Genetic Factors Governing Susceptibilities to Severe Infections

GSK-Chair of Infectious Diseases

Pr Jean-Paul MIRA

Potential Impact of Genomics on Therapeutic Concepts in Sepsis

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18 février 2005

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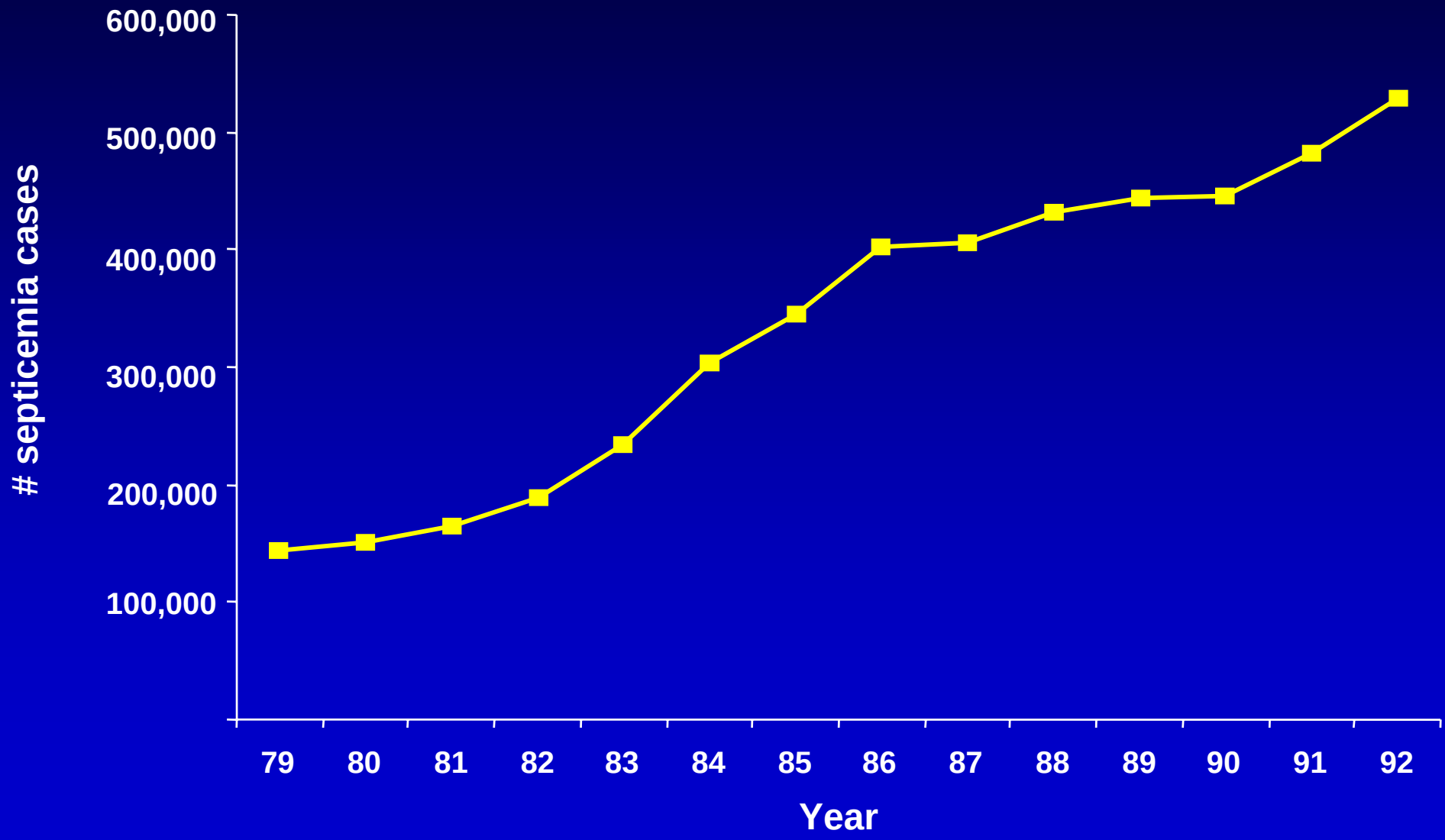
Medical ICU & Dept. of Cell Biology

Cochin University Hospital & Cochin Institute, Paris, F

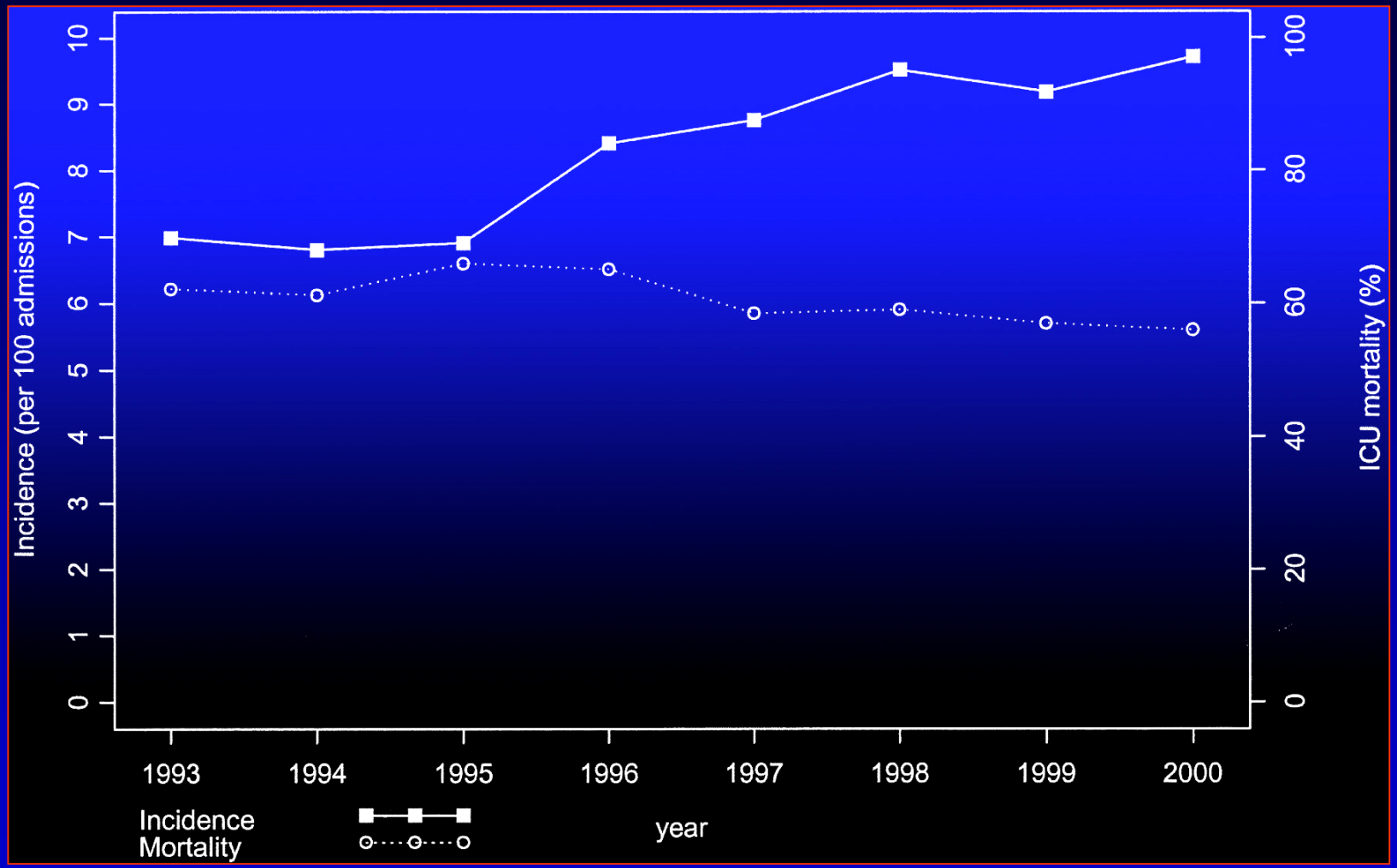
Humanity has but three great enemies:
fever, famine and war; of these,
by far the greatest, by far the most terrible,
is fever.

Sir William Osler

Incidence of sepsis in US (1979-1992)



Septic Shock Epidemiology



Annane D. Am J Respir Crit Care Med 2003;168:165–172

Incidence of severe sepsis in US

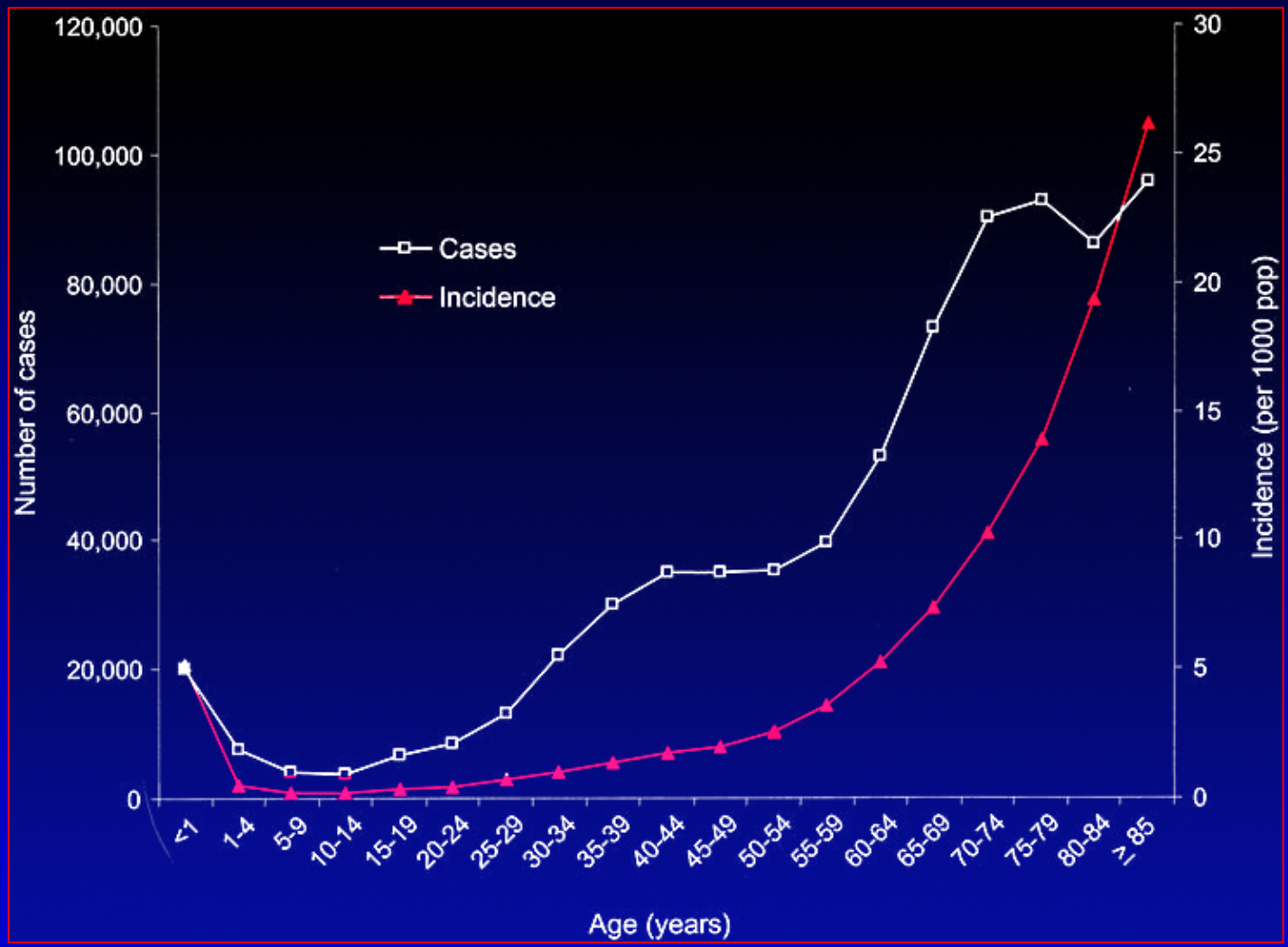
Seven states

6.6 M hospitalizations

200,000 severe sepsis

National 751,000 cases

51% Intensive Care



Angus DC et al. Crit Care Med 2001;29:1303

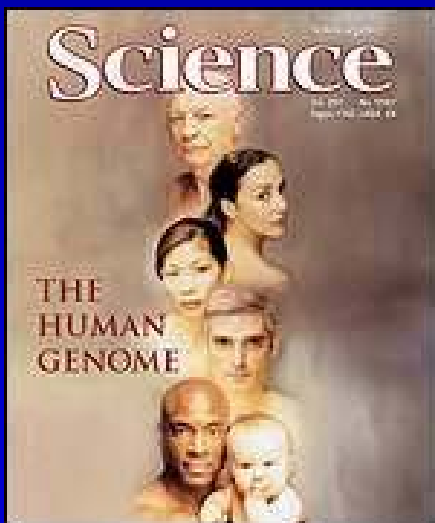
Completion of the Human Genome Project
- April 2003 -

- 12.5 years
- $ 2.7 billion
- International cooperative effort
  - 6 countries
  - 20 sequencing centers
- Full & immediate data release

http://www.ncbi.nih.gov/genome/guide/human

From Osler to Human Genome

1953	Watson and Crick: double helical structure of DNA
1960s	Role of RNA and Genetic Code
1970s	Recombinant DNA technology
1977	Sanger and Gilbert: DNA sequencing
1983	Mapping of disorders by linkage (Huntington disease)
1986	Polymerase Chain Reaction
1990	Human Genome Project
1995	*Haemophilus influenzae* genome
2003	Human genome sequence




ONE SMALL STEP FOR MAN...
HUMAN GENOME
REX BABIN


La Recherche
Génome humain
Les vrais enjeux d'un grand programme


TIME
LOTTE'S IMPEACHMENT PLAN
SPECIAL ISSUE
THE FUTURE OF MEDICINE
How genetic engineering will change us in the next century

