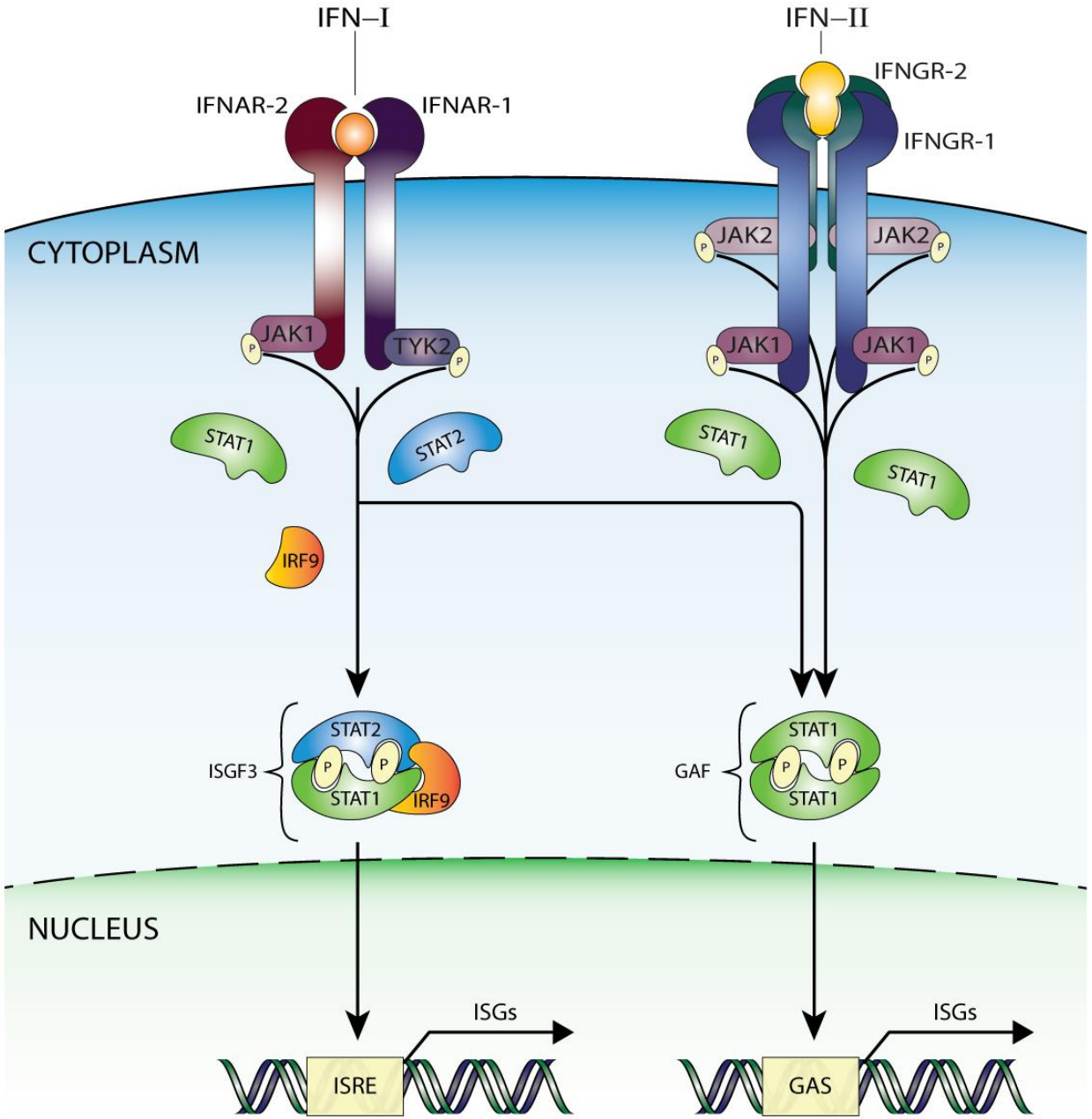


STATs in Health & Disease

STAT	Cellular functions	Major diseases
1	• Cell growth and apoptosis • T_H1 cell-specific cytokine production • Antimicrobial defence	• Atherosclerosis • Infection • Immune disorders
2	• Mediation of IFNα/IFNβ signalling	• Cancer • Infection • Immune disorders
3	• Cell proliferation and survival • Inflammation • Immune response • Embryonic development • Cell motility	• Cancer
4	• T_H1 cell differentiation • Inflammatory responses • Cell proliferation	• Experimental autoimmune encephalomyelitis (multiple sclerosis) • Systemic lupus erythematosus
5A	• Cell proliferation and survival • IL-2Rα expression in T lymphocytes • Mammary gland development • Lactogenic signalling	• Cancer • Chronic myelogenous leukaemia
5B	• Cell proliferation and survival • IL-2Rα expression in T lymphocytes • Sexual dimorphism of body growth rate • NK cell cytolytic activity	• Cancer • Chronic myelogenous leukaemia
6	• Inflammatory and allergic immune response • B cell and T cell proliferation • T_H2 cell differentiation	• Asthma • Allergy

Nature Reviews | Drug Discovery

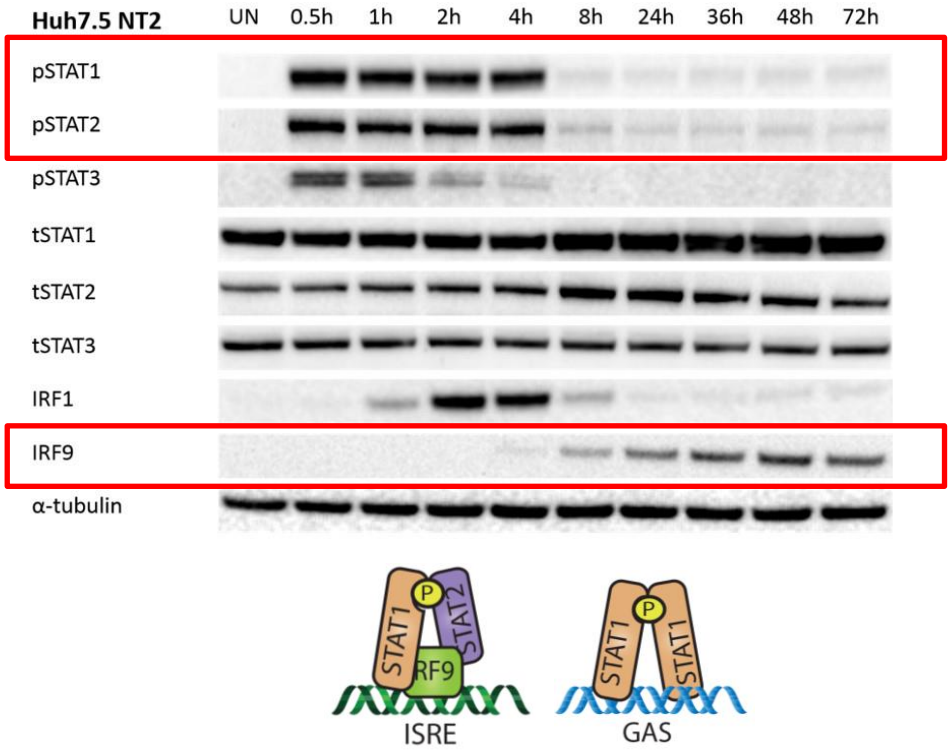
Canonical IFN-signaling (1990)



Sekrecka et al. Front in Immunol. 2018

IFN-I Signaling: pSTAT1, pSTAT2 & IRF9

IFN-I

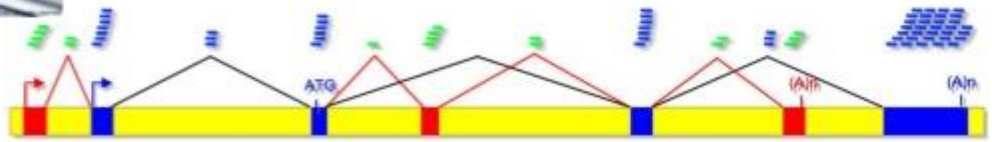


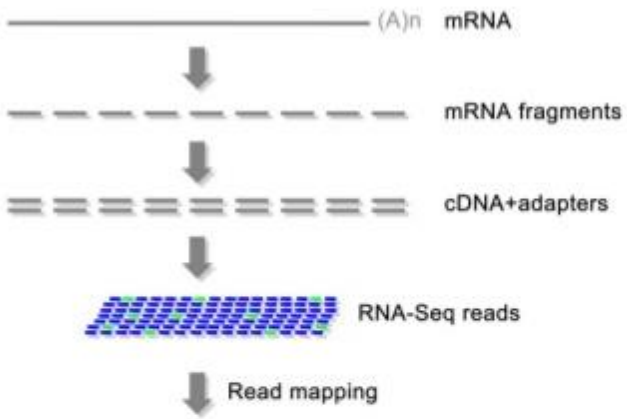
Yamauchi et al., Scientific Rep 2016

Sekrecka et al. 2018

RNA-seq Work Flow

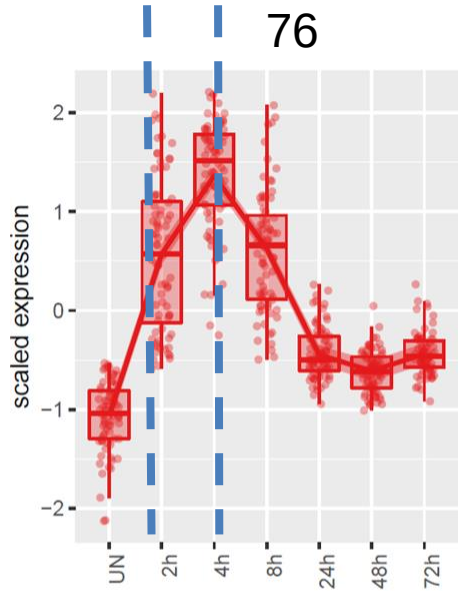
Illumina HiSeq

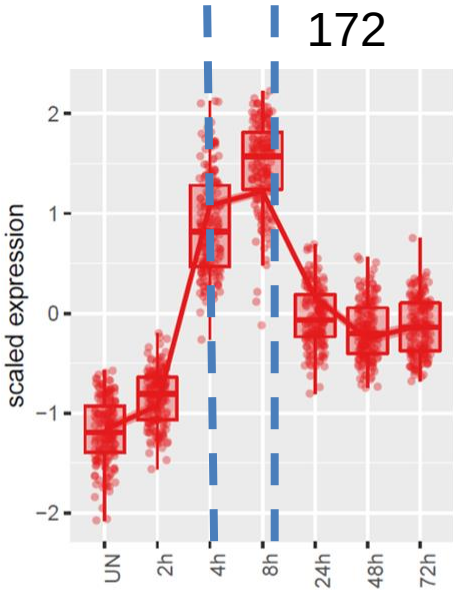


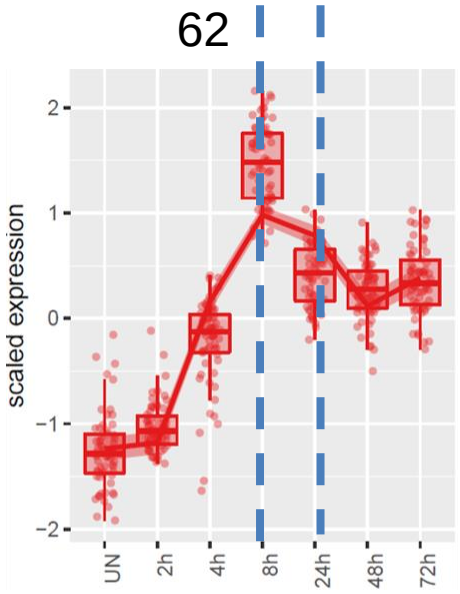


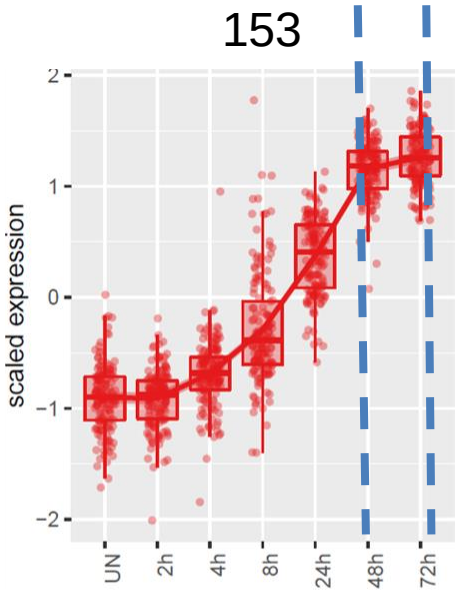
Blencowe B J et al. Genes Dev. 2009;23:1379-1386

