Pharmacokinetic and Pharmacodynamic Considerations in the Development of Macromolecules

April 10, 2008

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OUTLINE OF LECTURE TOPICS

* Macromolecules
* Interspecies Scaling
* Pharmacokinetic Characteristics
  - Scientific Issues
* Pharmacodynamics
* Monoclonal Antibodies

REPRESENTATIVE MARKETED MACROMOLECULES

Macromolecule	Trade Name
Erythropoietin	Epogen (Amgen)
Growth Hormone	Nutropin (Genentech)
G-CSF	Neupogen (Amgen)
IL-2	Proleukin (Chiron)
IL-11	Neumega (GI)
Factor IX	BeneFIX (GI)
rt-PA	Alteplase (Genentech)

APPROVED MONOCLONAL ANTIBODIES

Name	Approval	Indication
Avastin **Bevacizumab**	Feb, 2004	First line (with 5-FU) in metastatic colon CA
Erbitux **Cefuximab**	Feb, 2004	Alone or in combination in metastatic colon CA
Raptiva **Efalizumab**	Oct, 2003	Moderate to severe psoriasis
Xolair **Omalizumab**	June, 2003	Asthma
Humira **Adalimumab**	Dec, 2002	Prophylaxis of acute organ rejection
Campath **Alemtuzumab**	May, 2001	Second line treatment of β-cell CLL in patients

ASSAYS FOR MACROMOLECULES

* Immunoassays
  - ELISA (Enzyme-Linked Immuno-sorbent Assay)
  - RIA (Radioimmunoassay)
  - IRMA (Immunoradiometric Assay)
  - RRA (Radioreceptor Assay)

INTERSPECIES SCALING OF MACROMOLECULES

Factors to Consider

* Species specificity
* Glycosylation and sialation
* Binding proteins
* Size, shape and charge
* Relative abundance of tissue receptors

ALLOMETRIC EQUATIONS FOR SOME MACROMOLECULES

Macromolecule	Allometric Equations V_1	CL
Factor IX	$87\ W^{1.26}$	$14\ W^{0.68}$
Factor VIII	$44\ W^{1.04}$	$10\ W^{0.69}$
IL-12	$65\ W^{0.85}$	$8\ W^{0.62}$
GH	$68\ W^{0.83}$	$7\ W^{0.71}$
rt-PA	$91\ W^{0.93}$	$17\ W^{0.84}$

INITIAL COMPARTMENT VOLUME PREDICTED BY ALLOMETRIC SCALING COMPARED WITH OBSERVED V_1

Macromolecule	Human Parameter: V_1 Predicted (mL)	V_1 Observed (mL)
FIX	18,380	10,150
Factor VIII	3,617	3,030
IL-12	2,406	3,360
GH	2,243	2,432
rt-PA	5,814	4,450

ELIMINATION CLEARANCE PREDICTED BY ALLOMETRIC SCALING COMPARED WITH OBSERVED CL

Macromolecule	Human Parameter: Cl Predicted (mL/hr)	Cl Observed (mL/hr)
FIX	248	434
Factor VIII	195	174
IL-12	113	406
GH	148	175
rt-PA	646	620

ALLOMETRIC EQUATIONS for EGF Mab PK PARAMETERS

Parameter (Y)	Coefficient (*a*)	Exponent (*b*)	*r*
V_d (mL)	219	0.84	0.92
CL (mL/hr)	4.07	0.85	0.94

COMPARISON BETWEEN the PREDICTED EGF PK PARAMETERS and OBSERVED PK PARAMETERS

Parameter (Y)	Predicted PK Parameter Estimate	Observed PK Parameter in Cancer Patients
V_d (L/kg)	0.01	0.04
CL (mL/hr/kg)	0.22	0.98

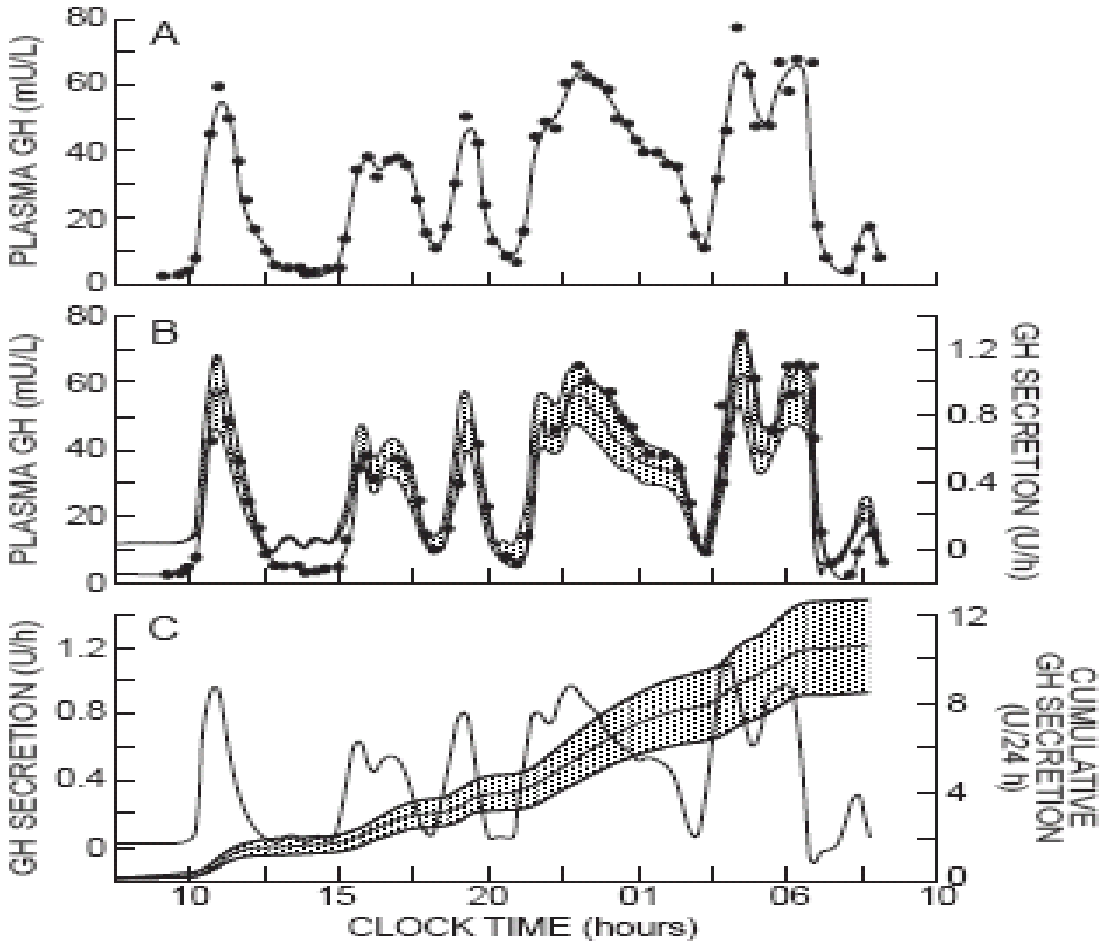
PHARMACOKINETIC CHARACTERISTIC OF MACROMOLECULES

* Endogenous concentrations
* Absorption
* Distribution
* Metabolism
* Elimination