Genetics and Therapeutic Concepts

Genetics and Therapeutic Concepts in Sepsis

- Variation in gene expression

- Variation in DNA sequence

Genetics and Therapeutic Concepts in Sepsis

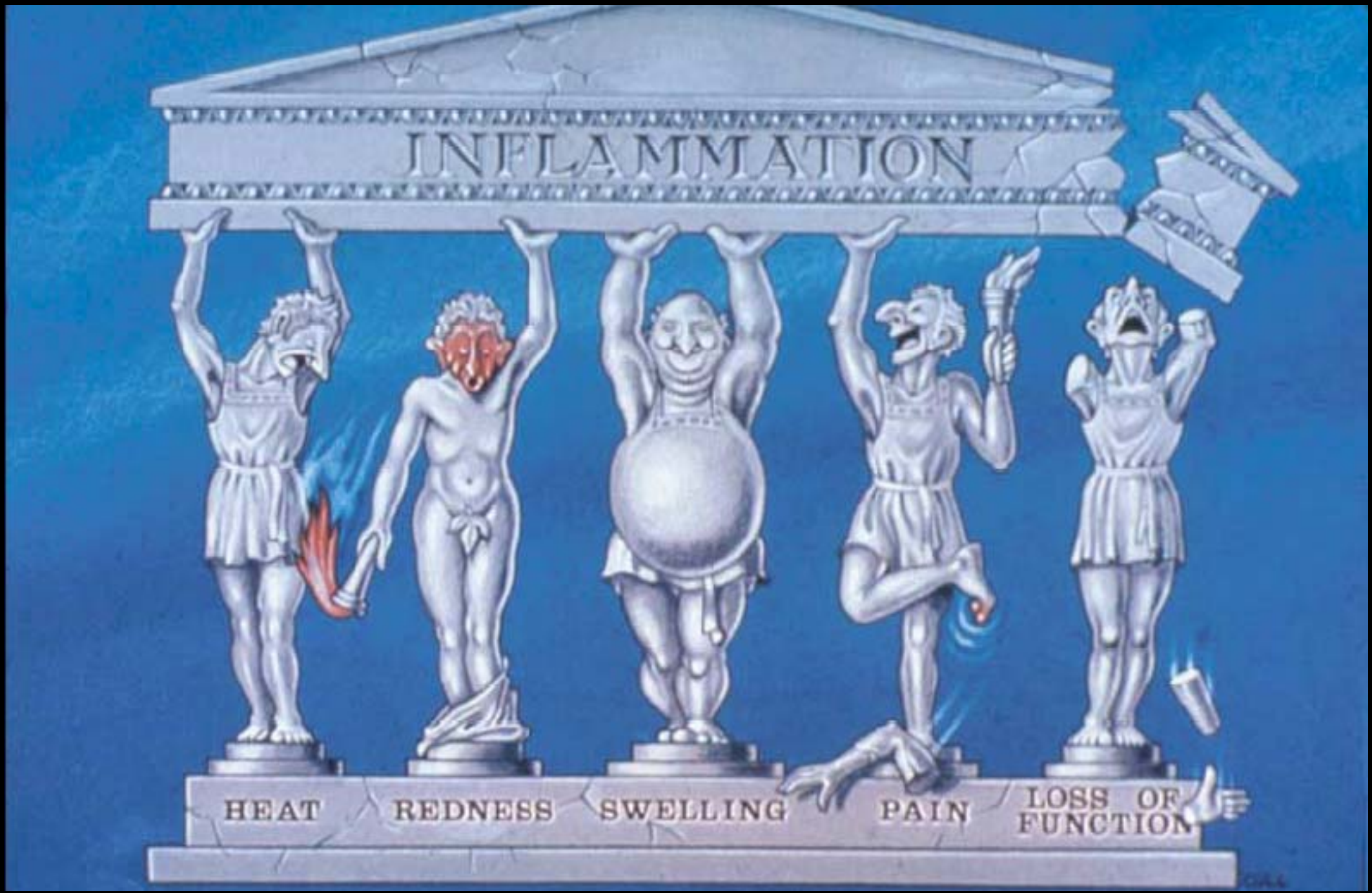
- Variation in gene expression

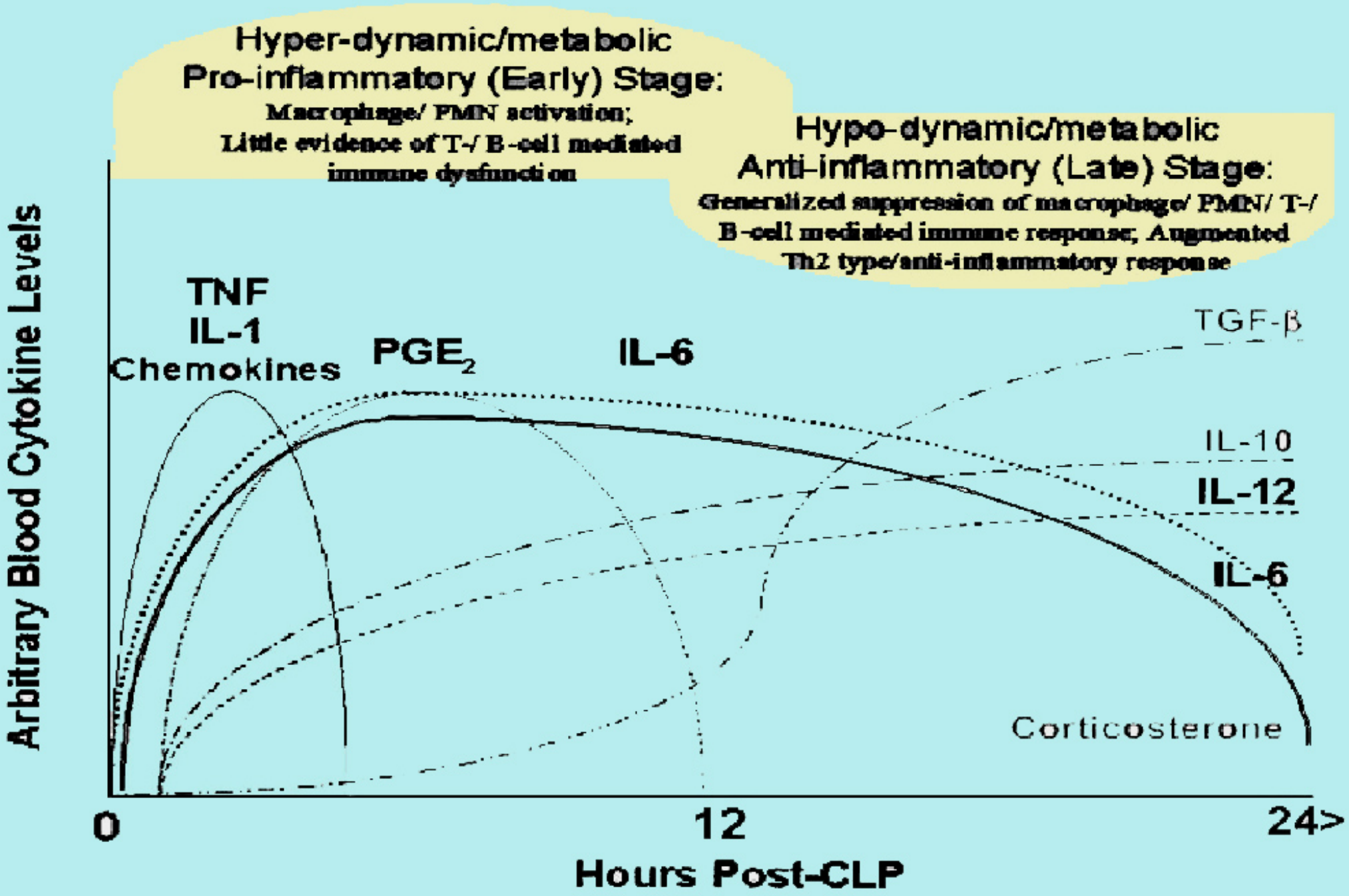
  → Functional Genomics

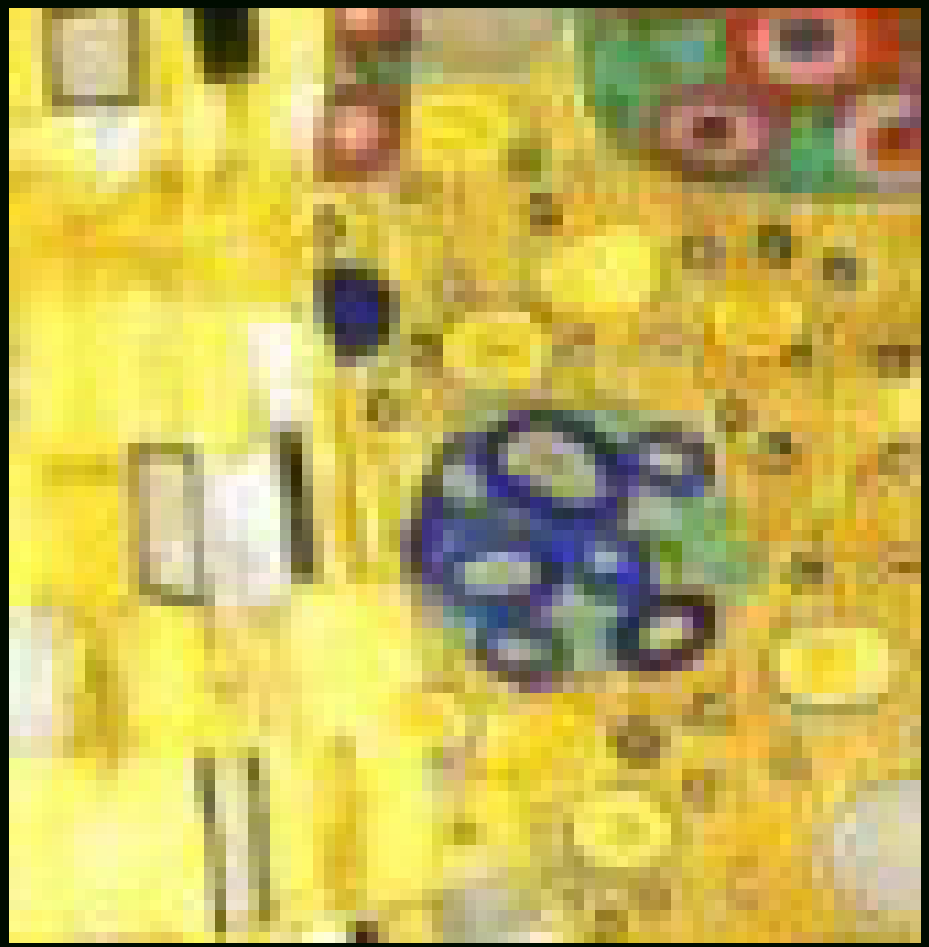
- Variation in DNA sequence

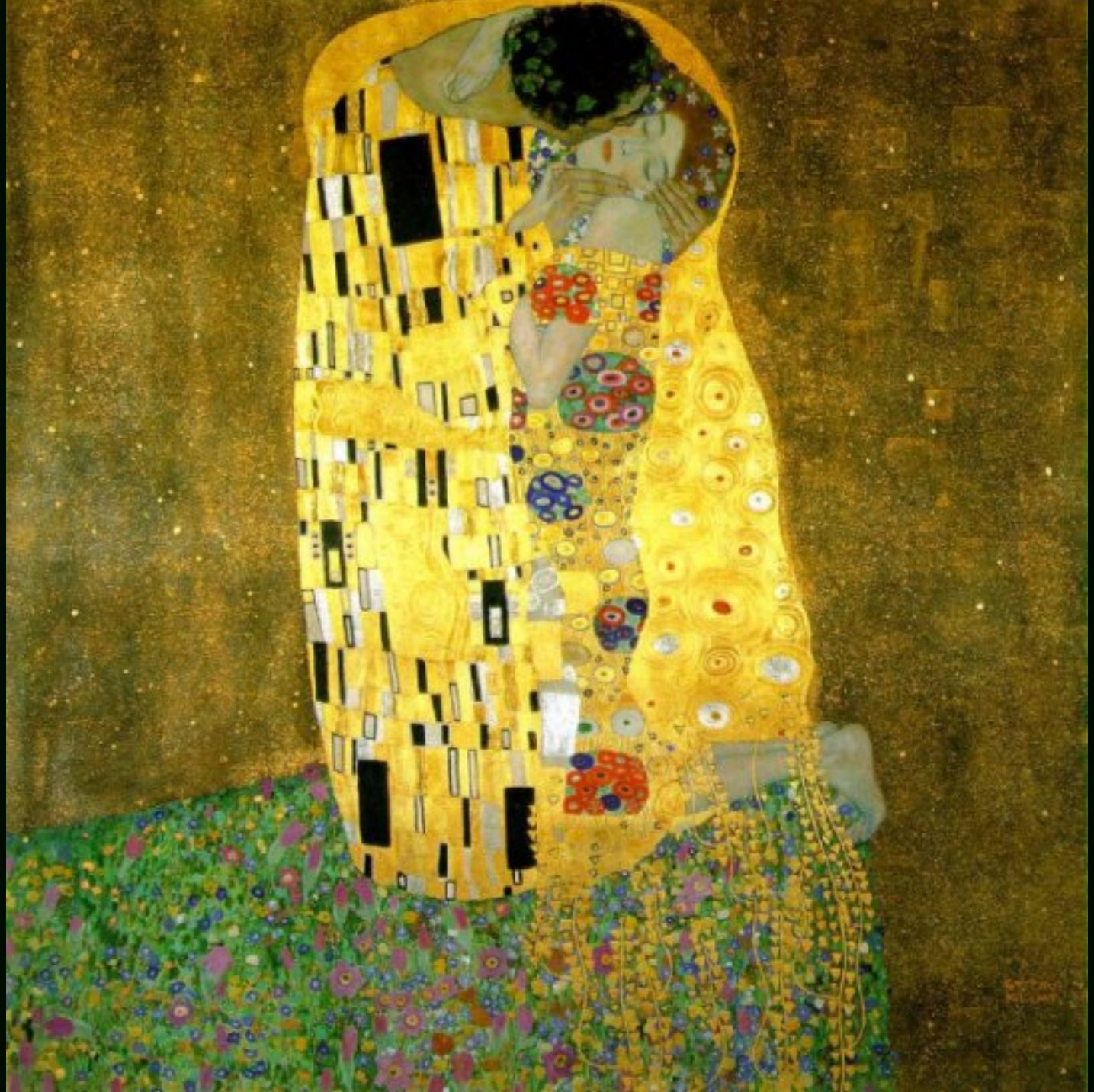
The first step in rationally treating a disease is to assess the patient against a classification of diseases, the results being used to predict the person's response to various therapies. The effectiveness of the process depends on the quality of the classification.

Claude Bernard

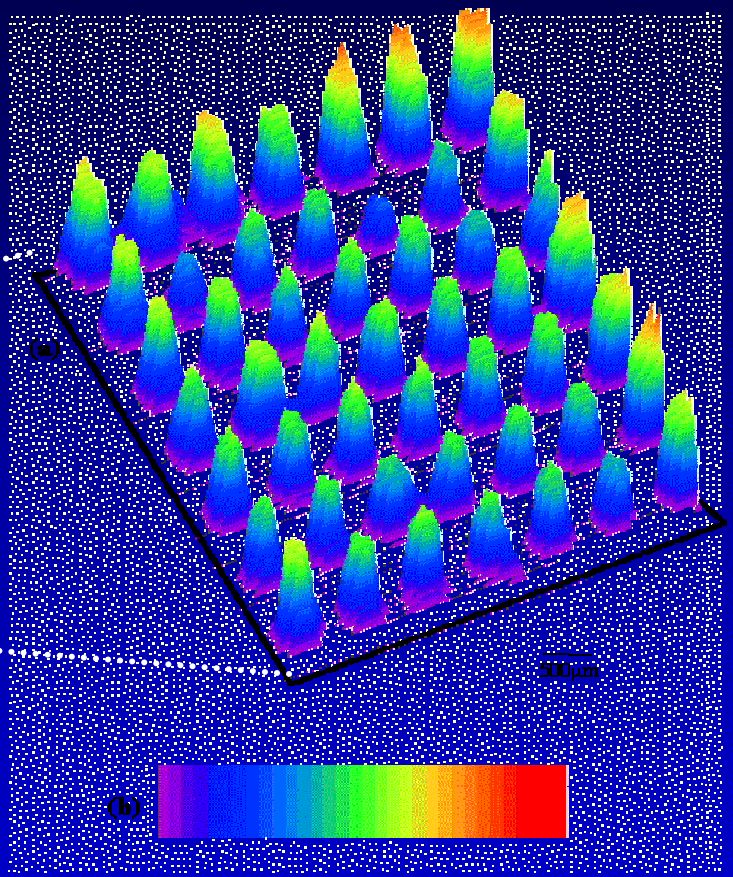

INFLAMMATION
HEAT
REDNESS
SWELLING
PAIN
LOSS OF FUNCTION


Hyper-dynamic/metabolic
Pro-inflammatory (Early) Stage:
Macrophage/ PMN activation;
Little evidence of T-/ B-cell mediated
immune dysfunction
Hypo-dynamic/metabolic
Anti-inflammatory (Late) Stage:
Generalized suppression of macrophage/ PMN/ T-/
B-cell mediated immune response; Augmented
Th2 type/anti-inflammatory response
TNF
IL-1
Chemokines
PGE2
IL-6
TGF-β
IL-10
IL-12
IL-6
Corticosterone
Arbitrary Blood Cytokine Levels
0
12
24>
Hours Post-CLP





DNA Microarray Technology

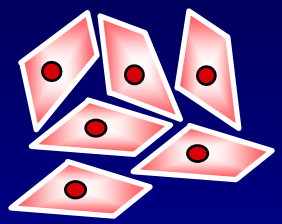


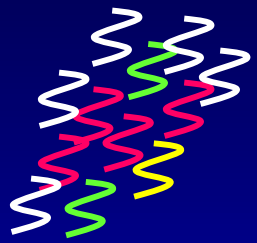
Genome-Wide Profiles of mRNA Expression

Biological samples

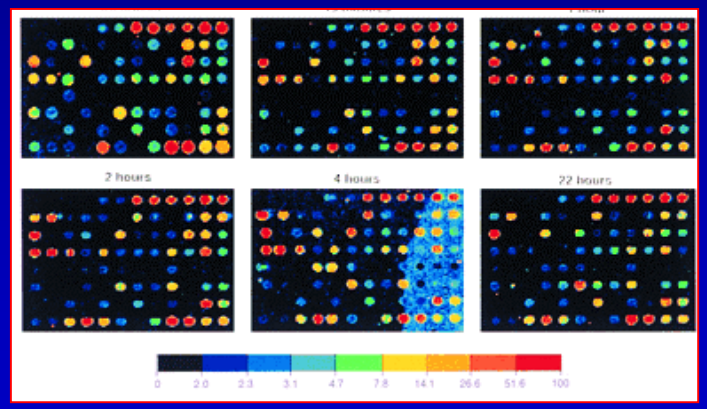
mRNA extraction

Target choice
(30,000/chips)





Analysis



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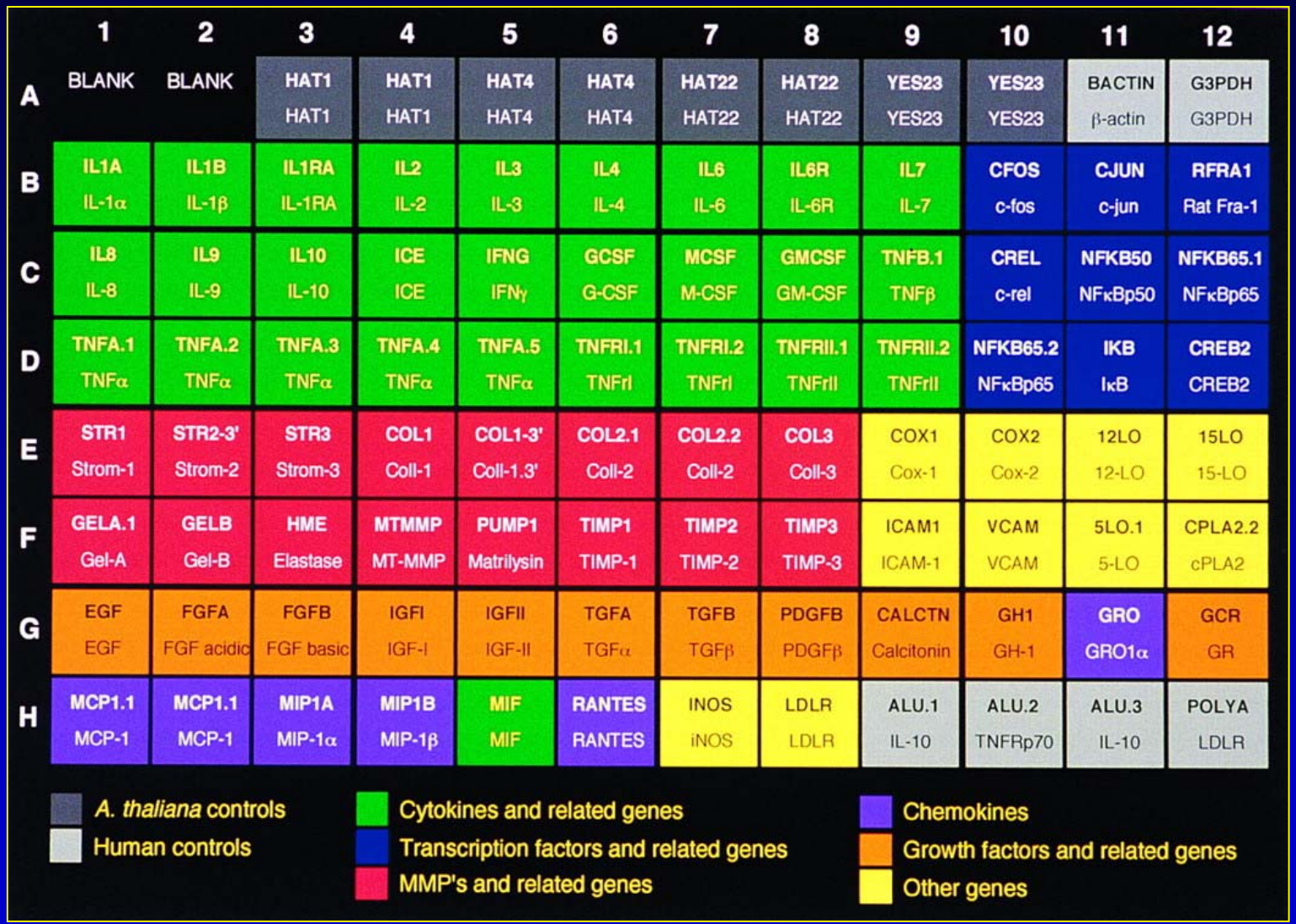
Hybridization

Analysis of sepsis-related genes using cDNA microarrays

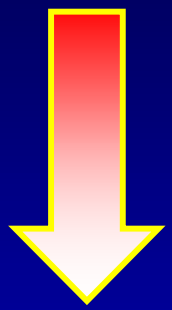


	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK	HAT1 HAT1	HAT1 HAT1	HAT4 HAT4	HAT4 HAT4	HAT22 HAT22	HAT22 HAT22	YES23 YES23	YES23 YES23	BACTIN β-actin	G3PDH G3PDH
B	IL1A IL-1α	IL1B IL-1β	IL1RA IL-1RA	IL2 IL-2	IL3 IL-3	IL4 IL-4	IL6 IL-6	IL6R IL-6R	IL7 IL-7	CFOS c-fos	CJUN c-jun	RFRA1 Rat Fra-1
C	IL8 IL-8	IL9 IL-9	IL10 IL-10	ICE ICE	IFNG IFNγ	GCSF G-CSF	MCSF M-CSF	GMCSF GM-CSF	TNFB.1 TNFβ	CREL c-rel	NFKB50 NFκBp50	NFKB65.1 NFκBp65
D	TNFA.1 TNFα	TNFA.2 TNFα	TNFA.3 TNFα	TNFA.4 TNFα	TNFA.5 TNFα	TNFRI.1 TNFrI	TNFRI.2 TNFrI	TNFRII.1 TNFrII	TNFRII.2 TNFrII	NFKB65.2 NFκBp65	IKB IκB	CREB2 CREB2
E	STR1 Strom-1	STR2-3' Strom-2	STR3 Strom-3	COL1 Coll-1	COL1-3' Coll-1.3'	COL2.1 Coll-2	COL2.2 Coll-2	COL3 Coll-3	COX1 Cox-1	COX2 Cox-2	12LO 12-LO	15LO 15-LO
F	GELA.1 Gel-A	GELB Gel-B	HME Elastase	MTMMP MT-MMP	PUMP1 Matrilysin	TIMP1 TIMP-1	TIMP2 TIMP-2	TIMP3 TIMP-3	ICAM1 ICAM-1	VCAM VCAM	5LO.1 5-LO	CPLA2.2 cPLA2
G	EGF EGF	FGFA FGF acidic	FGFB FGF basic	IGFI IGF-I	IGFII IGF-II	TGFA TGFα	TGFB TGFβ	PDGFB PDGFβ	CALCTN Calcitonin	GH1 GH-1	GRO GRO1α	GCR GR
H	MCP1.1 MCP-1	MCP1.1 MCP-1	MIP1A MIP-1α	MIP1B MIP-1β	MIF MIF	RANTES RANTES	INOS iNOS	LDLR LDLR	ALU.1 IL-10	ALU.2 TNFRp70	ALU.3 IL-10	POLYA LDLR

A. thaliana controls
Human controls
Cytokines and related genes
Transcription factors and related genes
MMP's and related genes
Chemokines
Growth factors and related genes
Other genes

Heller RA. Proc. Natl. Acad. Sci. USA 1997;94: 2150

Functional Genomics

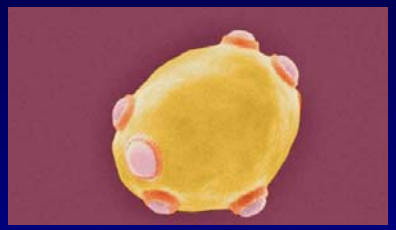


Molecular Signature of Sepsis

Which genes are expressed and to what magnitude?