IFN-I Signaling:
ISG expression ~ different clusters





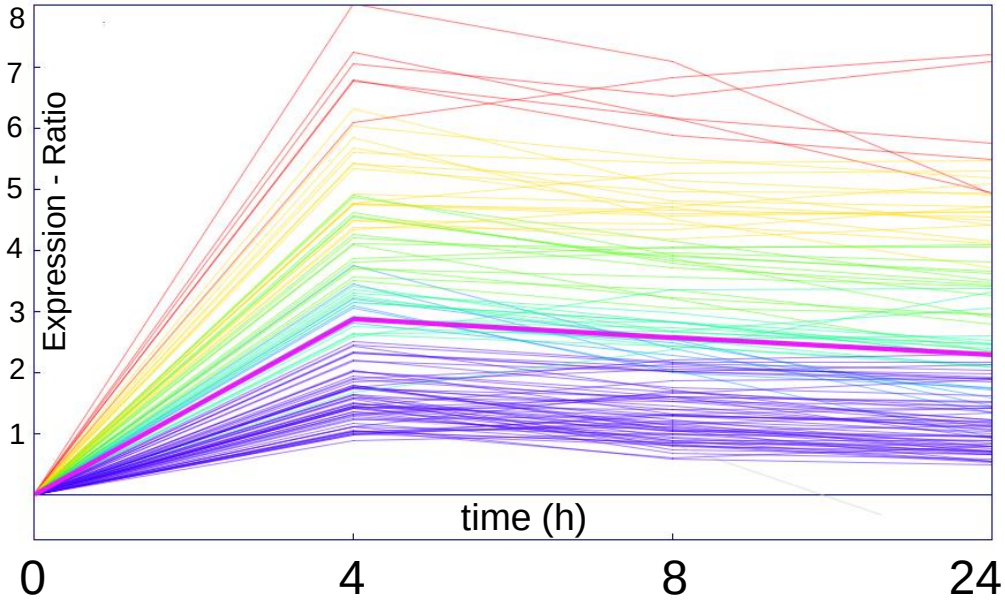




RNAseq on Huh-7.5 -/+ IFN-I

Sekrecka et al. 2019

ISG Expression in IFN-I treated cells



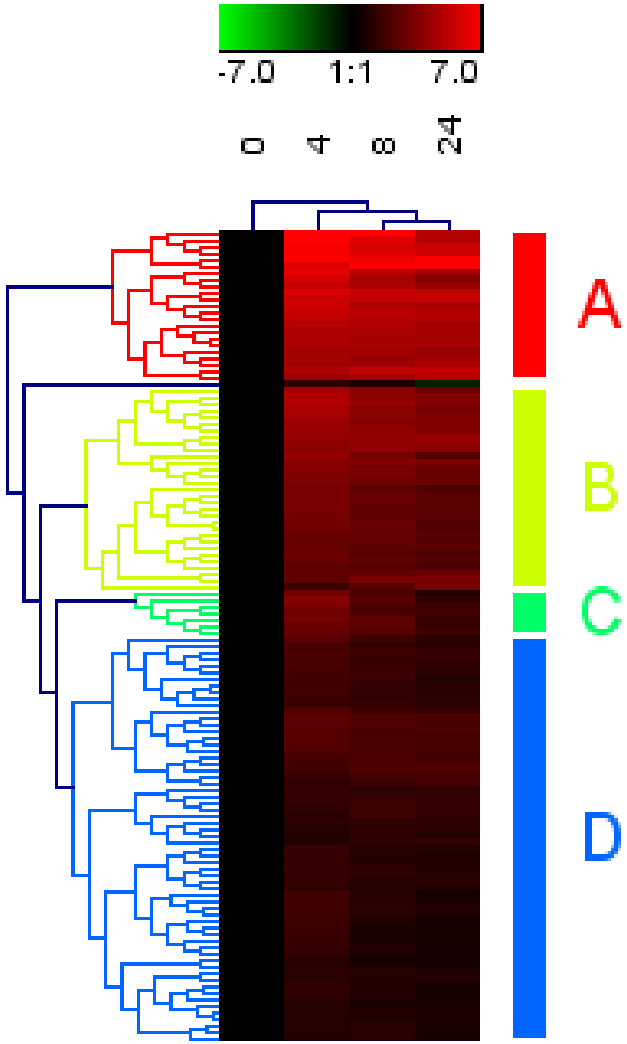
Expression view – 124 up regulated genes

Gene Ontology

ter_ID	Description	log_{10} p-value
GO:0002376	immune system process	-29.12
GO:0051607	defense response to virus	-28.38
GO:0002252	immune effector process	-25.61
GO:0006955	immune response	-21.24
GO:0045087	innate immune response	-19.97
GO:0019882	antigen processing and presentation	-10.24
GO:0002682	regulation of immune system process	-10.01
GO:0042089	cytokine biosynthetic process	-5.04
GO:0042107	cytokine metabolic process	-4.91
GO:0009617	response to bacterium	-4.44
GO:0032608	interferon-beta production	-4
GO:0032606	type I interferon production	-4
GO:0045343	regulation of MHC class I biosynthetic process	-3.23

Anti viral response

ISG Expression in IFN-I treated cells



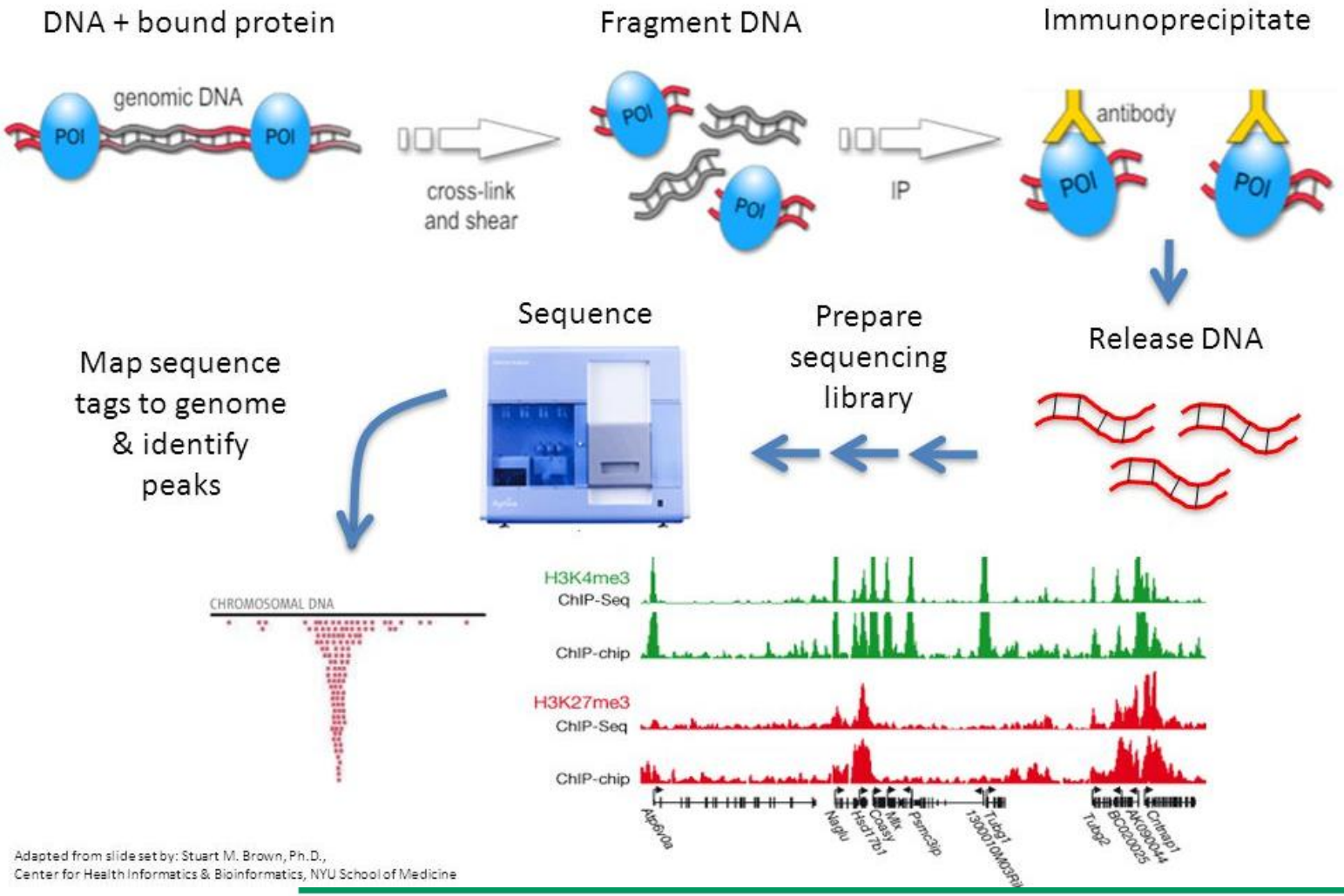
Er ific cluster

Cluster	Genes
A	Batf2, Oasl1, Rsad2, Oas1a, Oas2, Ifi47, Cxcl10, Tyki, Gbp10, LOC435565, Gbp6, D14Ertd668e, Mx2, Oas1b, Irf7, LOC100048346, Usp18, LOC667370, Trim30, Oasl2, Sp100, Oasl1
B	
C	
D	

Cluster	GO Term	p-value
A	defense response	4.72E-17
	defense response to virus	1.63E-15
	response to virus	1.39E-14
	cellular response to interferon-beta	5.73E-13
	response to interferon-beta	1.86E-12
	Response to type I interferon	4.85E-07
	immune system process	3.76E-06
	immune response	3.36E-04
	negative regulation of type I interferon-mediated signaling pathway	8.17E-04
	antigen processing and presentation of endogenous peptide antigen	2.44E-09
	antigen processing and presentation of peptide antigen via MHC class I	2.44E-09
	regulation of T cell mediated cytotoxicity	7.26E-07

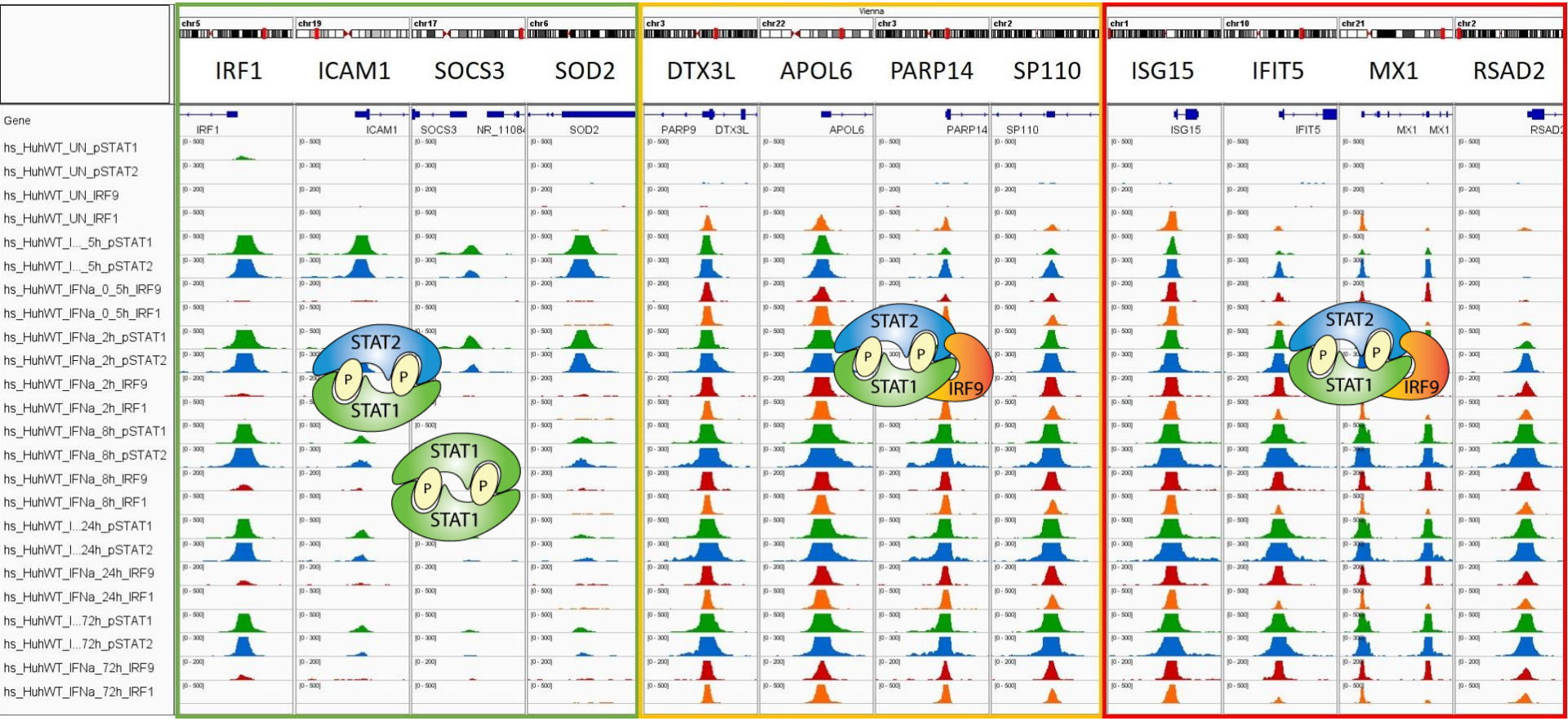
on against viral infection

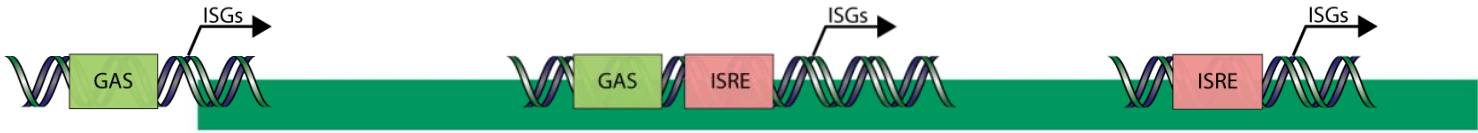
ChIPseq Work Flow



Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

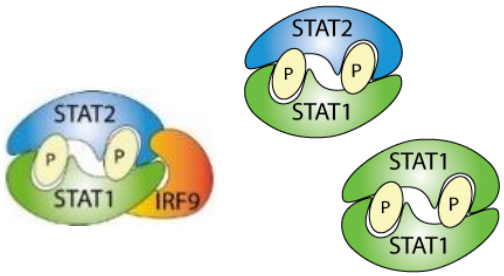
IFN-I signaling: pSTAT1, pSTAT2 & IRF9 DNA-binding