THE PROBLEM OF ENDOGENOUS CONCENTRATIONS OF MACROMOLECULES

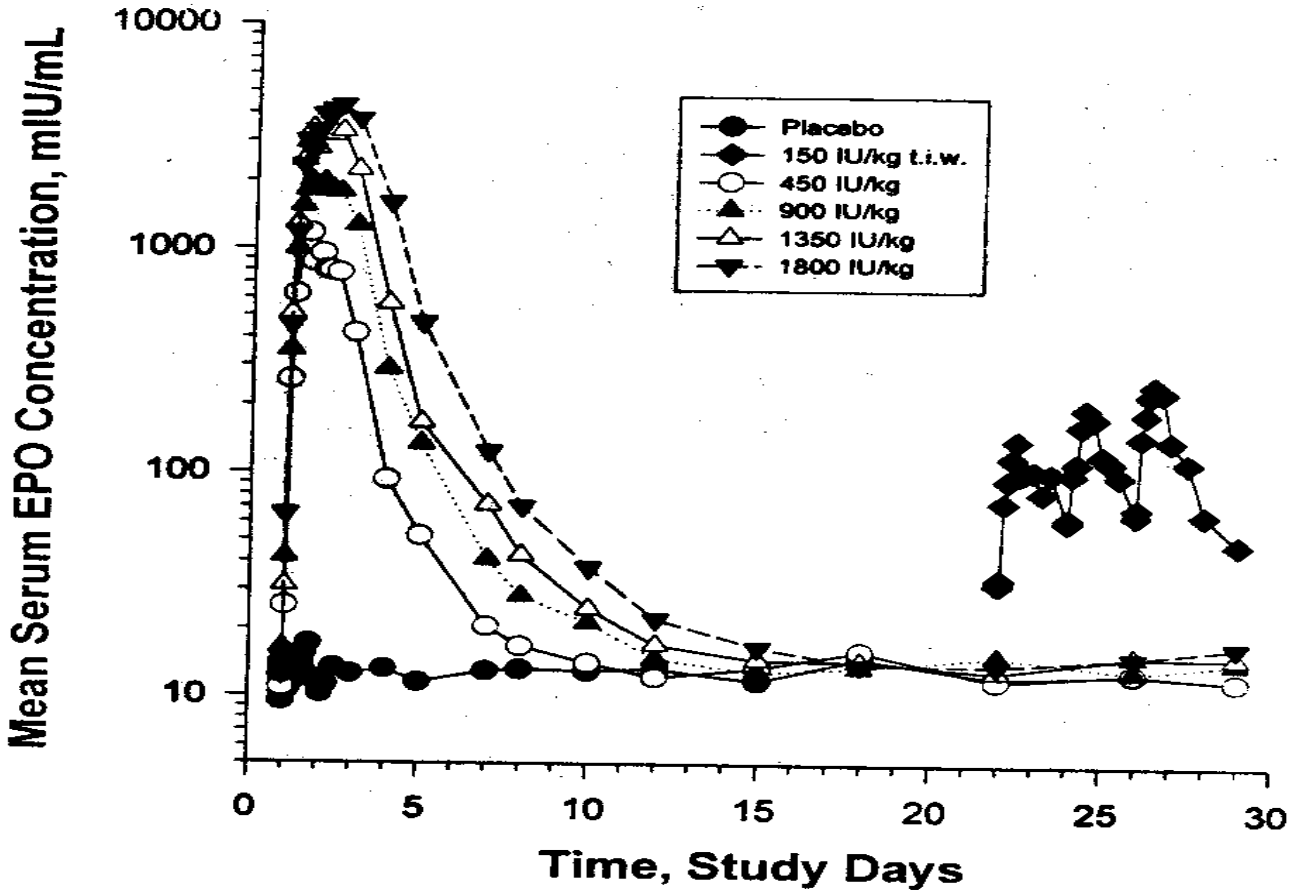
* Endogenous concentrations - What do you do with them?
* Two examples
  - Growth Hormone
  - Erythropoietin

Growth Hormone



Albertsson-Wikland K, et al. Am J Physiol 1989;257:E809-14.)