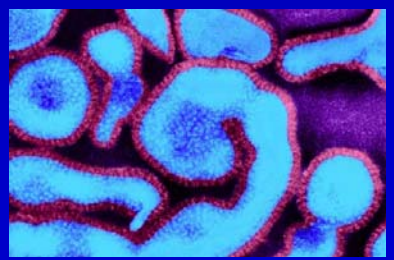
Dendritic Cell Responses to Pathogens

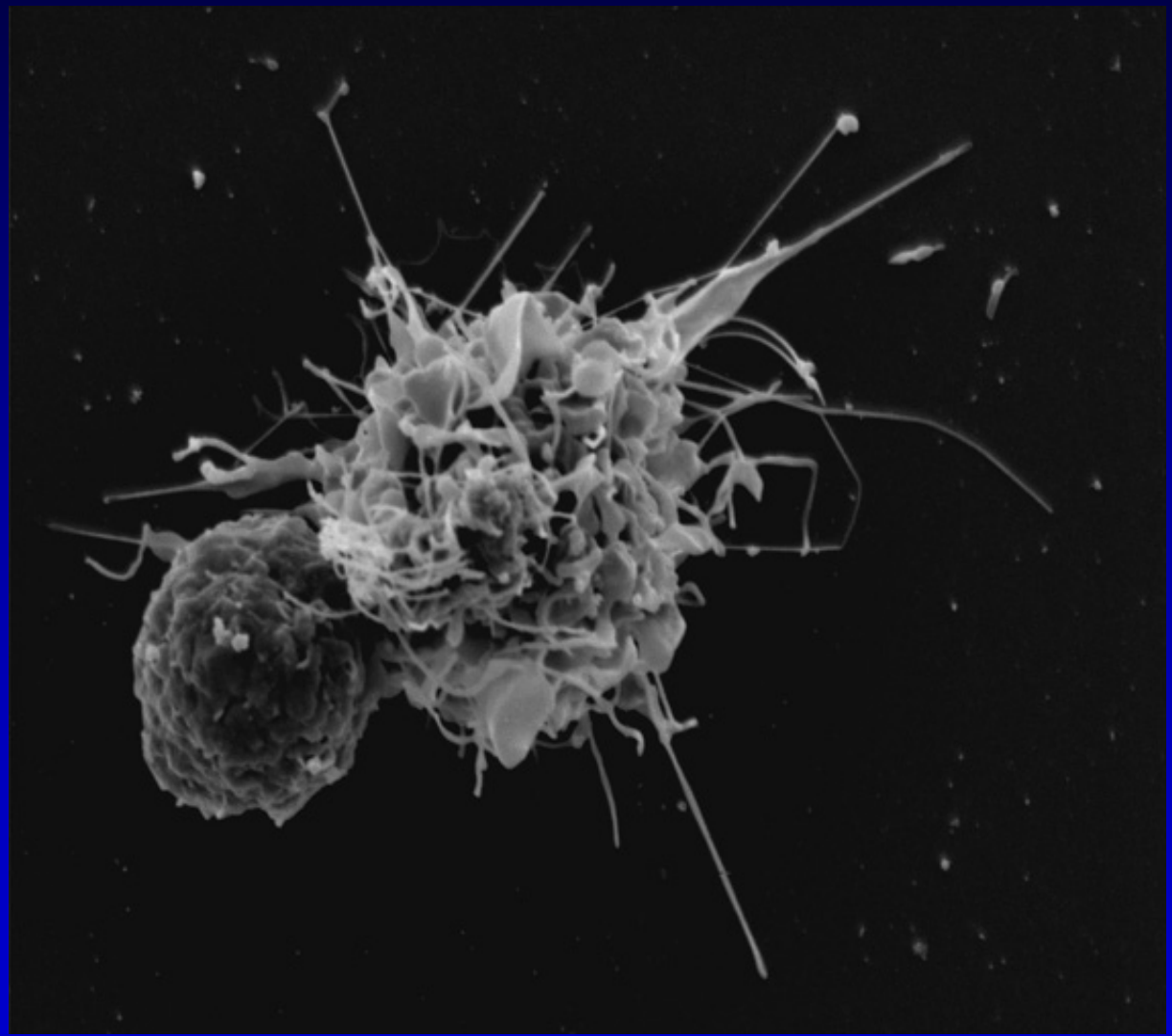
Candida



E. coli

Influenzae Virus

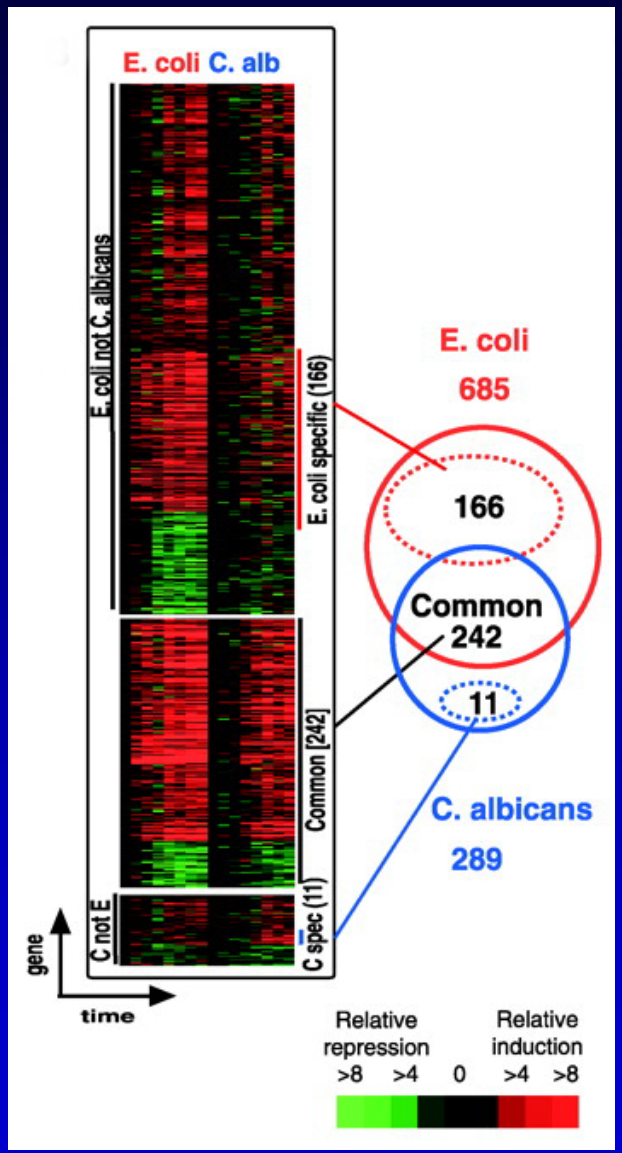


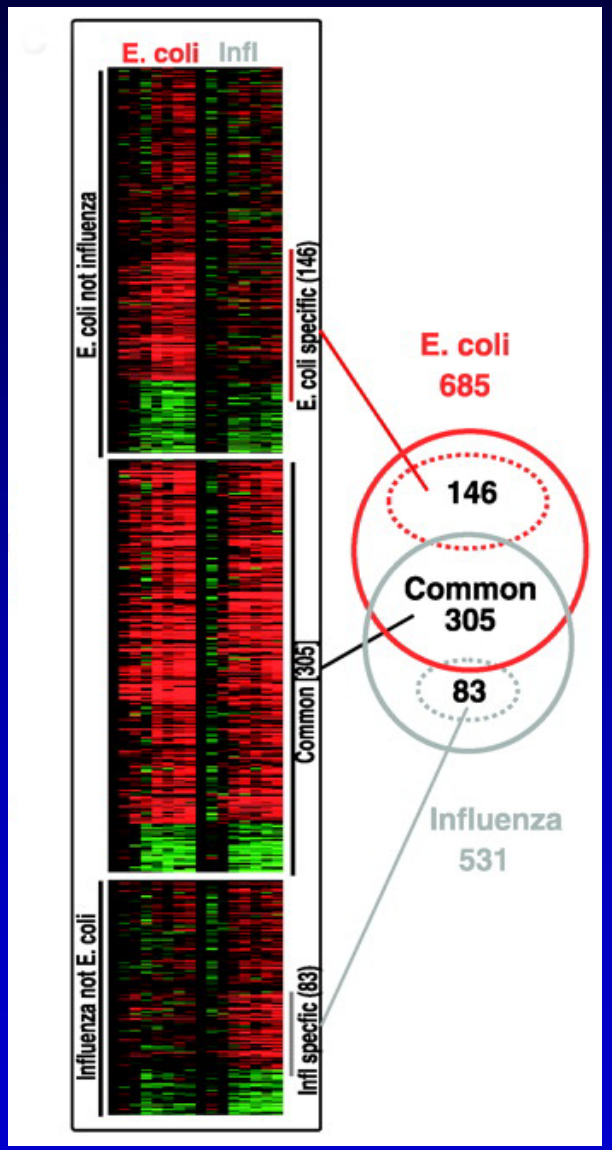


Huang Q. Science 2001;294:870

Cell Response is Pathogen-Dependent

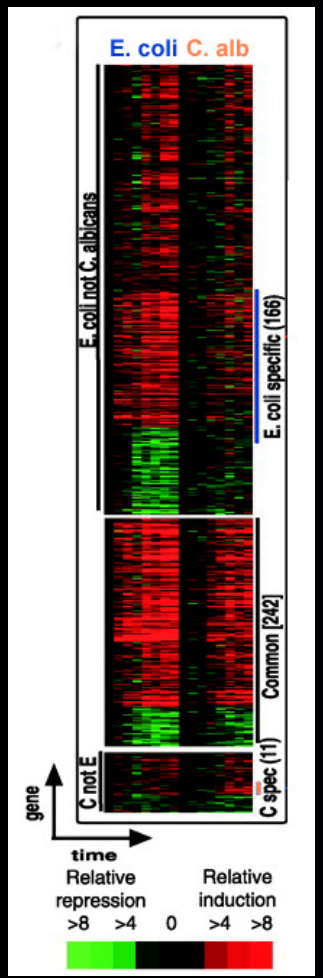
6800 genes tested

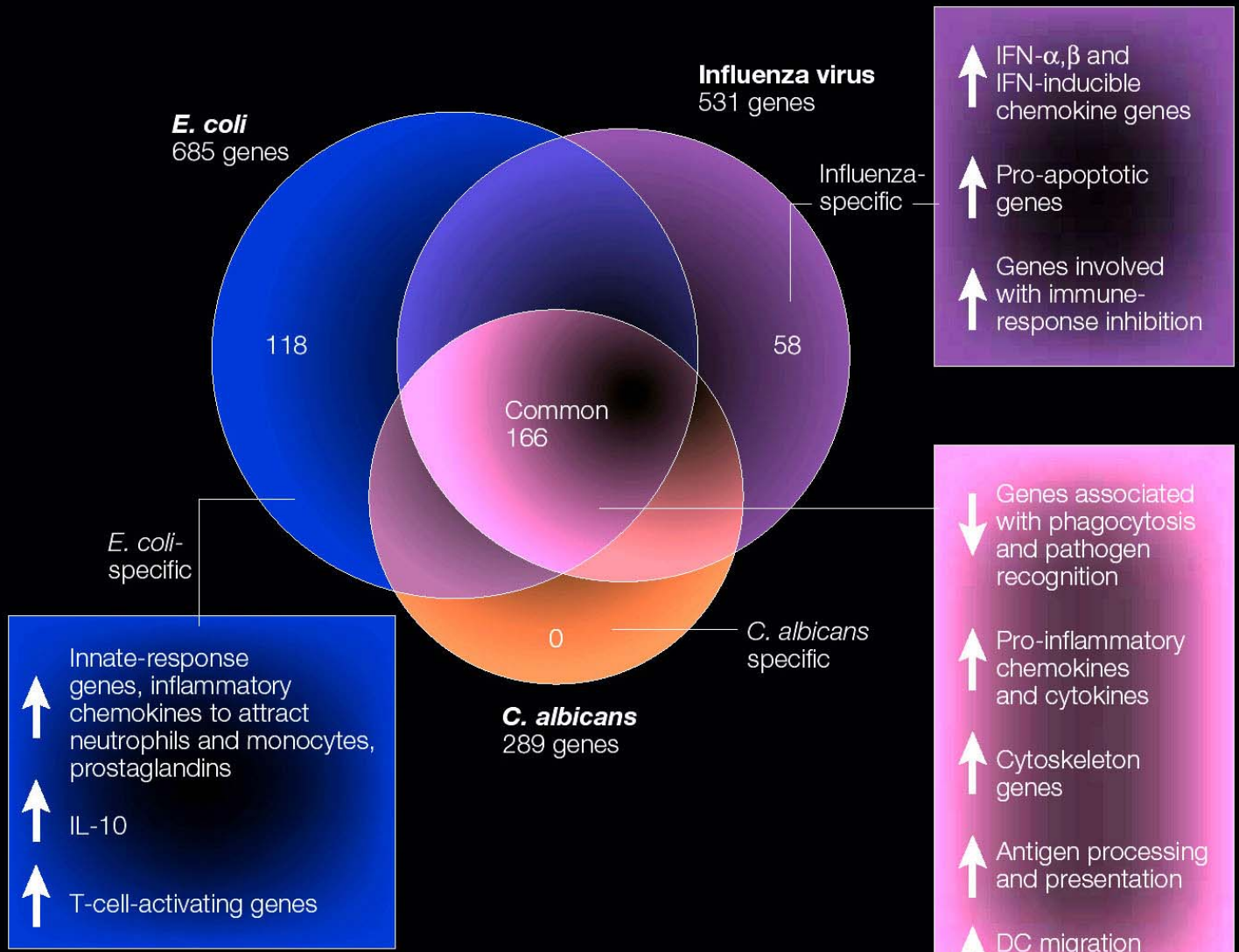


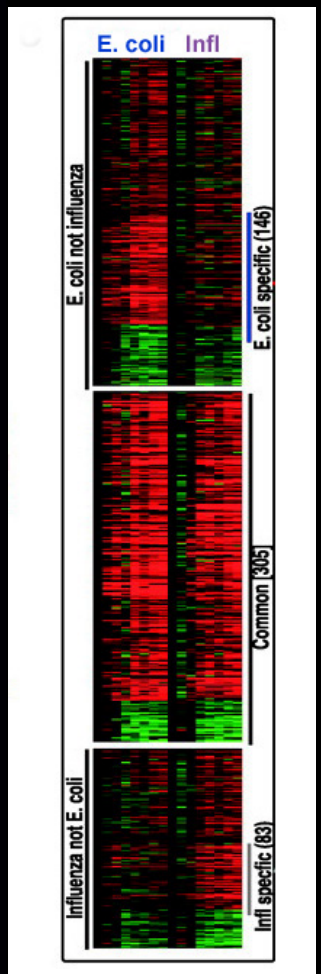


Huang Q. Science 2001;294:870

Dendritic cells ellicit a pathogen-specific immune response

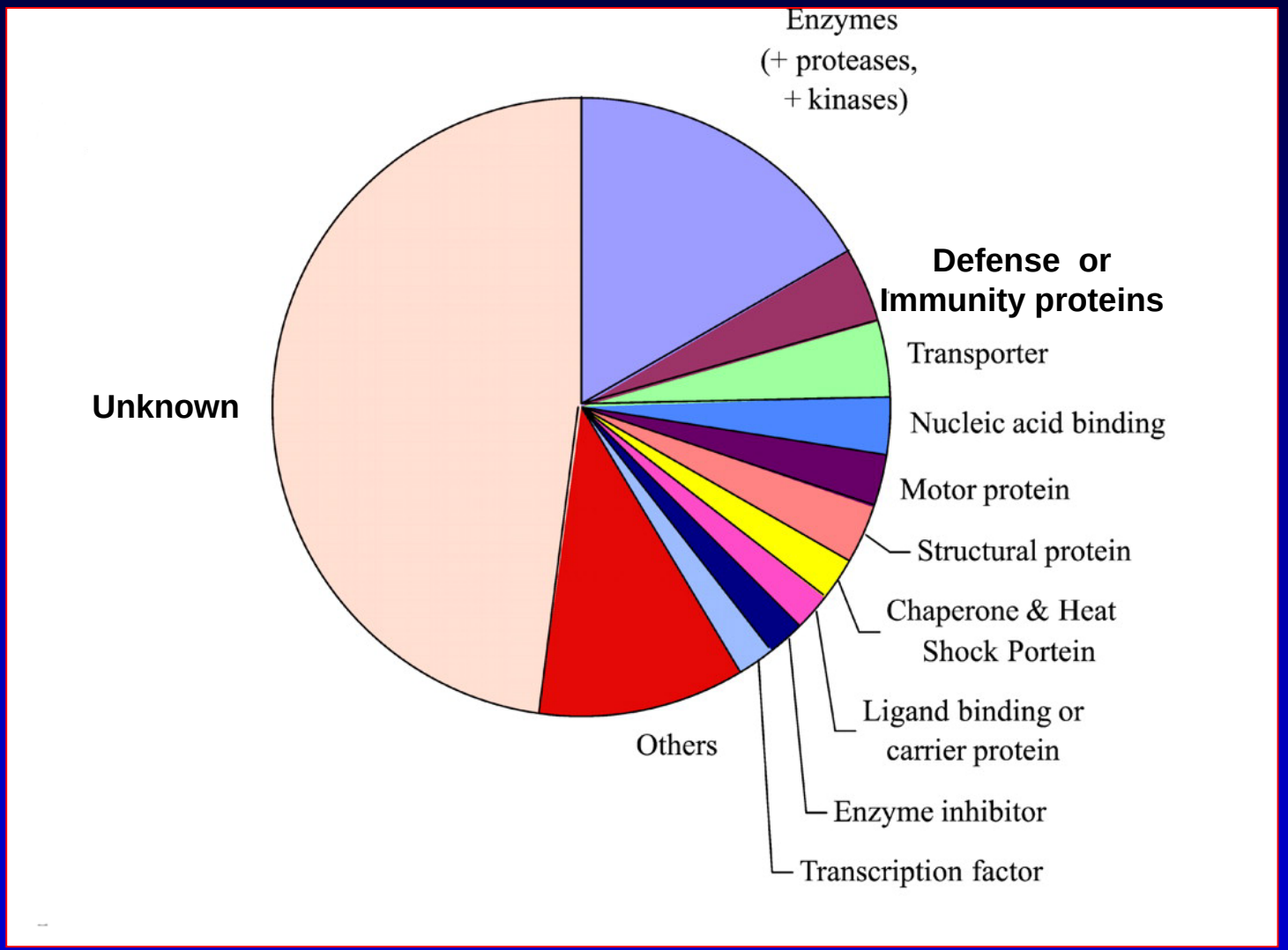






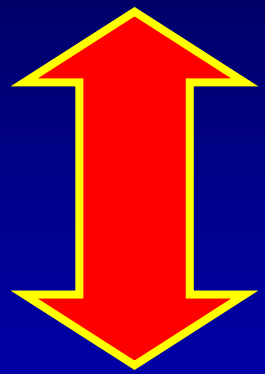
Huang Q. Science 2001;294:870

Identification of New Pathways



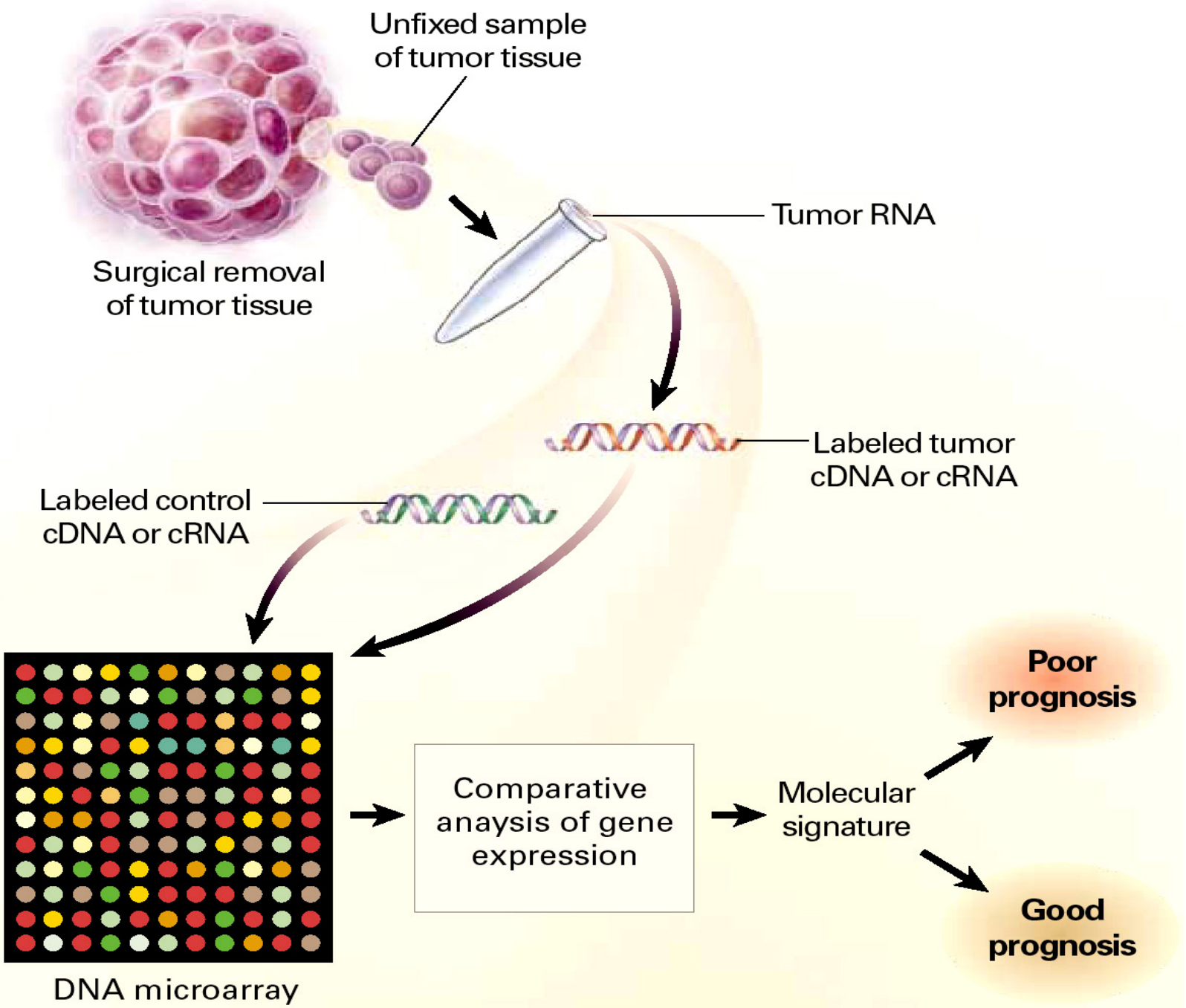
Irving P. PNAS 2001;98:15119

SEPSIS



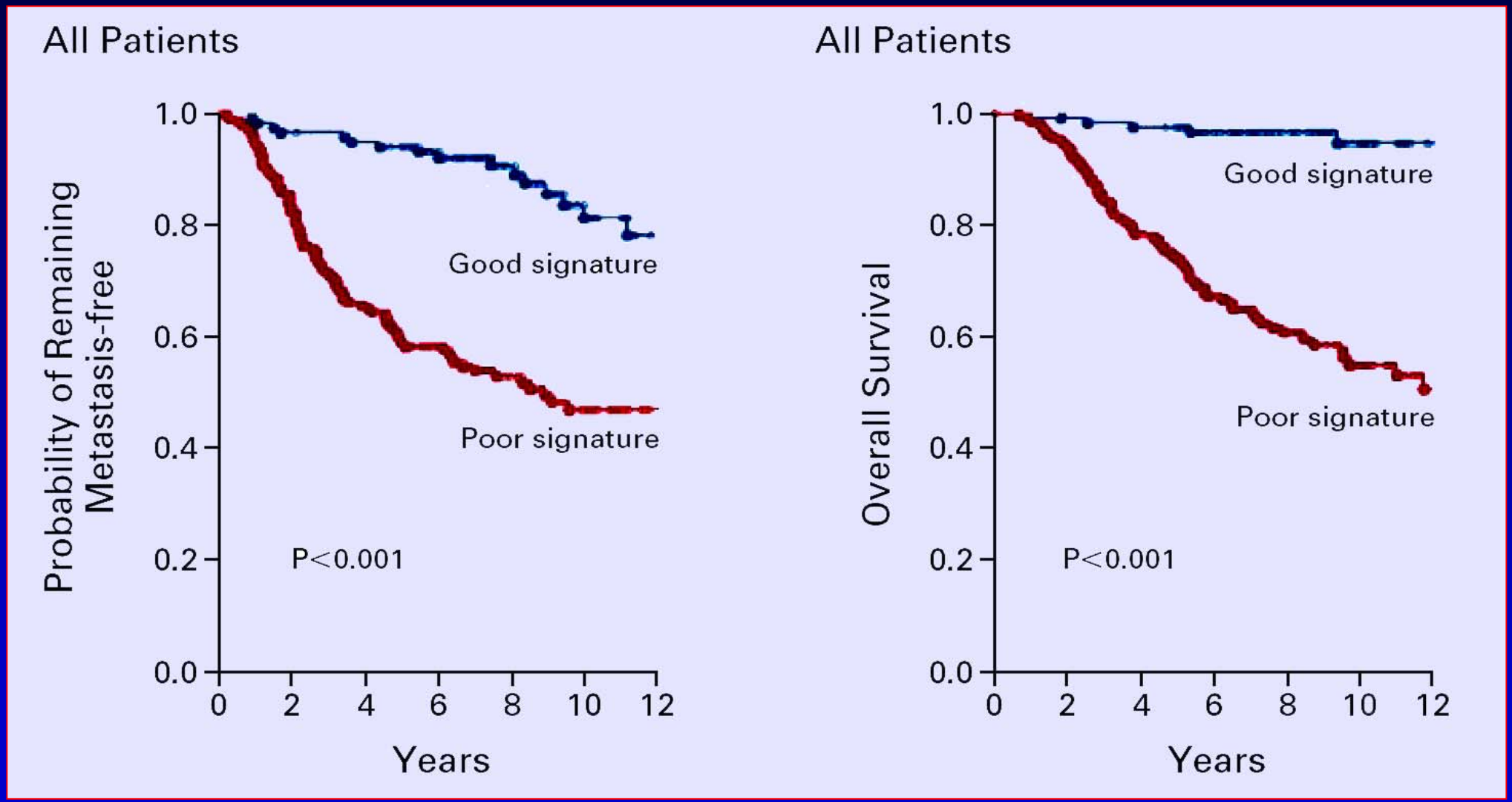
CANCER

Marshall J.