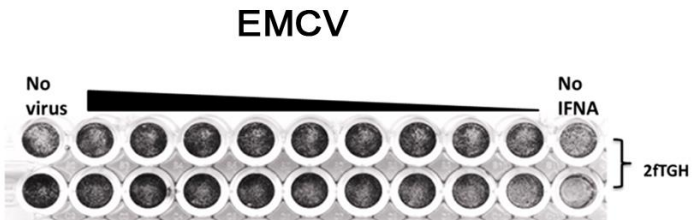


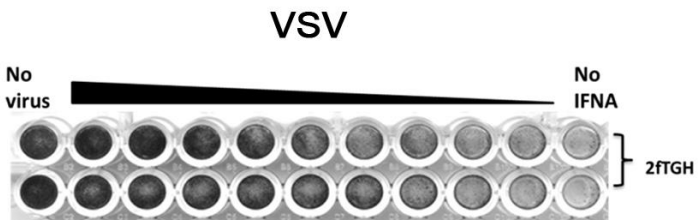


Sekrecka et al. 2019

IFN-I-mediated Anti-viral responses







MOI 0.3

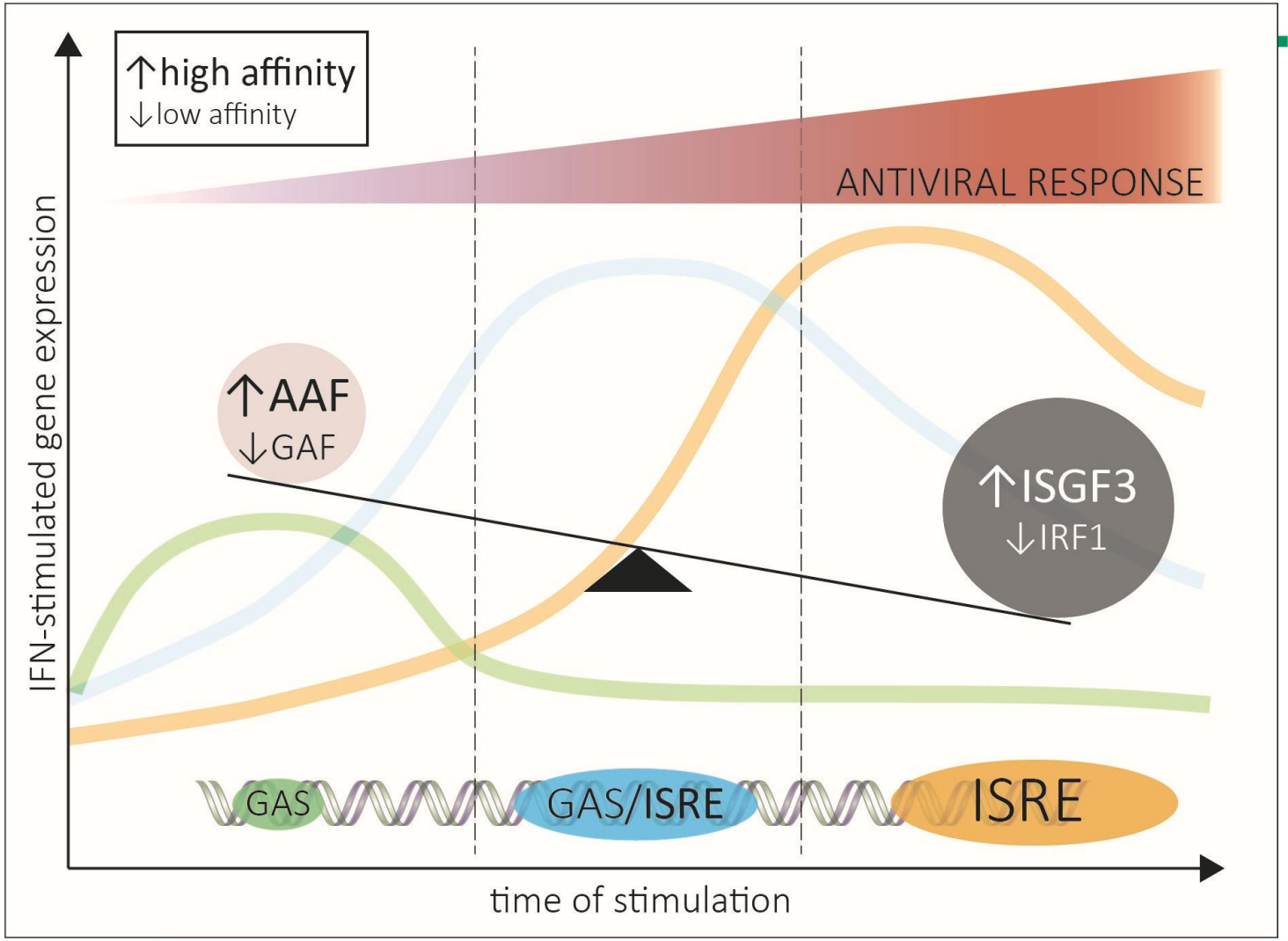
Blaszczyk et al., Biochem J, 2015

IFN-I signaling & action

Time-dependent IFN-induced

-Complex formation
-DNA-binding &
-ISG transcription

Antiviral Response



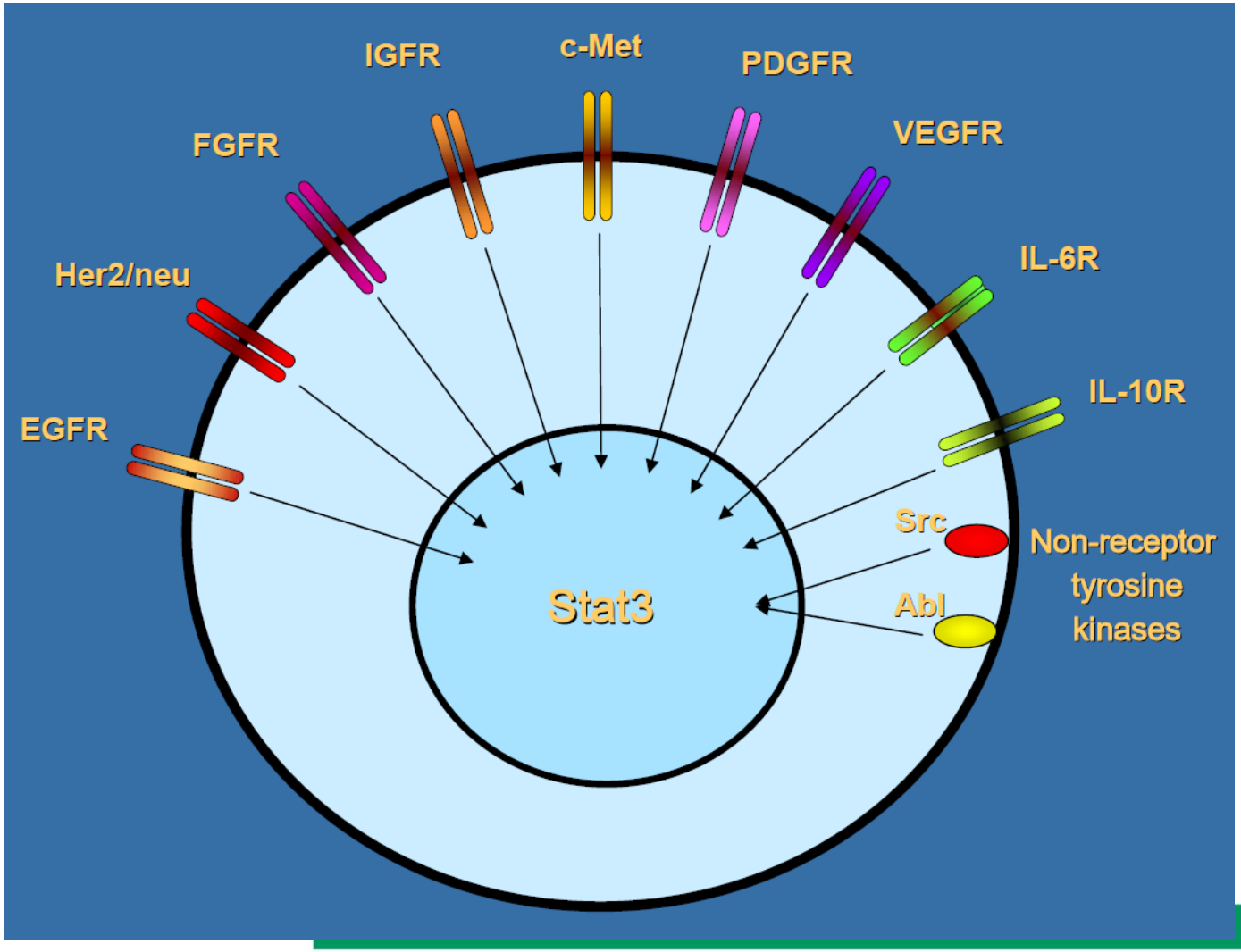
STAT3: A target for many human cancers

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

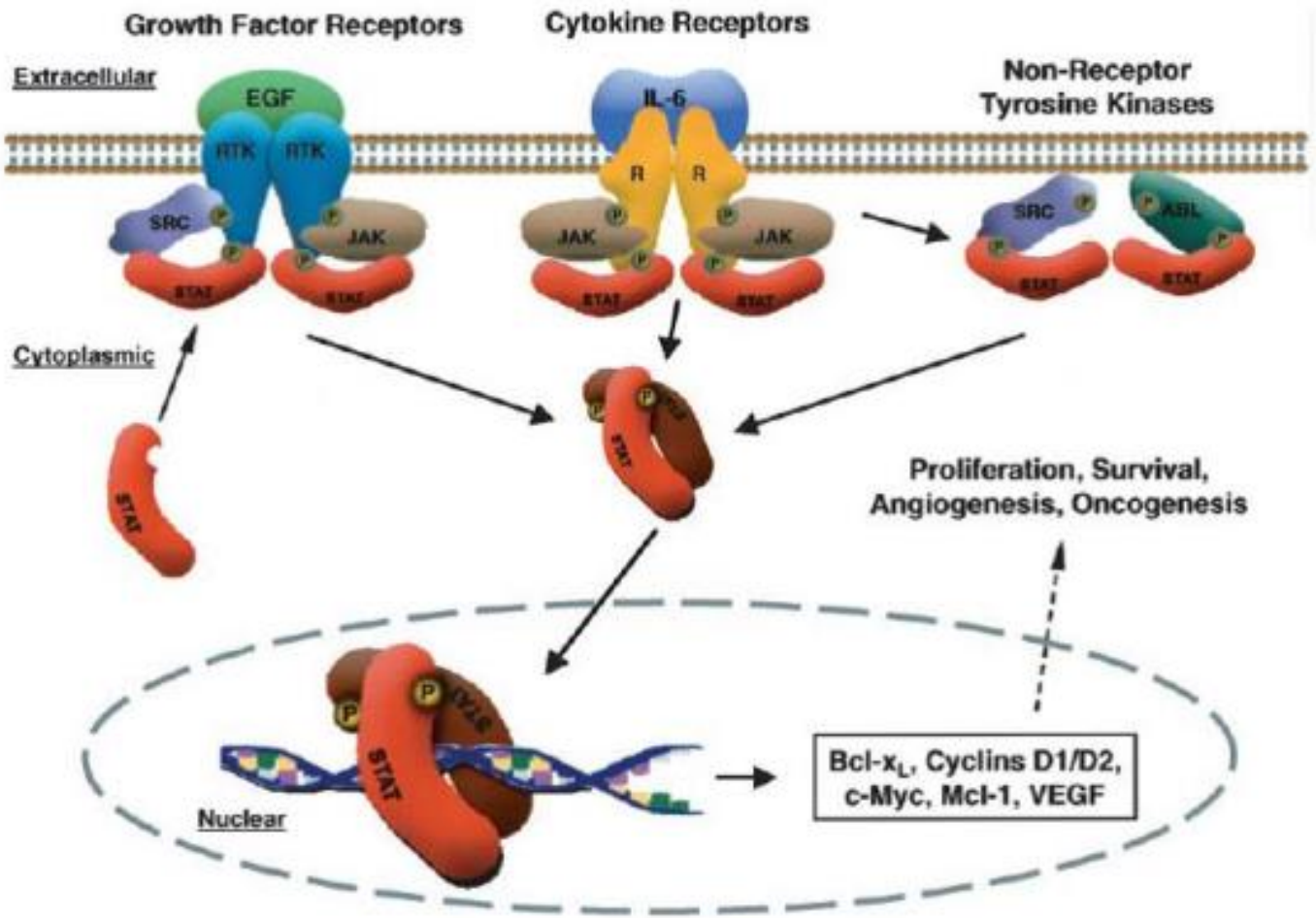
Hua Yu - Marcin Kortylewski et al., 2007