ERYTHROPOIETIN

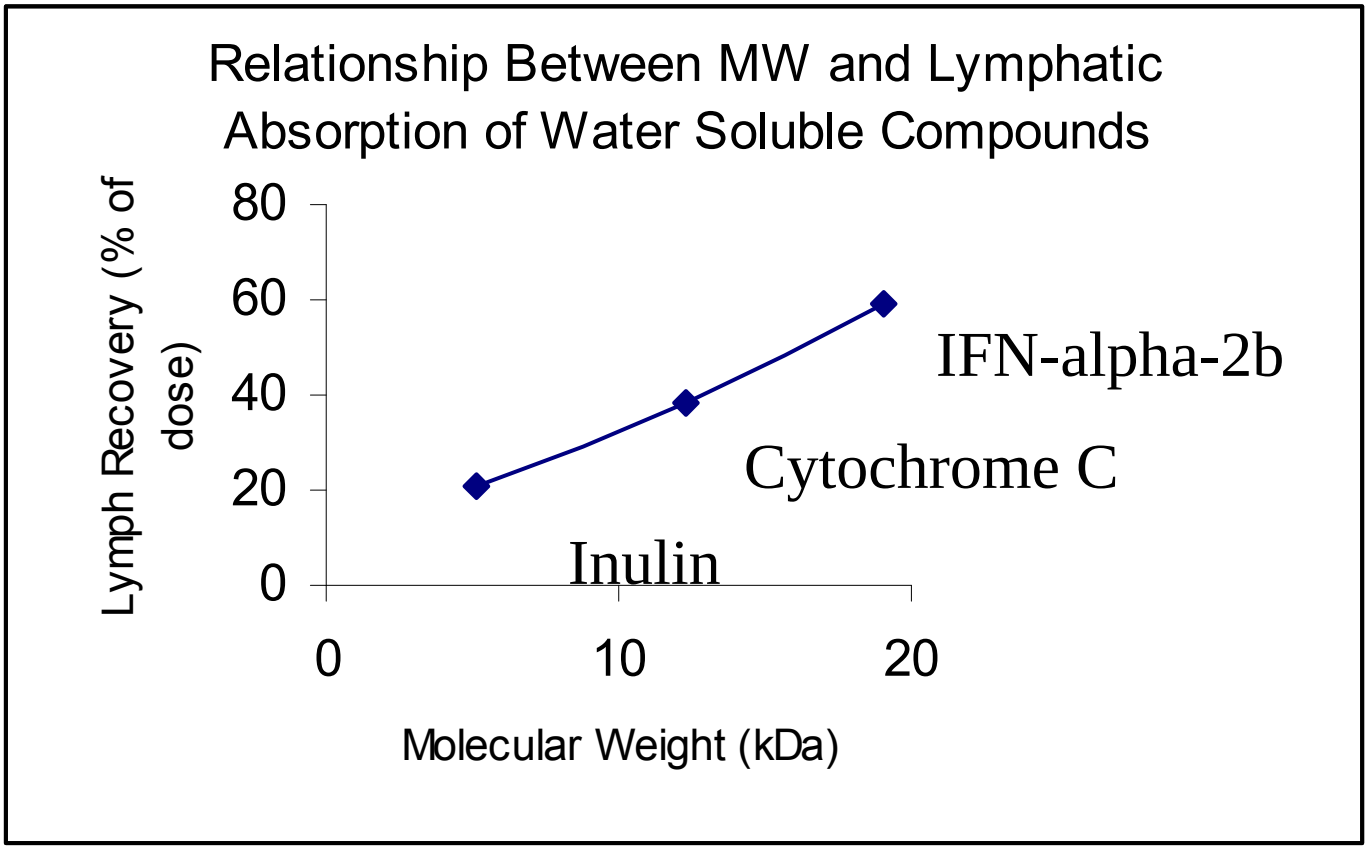


Cheung et al CPT 1998; 64:412-423

ABSORPTION OF MACROMOLECULES

* Flip-flop model
* Site of administration

RELATIONSHIP BETWEEN MW AND LYHMPHATIC ABSORPTION OF WATER SOLUBLE COMPOUNDS

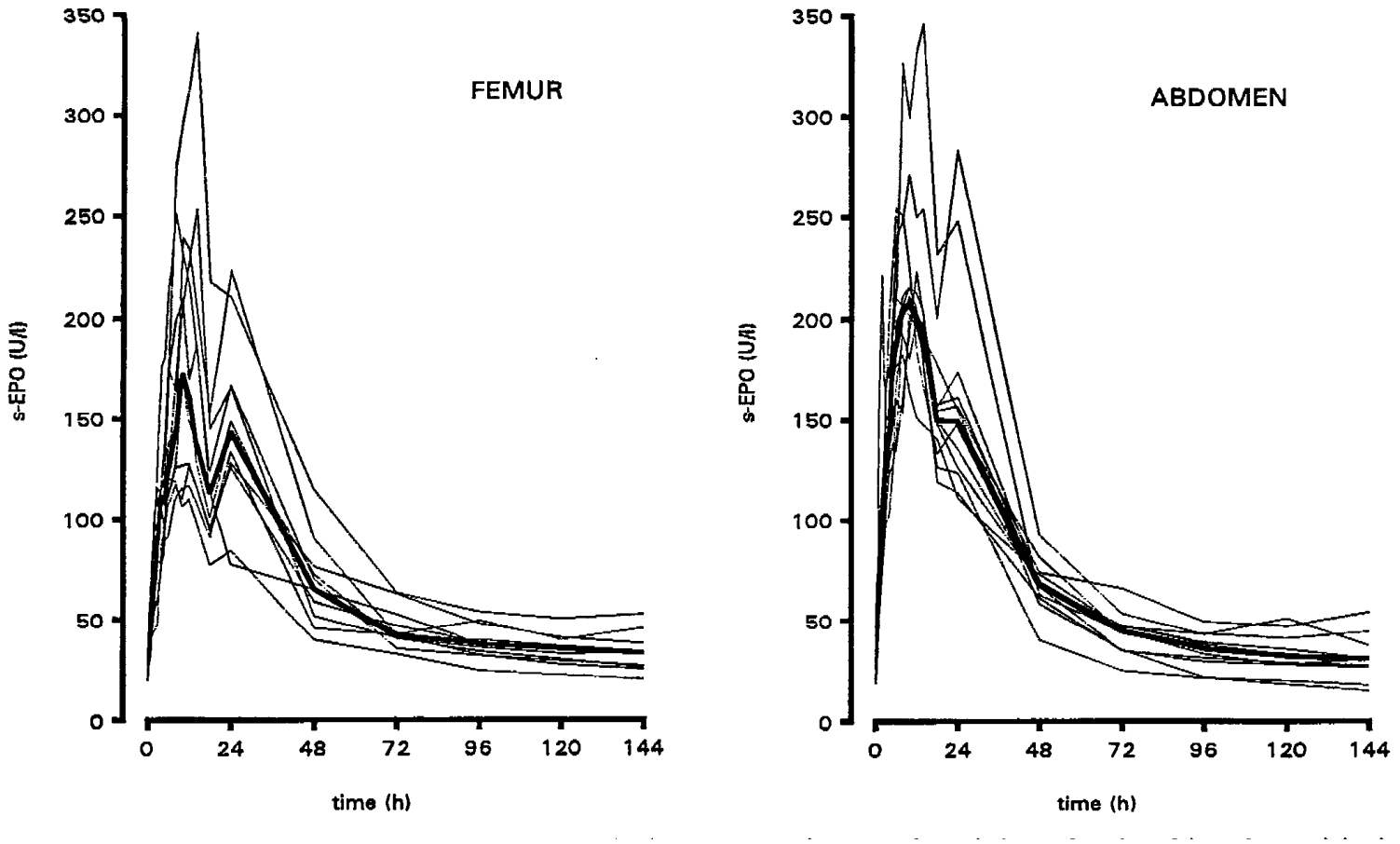


Supersaxo A et al. Pharm Res 1990; 7:167-169

COMPARISON OF ABSORPTION AND ELIMINATION RATE CONSTANTS

Macromolecule	Route of Administration	K_a (hr^{-1})	K_e (hr^{-1})
GH	SC	0.23 ± 0.04	0.43 ± 0.05
	IV		2.58
IFN-α-2b	SC	0.24	0.13
	IV		0.42
Erythropoietin	SC	0.0403 ± 0.002	0.206 ± 0.004
	IV		0.077

SITE OF INJECTION EFFECTS ON EPO ABSORPTION



Jensen JD et al Eur J Clin Pharmacol 1994; 46:333-337

DISTRIBUTION OF MACROMOLECULES

* Volume of Distribution
* Binding Proteins

DISTRIBUTION VOLUMES OF REPRESENTATIVE MACROMOLECULES

Macromolecule	MW (kDa)	V_1 (mL/kg)	V_{ss} (mL/kg)
Inulin	5.2	55	164
Factor IX	57	136*	271*
IL-2	15.5	60	112
IL-12	53	52	59
G-CSF	20	44	60
rt-PA	65	59	106

* Calculated from literature

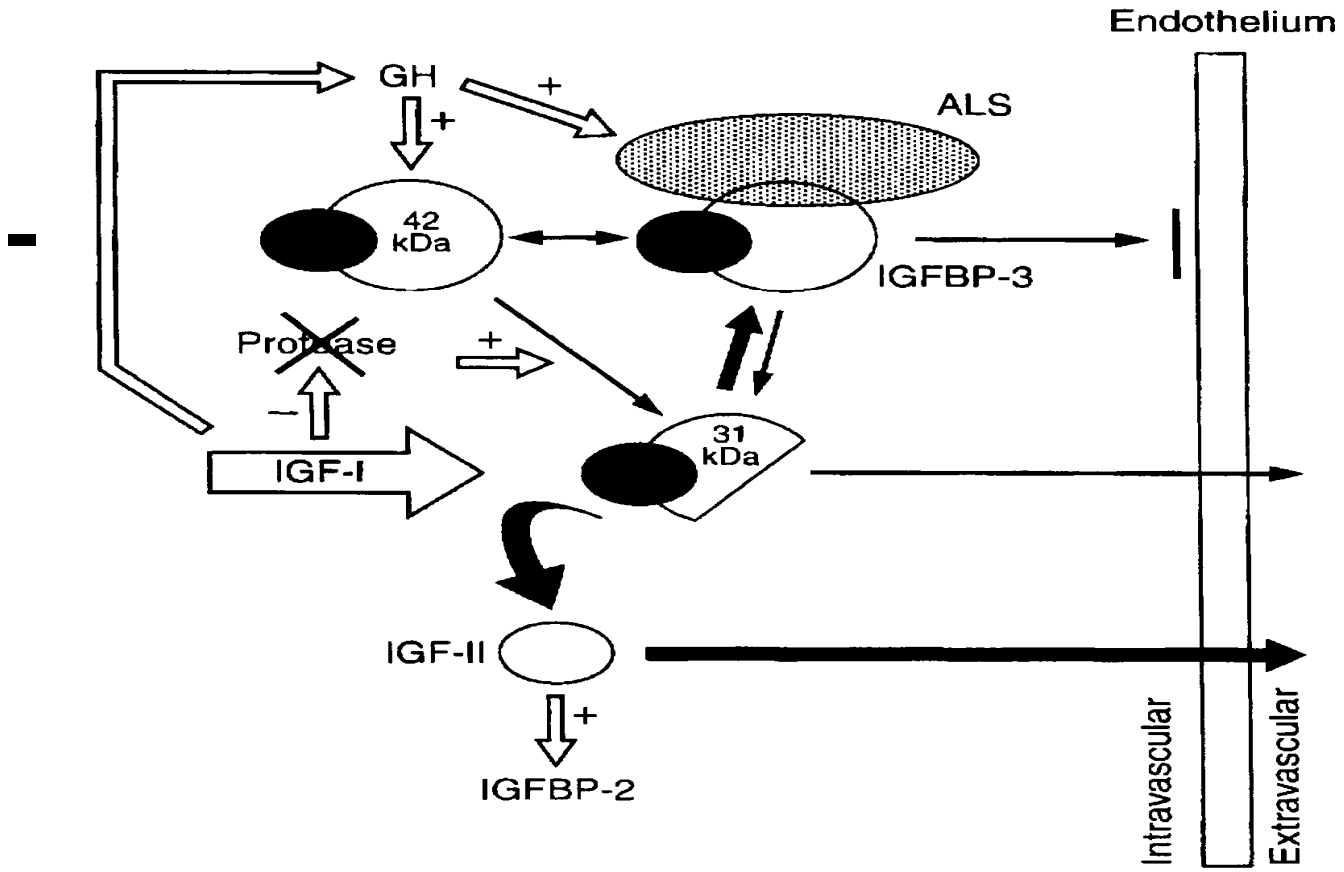
PHARMACOKINETICS of MARKETED MONOCLONAL ANTIBODIES

Mabs	Molecular Weight (kD)	$T_{1/2}$[a] (Days)	V_1[a] (L)	Vss[a]
Avastin	149	13-15	3	3.5-4.5 L
Erbitux	152	ND[b]	2.7-3.4	2-3 L/m^2
Raptiva	150	6-7.5[c]	NR[d]	9 L[e]
Humira	148	12-18	3	5 L
Campath	150	1-14[f]	NR[d]	7-28 L

EFFECTS & RELEVANCE OF MACROMOLECULE BINDING TO α_2-MACROGLOBULIN

Macromolecule	Effect	Relevance
NGF		Assay inteference
IL-1	Regulation of proliferation of thymocytes	Regulatory protein
IL-2	Impaired proliferation of T-cells	Inactivation
TGF_β	Growth of kidney fibroblasts	Clearance