Unfixed sample of tumor tissue
Surgical removal of tumor tissue
Tumor RNA
Labeled tumor cDNA or cRNA
Labeled control cDNA or cRNA
DNA microarray
Comparative anaysis of gene expression
Molecular signature
Poor prognosis
Good prognosis

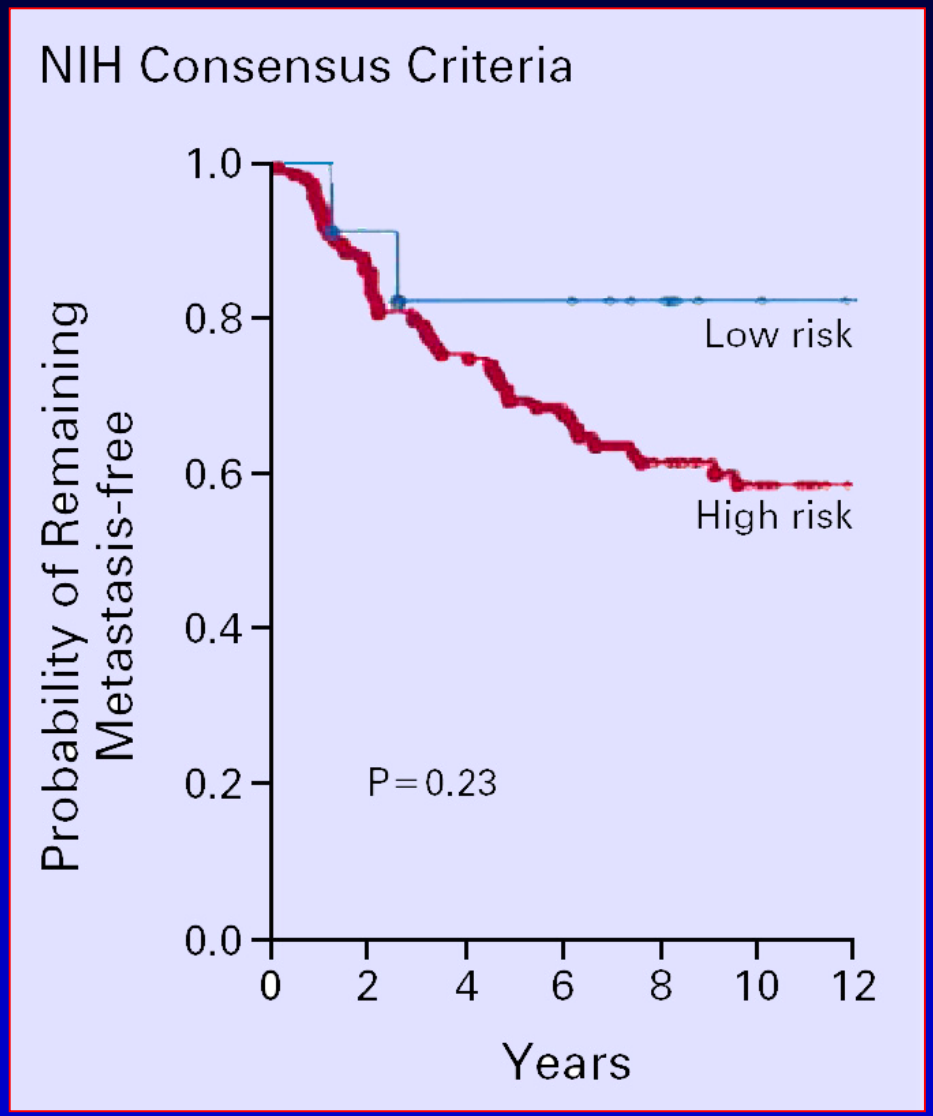
Molecular Profiling by Microarrays ➔ Cancer Prognostic

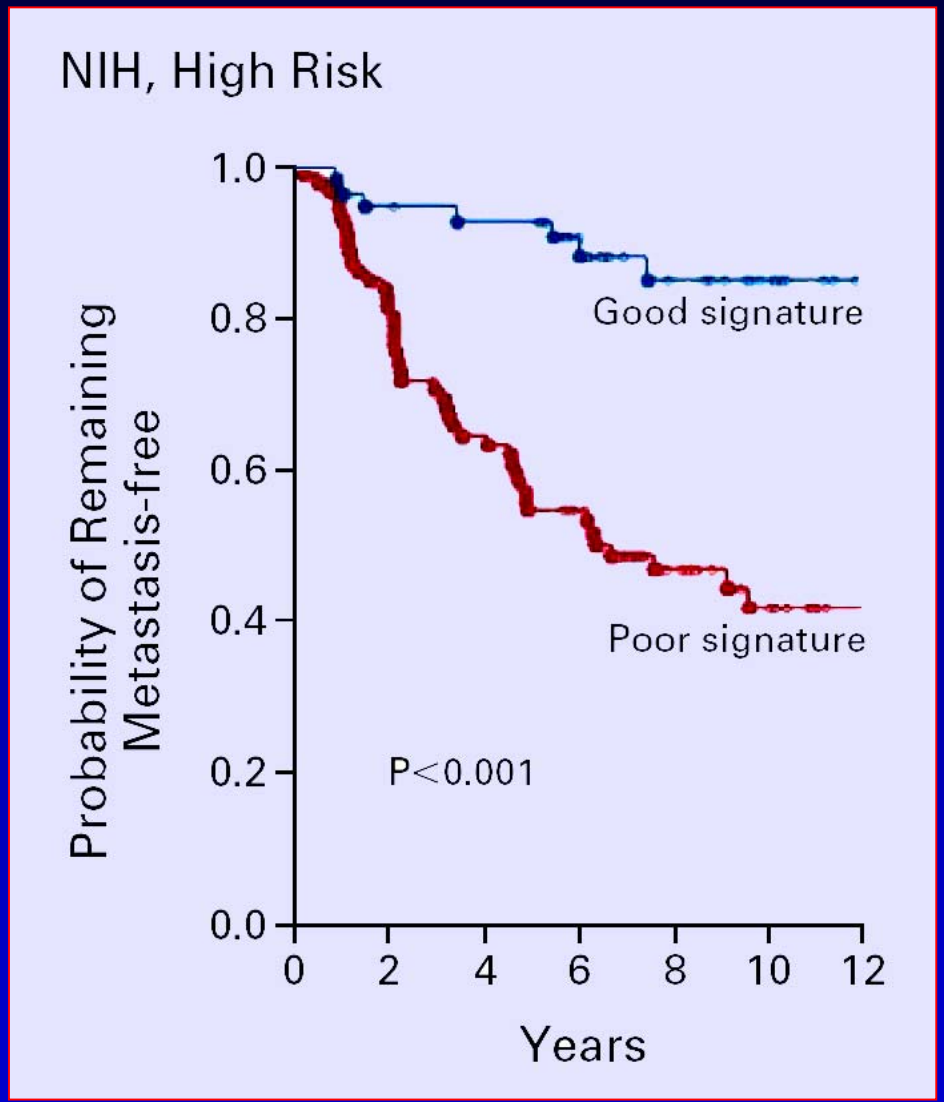
295 primary breast carcinomas – Cluster of 70 genes



Van't Veer LJ. N Engl J Med 2002;347:1999

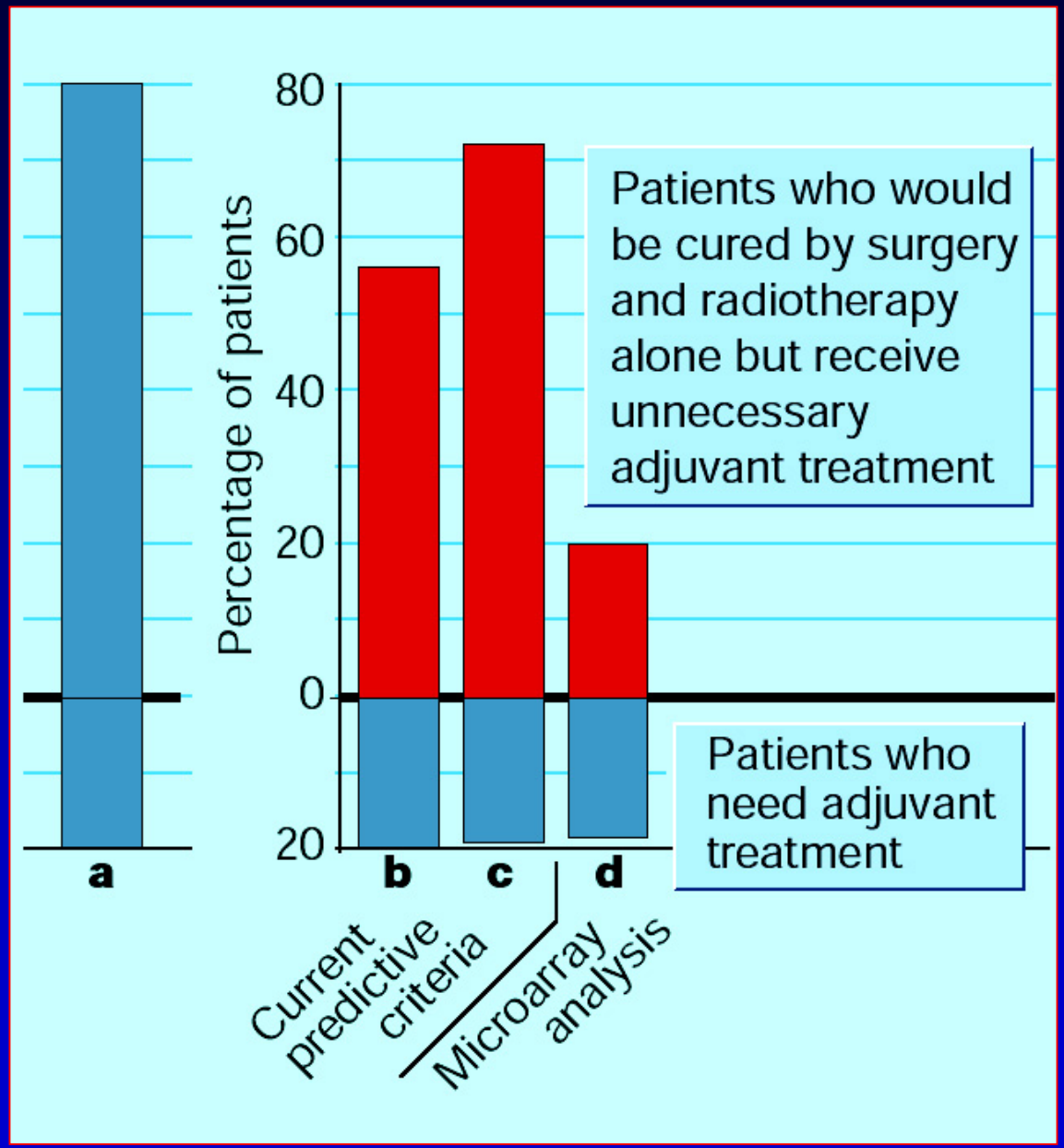
Molecular Profiling by Microarrays ➔ Cancer Prognostic





Van't Veer LJ. N Engl J Med 2002;347:1999

Molecular Profiling by Microarrays ➔ Cancer Classification



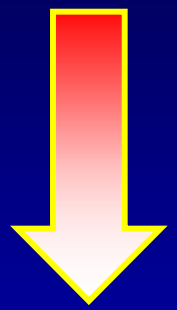
b: NIH consensus

c: St Gallen classification

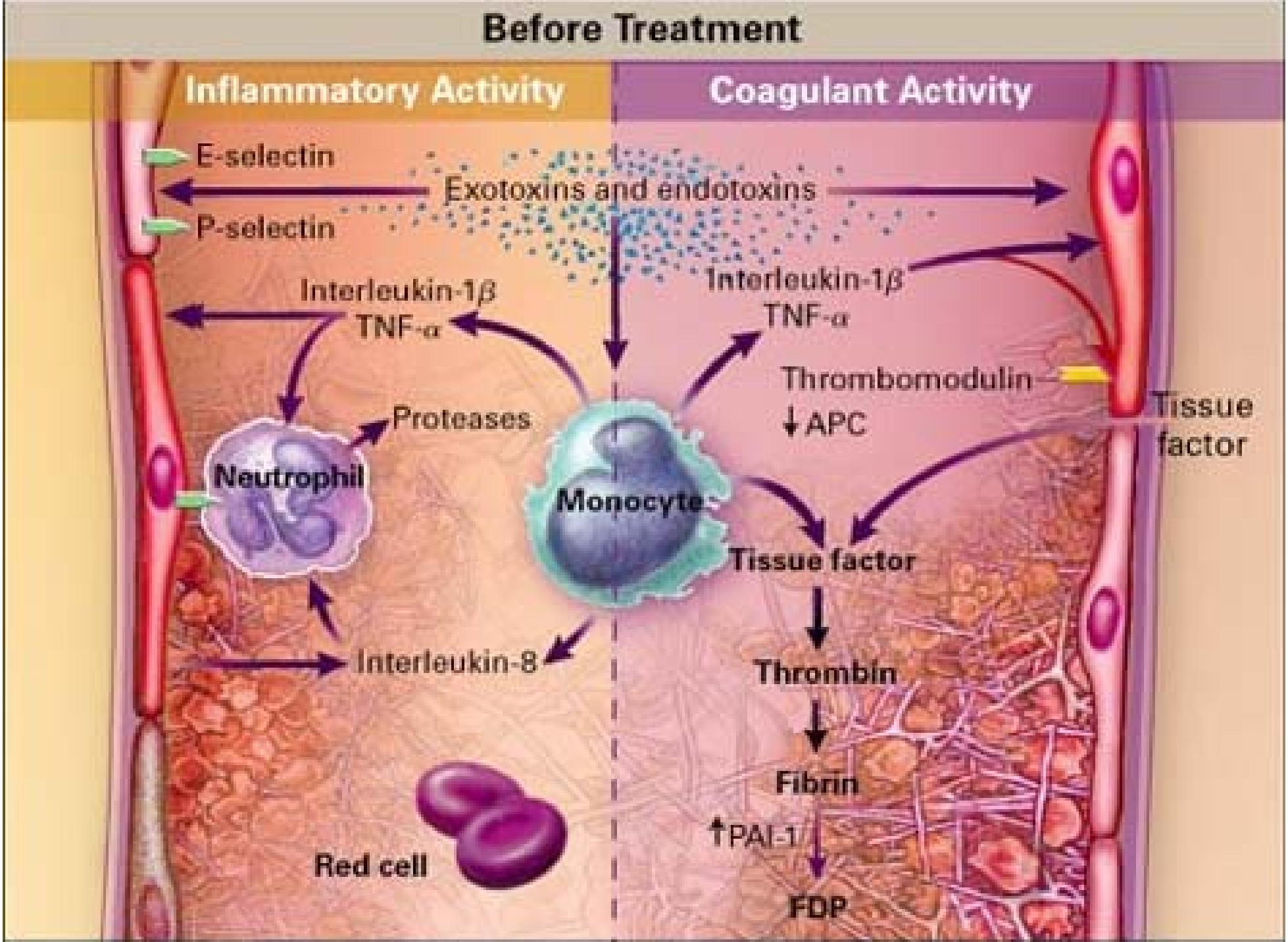
d: Microarray analysis (70 marker genes)

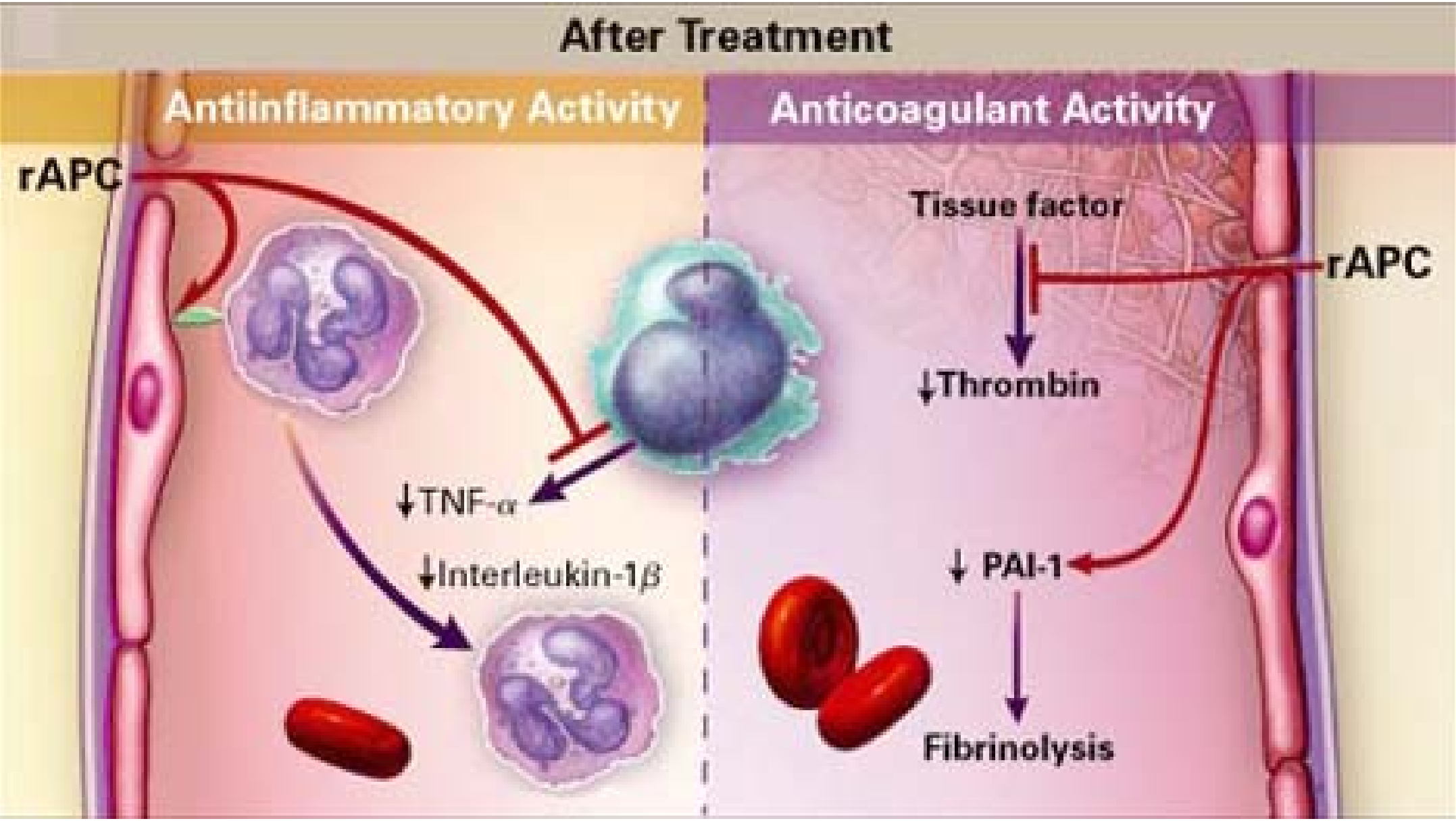
Van't Veer LJ. Nature 2002;415:530

Functional Genomics



Identification of Drug Targets


Before Treatment
Inflammatory Activity
Coagulant Activity
E-selectin
P-selectin
Exotoxins and endotoxins
Interleukin-1β
TNF-α
Interleukin-1β
TNF-α
Thrombomodulin
↓APC
Tissue factor
Proteases
Neutrophil
Monocyte
Tissue factor
Interleukin-8
Thrombin
Fibrin
↑PAI-1
Red cell
FDP


After Treatment
Antiinflammatory Activity
Anticoagulant Activity
rAPC
Tissue factor
rAPC
↓Thrombin
↓TNF-α
↓Interleukin-1β
↓ PAI-1
Fibrinolysis

The New England Journal of Medicine

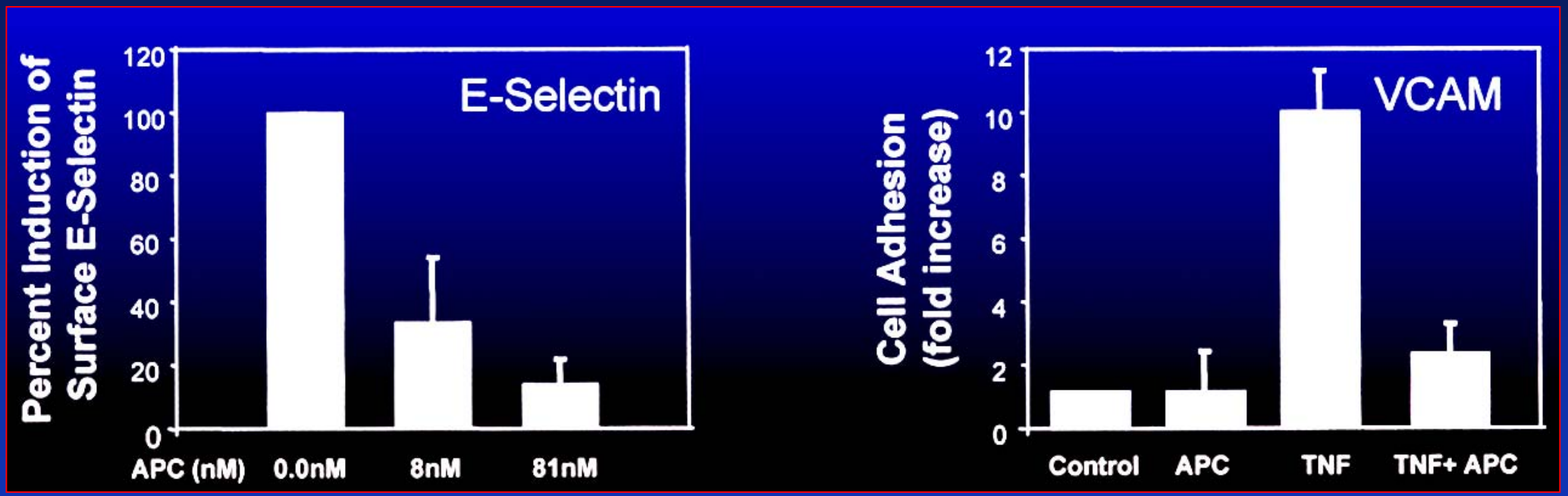
VOLUME 344 MARCH 8, 2001 NUMBER 10

EFFICACY AND SAFETY OF RECOMBINANT HUMAN ACTIVATED PROTEIN C FOR SEVERE SEPSIS

GORDON R. BERNARD, M.D., JEAN-LOUIS VINCENT, M.D., PH.D., PIERRE-FRANCOIS LATERRE, M.D., STEVEN P. LAROSA, M.D., JEAN-FRANCOIS DHAINAUT, M.D., PH.D., ANGEL LOPEZ-RODRIGUEZ, M.D., JAY S. STEINGRUB, M.D., GARY E. GARBER, M.D., JEFFREY D. HELTERBRAND, PH.D., E. WESLEY ELY, M.D., M.P.H., AND CHARLES J. FISHER, JR., M.D., FOR THE RECOMBINANT HUMAN ACTIVATED PROTEIN C WORLDWIDE EVALUATION IN SEVERE SEPSIS (PROWESS) STUDY GROUP*