STAT3: Point of convergence in oncogenic signaling



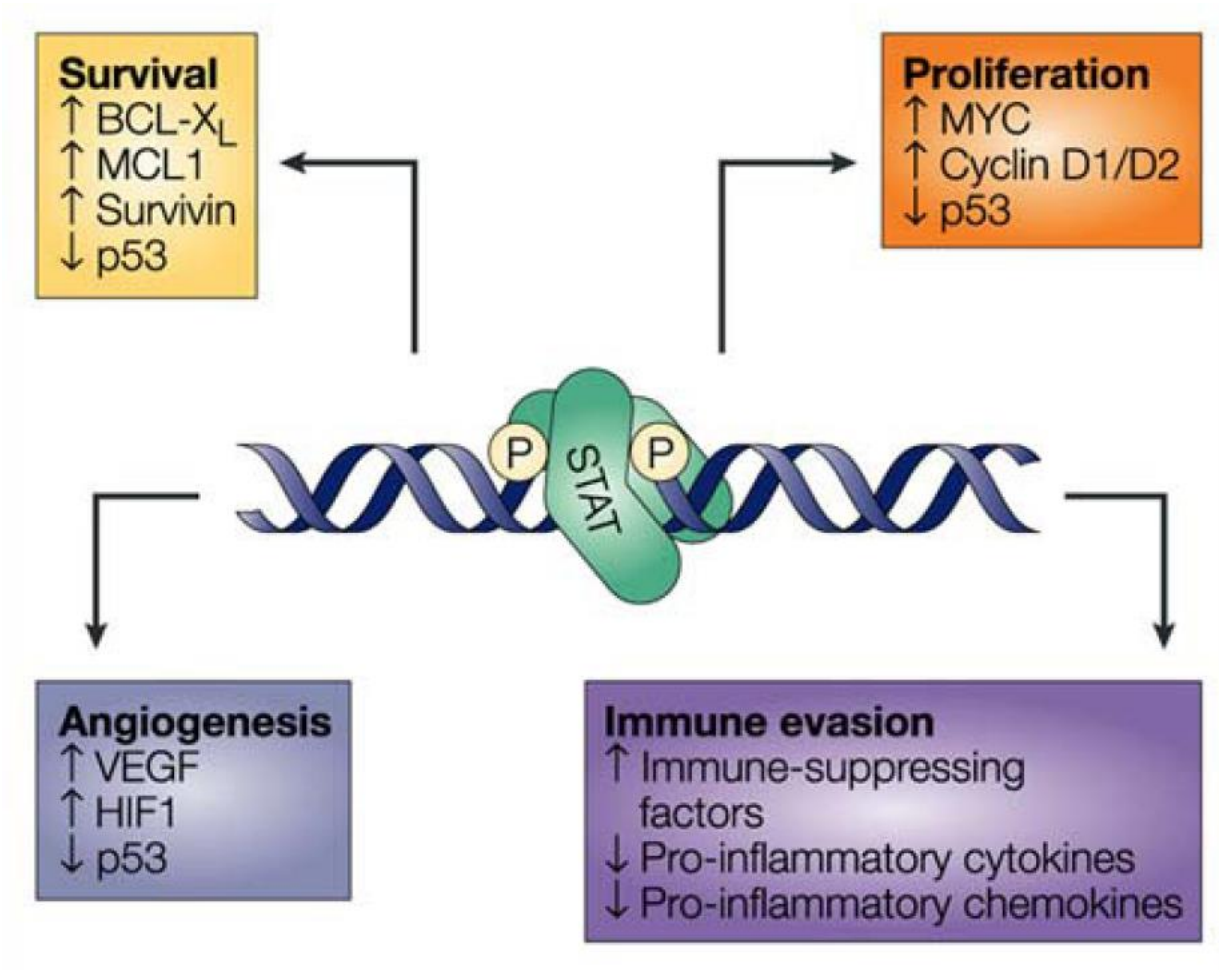
Yu et al., 2007

STAT3 activation in Cancer



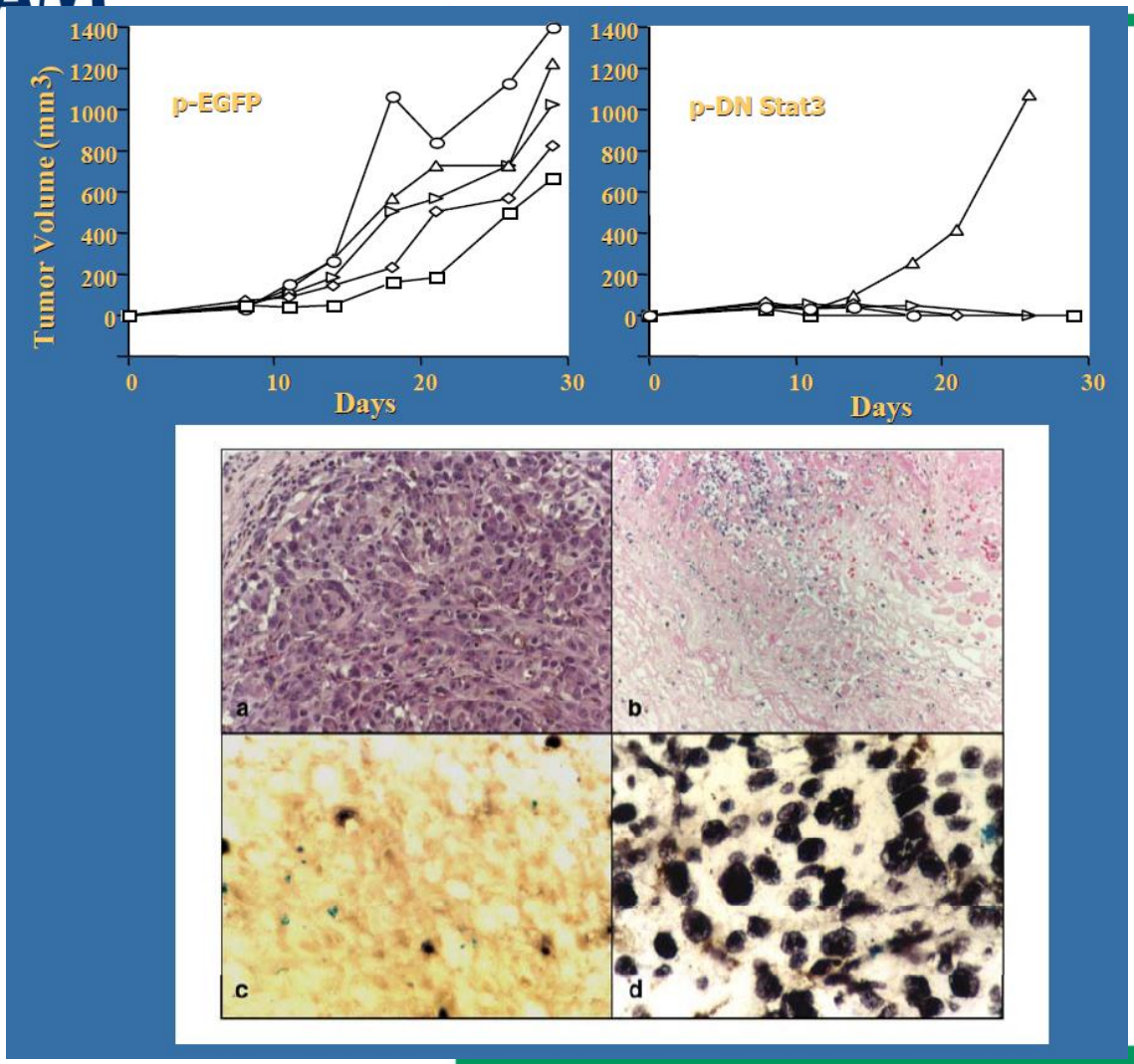
Kortylewski M. and Yu H., 2007

Multiple roles of STAT3 activation in tumor cells



Yu et al., 2007

Gene therapy with DN-STAT3 Involves “Bystander effects”



Murine Melanoma
B16 Tumors

15% electroinjected

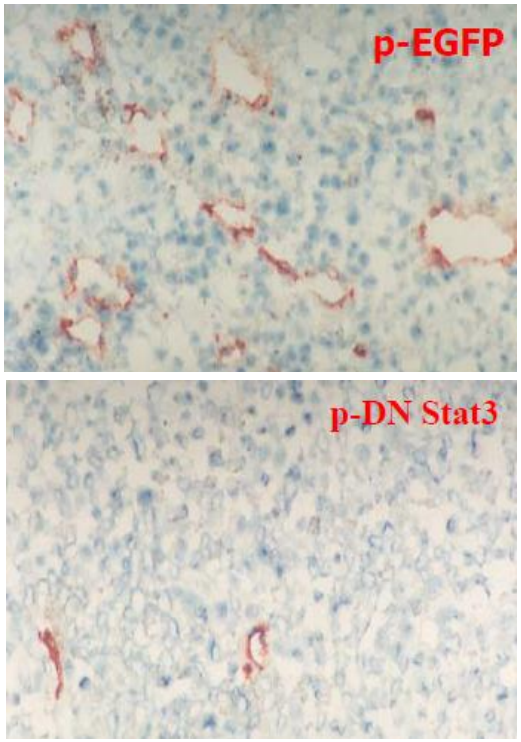
50-90% apoptotic

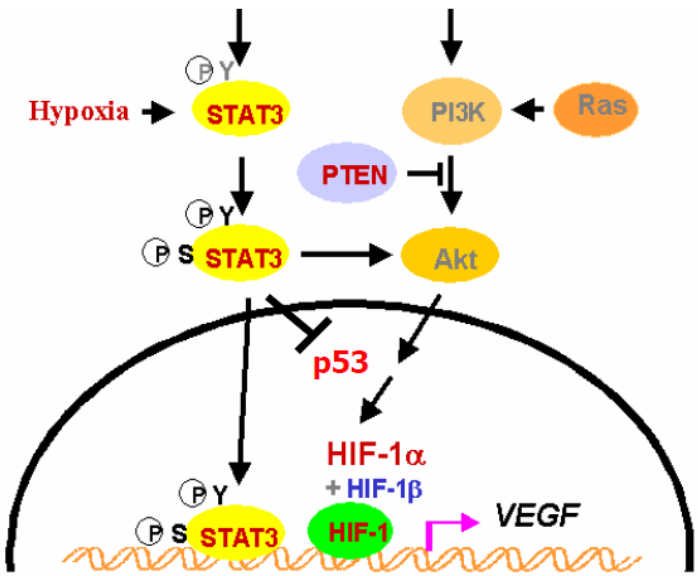
Influx immune cells

pEGFP pDN-STAT3

Niu et al., 1999

STAT3 is critical for Tumor Angiogenesis



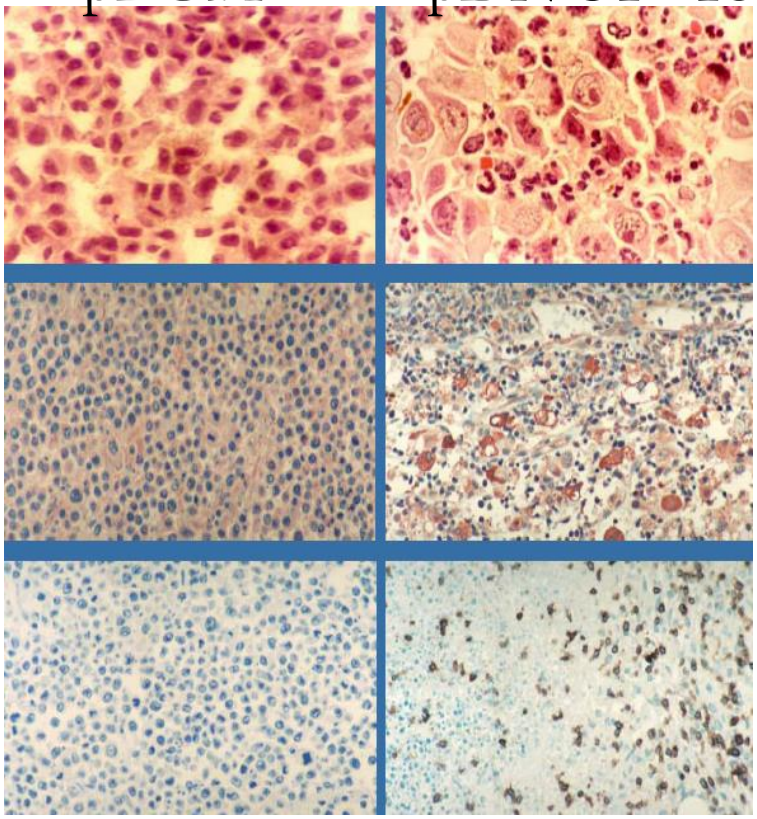


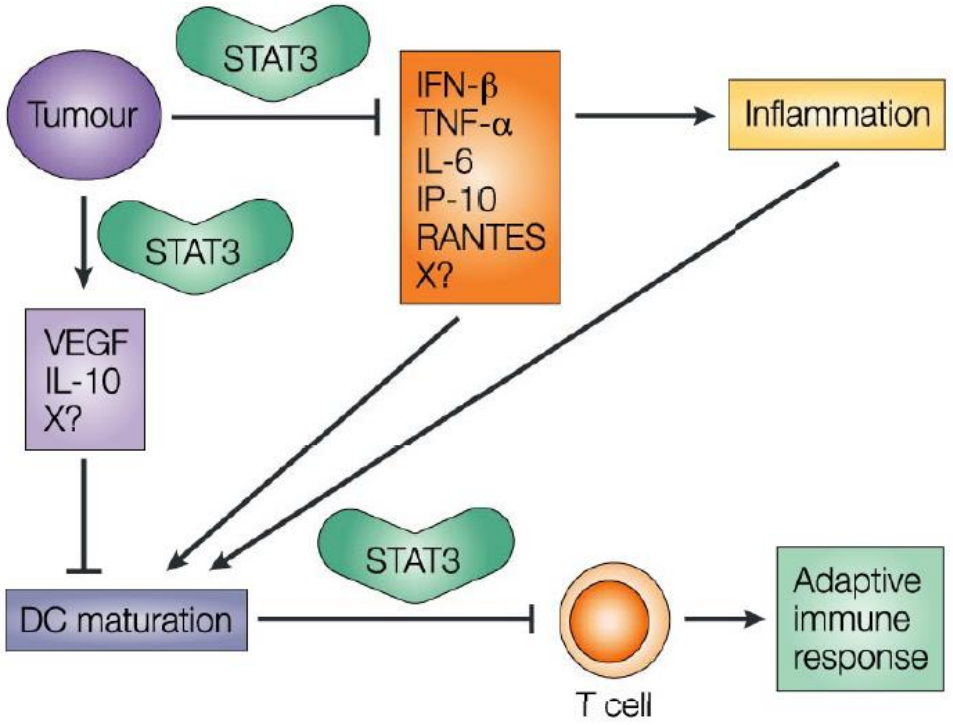
Angiogenesis

↑ VEGF	↓ IFNγ
↑ bFGF	↓IL-12
↑ HGF	↓IP-10
↑ HIF-1	↓Stat1
↑ MMP-2	↓IFNβ
↑ MMP-9	↓p53

Niu et al, *Oncogene*, 21:2000-2008, 2002
Xu et al, *Oncogene*, 25:5552-5560, 2005
Niu et al, *Mol Cell Biology*, 25, 7432-7440, 2005