HYPOTHETICAL MODEL of the BINDING EFFECTS of IGF-1



METABOLIC EFFECTS OF MACROMOLECULES

* Effects on P450s

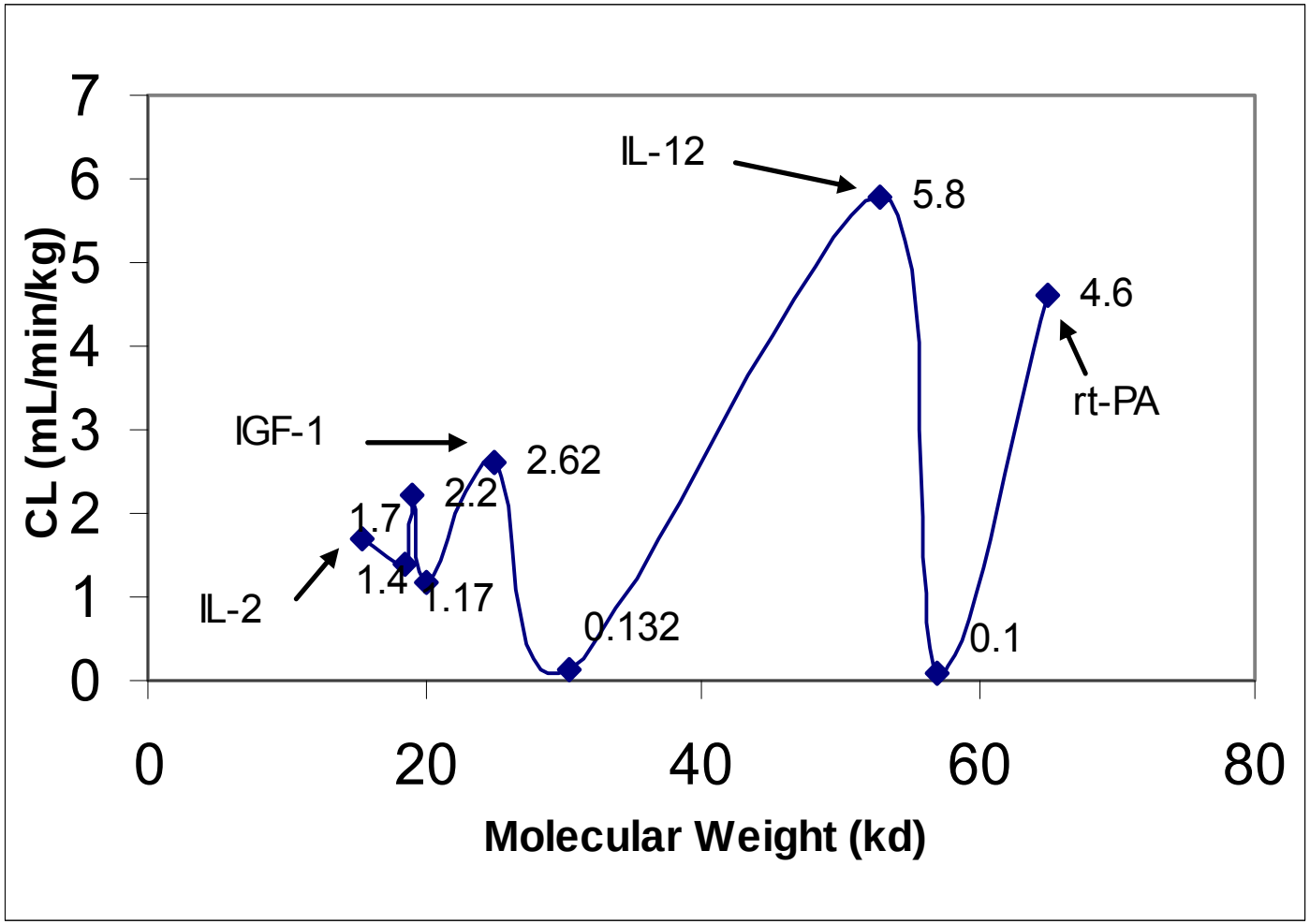
EFFECTS OF MACROMOLECULES ON P450 CYP ENZYMES

Macromolecule	Isoenzyme	Effects
IFN-γ	CYP2C11	Decreased mRNA and enzyme levels
IL-1	CYP2C11	Decreased mRNA and enzyme levels
	CYP 2D	Decreased mRNA and enzyme levels
IL-2	CYP2D1	Increased mRNA and enzyme levels
IL-6	CYP2C11	Decreased mRNA and enzyme levels
TNF	CYP2C11	Decreased enzyme levels

EXCRETION OF MACROMOLECULES

* Contributions of kidney and liver
* CHO vs E. Coli produced
* Receptor mediated clearance

RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND ELIMINATION CLEARANCE



LIVER CELL SURFACE RECEPTORS FOR CLEARANCE OF CARBOHYDRATES & MONOSACCHARIDES

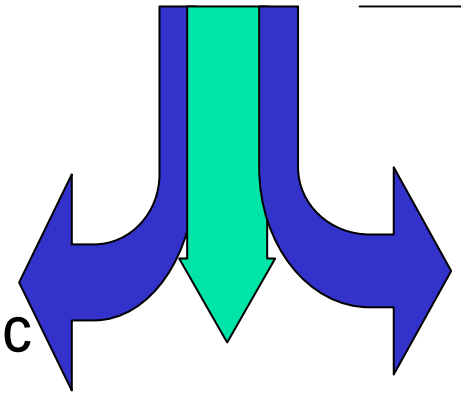
Specificity	Cell Type
Gal/Gal/NAc	Liver parencymal cells
Gal/GalNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Man/GlcNAc	Liver Kupffer and endothelial cells Peritoneal macrophages
Fuc	Liver Kupffer cells

DIFFERENCES BETWEEN rhEPO AND NESP (NOVEL ERYTHROPOIESIS-STIMULATING PROTEIN)

* rhEPO
  - 165 normal amino acid sequence
  - Up to 40% carbohydrate
  - 3 N-linked sugar chains
  - Up to 14 sialic acids
  - 30.4 Kd
  - Plasma $T_{1/2}$= 4-8 hrs

* NESP
  - 5 amino acid exchanges
  - Up to 52% carbohydrate
  - 5 N-linked sugar chains
  - Up to 22 sialic acids
  - 38.5 Kd
  - Plasma $T_{1/2}$= 24 hrs