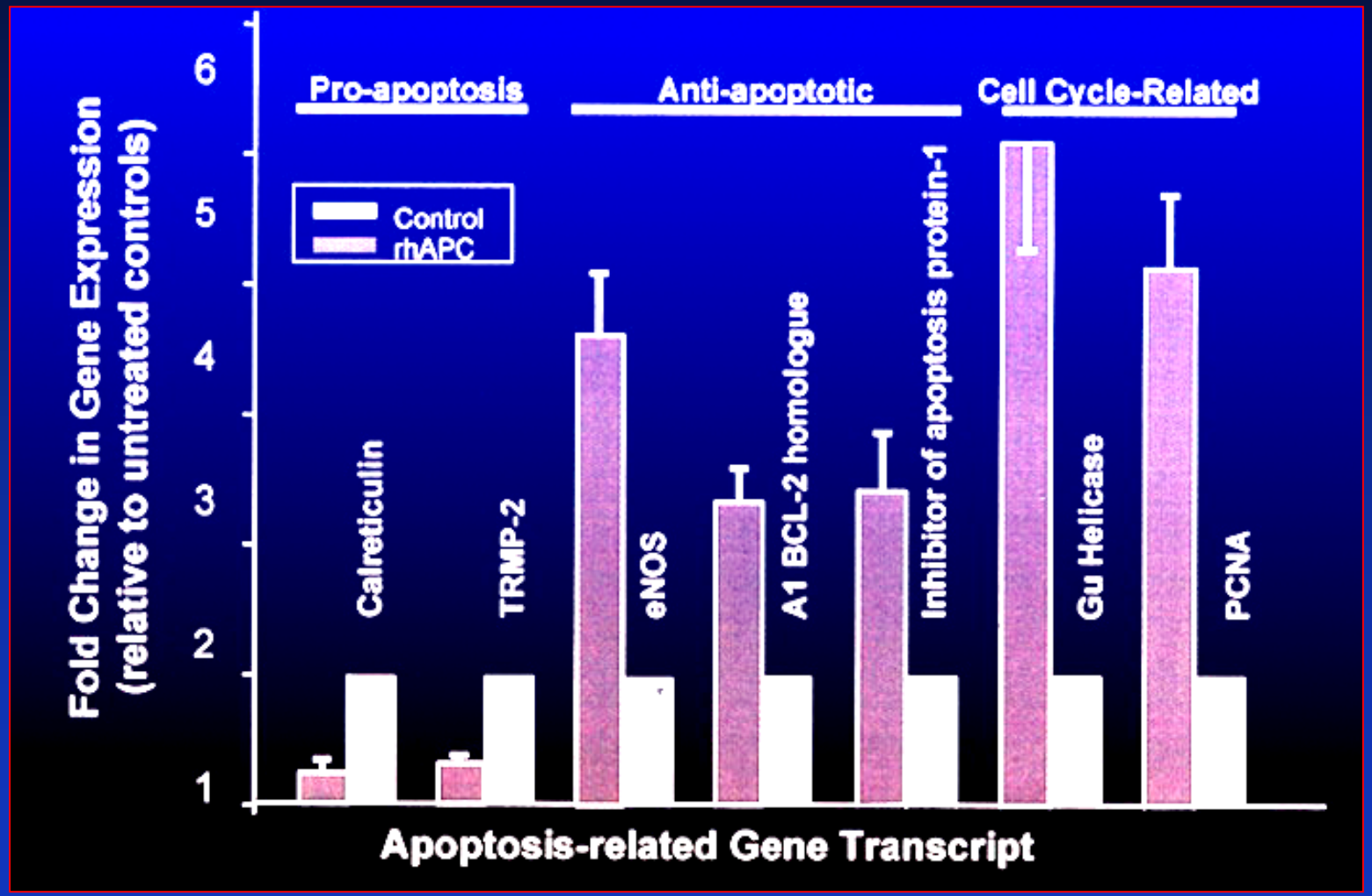
Drotrecogin alfa (activated) on Stimulated Endothelial Cells

Microarray: 6800 gènes

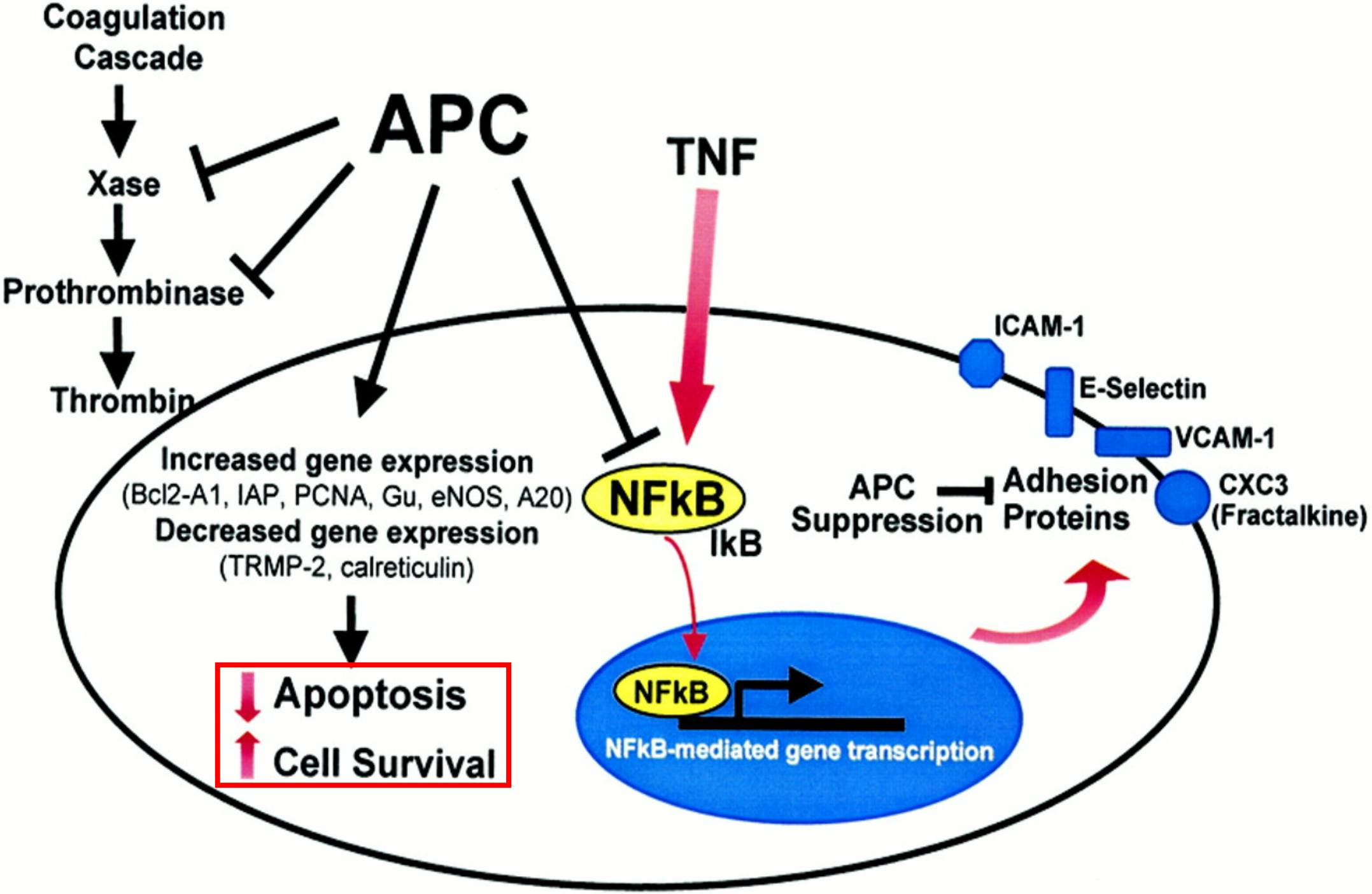


Joyce DE. J Biol Chem 2001;276:11199

Drotrecogin alfa (activated) on stimulated endothelial cells



Joyce DE. J Biol Chem 2001;276:11199


Coagulation Cascade
APC
TNF
Xase
Prothrombinase
Thrombin
ICAM-1
E-Selectin
VCAM-1
CXC3 (Fractalkine)
Increased gene expression
(Bcl2-A1, IAP, PCNA, Gu, eNOS, A20)
Decreased gene expression
(TRMP-2, calreticulin)
NFkB
IkB
APC Suppression
Adhesion Proteins
Apoptosis
Cell Survival
NFkB
NFkB-mediated gene transcription

Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective

Tong Cheng[1], Dong Liu[1], John H. Griffin[2], José A. Fernández[2], Francis Castellino[3], Elliot D. Rosen[3], Kenji Fukudome[4] & Berislav V. Zlokovic[1,5]

Cheng T. Nature Medicine 2003

Genetics and Potential Therapeutic Concepts in Sepsis

→ Understanding host response to pathogens

Identification of new pathways involved in sepsis

Drug target validation

→Diagnostic expression markers (Fingerprint)

→Prognostic expression markers

→Drug efficacy markers

Genetics and Therapeutic Concepts in Sepsis

- Variation in gene expression

  → Functional Genomics

- **Variation in DNA sequence**

  → **Genetic Susceptibility to Sepsis**

  → **Pharmacogenomics**

WE ALL, AS HUMANS, SHARE

THE SAME BASIC GENES

BUT…











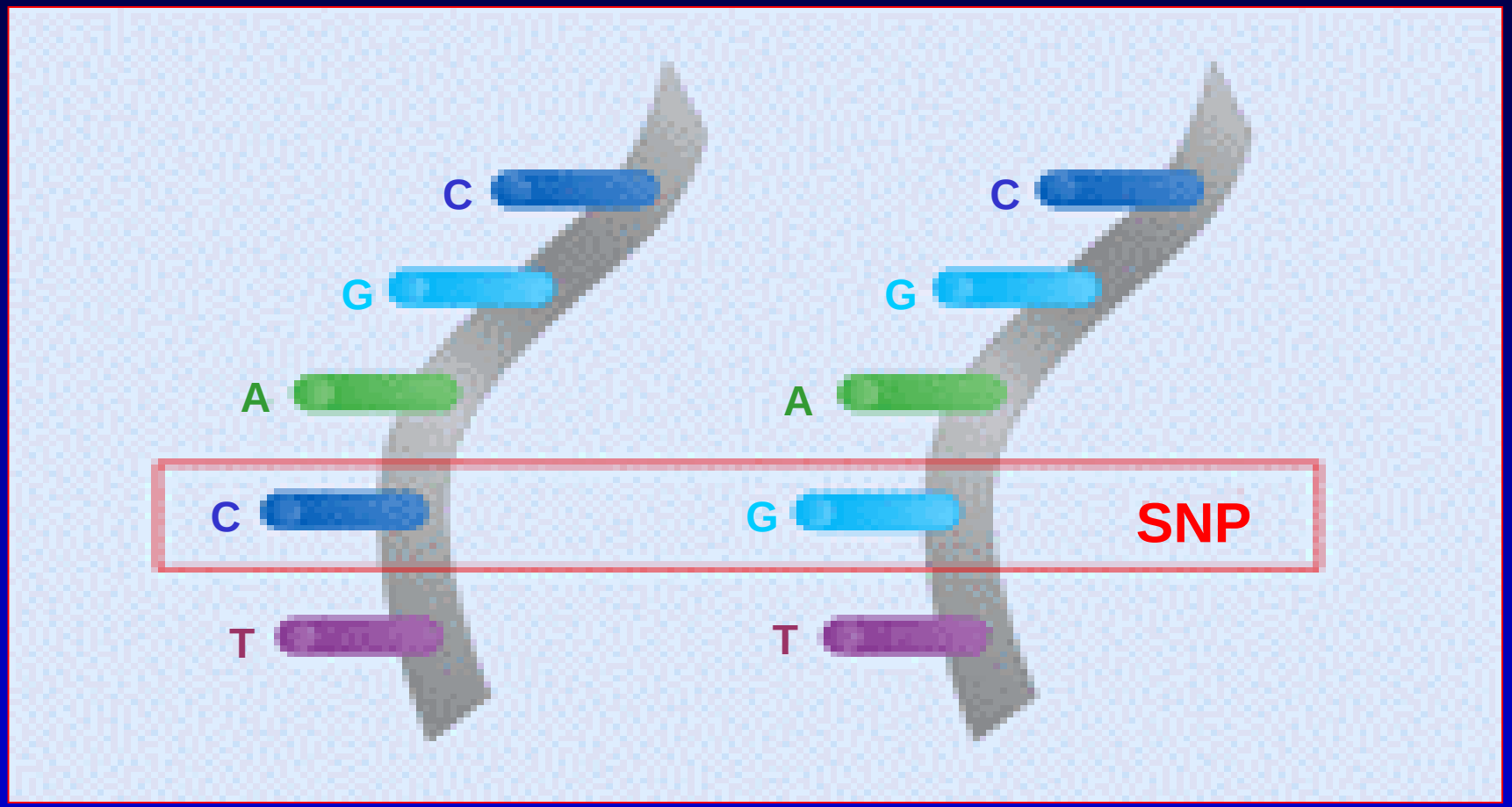

I apologize for this latest entry. I can't find a chimp making a face as dumb as this one. -Rich








Single Nucleotide Polymorphism



http://snp.cshl.org

DNA
EVIDENCE

Evidences for a genetic component to sepsis

Animal Studies

- Susceptibility/resistance to certain infection in mice

  C3H/HeN *vs.* C3H/HeJ

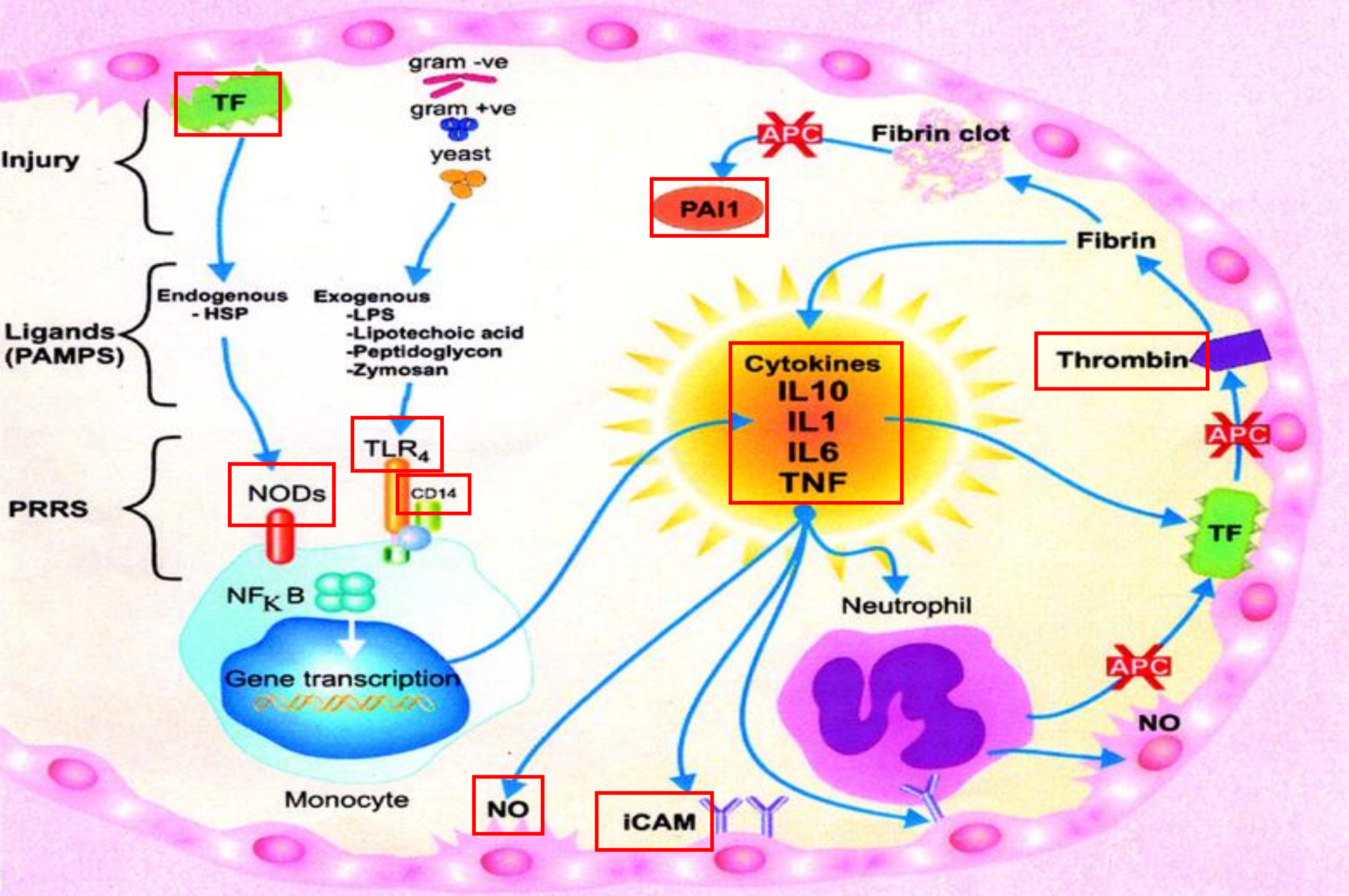
- Susceptibility/resistance phenotypes of knockout mice

Human Studies

- Ethnic differences

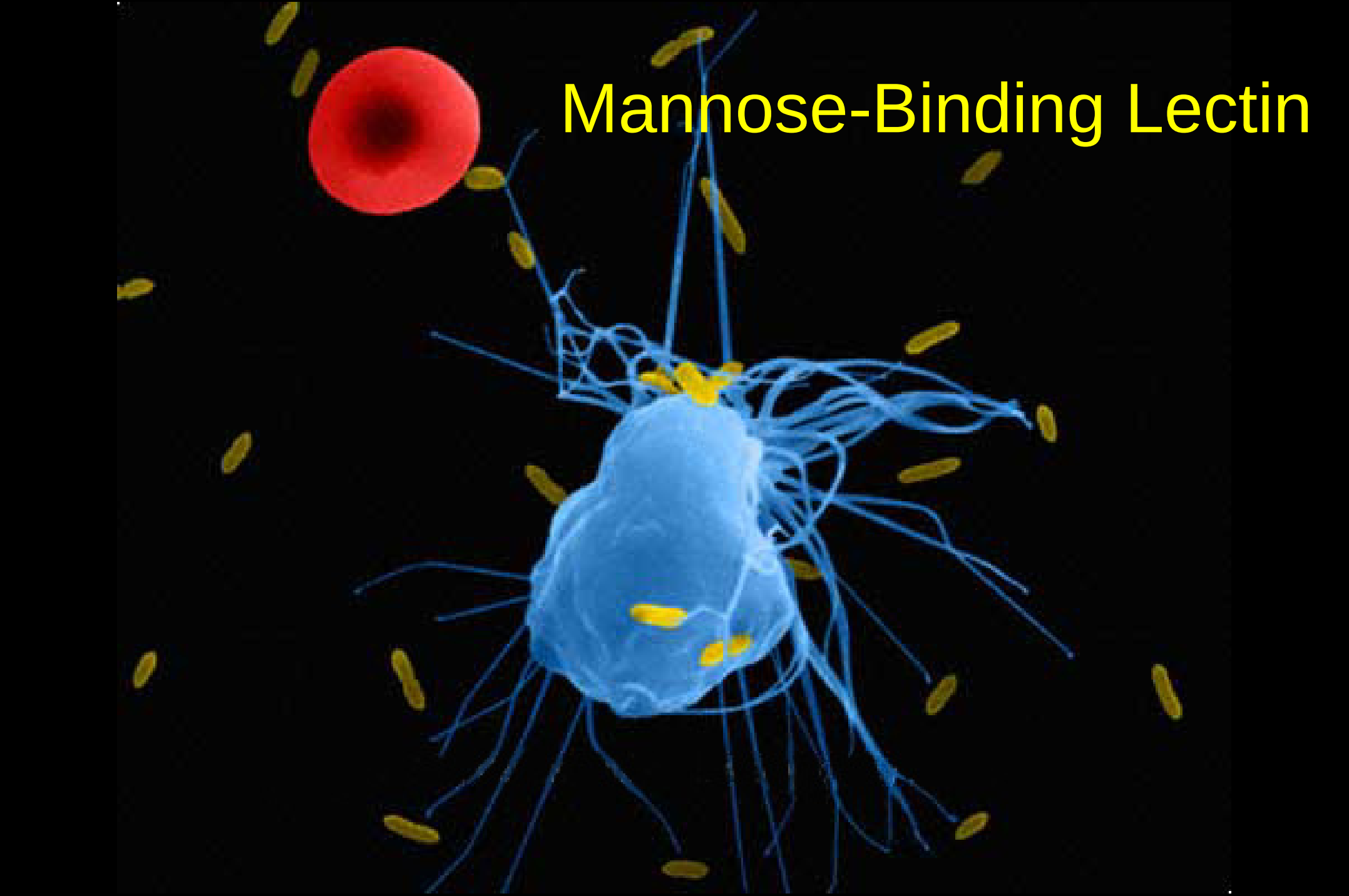
- Twin Studies

- Adoptee Studies

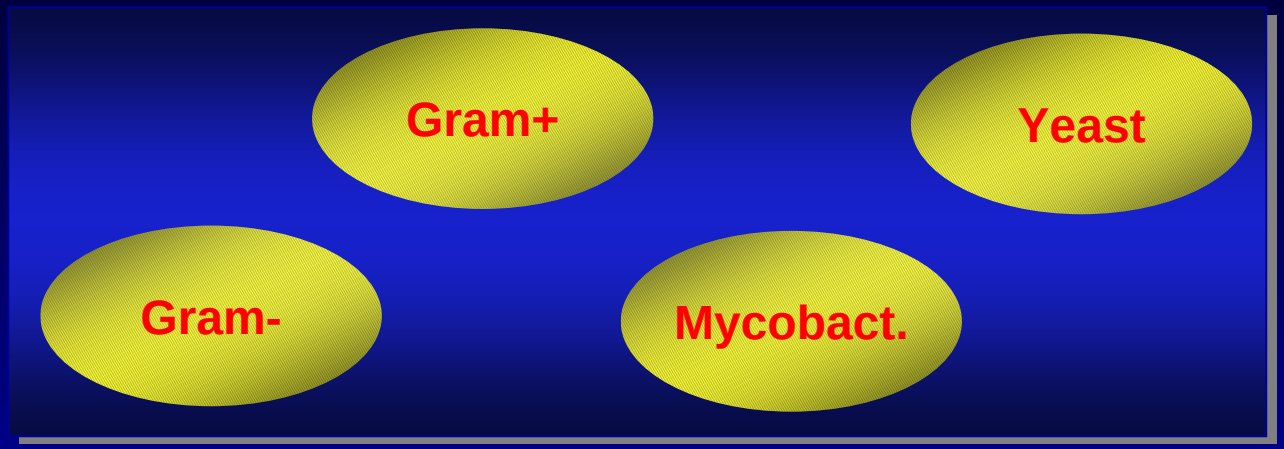

gram -ve
gram +ve
yeast
TF
Injury
Endogenous
- HSP
Exogenous
-LPS
-Lipotechoic acid
-Peptidoglycon
-Zymosan
Ligands
(PAMPS)
PRRS
NODs
TLR4
CD14
NFK B
Gene transcription
Monocyte
NO
iCAM
APC
Fibrin clot
PAI1
Fibrin
Cytokines
IL10
IL1
IL6
TNF
Thrombin
APC
TF
Neutrophil
APC
NO