Critical role of tumor STAT3 Activation in Immune Evasion

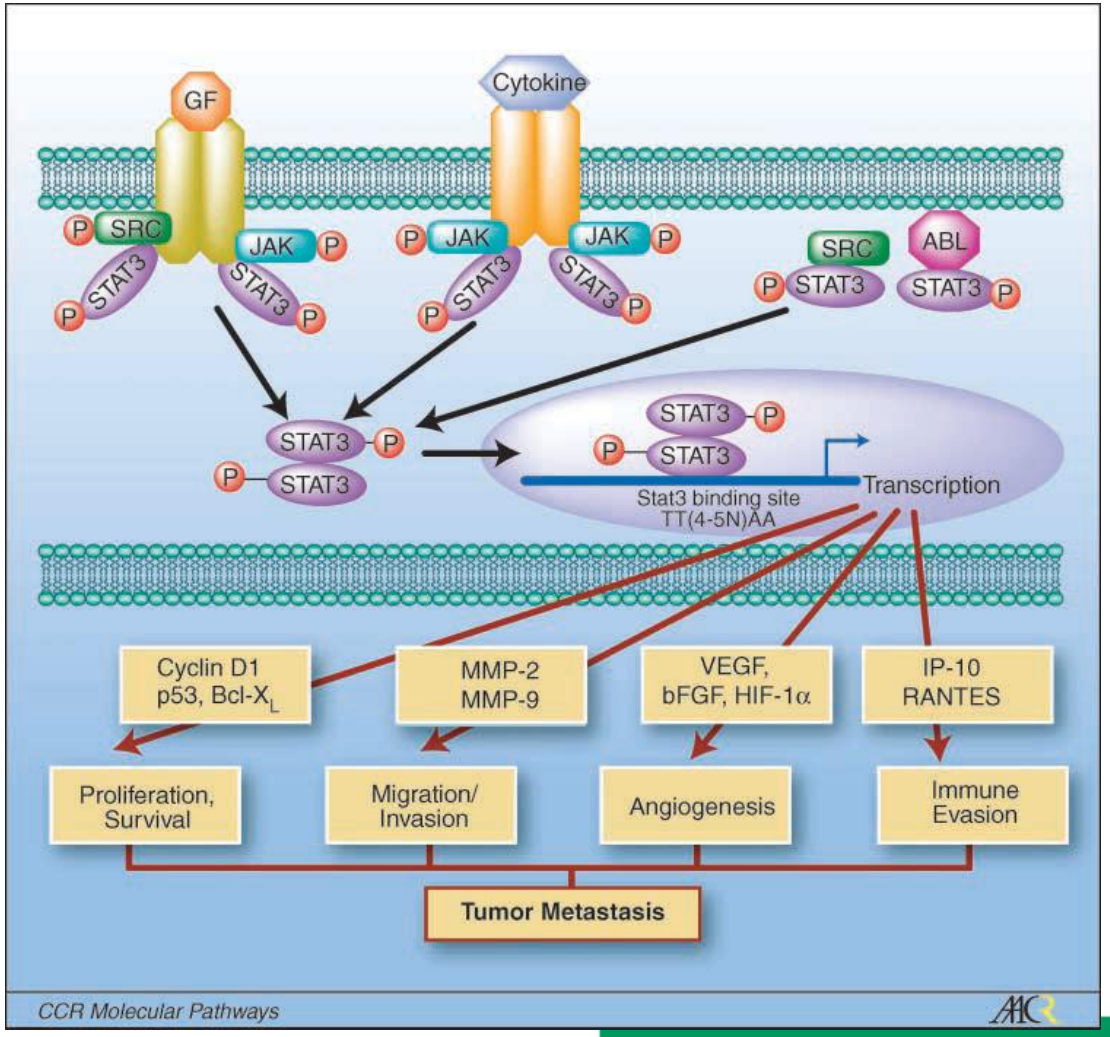




Influx of immune cells
Increased apoptosis

Wang, Niu, Kortylewski and Burdelya et al, *Nature Medicine;* Jan 2004
Burdelya and Kujawski et al, *J. Immunol,* Jan 2005
Yu and Jove, *Nature Reviews Cancer,* Feb 2004

Role of STAT3 in Oncogenesis & Tumor Metastasis



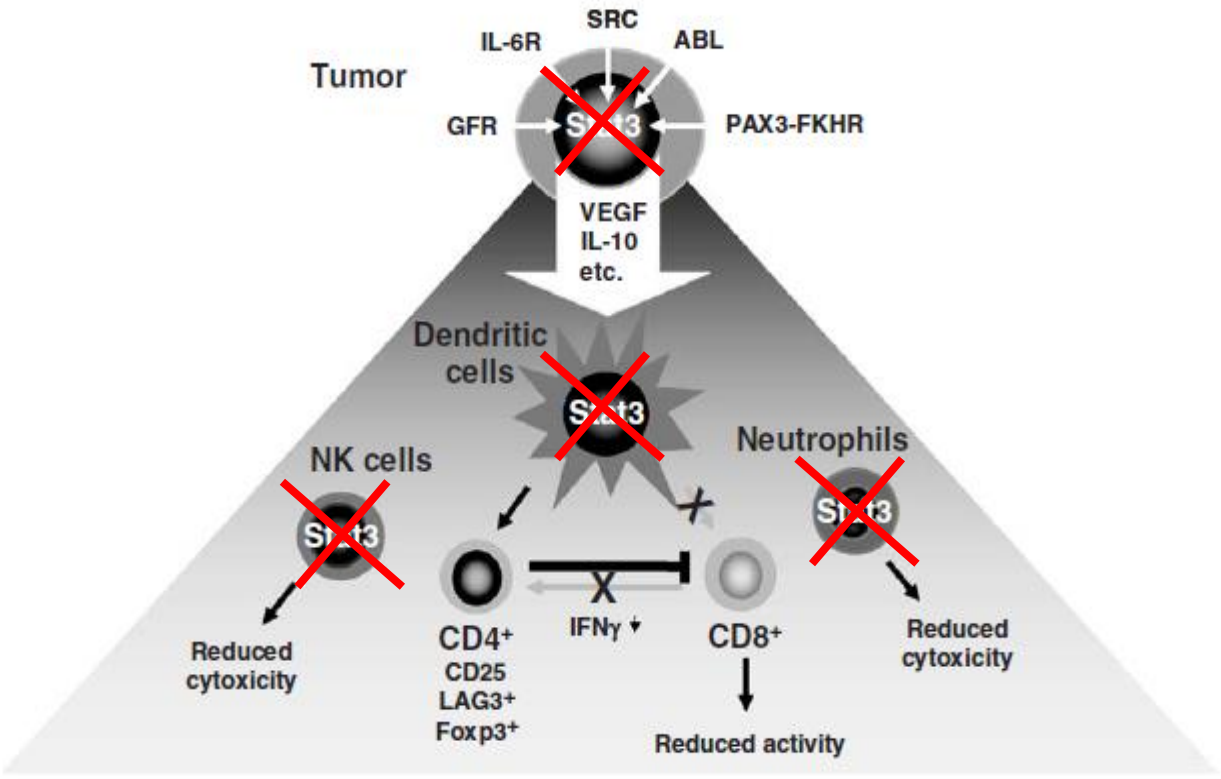
STAT3:
a novel multi-functional protein involved in