METABOLIC FATE OF EPO



Extracellular deglycosylation and proteolytic cleavage

Hepatocytic galactose receptor mediated uptake

↓

Intracellular degradation

Renal Filtration

↓

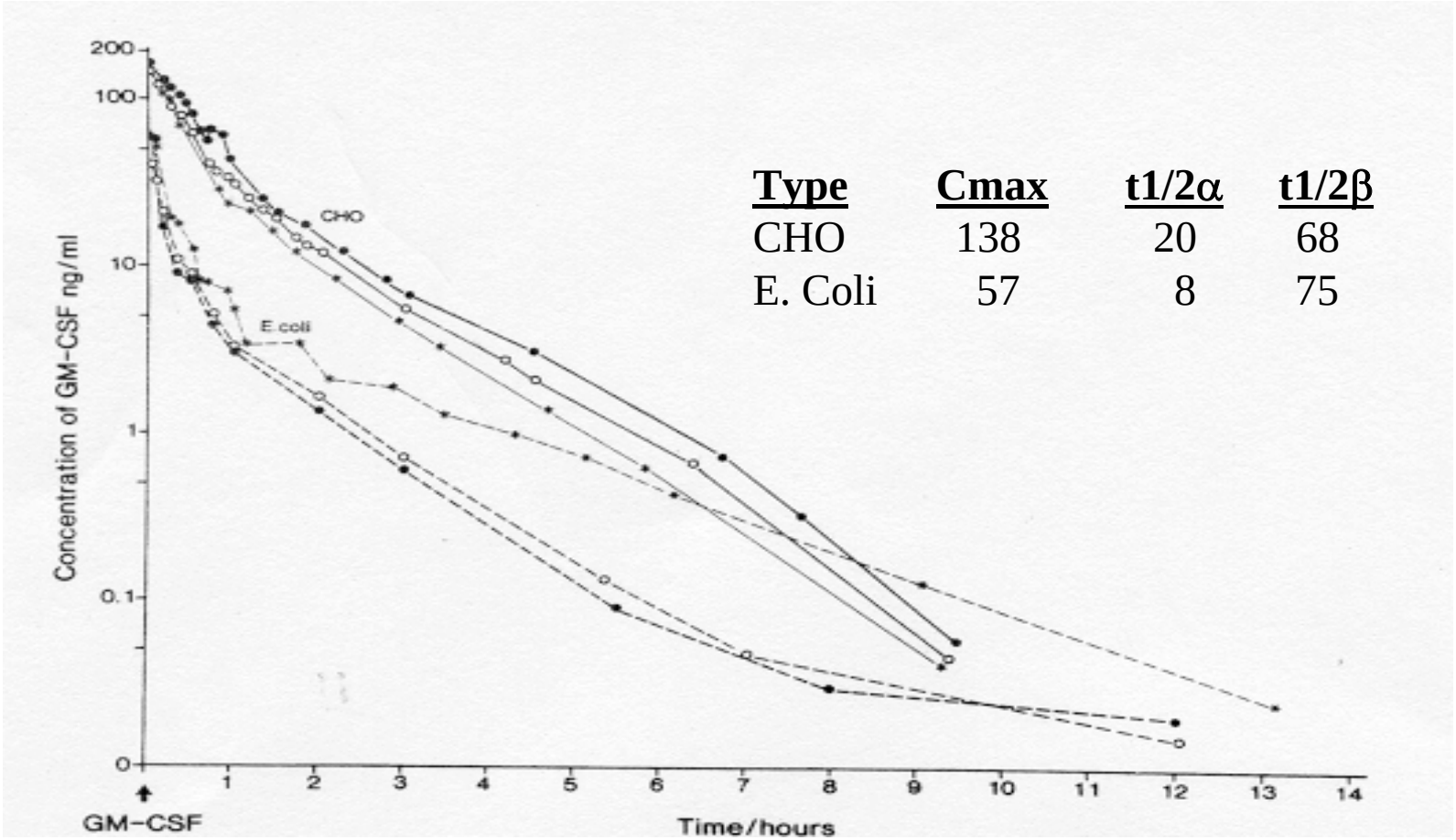
Tubular reabsorption and degradation

EPO receptor mediated uptake

↓

Intracellular degradation

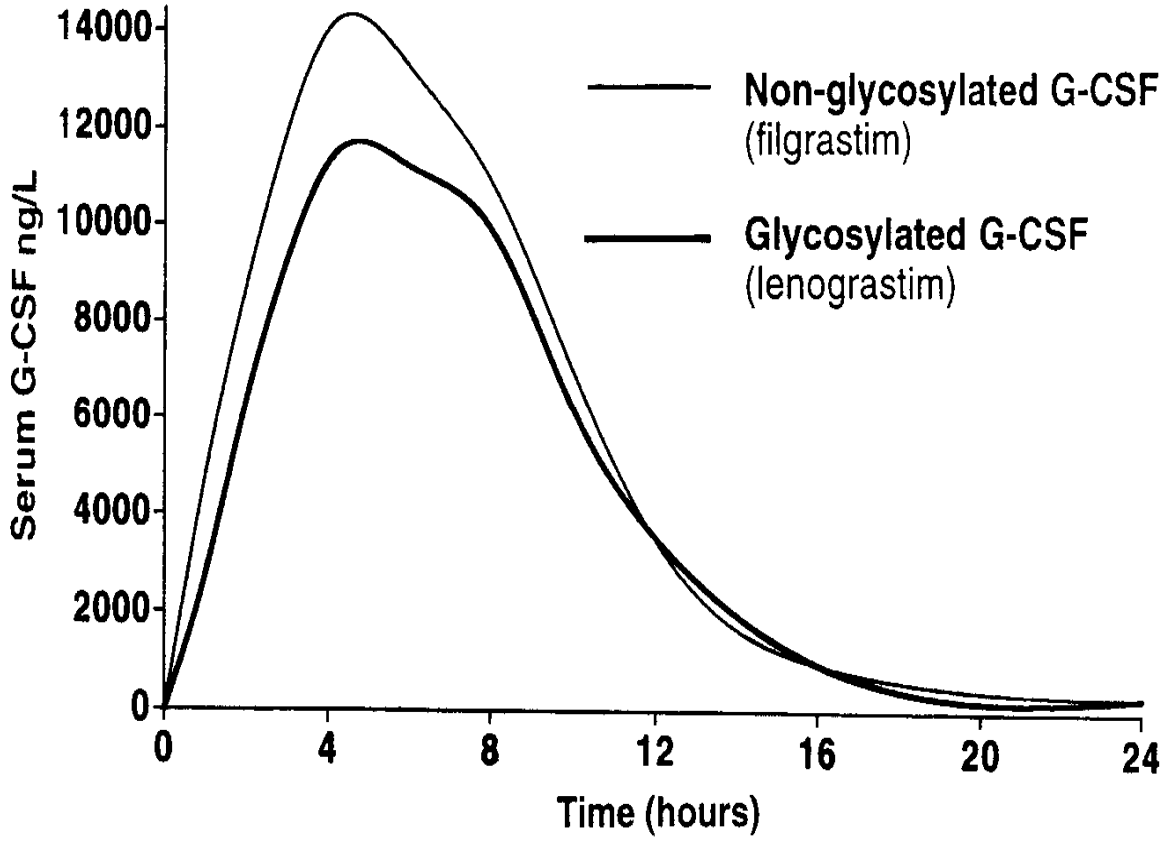
SERUM CONCENTRATION-TIME PROFILES FOR CHO VS. E. Coli PRODUCED GM-CSF



Type	Cmax	t1/2α	t1/2β
CHO	138	20	68
E. Coli	57	8	75

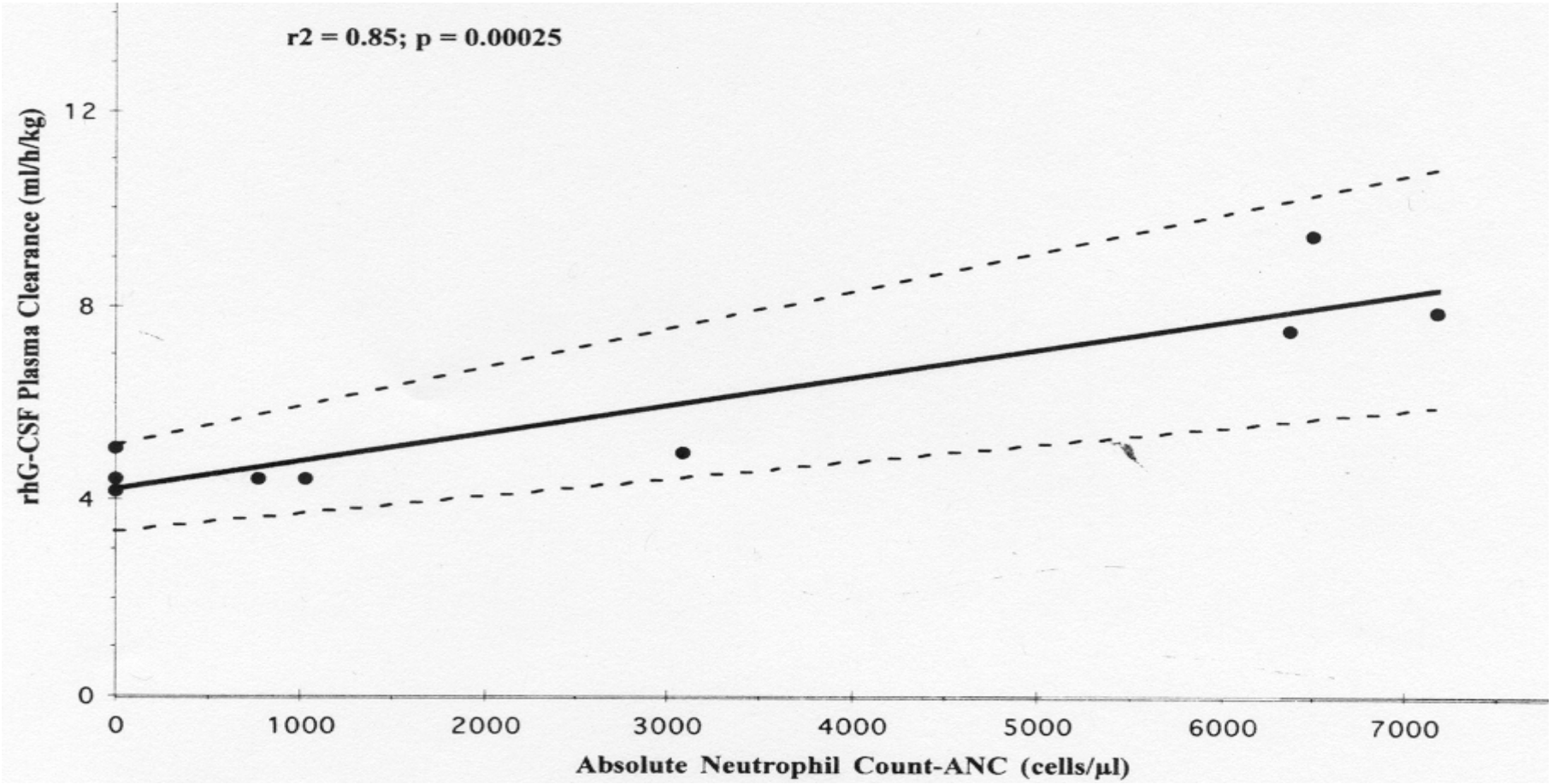
Mortensen HD et al. Eur J Haematol 1993; 50:32-36