Genetic Polymorphisms and Severe Sepsis

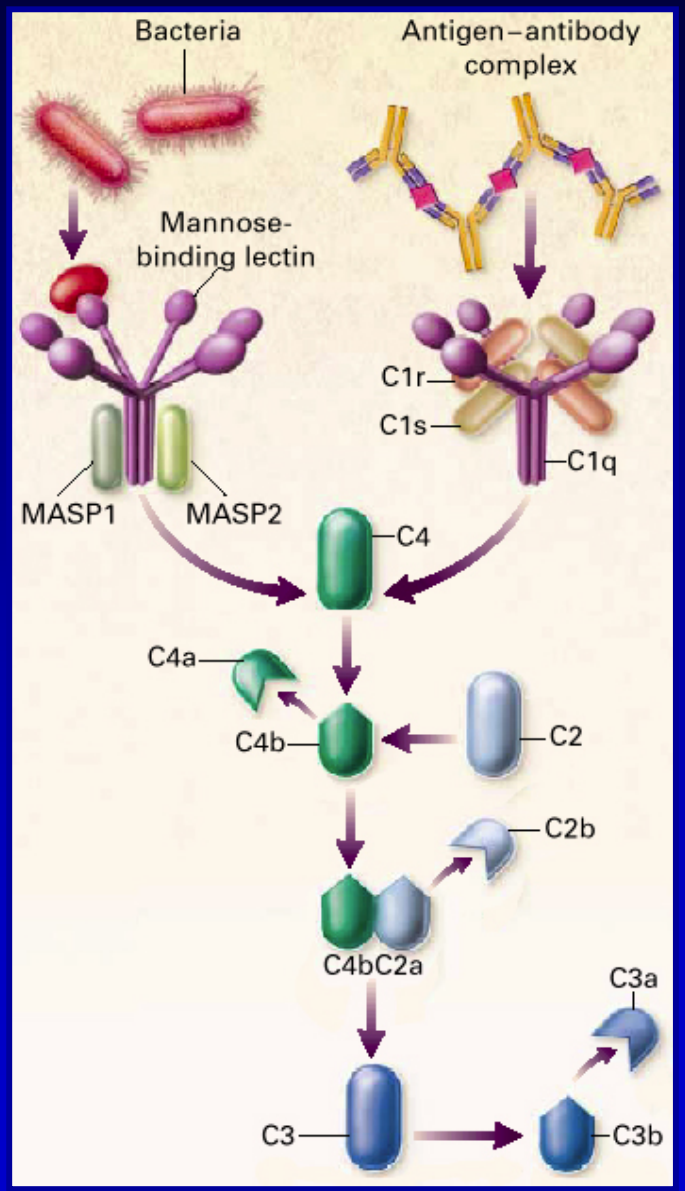
Gene	Susceptibility and/or Outcome
Mannose Binding Lectin	Meningococcemia, Pneumococcemia Severe sepsis
Toll-Like Receptor 4/2	Gram negative/positive Septic Shock
Toll-Like Receptor 5	Legionnaire's Disease
CD14	Septic Shock
FCγRII Receptor	Meningococcemia; Pneumococcemia
TNF locus	Meningococcemia Septic Shock; Cerebral Malaria
IL-18	Severe Sepsis
IL-10	Severe Sepsis, Meningococcemia
IL-6	Severe sepsis
IL-1 locus	Severe Sepsis
IL-4	Viral Pneumonia
PAI-1	Meningococcemia; Severe sepsis
FactorV Leiden	Meningococcemia; Severe sepsis


Mannose-Binding Lectin

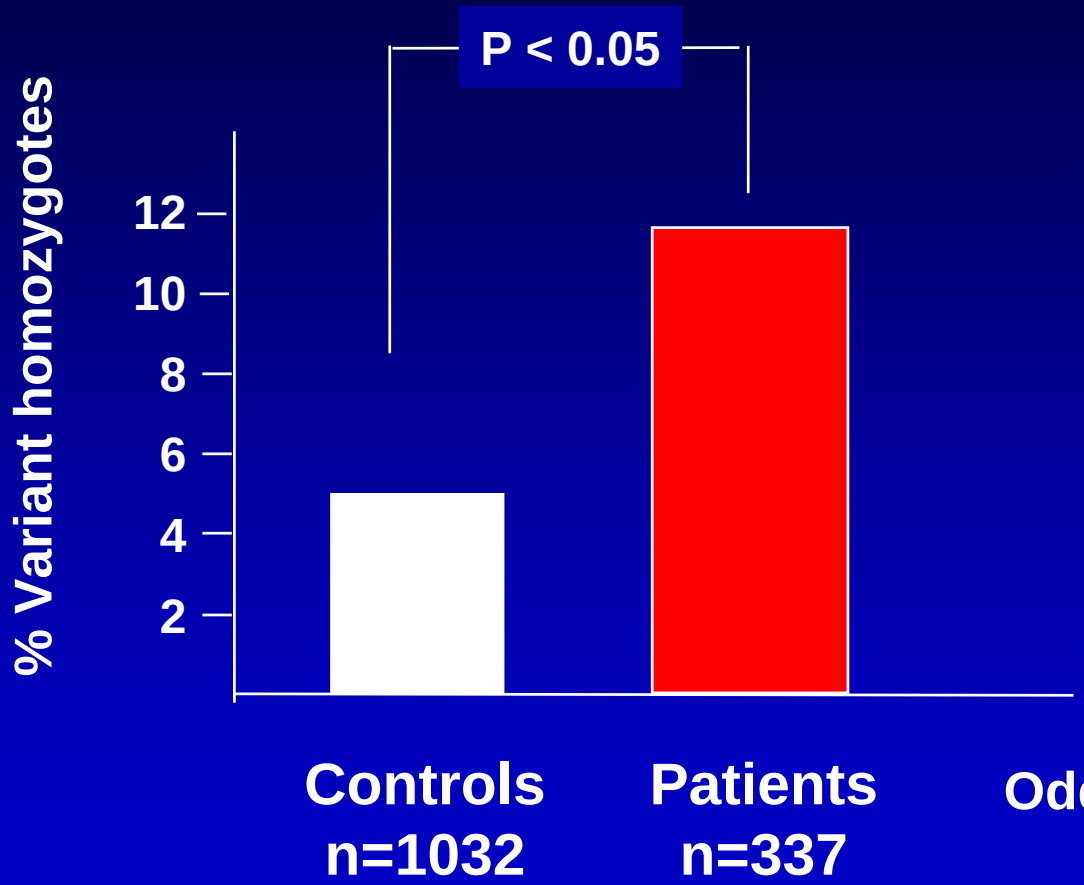
Mannose-Binding Lectin



- Collectin
- Structural homology with C1q
- Associated to 2 serine proteases
- Variability:
  - Point mutations codons 52, 54, 57
  - Polymorphisms in the promoter



MBL genotype and risk of invasive pneumococcal disease



Odds ratio 3.48 (1.51 – 8.01); p=0,003

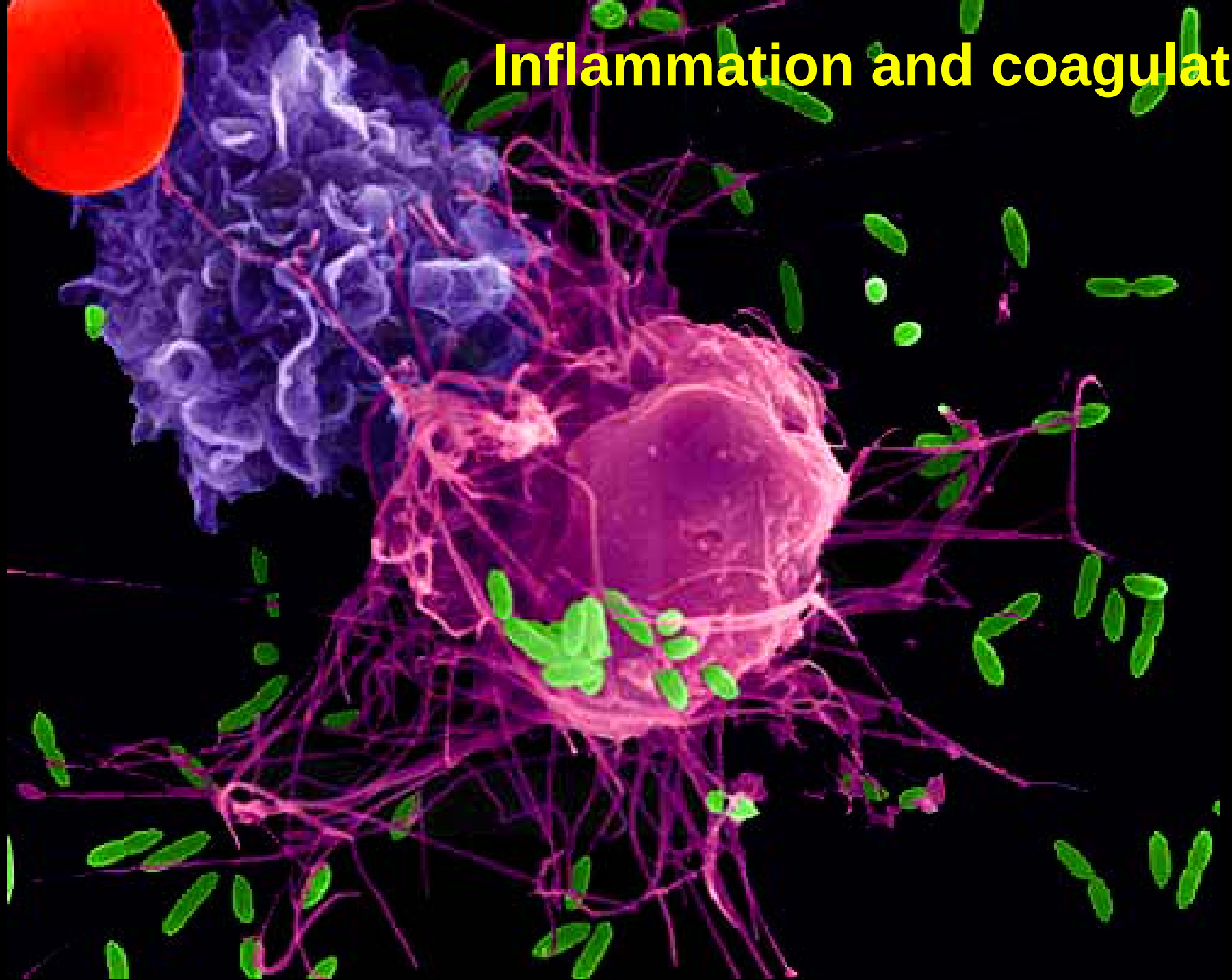
Roy et al. Lancet 2002; 359: 1569

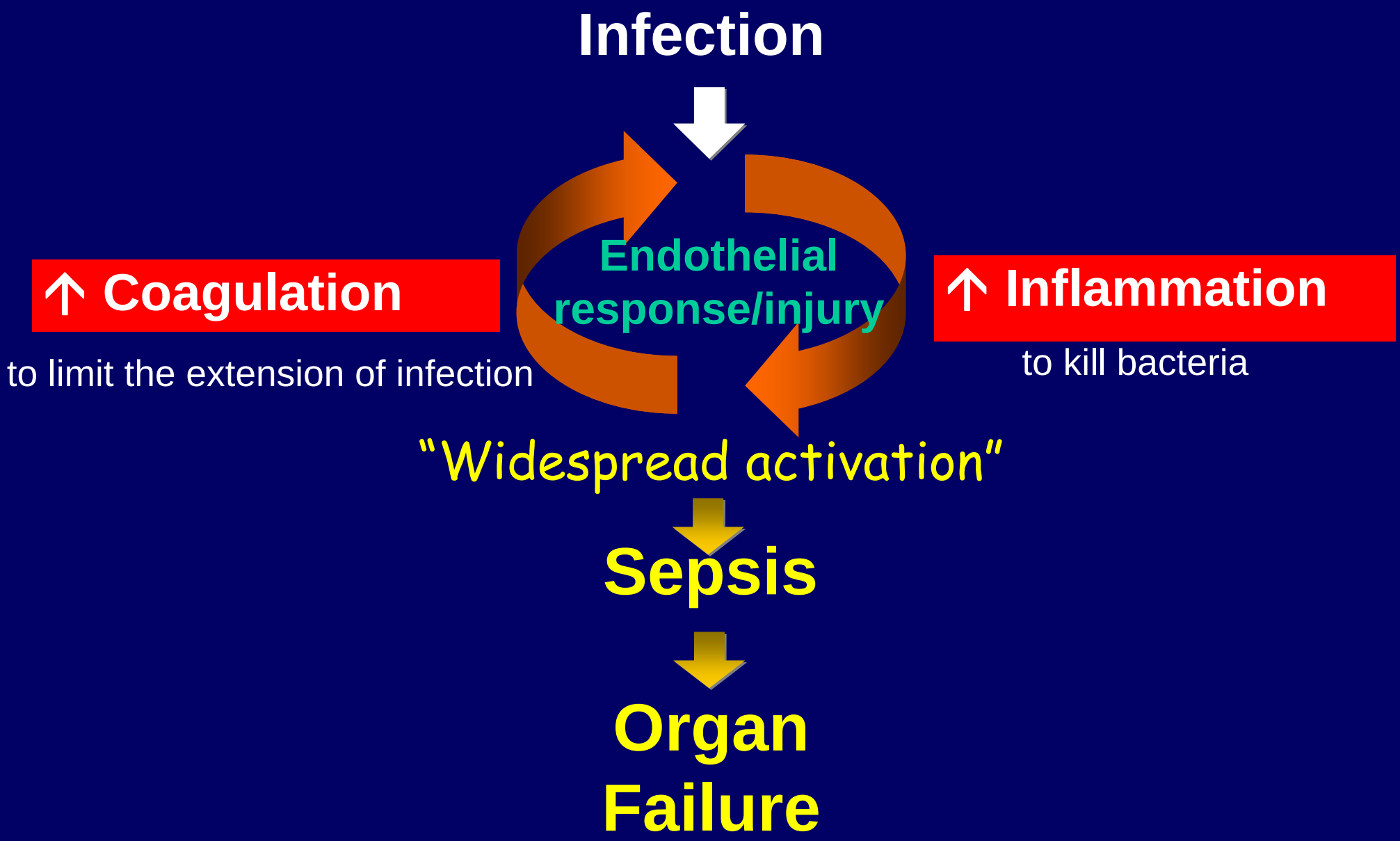
Clinical potential of mannose-binding lectin-replacement therapy

J.A. Summerfield[1]

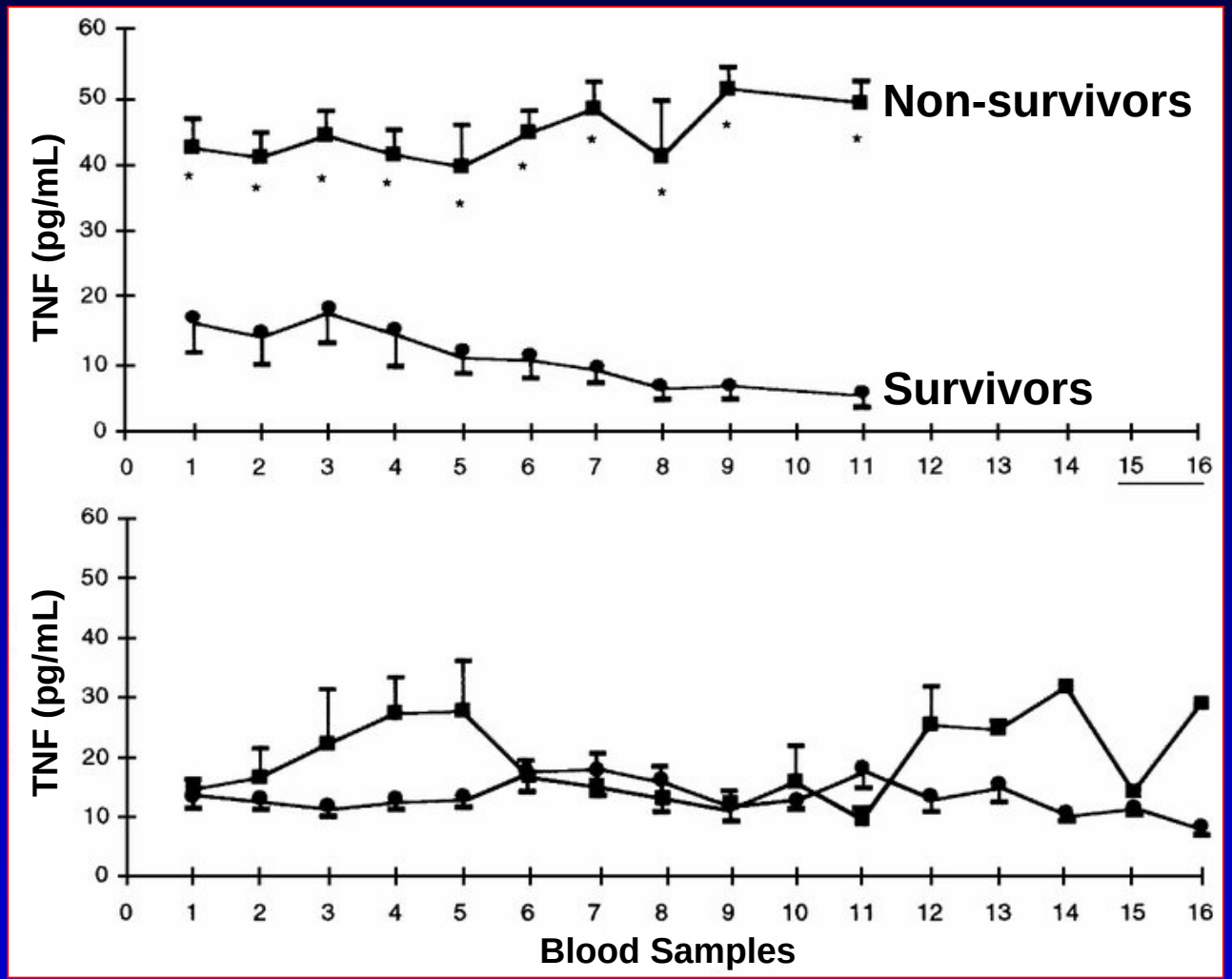
Division of Medicine, Faculty of Medicine, Imperial College London, St Mary's Campus, London W2 1NY, U.K.