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

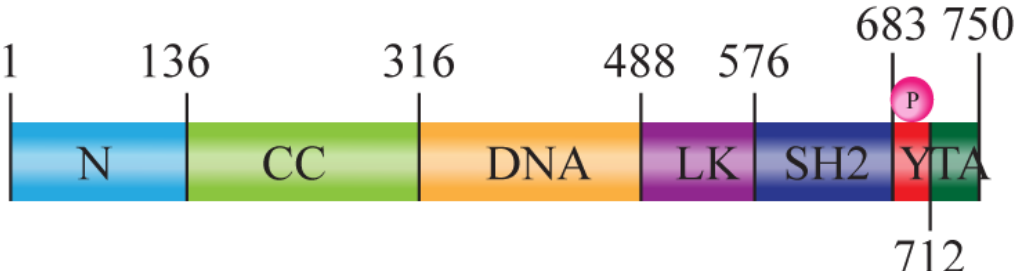
- Gene therapy
  - DN-STAT3
  - SOCS3

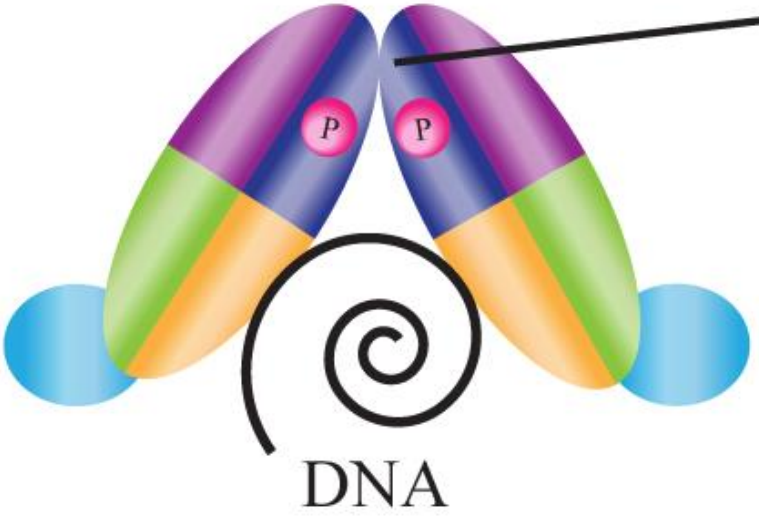
- RNAi + targeting vectors

- Combination therapy
  - Immune therapy

Inhibition of tumor development + progression

STAT Structure & Dimerization



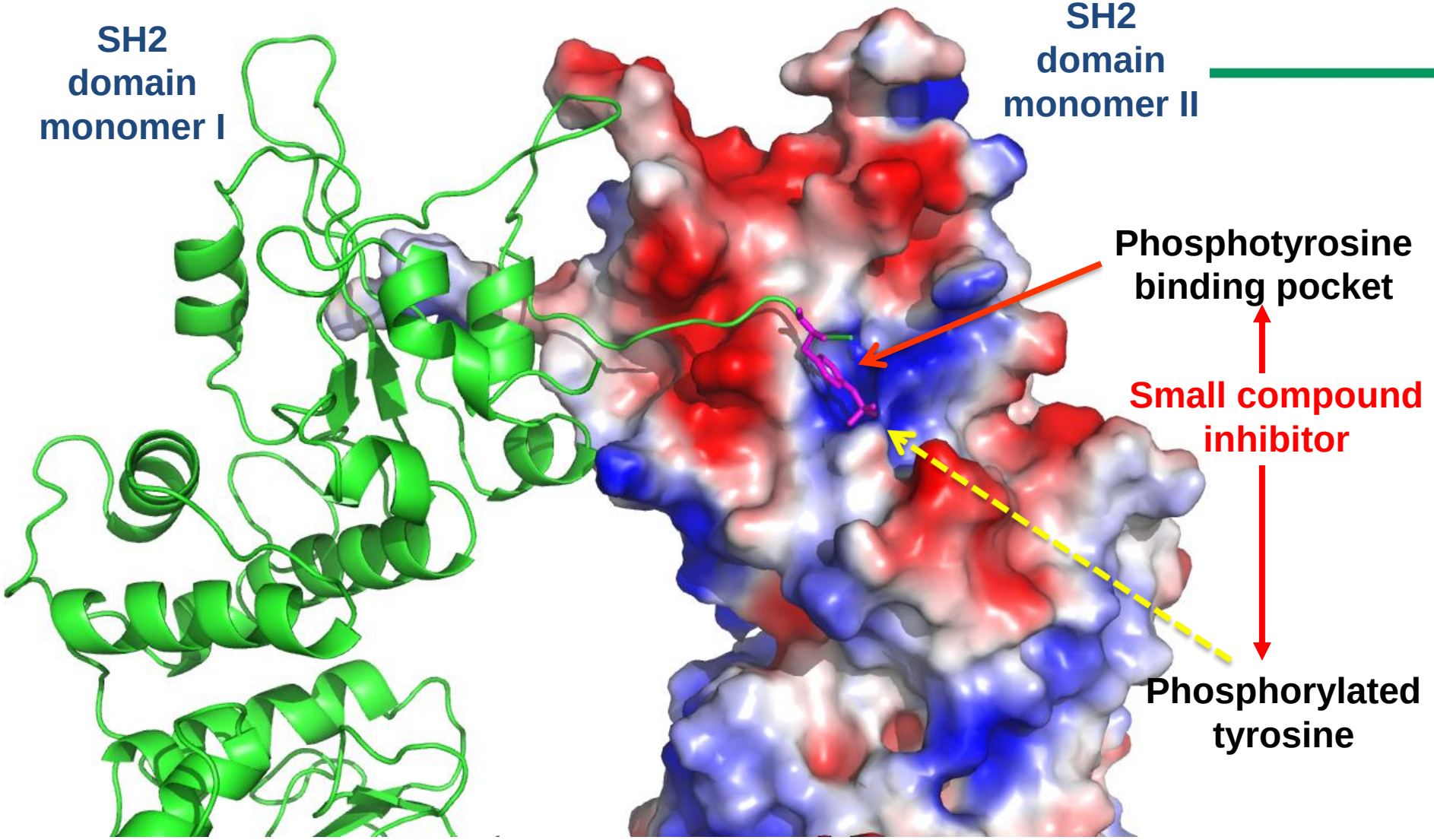


Interacting
SH2-domain

STAT-dimer

Sikorski et al., CGFR 2011

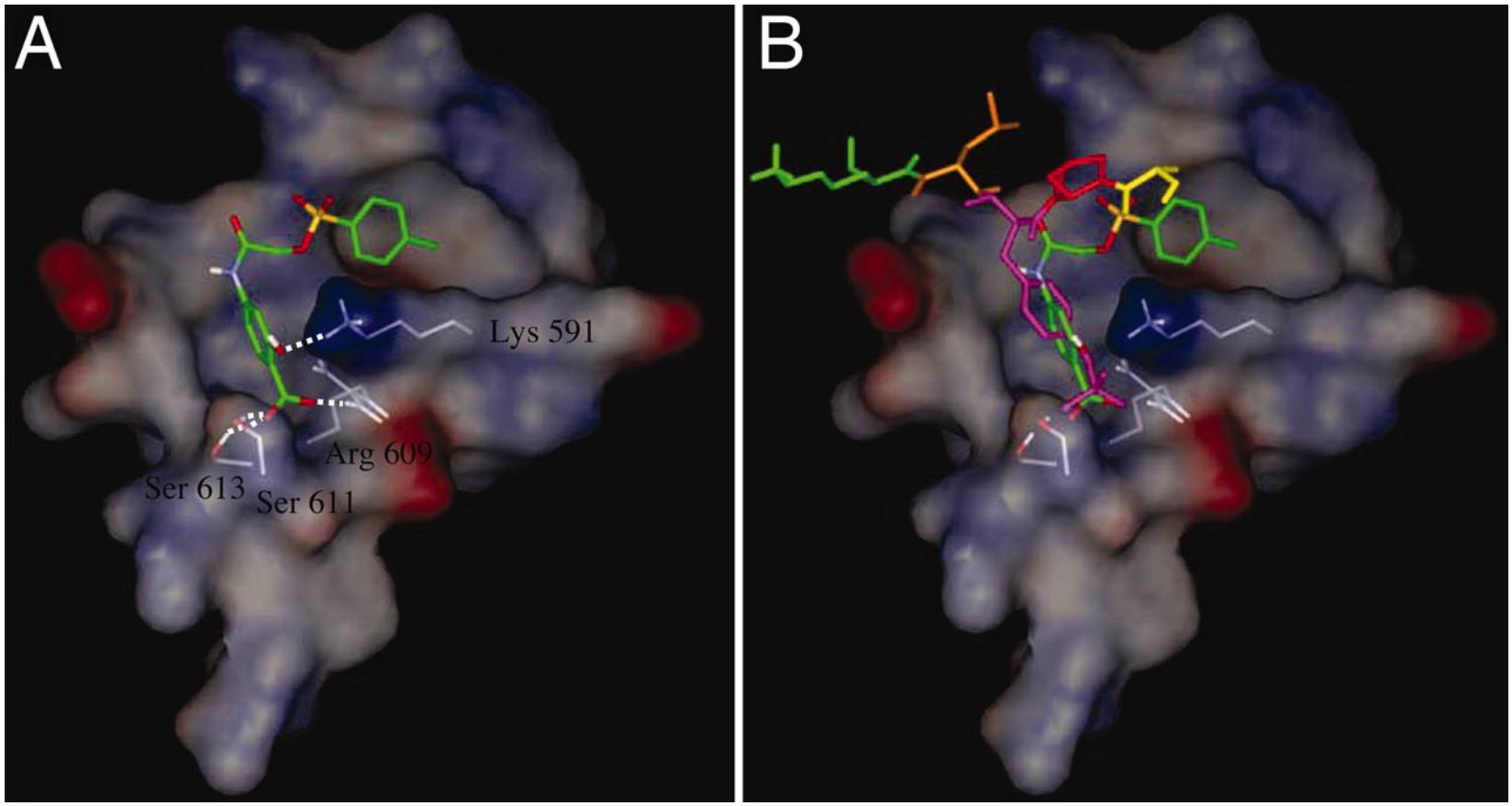
Structural information: STAT1-STAT3



Chen et al., 1998, Cell, 93:827

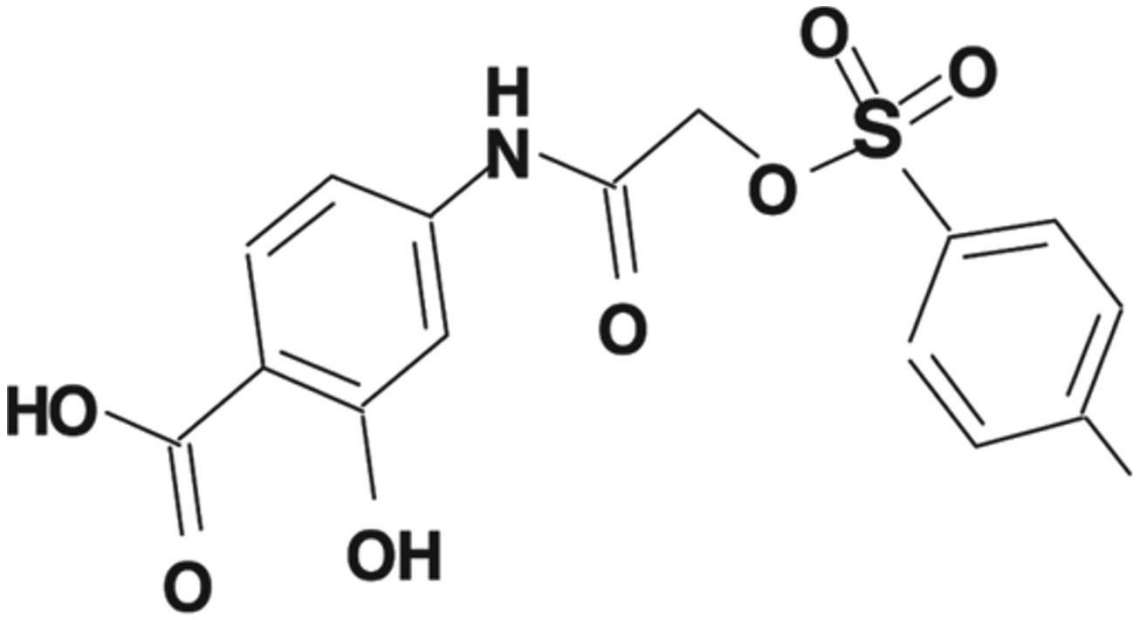
Becker et al., 1998, Nature, 394:145

Application of computational modelling 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



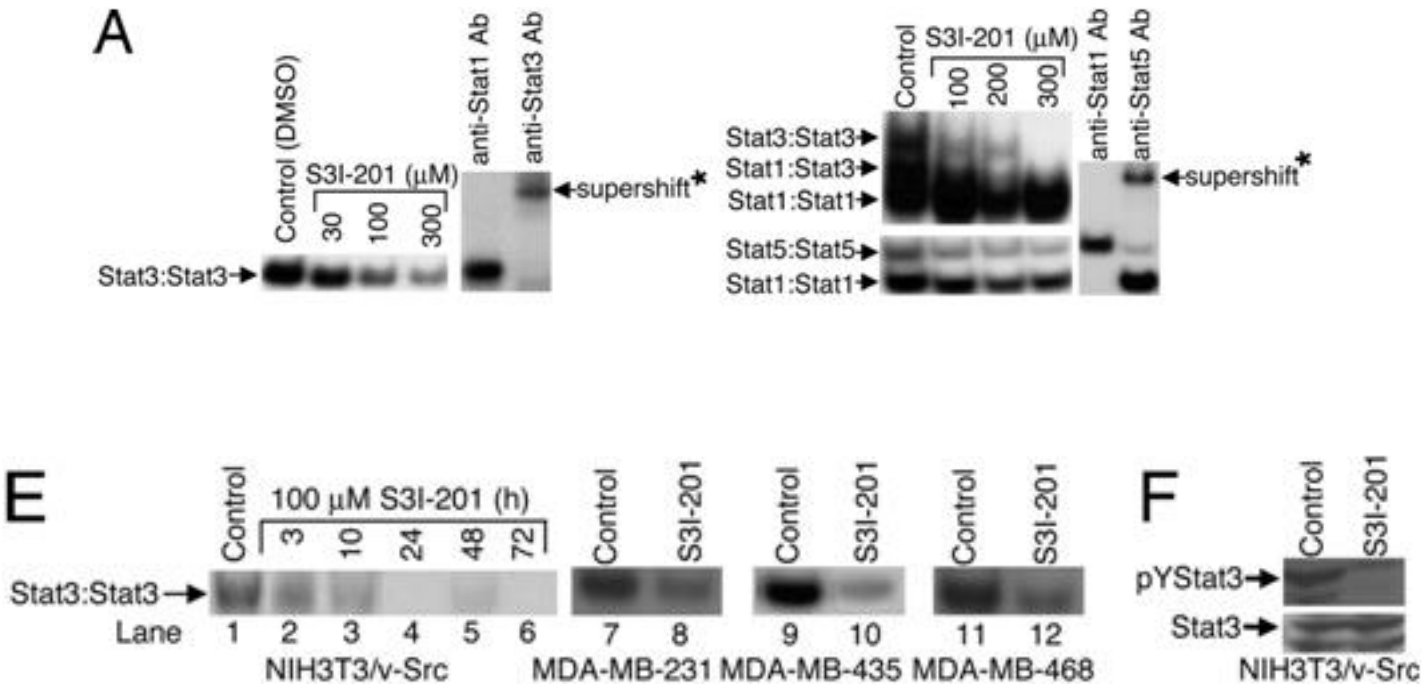
NSC 74859 (S3I-201)

Dimerization

DNA binding

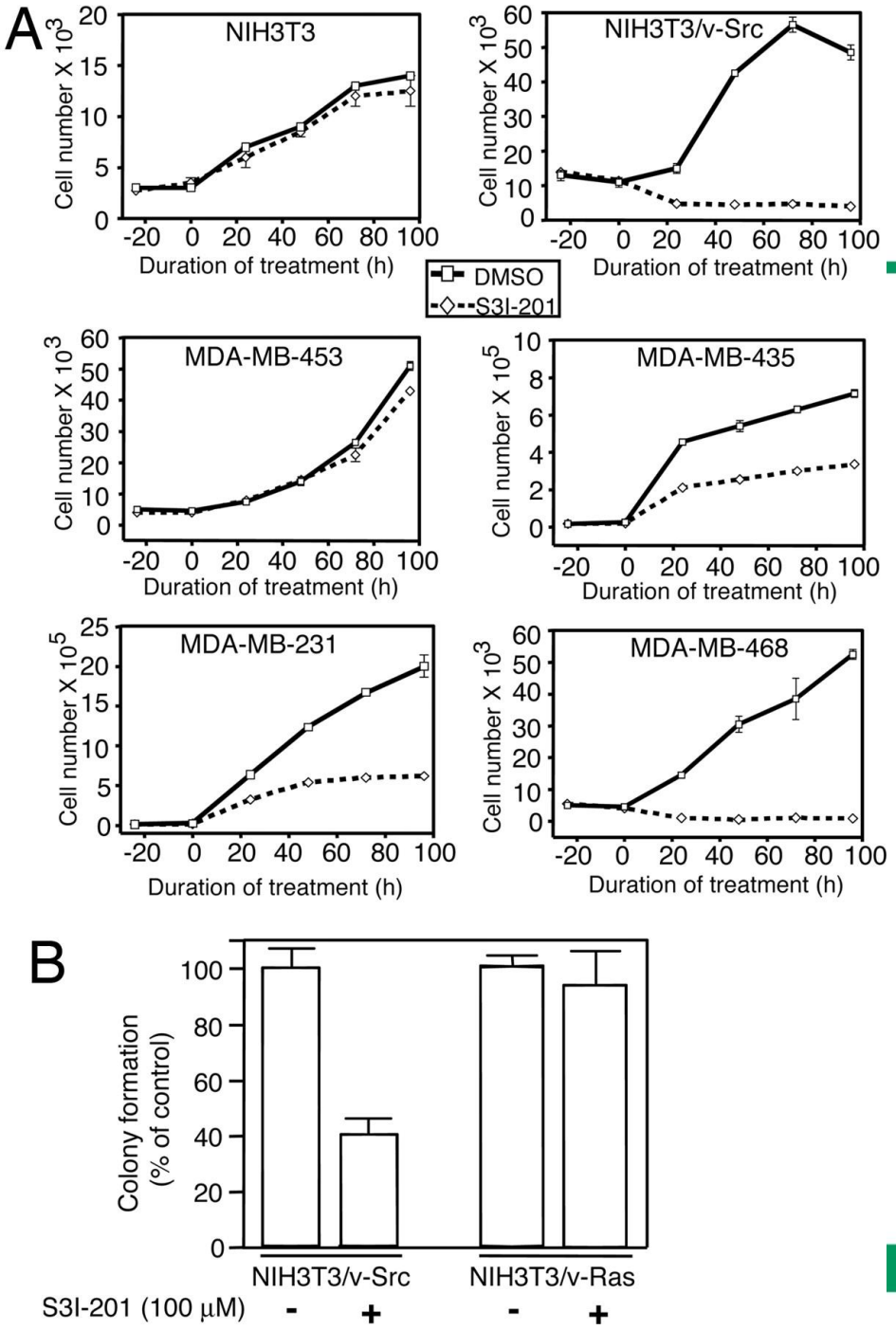
Siddiquee K. et.al. PNAS 2007;104:7391-7396

Evaluation for effects of S3I-201 on STAT-DNA binding



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

S3I-201 inhibits anchorage-dependent and -independent growth only of cells that contain persistently active Stat3

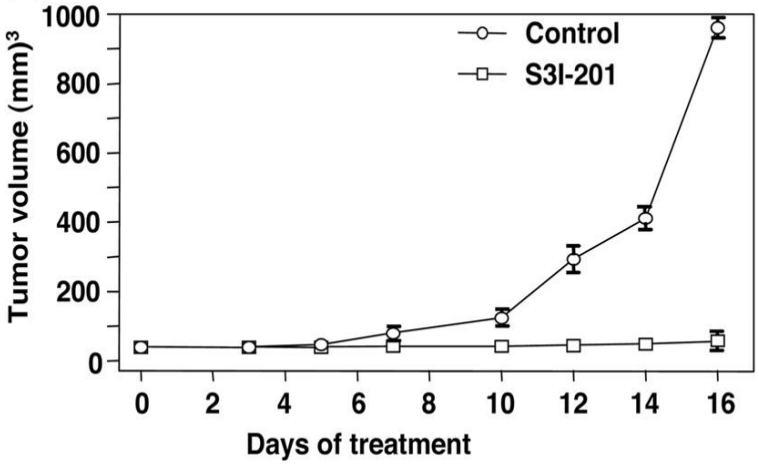


Siddiquee K. et.al.
PNAS 2007;104:7391-7396

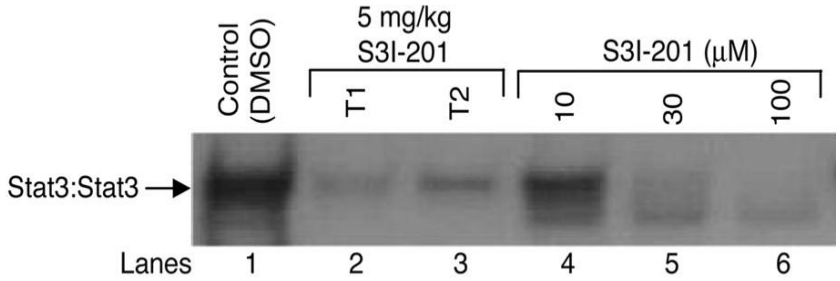
In vivo Tumor growth inhibition by S3I-201

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

A



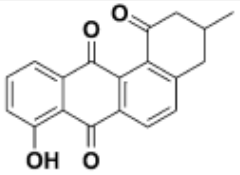
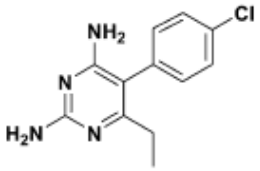
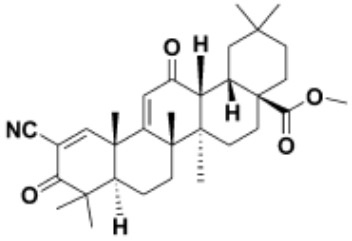
B