SERUM CONCENTRATION-TIME PROFILES FOR NON-GLYCOSYLATED VS. GLYCOSYLATED G-CSF



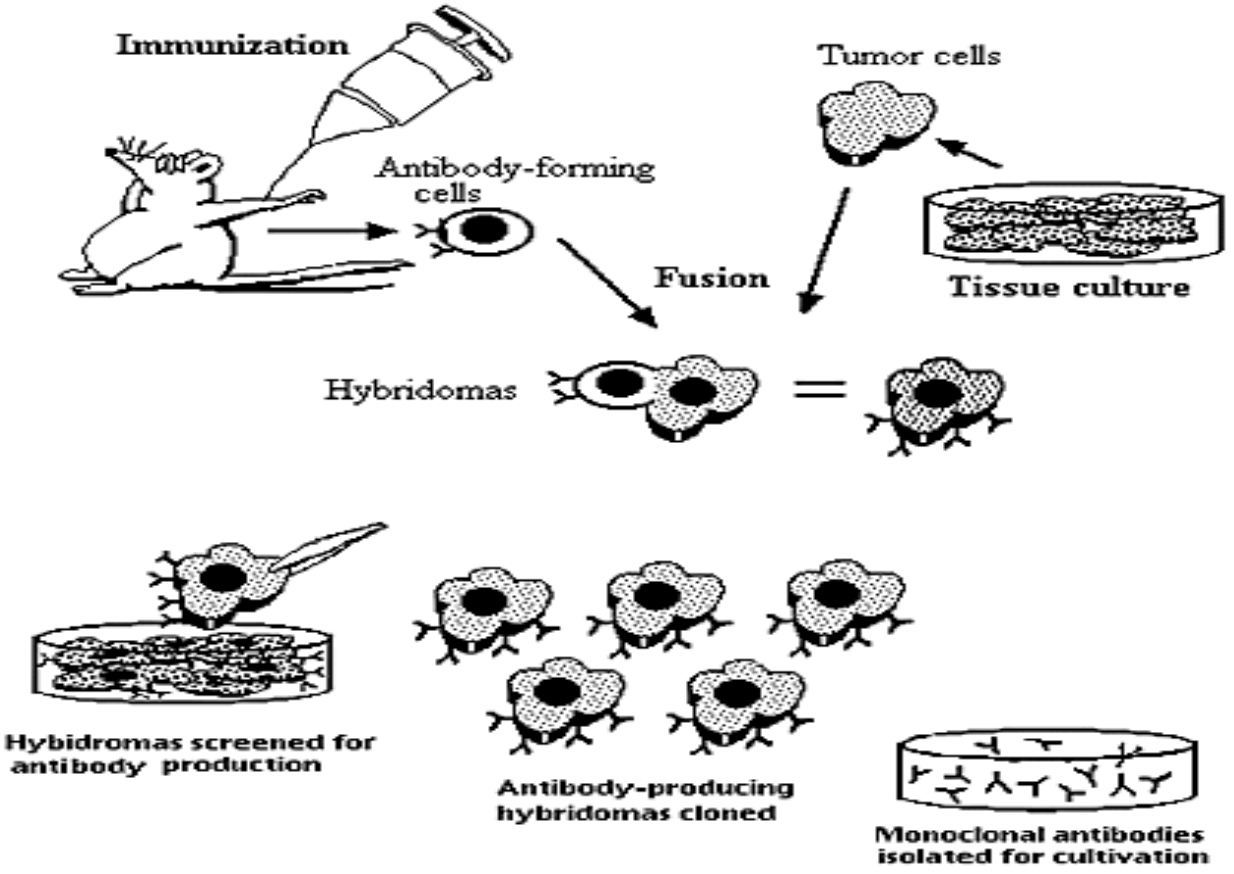
Watts et al. Br J Haematol 1997; 98:474-479