Inflammation and coagulation




Infection
↑ Coagulation
to limit the extension of infection
Endothelial response/injury
↑ Inflammation
to kill bacteria
"Widespread activation"
Sepsis
Organ Failure

TNF plasma levels and mortality



Martin C et al. Crit Care Med 1997;25:1813

Clinical Trails for Neutralization of TNF

Agent	Study	Control mortality	Anti-TNF mortlaity	Benefit
1. Monoclonal antibodies				
CB006	Fisher et al 1993	6/19 (32%)	27/61 (44%)	- 12%
CDP571	Dhainaut 1995	6/10 (60%)	20/32 (63%)	- 3%
Bay 1351	Abraham 1995	108/326 (33%)	196/645 (30%)	+ 3%
Bay 1351	Cohen 1996	66/167 (40%)	144/386 (37%)	+ 3%
Bay 1351	Abraham 1998	398/930 (43%)	382/948 (40%)	+3%
MAK195 (Afelimomab)	Reinhart 1996	12/29 (41%)	44/93 (47%)	- 4%
MAK195 (Afelimomab)	Reinhart 2000	128/222 (58%)	121/224 (54%)	+ 4%
MAK195 (Afelimomab)	Panacek 2000*	243/510 (48%)	213/488 (44%)	+ 4% (6,9%)**
2. Soluble receptors				
P 75 fusion protein	Fisher 1996	10/33 (30%)	49/108 (45%)	- 15%
P 55 fusion protein	Abraham 1997	54/140 (39%)	136/358 (38%)	+ 1%
P 55 fusion protein	Abraham 1998	192/680 (28%)	177/682 (27%)	+ 1%

* pat. with IL-6 > 1000; **risk adjusted mortality

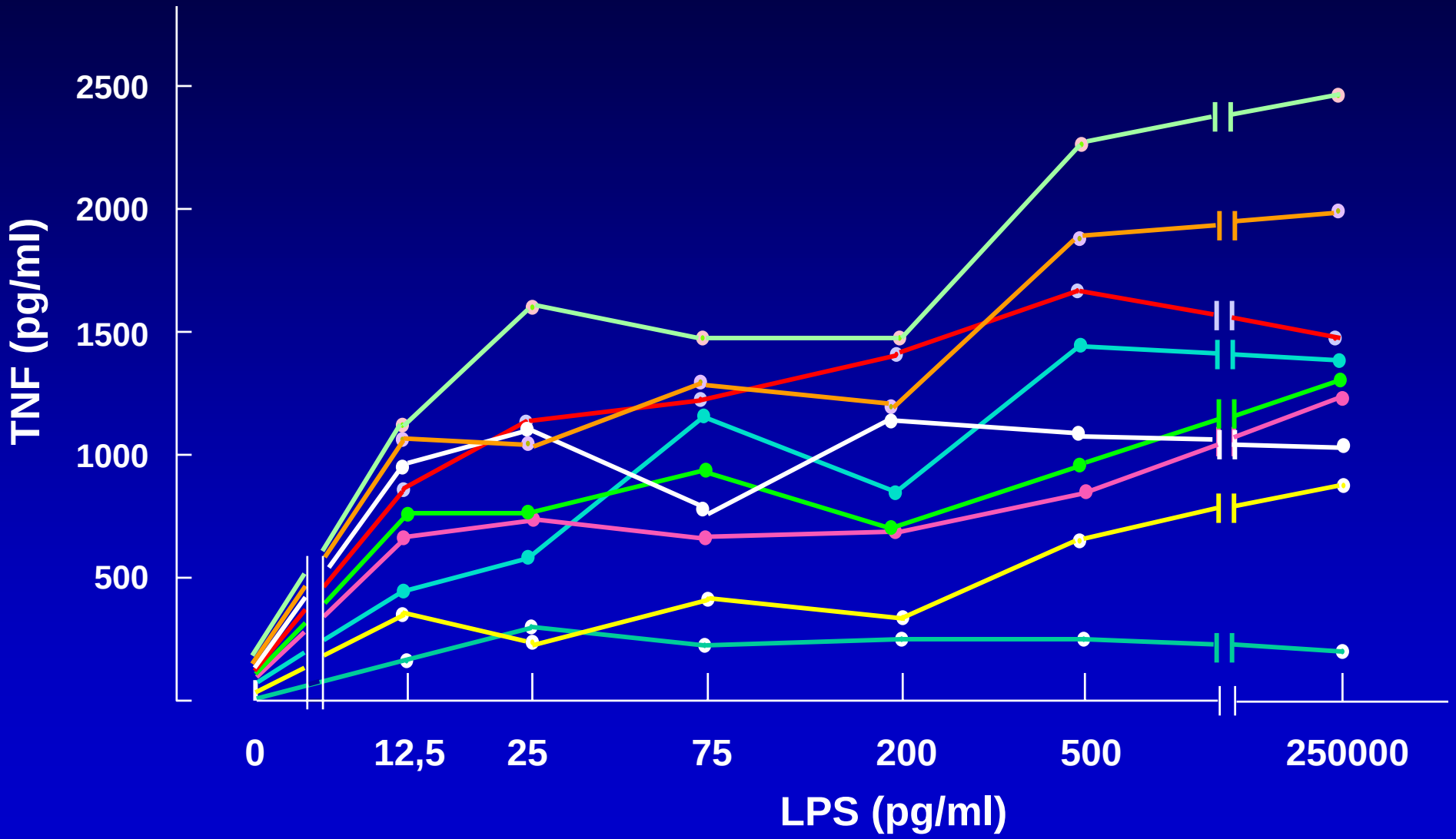
Reinhart K. Crit Care Med 2001;29:S121

Possible beneficial effects of anti-TNF strategies in sepsis

	Clinical effects	P-value	Trials/Authors
Patients with TNF > 50 pg/ml	Trend in mortality reduction	(n.s.)	CB 006 Fisher 1993
Patients with shock	More rapid reversal of shock	P < 0.05	Bay 1351 Abraham 1996
	More rapid reversal of shock	P < 0.007	Bay 1351 Cohen 1996
	Delay in onset of organ failure	P < 0.03	Bay 1351 Cohen 1996
Patients with early shock and severe sepsis	Decreased incidence of new organ dysfunctions	P < 0.0001	Ro p. 55-IgG Pittet 1999
	More organ failure free days	n.s.	
	Shorter time on the ventilator (3.2 days)	n.s.	
	Shorter ICU stay (4.1 days)	n.s.	
Patients with IL-6 > 1000	Trend in mortality reduction	n.s	MAK 195F, Reinhart 1996
	Trend in mortality reduction	n.s	MAK 195F, Reinhart 2001

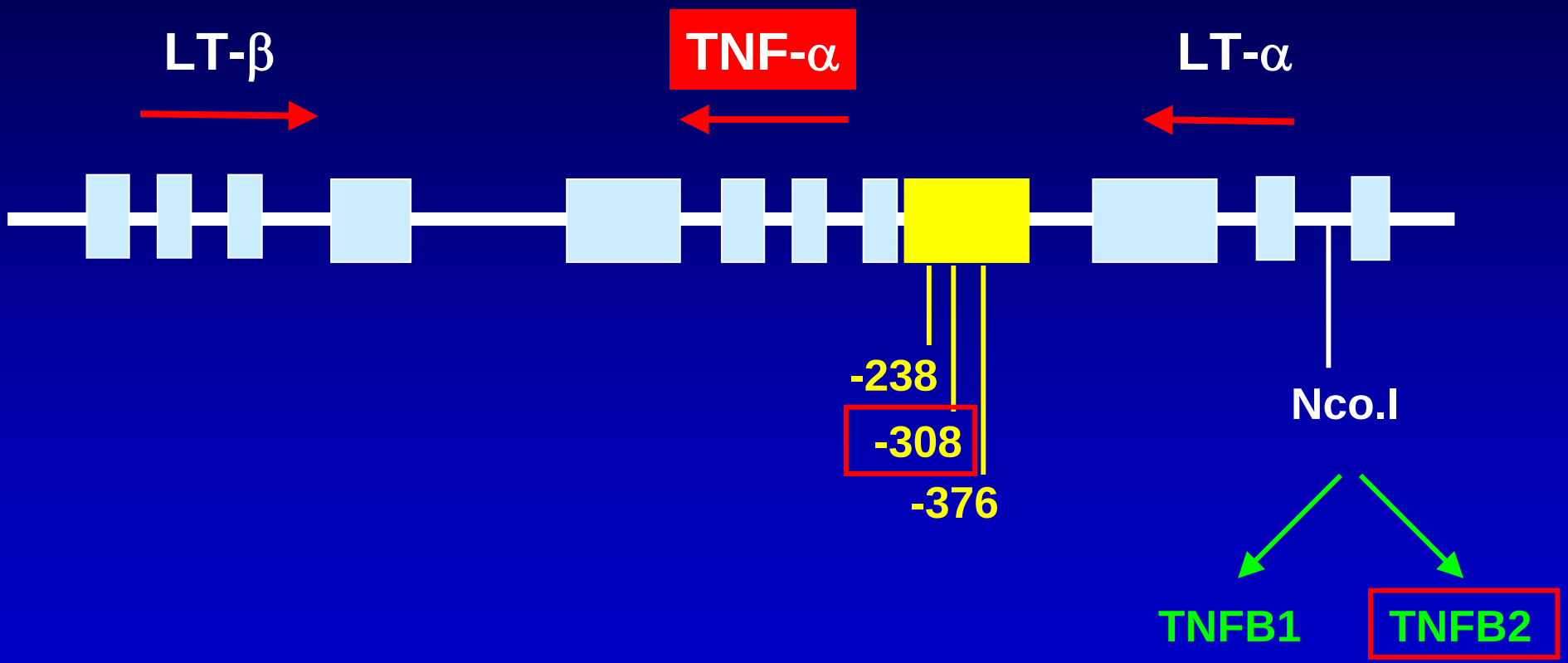
Reinhart K. Crit Care Med 2001;29:S121

Interindividual differences in TNF-α secretion

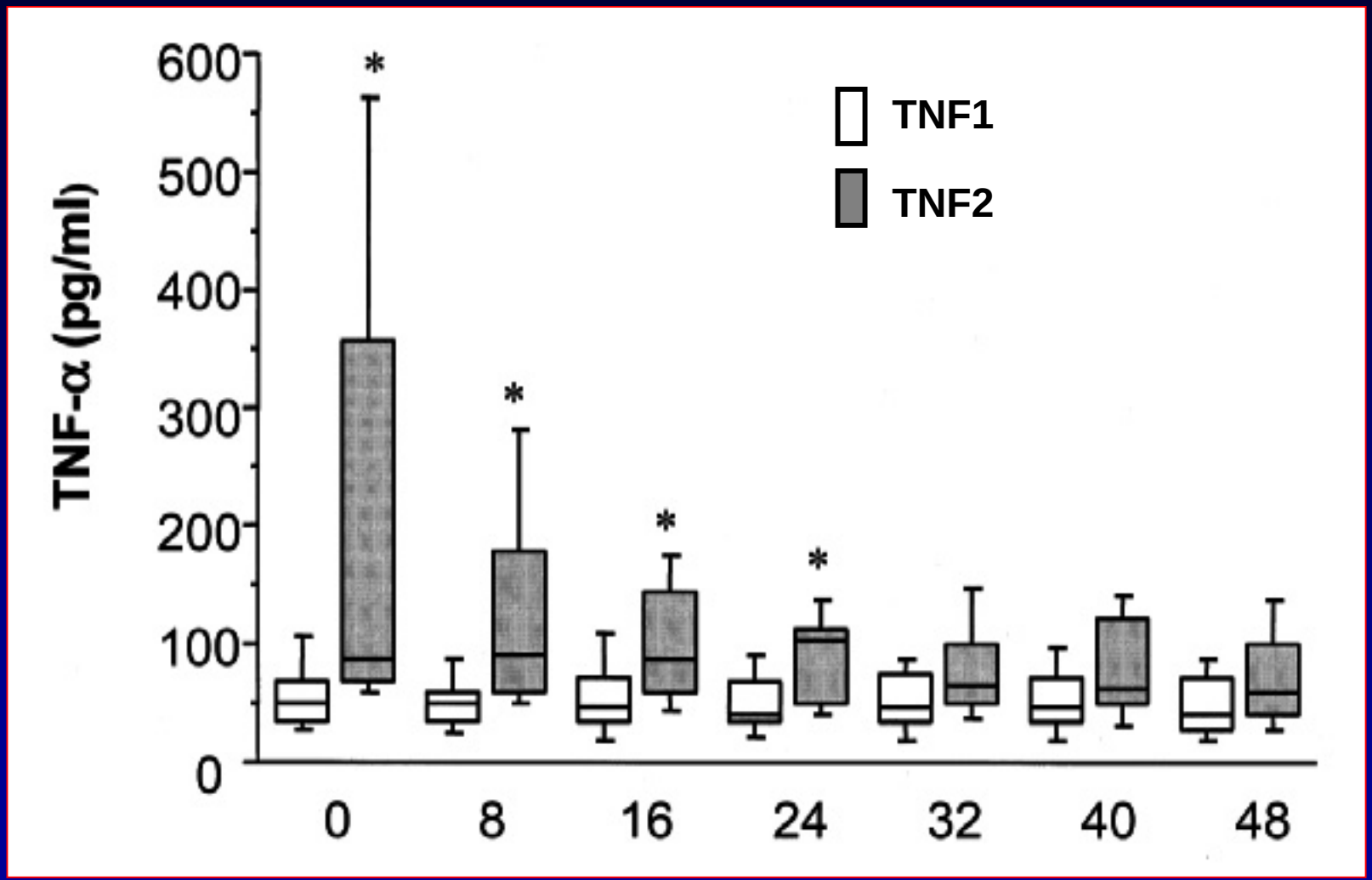


Molvig, 1988

TNF locus

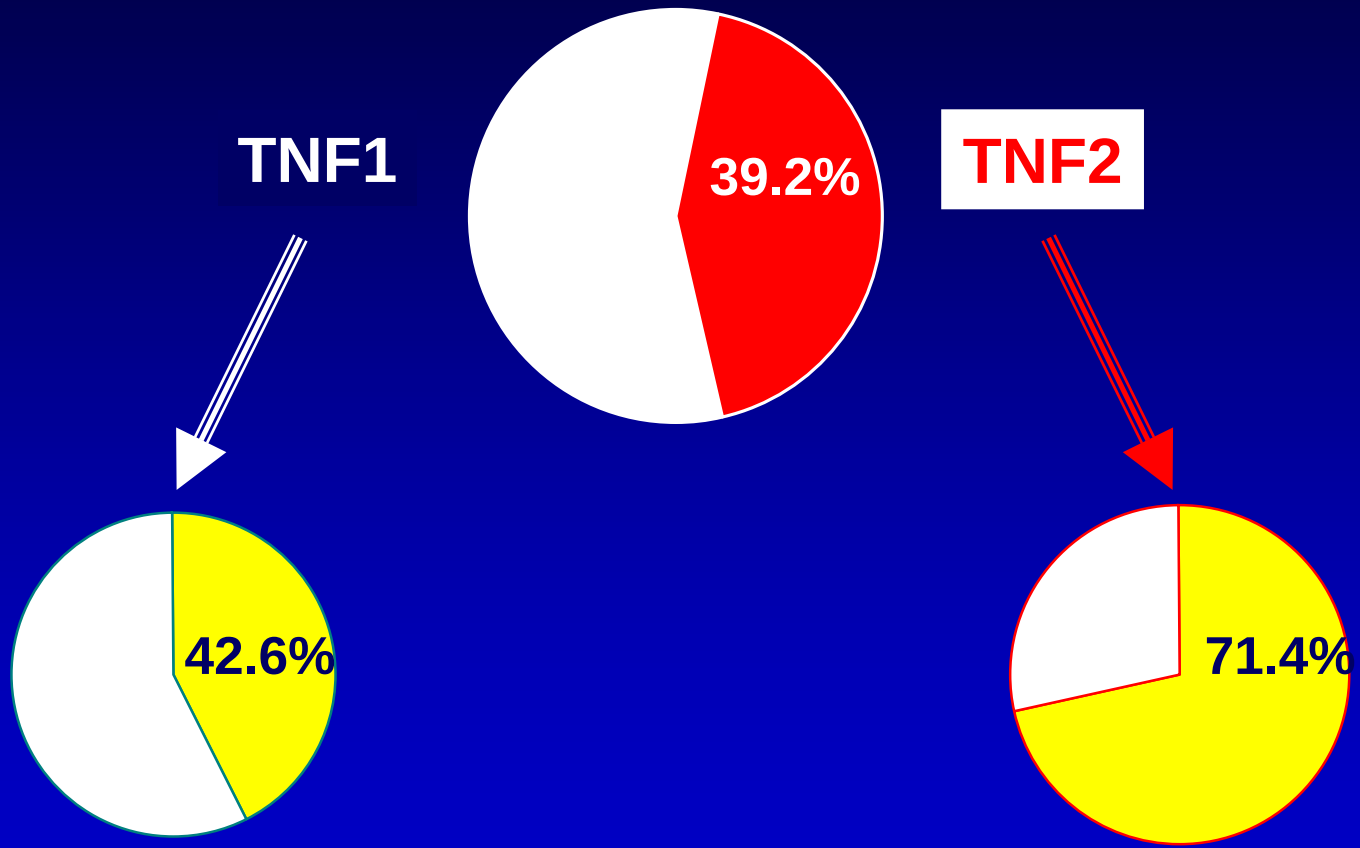


Association of TNF2 with TNF levels in Septic Shock



Appoloni O. Am J Med 2001; 110:486

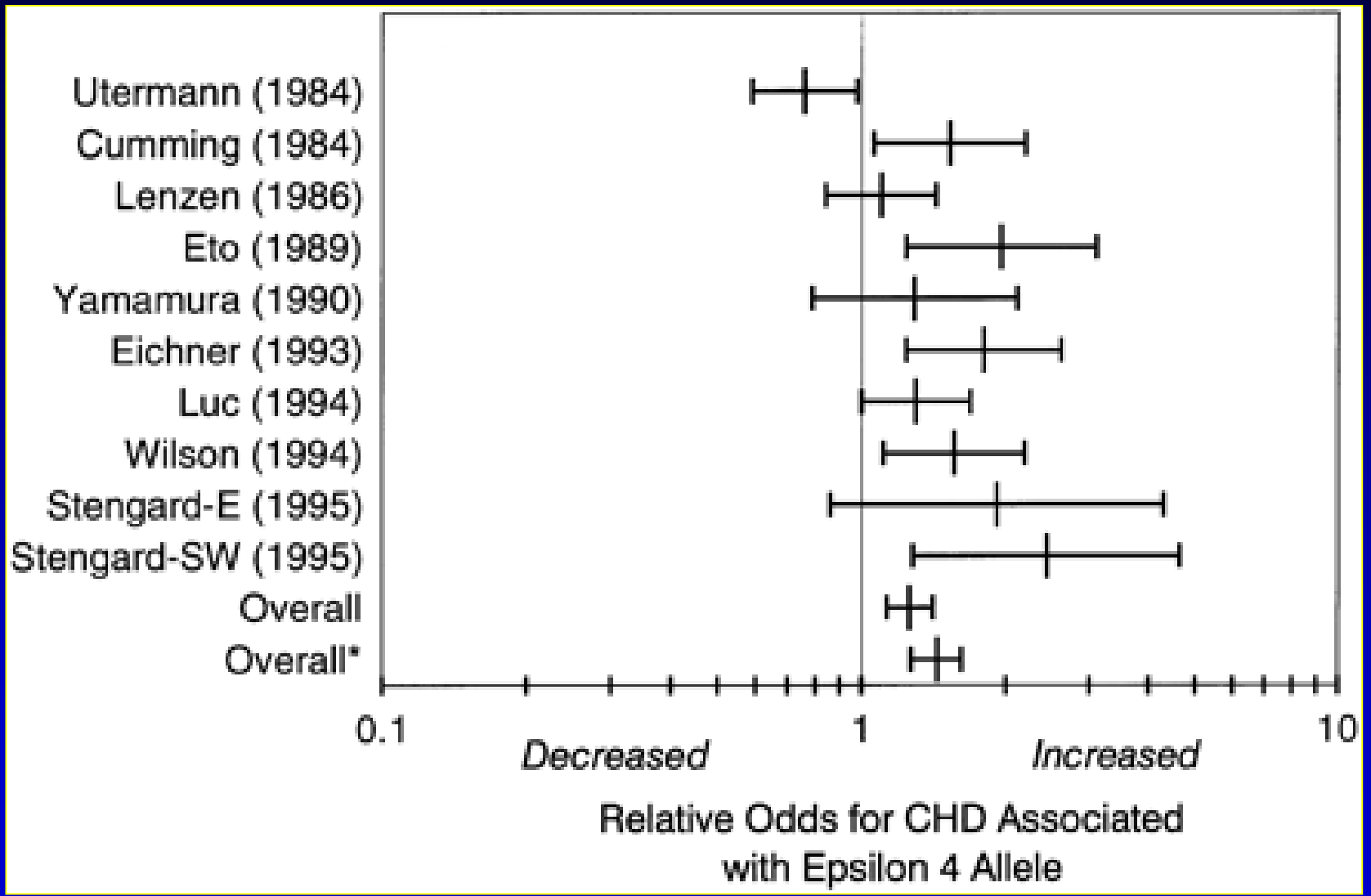
TNF2 Polymorphism and Septic Shock Outcome



JAMA 1999;282:561

Genetics Markers For Treatment Design

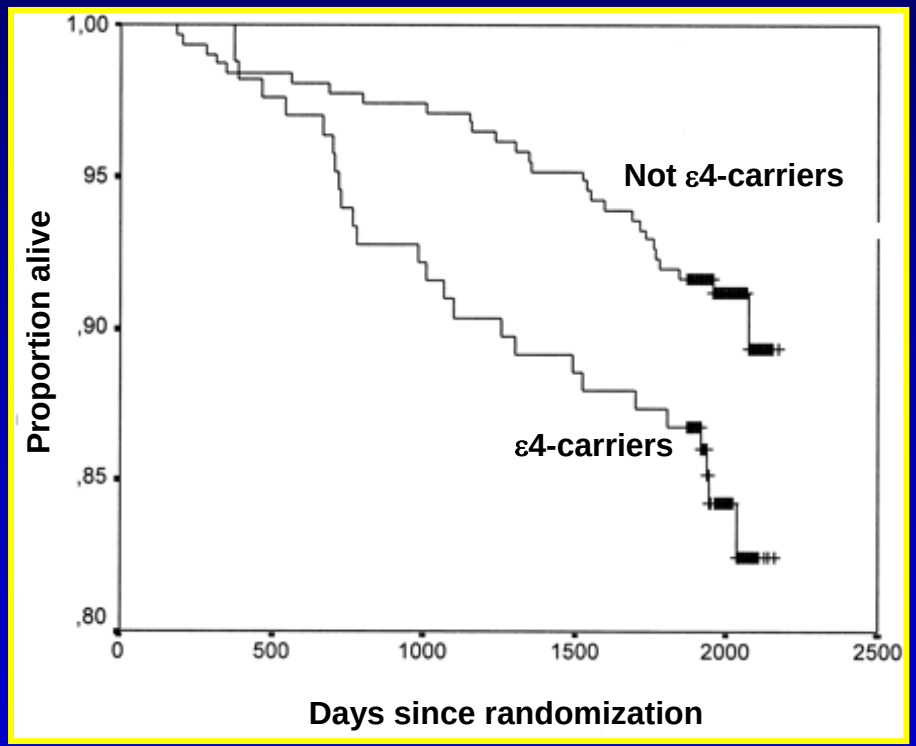
E4 allele of *apolipoprotein E* gene and coronary heart disease risk