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

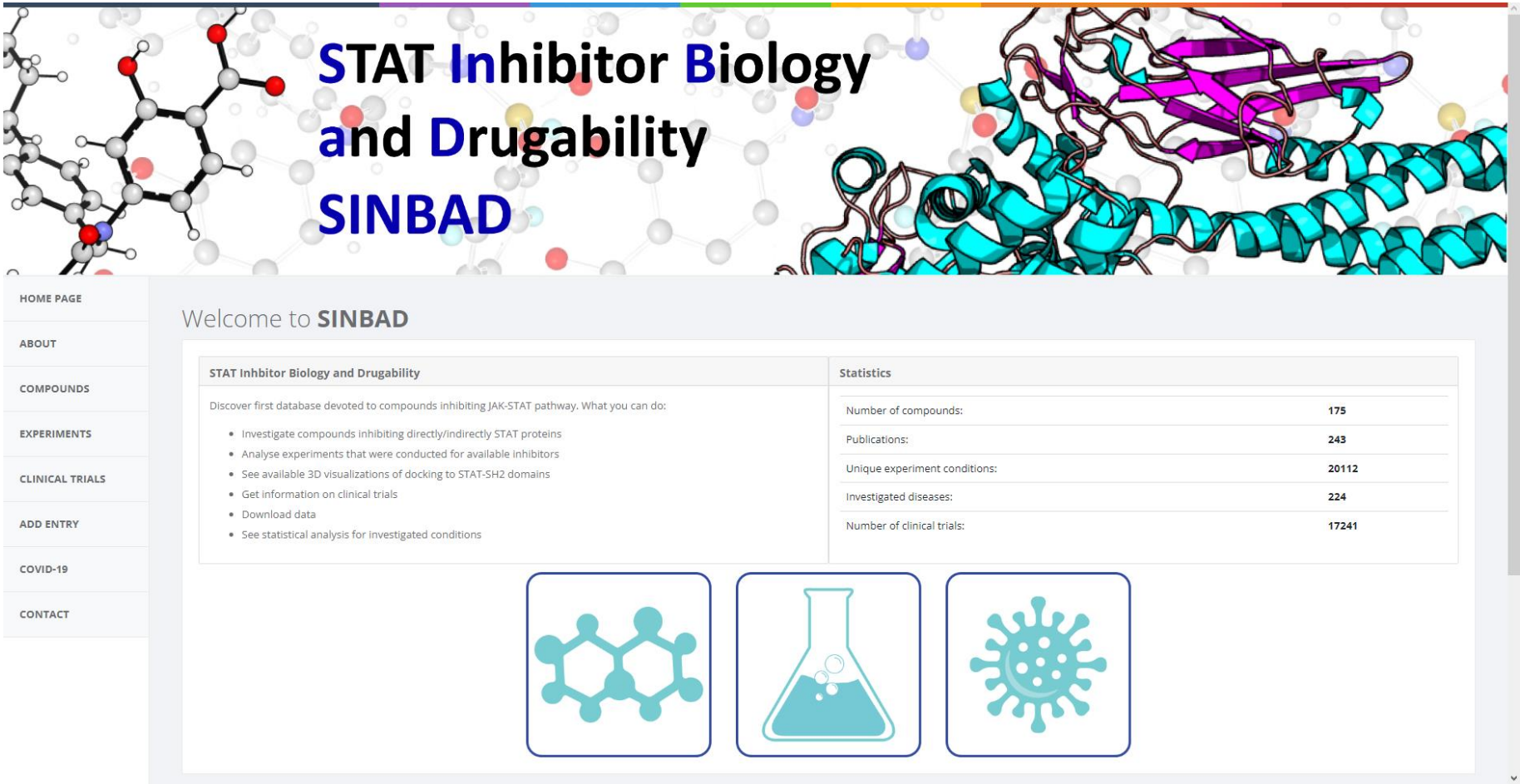
STAT3 inhibitors in clinical trials

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

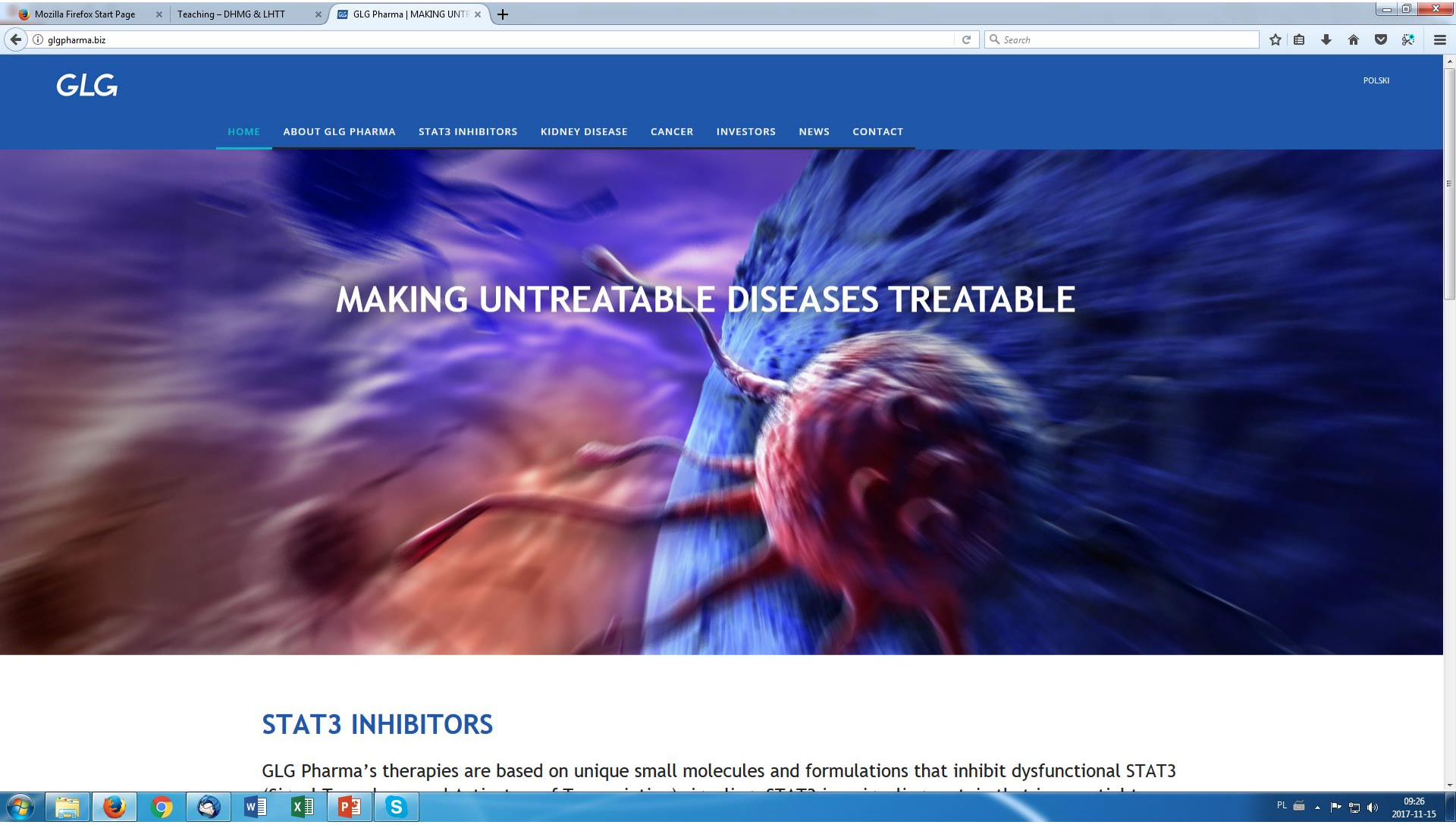
Debnath et al., 2012

SINBAD Database



Plens-Gałąska et al. 2022 Scientific Data

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

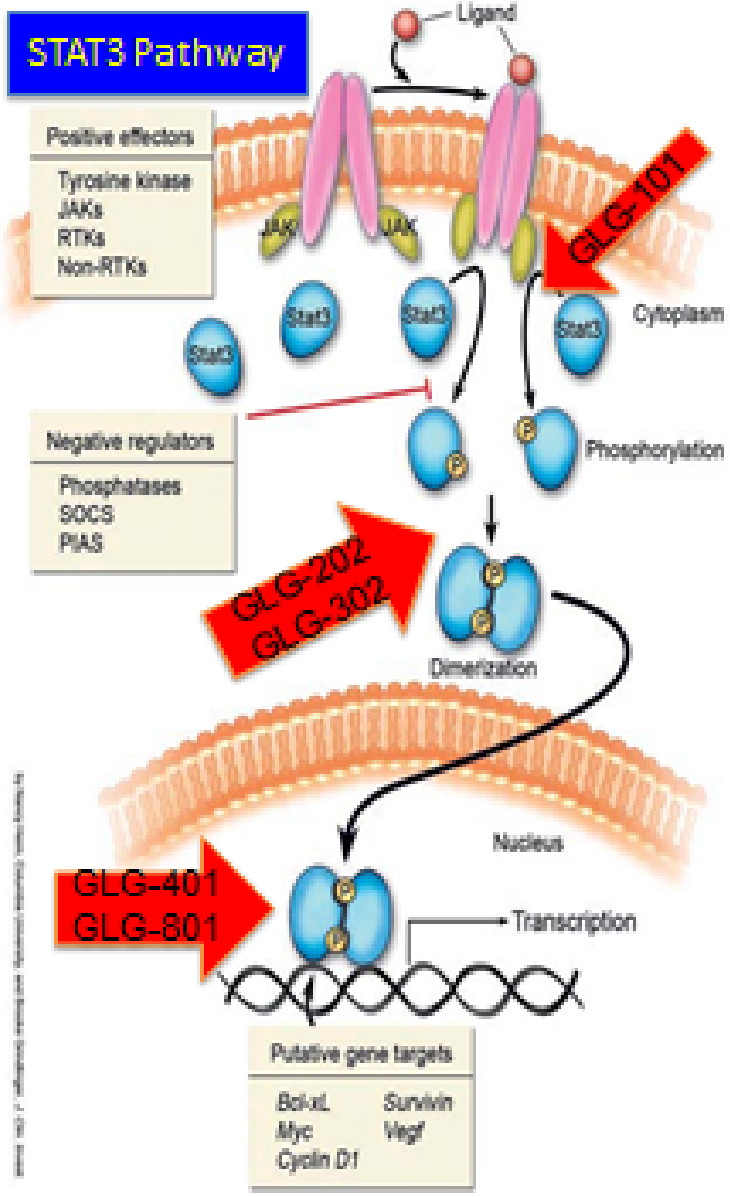
Activation of STAT3 PROTEIN is essential for

- 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.

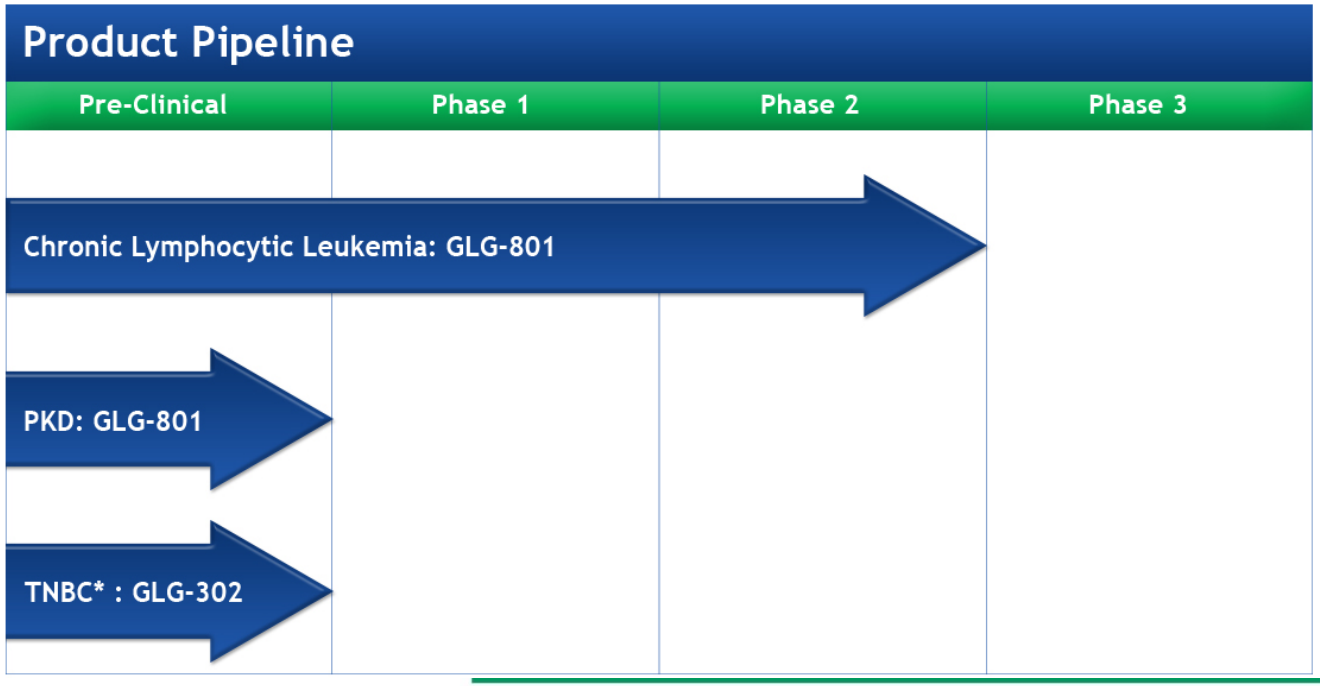
Pre-clinical work on GLG-801 has been completed for polycystic kidney disease and Phase I clinical trials are planned for 2016.

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.