RELATIONSHIP BETWEEN G-CSF CLEARANCE AND ABSOLUTE NEUTROPHIL COUNT

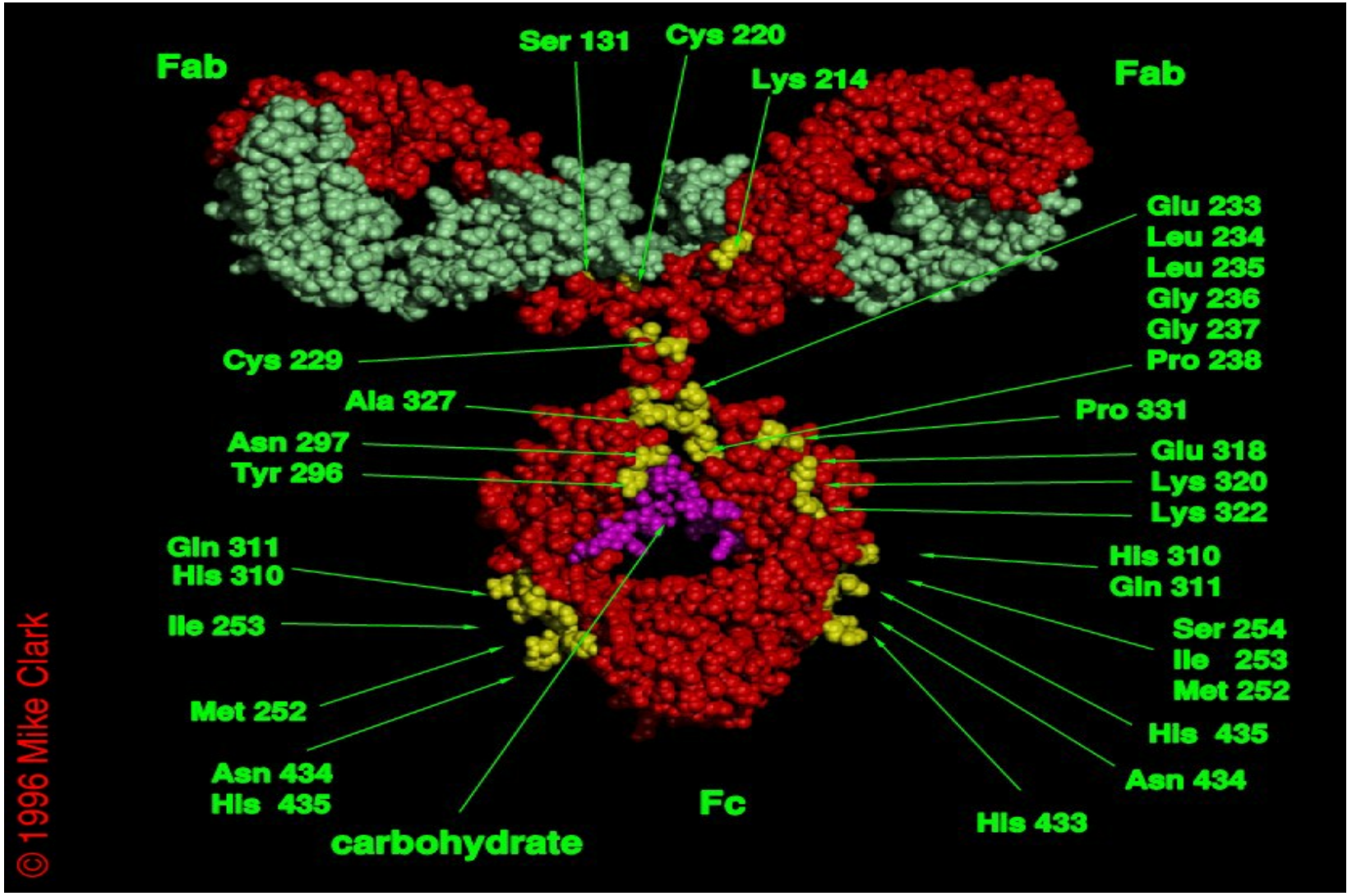


Ericson SG et al. Exper Hematol 1997; 25:1313-1325

MONOCLONAL ANTIBODY PRODUCTION



HUMAN IgG


Fab
Ser 131
Cys 220
Lys 214
Fab
Glu 233
Leu 234
Leu 235
Gly 236
Gly 237
Pro 238
Cys 229
Ala 327
Pro 331
Asn 297
Tyr 296
Glu 318
Lys 320
Lys 322
Gln 311
His 310
His 310
Gln 311
Ile 253
Ser 254
Ile 253
Met 252
Met 252
His 435
Asn 434
Asn 434
His 435
Fc
His 433
carbohydrate
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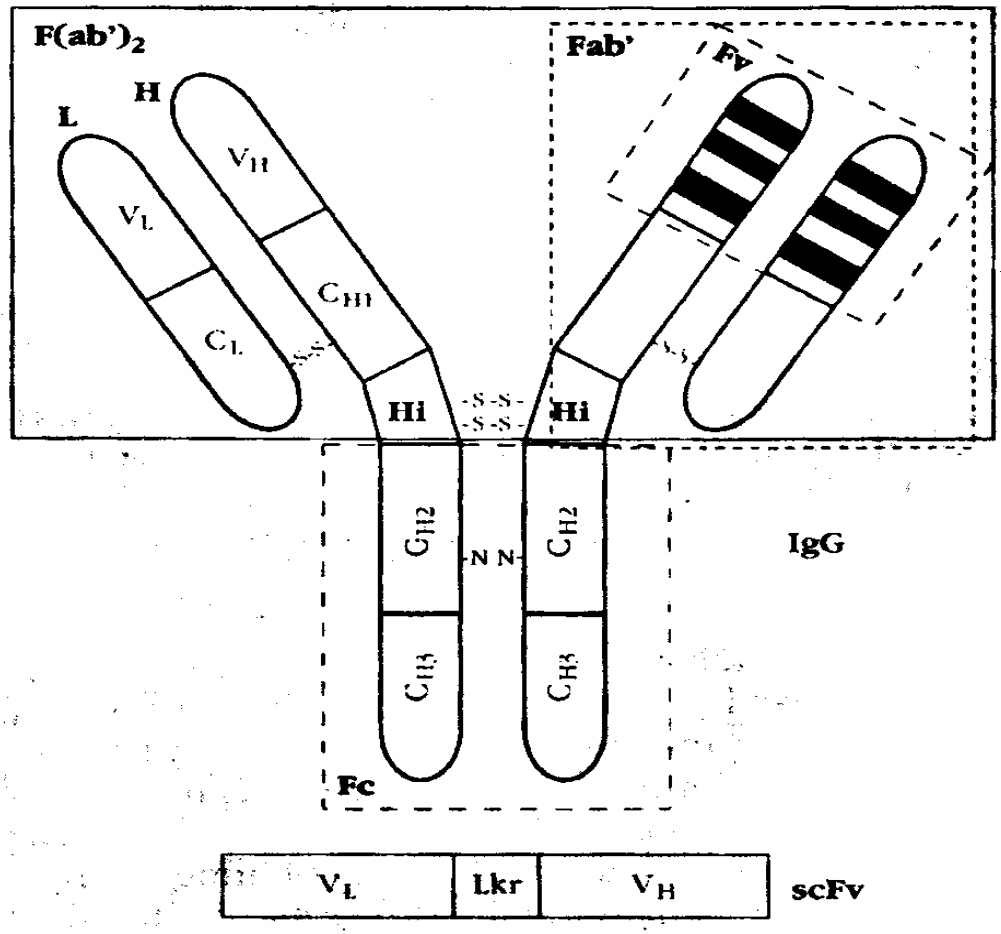
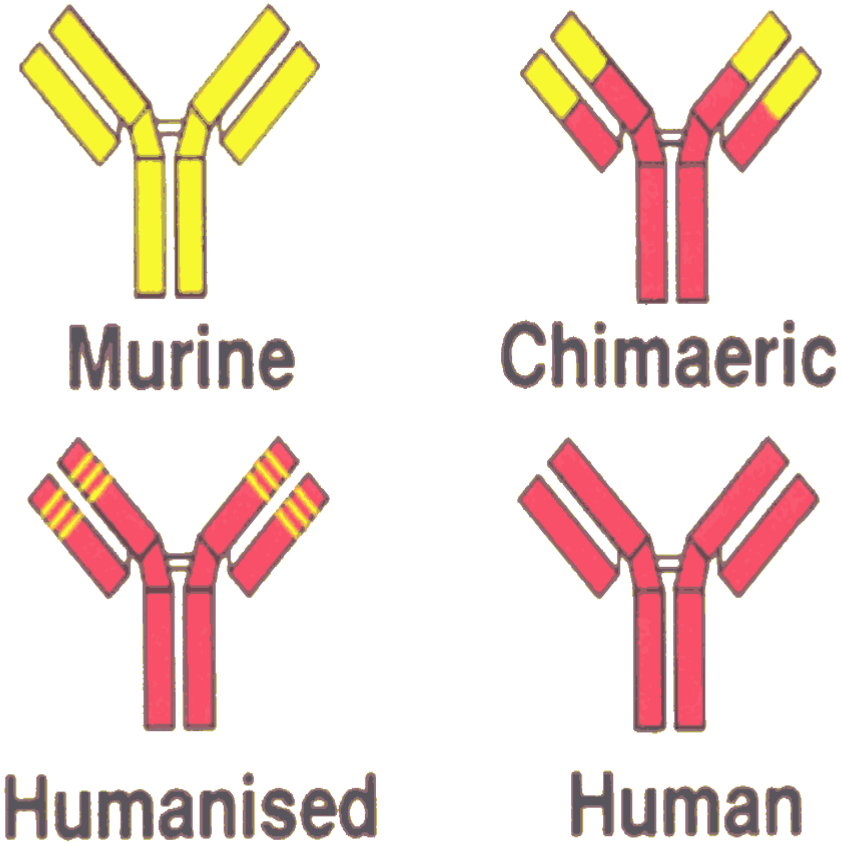
IgG and SINGLE-CHAIN Fv



CONCEPT OF ANTIBODIES



PROPOSED HUMAN PLASMA CLEARANCE of DIFFERENT ANTIBODY MOLECULES

Antibody Molecule	Molecular Weight (kD)	Relative Plasma Clearance (Cl)
Native intact human IgG	150	≈ 21 days
Fully human/humanized	150	
Chimeric human-mouse IgG	150	
Whole mouse IgG	150	
F $(ab)_2$	110	
Fab'	50	
Single chain FV (scFV)	25	≈ 1 day

DESIGN OF ANTIBODIES

* Molecules that can be attached:
  - Enzymes
  - Toxins
  - Viruses
  - Cationic tails
  - Biosensors

CHARACTERISTICS THAT AFFECT THE PHARMACOKINETICS OF MACROMOLECULES

* Physical characteristics
* Post-translational modification
* Binding
* Route of administration
* Duration of administration
* Frequency of administration

PATIENT CHARACTERISTICS THAT AFFECT PHARMACOKINETICS OF MACROMOLECULES

* Age
* Gender
* Disease
* Concurrent drugs

EFFECTS OF GENDER ON GROWTH HORMONE PK/PD

* Daily rhGH dose/kg required to normalize IGF-1 response in GH deficient women is higher than in men
  - Estrogen replacement also significantly increases rhGH dose requirement

Drug-Drug Interactions

The Journal of Clinical Pharmacology
http://www.jclinpharm.org

Drug Interaction Studies of Therapeutic Proteins or Monoclonal Antibodies

Iftekhar Mahmood and Martin David Green

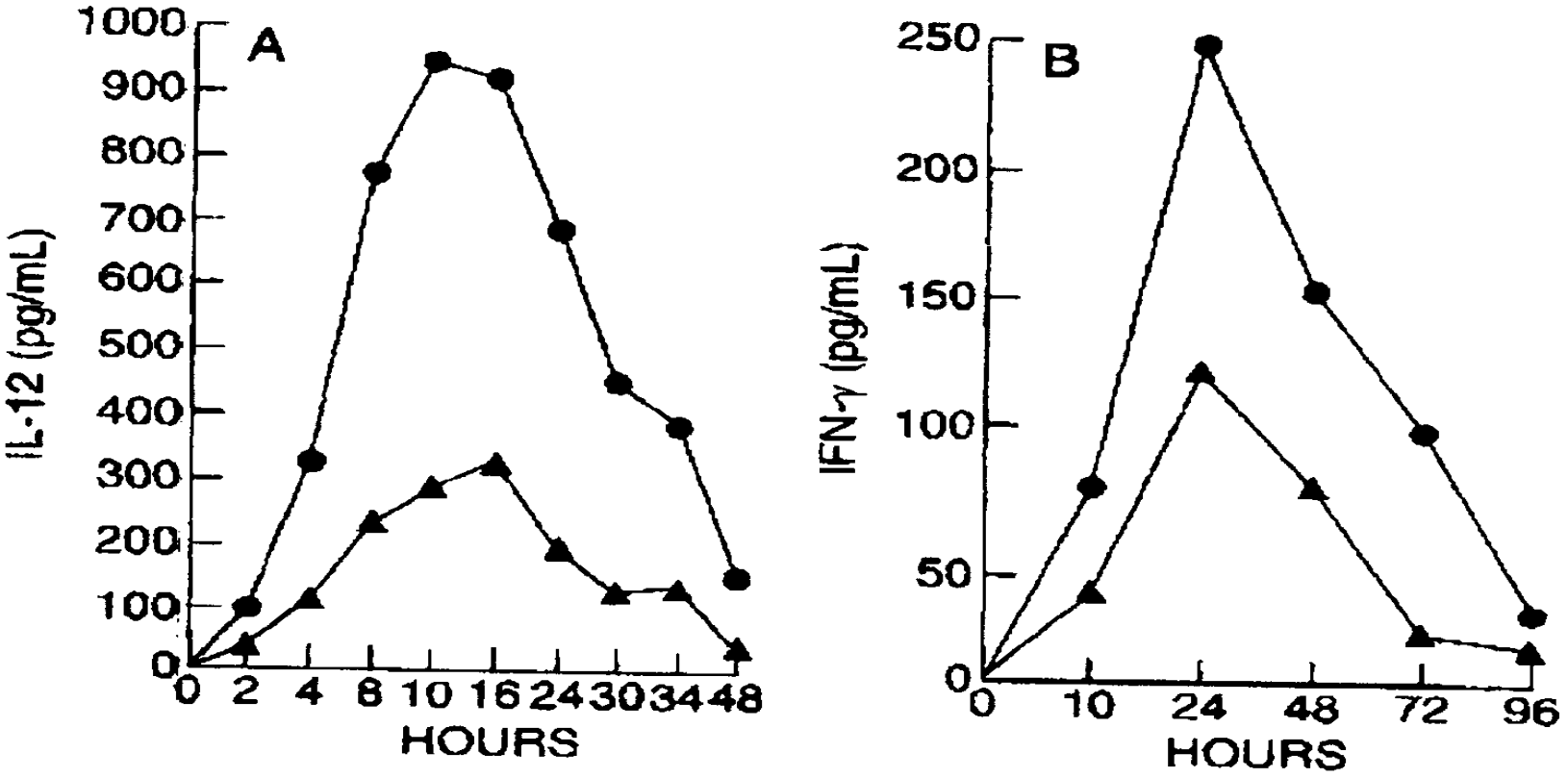
J. Clin. Pharmacol. 2007; 47; 1540 originally published online Oct 25, 2007;
DOI: 10.1177/0091270007308616

The online version of this article can be found at:
http://www.jclinpharm.org/cgi/content/abstract/47/12/1540