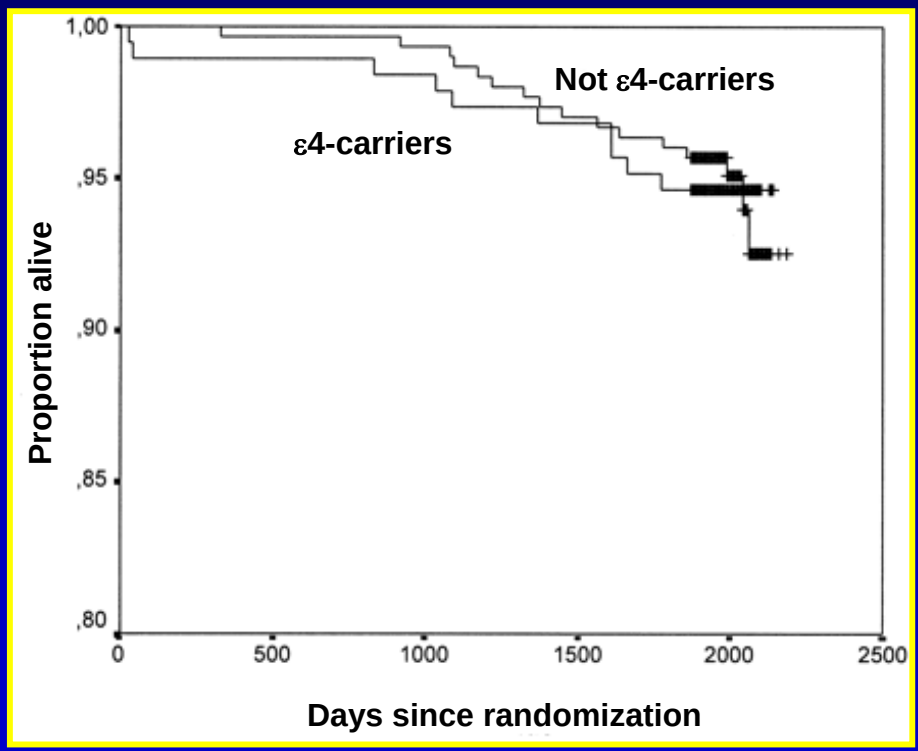
Wilson PWF. Arterioscler Thromb Vasc Biol 1996;16:1250

ε4 allele of *apolipoprotein E* gene and Simvastatin trial

Placebo Group

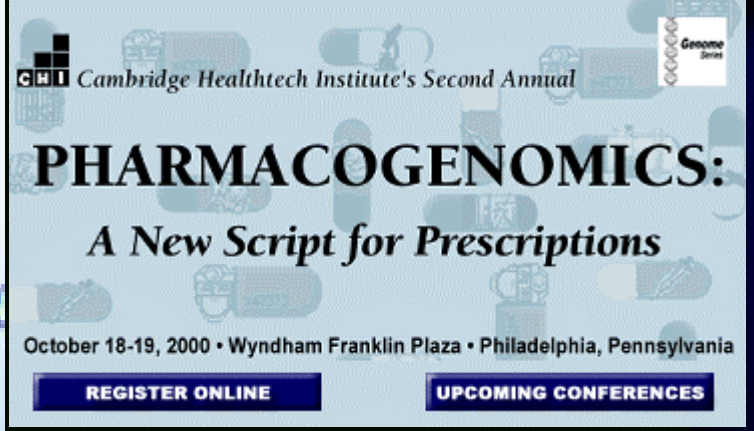


Simvastatin Group



Gerdes LU. Circulation 2000;101:1366


The Pharmacogenomics Journal


Cambridge Healthtech Institute's Second Annual
PHARMACOGENOMICS:
A New Script for Prescriptions
October 18-19, 2000 • Wyndham Franklin Plaza • Philadelphia, Pennsylvania
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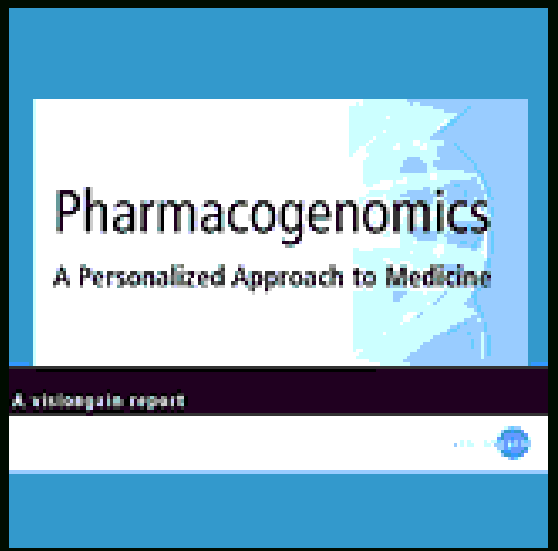

Pharmacogenomics
A Personalized Approach to Medicine
A visiongain report


PharmacoGenomics
online.com


PHARMACOGENOMICS
the Legal, Ethical & Clinical Challenges
University of Minnesota

Drug response variability

- Drug-drug interactions
- Patient ’s age
- Renal and liver functions
- Lifestyle variables (alcohol consumption,…)
- Genetics factors

Adverse Drug Reactions

- 2 million people hospitalized per year for ADR (6.7% hospitalization)
- 4th-6th leading cause of death among adults in the USA
- 12 billions US $ / year

Lazarou J. JAMA 1998;279:1200

- 7% top-selling US drugs are metabolized by enzymes with genetic variability
- 22% of US drug sales are metabolized by enzymes with genetic variability
- 59% od drug-related ADR are metabolized by enzymes with genetic variability

Phillips KA. JAMA 2001;286-2270

Interindividual variability in dose requirement

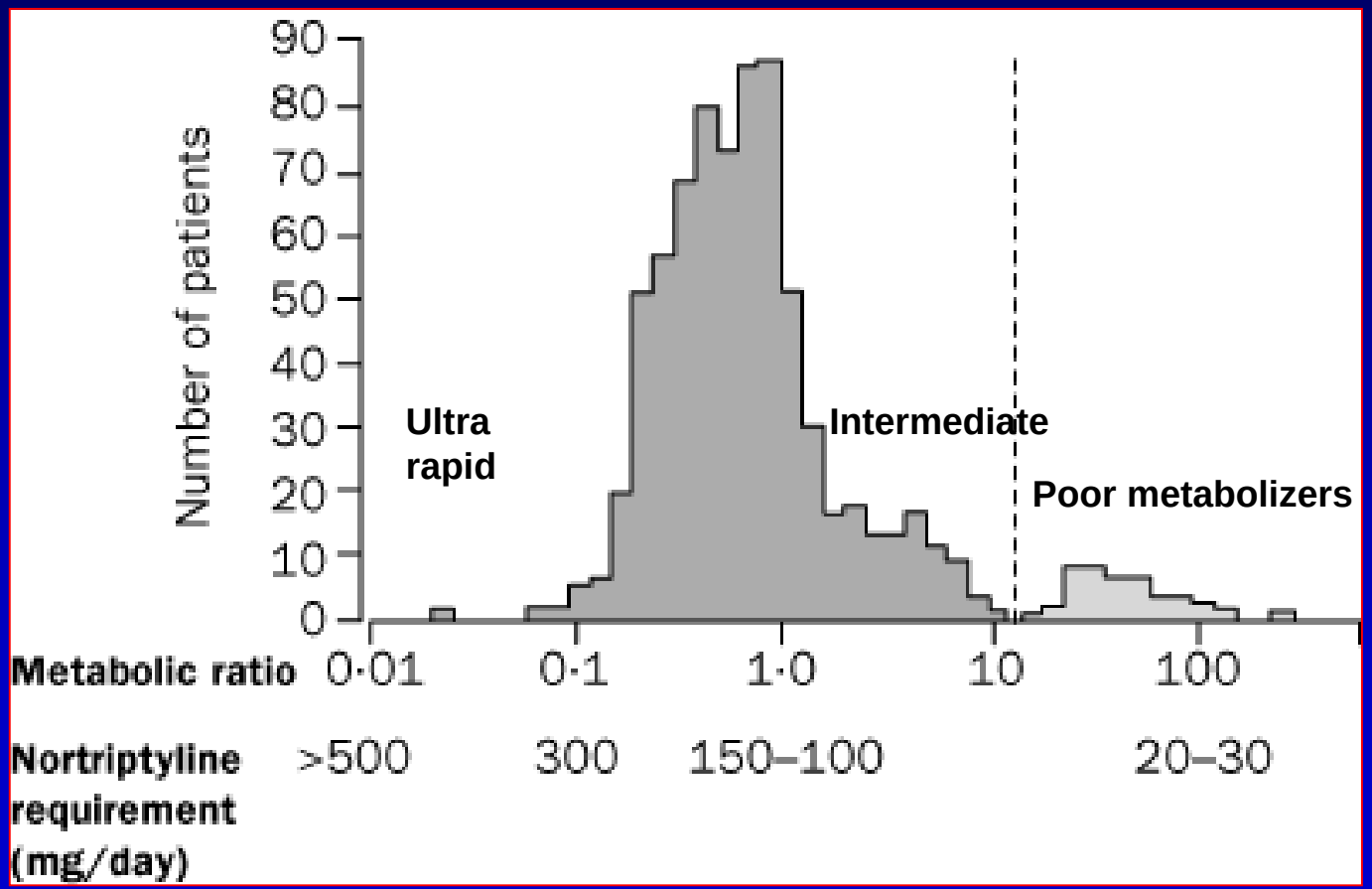
Genotype

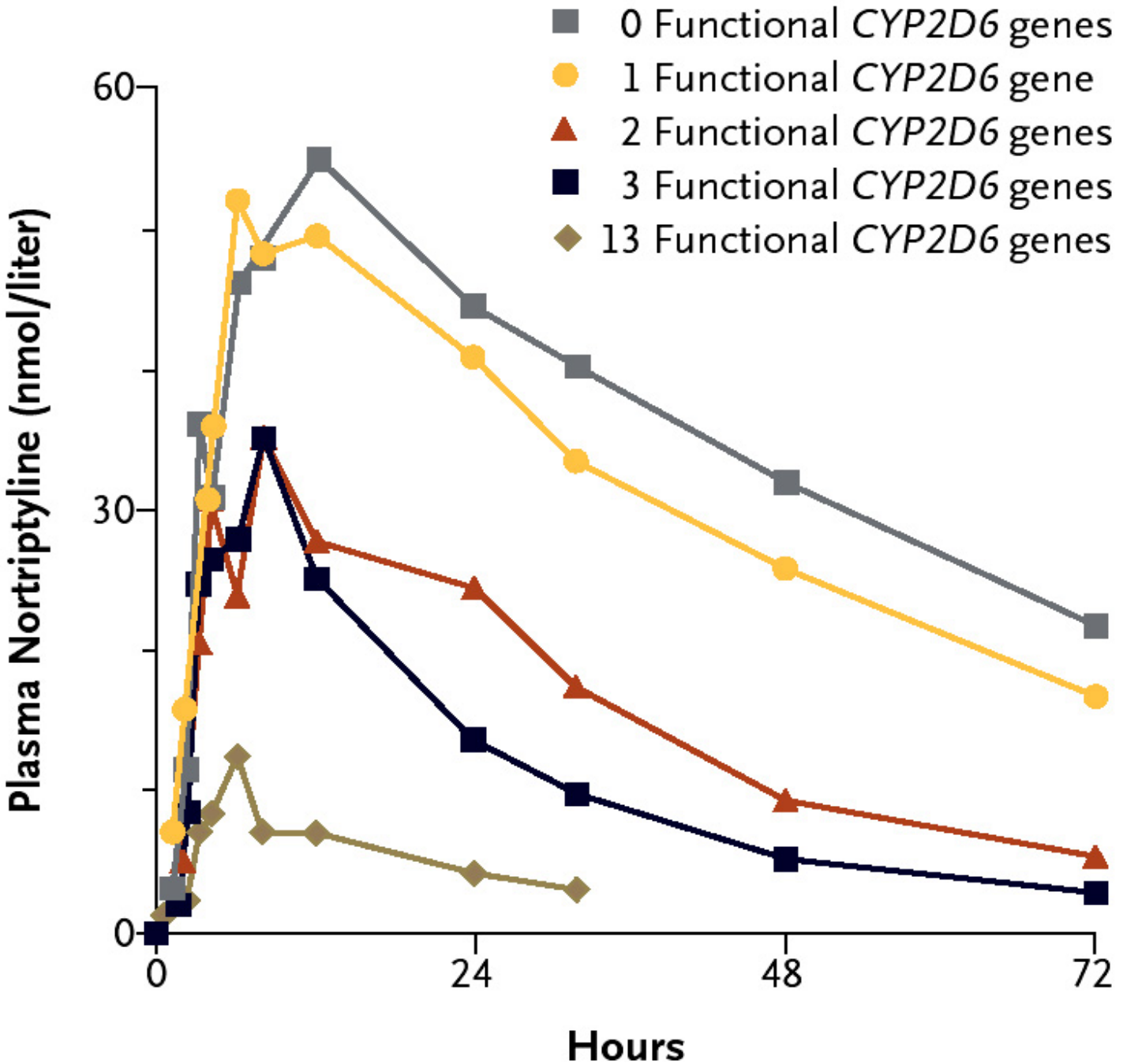
↓

Expression CYP2D6

↓

Phenotype




0 Functional CYP2D6 genes
1 Functional CYP2D6 gene
2 Functional CYP2D6 genes
3 Functional CYP2D6 genes
13 Functional CYP2D6 genes
Plasma Nortriptyline (nmol/liter)
60
30
0
0
24
48
72
Hours

Genetics and Therapeutic Concepts in Sepsis

- More powerful medicines
- Better, safer drugs the first time
- More accurate methods of determining appropriate drug dosages
- Advance screening for disease
- Drug discovery
- Decrease in the overall cost of health care

Conclusions

GENOMICS ⇒ **Reproductive Medicine**

- **Specific diagnosis**
- **Target therapy**

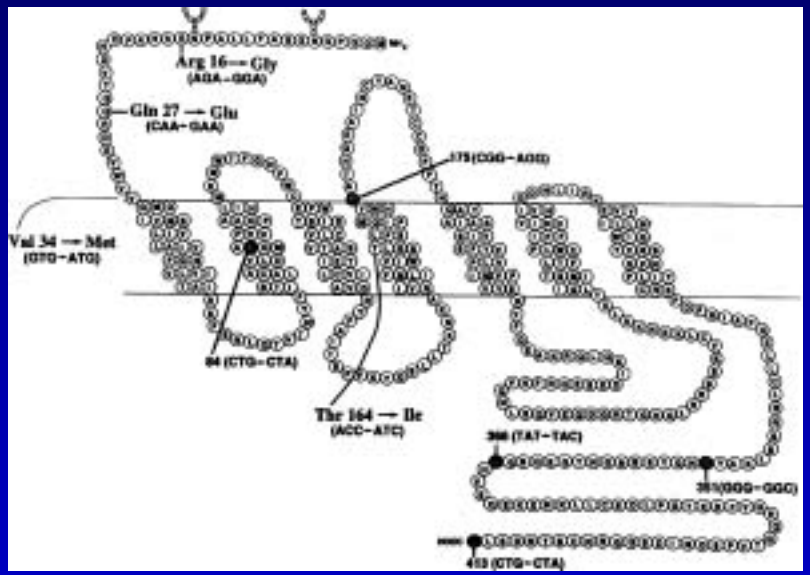
⇒ **Genome screens**

- **Susceptibility to disease**
- **Prediction of the drug response**

Genomic-based individualization of treatment

Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial

Elliot Israel, Vernon M Chinchilli, Jean G Ford, Homer A Boushey, Reuben Cherniack, Timothy J Craig, Aaron Deykin, Joanne K Fagan, John V Fahy, *Lancet* 2004; 364: 1505–12

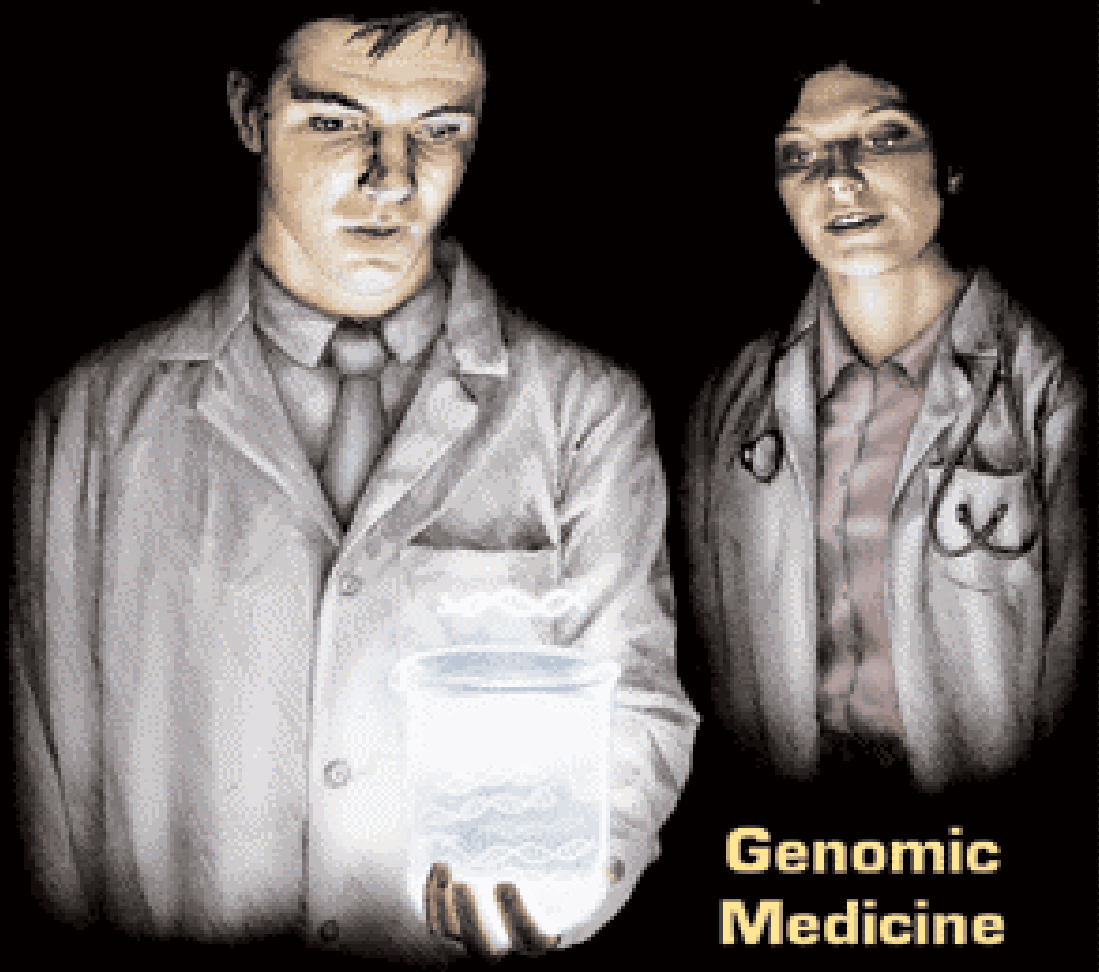


Interpretation Genotype at the 16th aminoacid residue of the β_2-adrenergic receptor affects the long-term response to albuterol use. Bronchodilator treatments avoiding albuterol may be appropriate for patients with the Arg/Arg genotype.

24 October 2003

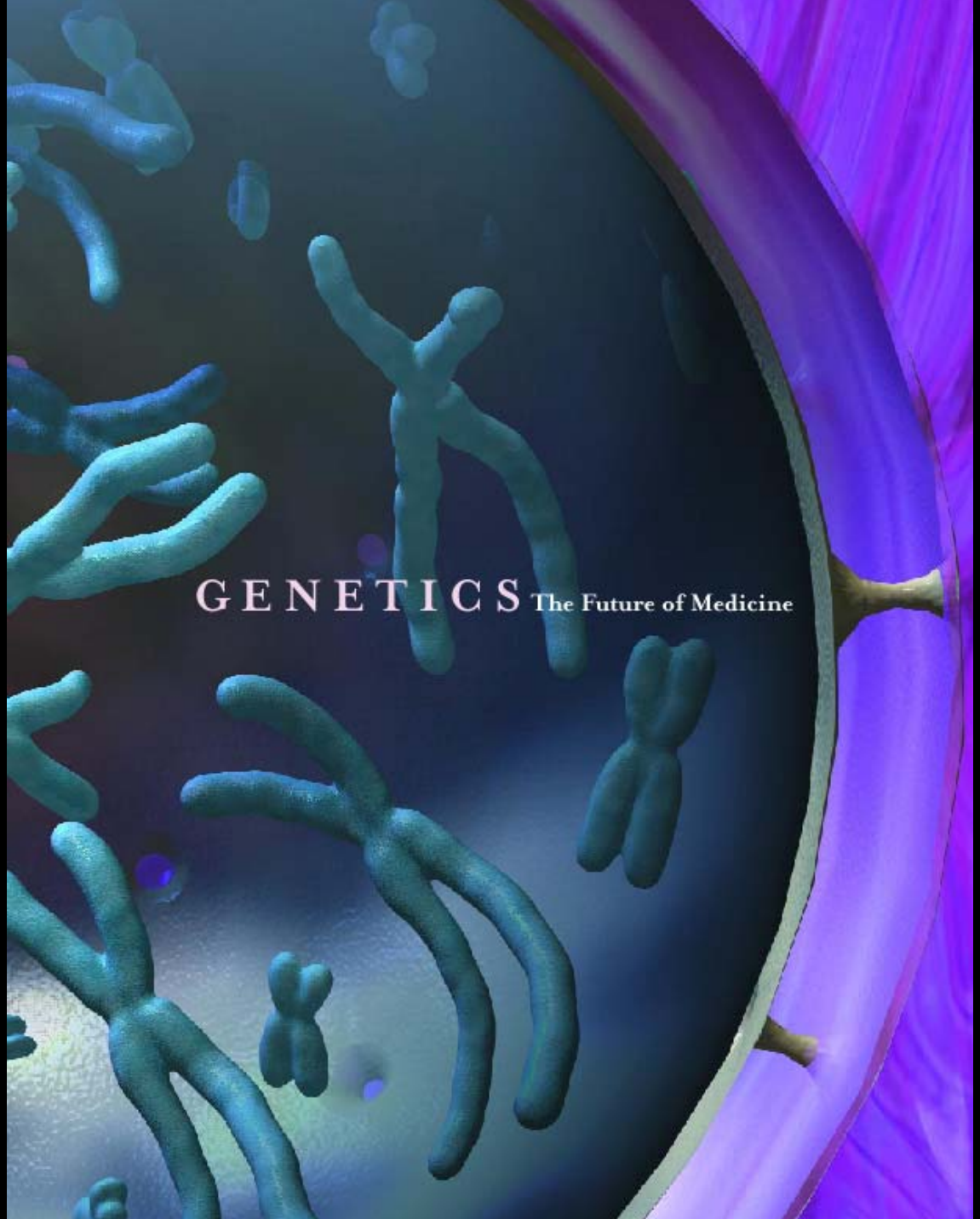
Science

Vol. 302 No. 5645
Pages 517–728 $10



Genomic Medicine

American Association for the Advancement of Science


GENETICS The Future of Medicine