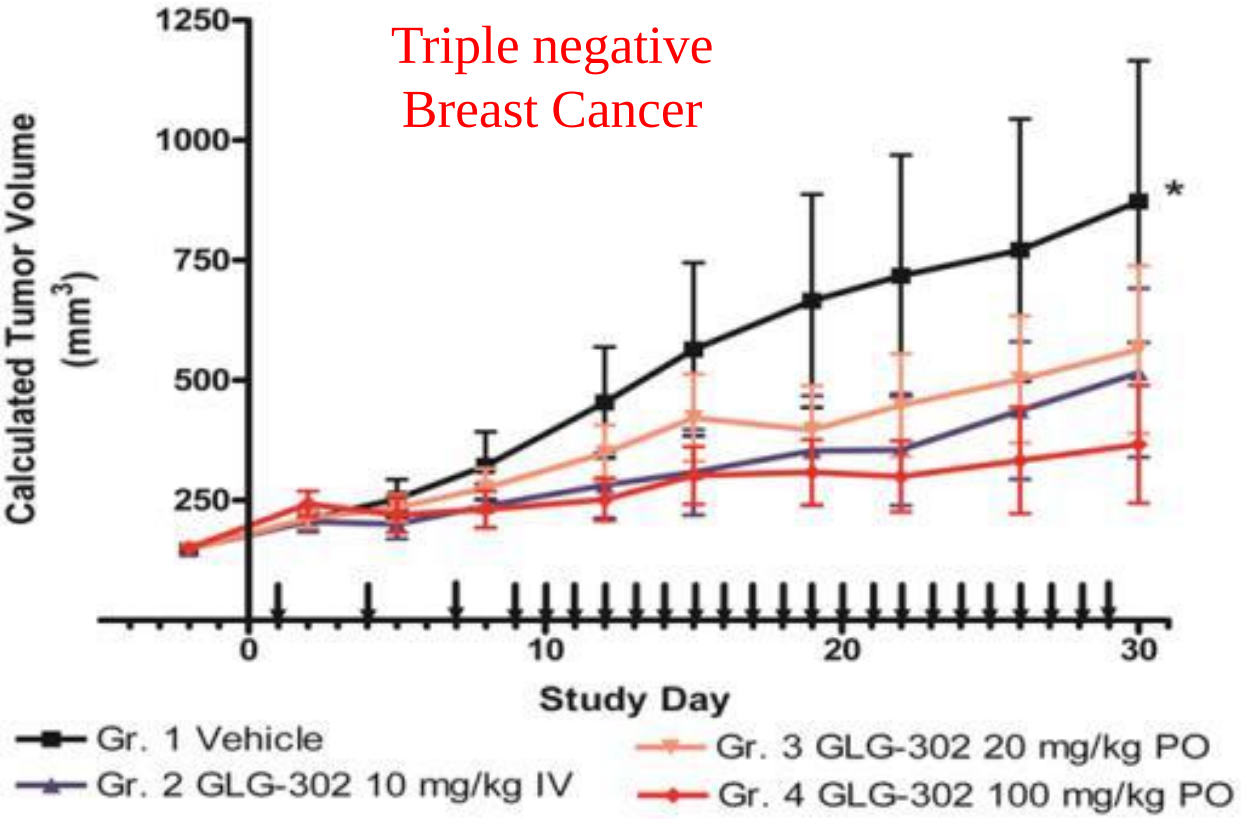
Because there is no current effective treatment for these diseases, regulatory approval processes should be accelerated



GLG Pharma: Pipeline

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

Triple negative
Breast Cancer



↓ = 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

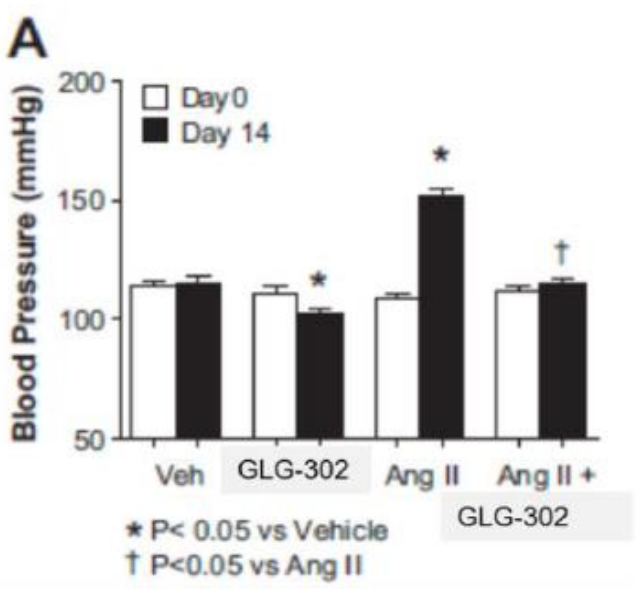
GLG Pharma: Pipeline

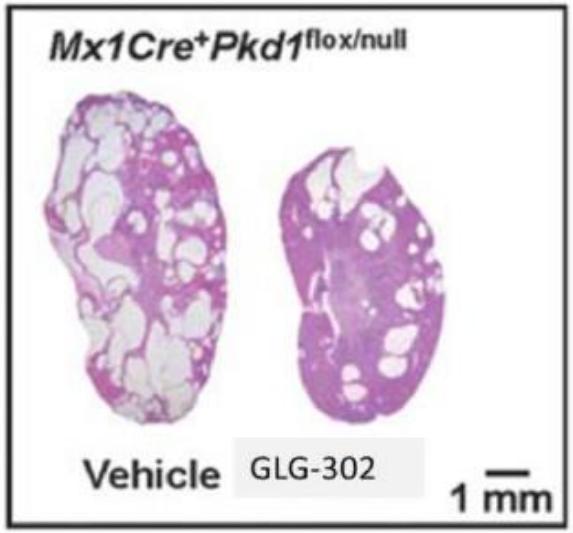
- **Kidney disease**

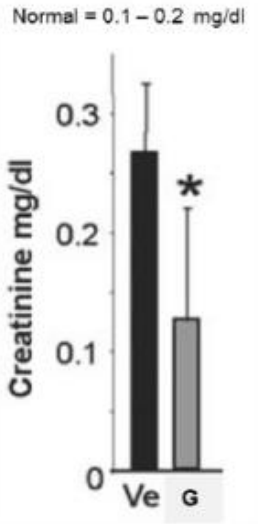
Polycystic Kidney disease

GLG-302 demonstrated efficacy in animal models of ADPKD

- It showed: decreased kidney size, number of cysts and normalized kidney function.





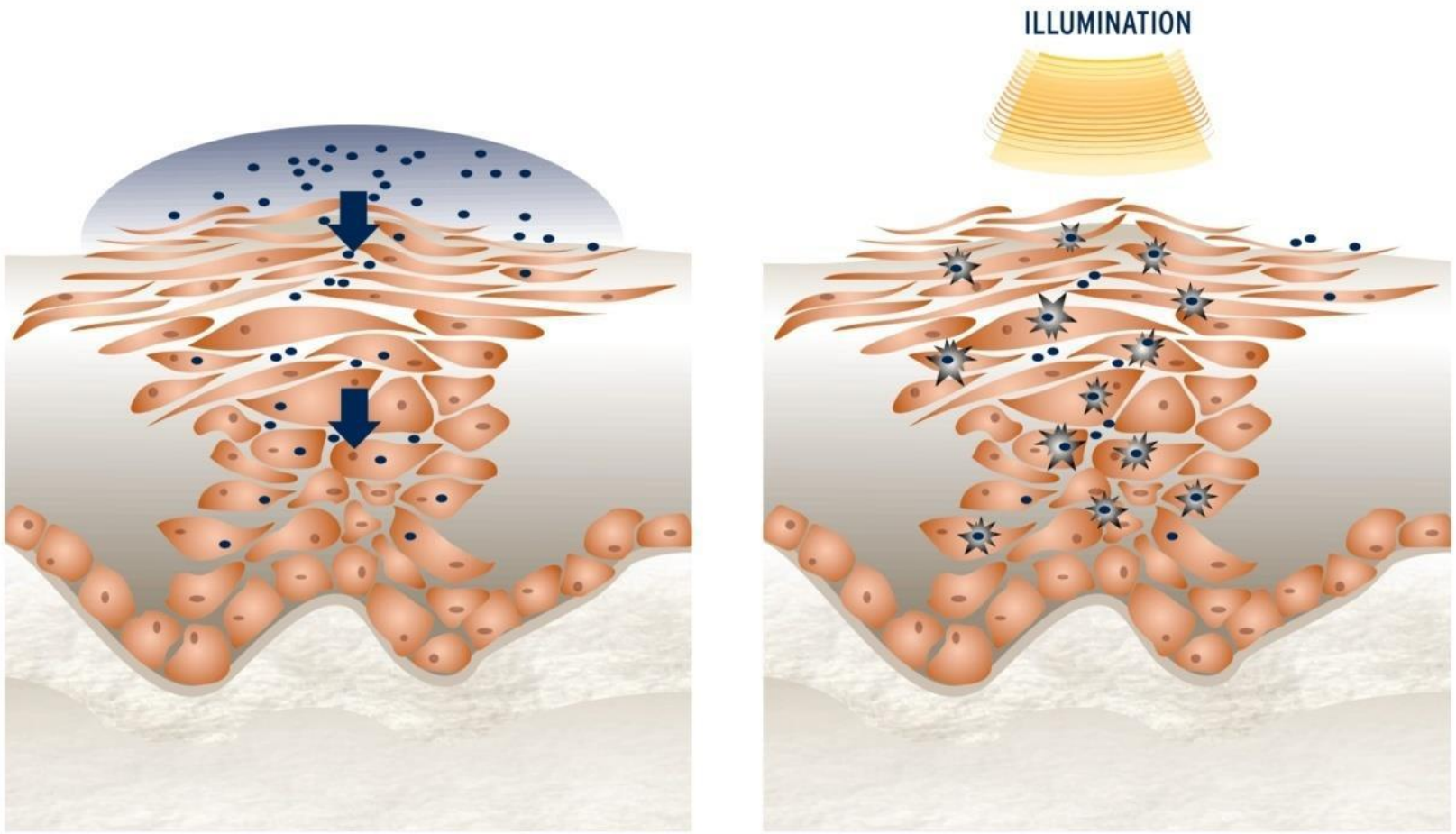


Mouse Polycystic Kidney treated with vehicle or GLG-302

Johnson: Hypertension 61:437-442, 2013

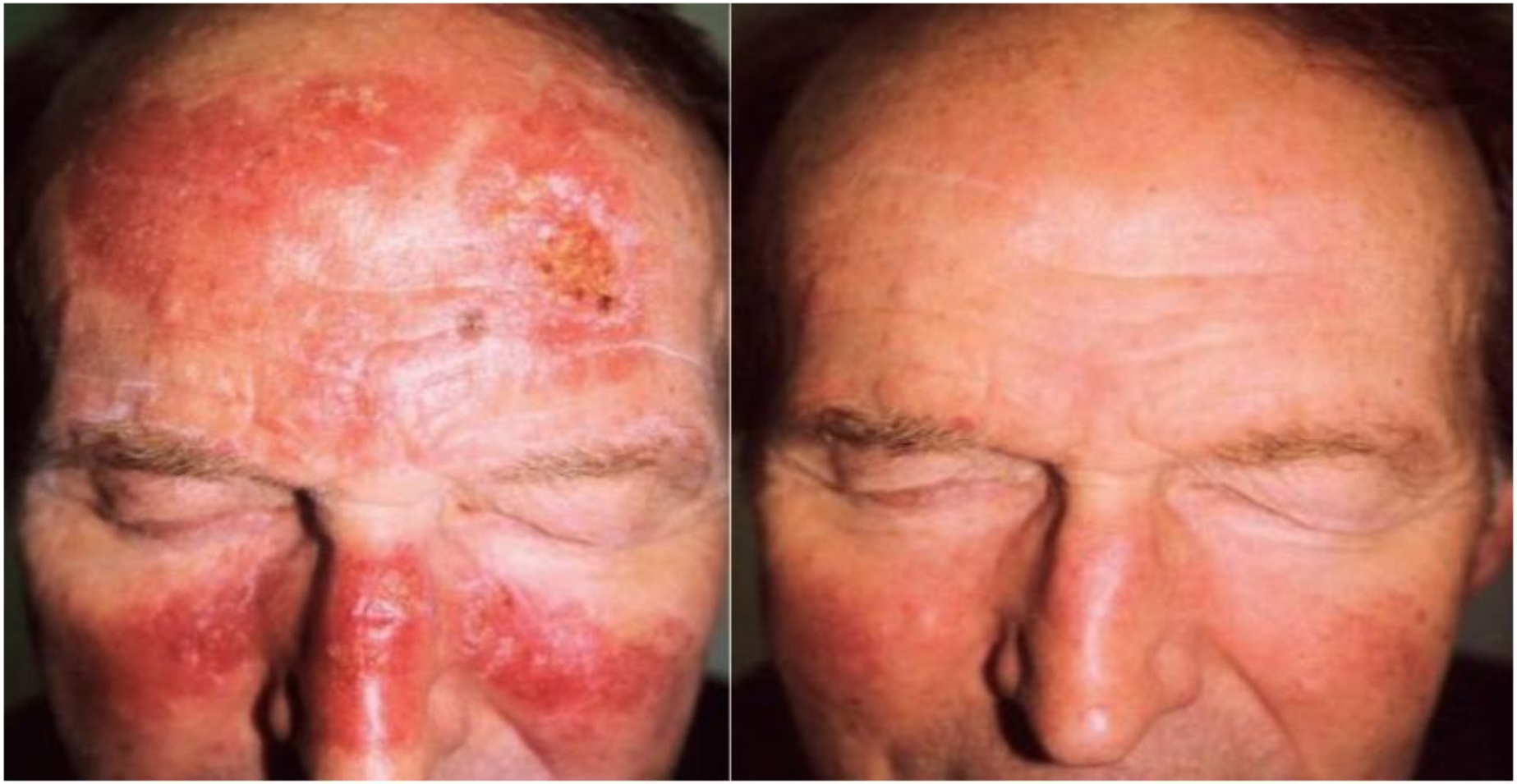
Takakura: Human Molecular Genetics, 20(21): 4143–4154, 2011

GLG Pharma: Pipeline
GLG-302



GLG Pharma: Pipeline

Actinic keratosis Squamous cancer Treatment **7 day follow-up visit**



GLG Pharma: Pipeline

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

- GLG-801, GLG-302, GLG-202
- 52 Other New Chemical Entities
- Rational Design Libraries
- Further Screening and Testing

Product Engine

Disease Targets

- Alpha-1 and Liver Cancer
- Polycystic Kidney Disease and Hypertension
- Liver Cancer Prevention
- Breast Cancer Treatment
- Cancer Stem Cell Treatment
- Proliferative Diseases, STAT3

- GLG-801 and GLG-302 to Market
- GLG-302 for Cancer Prevention
- Diagnostic Treatment and Monitoring
- Pipeline of New Drugs

Drug & Diagnostic

GLG PHARMA

Lead GLG Clinical Development Program for GLG-302 and GLG-801

Diagnostic Determines level of STAT3 Up-regulation and other circulating Disease Markers

Treat Targeted Disease with Single or Combination Therapies

Monitor Treatment Program if Tissue sample is available or Blood Circulating Markers

GLG PHARMA