PHARMACODYNAMICS OF MACROMOLECULES

* Important considerations
  - Regimen dependency
  - Endpoints
  - Models

REGIMEN DEPENDENCY OF IL-12 PHARMACOKINETICS AND IFN-γ STIMULATION

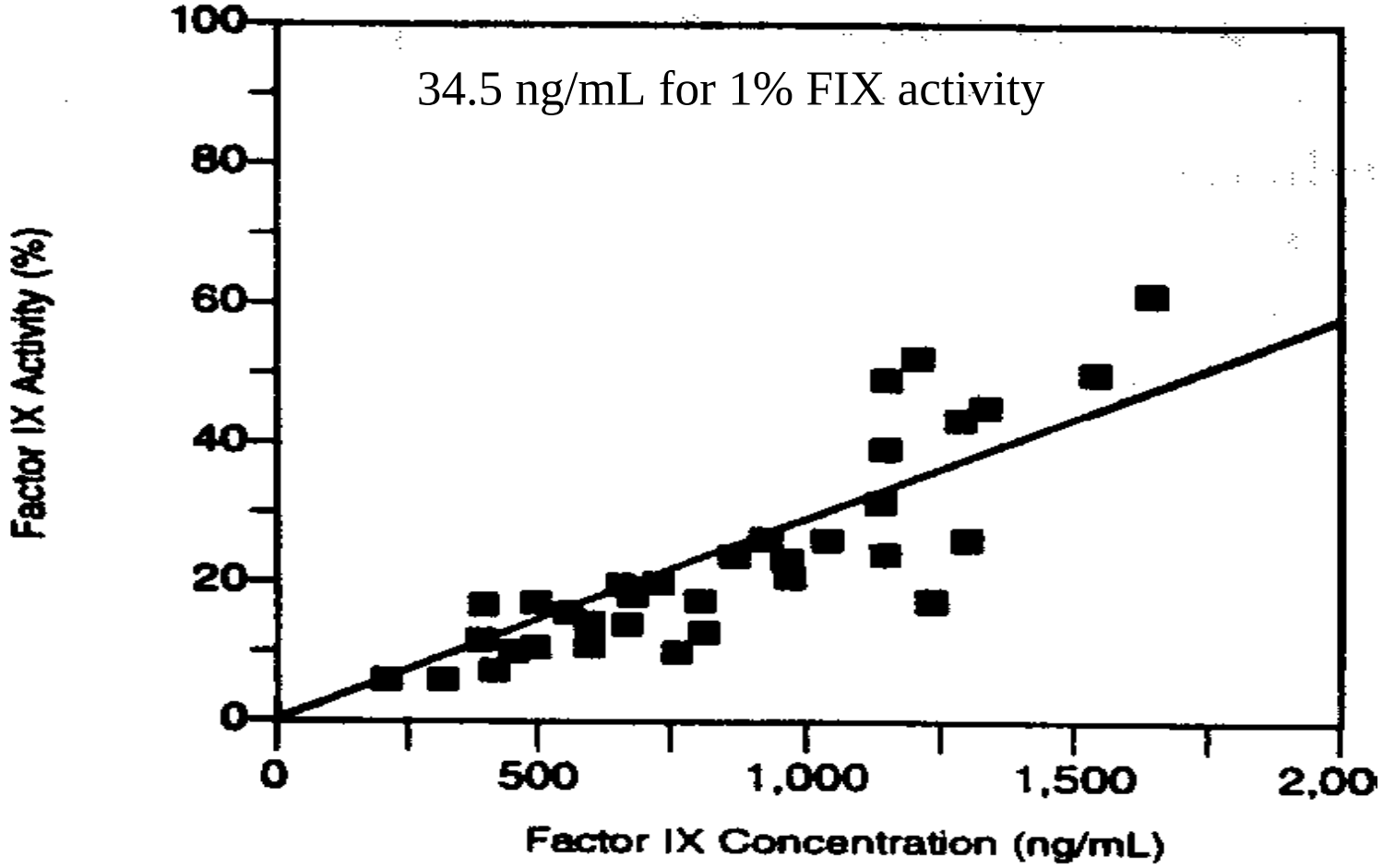


Motzer RJ et al. Clin Cancer Res 1998;4:1183-1191

PHARMACODYNAMIC ENDPOINTS

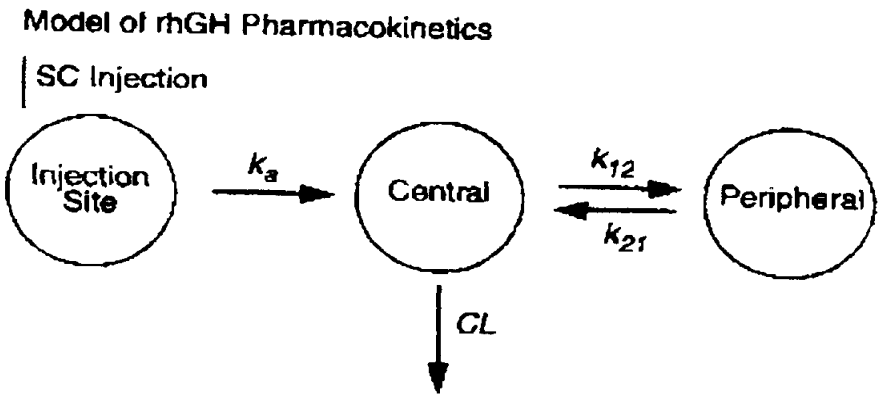
* Easy - replacement proteins
  - rFIX
* Difficult- casade of events
  - IGF-1

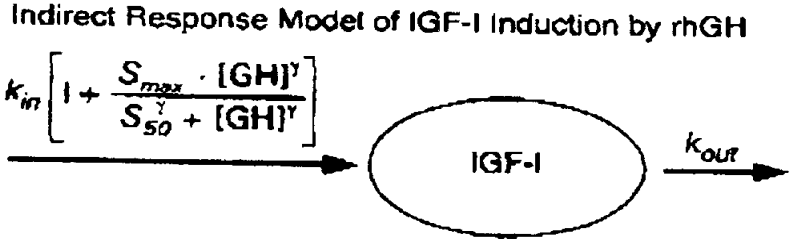
RELATIONSHIP BETWEEN rFIX CONCENTRATION AND ACTIVITY

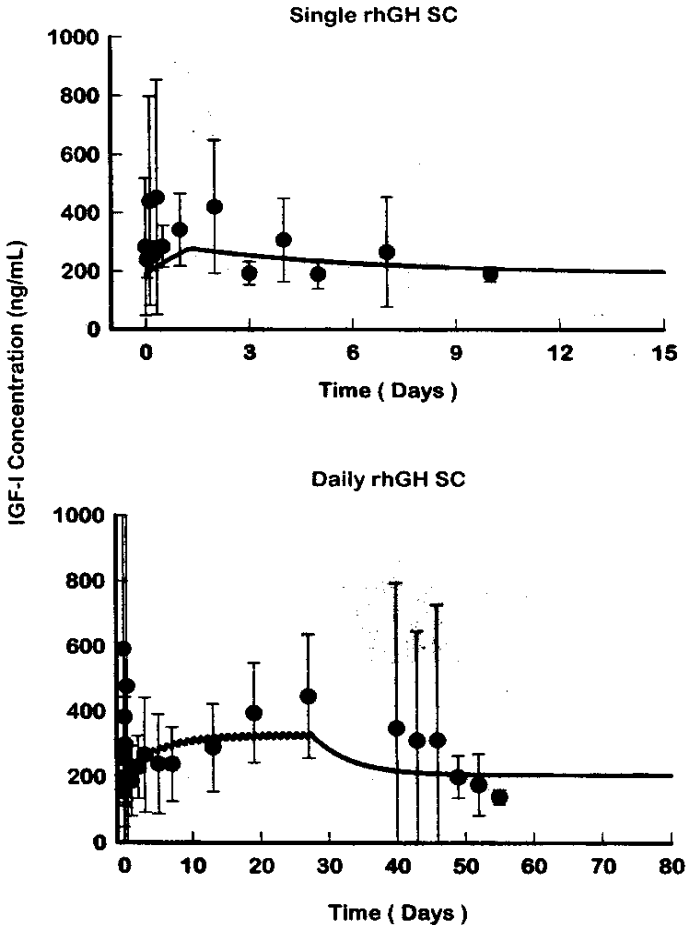


Schaub et al. Seminars in Hematology 1998; 35:28-32

PK-PD MODEL OF rhGH WITH MEASURED VS. PREDICTED [IGF-1] AFTER SINGLE AND DAILY SC rhGH INJECTIONS







Sun YN et al. JPET 1999; 289:1523-1532

PHARMACODYNAMIC ENDPOINTS

Omalizumab: Free IgE levels

Clinical outcomes

Basiliximab: Soluble IL-2 receptor

CD25+ T lymphocytes 1%

Summary

* Use scientific judgement and good sense in the interpretation of PK/PD results with macromolecules
* Application of PK principles that have been developed work with macromolecules
* Difficult to select the most appropriate pharmacodynamic endpoint

Acknowledgements

Genetic Institute

PK/PD Sciences

Dr. Joyce Mordenti

Dr. Art Atkinson

Dr. Juan Lertora