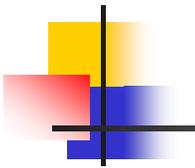


# Pharmacokinetic and Pharmacodynamic Considerations in the Development of Macromolecules

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Pamela D. Garzone, Ph.D.

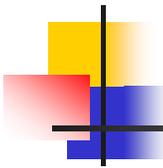
April 22, 2010



## OUTLINE OF LECTURE TOPICS

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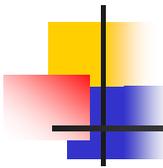
- Macromolecules
- Interspecies Scaling
- Pharmacokinetic Characteristics
  - Scientific Issues
- Pharmacodynamics
- Monoclonal Antibodies



## REPRESENTATIVE MARKETED MACROMOLECULES

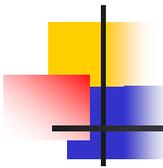
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<u>Macromolecule</u>	<u>Trade Name</u>
Erythropoietin	Epogen (Amgen)
Growth Hormone	Nutropin (Genentech)
G-CSF	Neupogen (Amgen)
IL-2	Proleukin (Chiron)
IL-11	Neumega (GI)
Factor IX	BeneFIX (GI)
rt-PA	Alteplase (Genentech)



## APPROVED MONOCLONAL ANTIBODIES

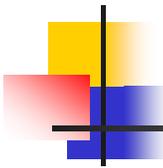
Name	Approval	Indication
Avastin Bevacizumab	Feb, 2004	First line (with 5-FU) in metastatic colon CA
Erbitux Cefuximab	Feb, 2004	Alone or in combination in metastatic colon CA
Raptiva Efalizumab	Oct, 2003	Moderate to severe psoriasis
Xolair Omalizumab	June, 2003	Asthma
Humira Adalimumab	Dec, 2002	Prophylaxis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



## ASSAYS FOR MACROMOLECULES

---

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

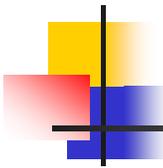


## INTERSPECIES SCALING OF MACROMOLECULES

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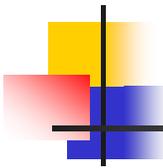
### Factors to Consider

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



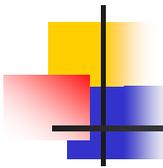
## ALLOMETRIC EQUATIONS FOR SOME MACROMOLECULES

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



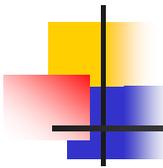
**INITIAL COMPARTMENT VOLUME  
PREDICTED BY ALLOMETRIC SCALING  
COMPARED WITH OBSERVED  $V_1$**

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



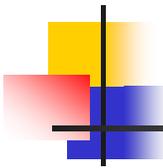
**ELIMINATION CLEARANCE  
PREDICTED BY ALLOMETRIC SCALING  
COMPARED WITH OBSERVED CL**

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



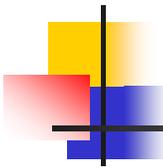
## ALLOMETRIC EQUATIONS for EGF Mab PK PARAMETERS

<b>Parameter (Y)</b>	<b>Coefficient (a)</b>	<b>Exponent (b)</b>	<b><i>r</i></b>
<b>V<sub>d</sub> (mL)</b>	<b>219</b>	<b>0.84</b>	<b>0.92</b>
<b>CL (mL/hr)</b>	<b>4.07</b>	<b>0.85</b>	<b>0.94</b>



## COMPARISON BETWEEN the PREDICTED EGF PK PARAMETERS and OBSERVED PK PARAMETERS

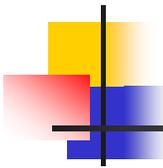
Parameter (Y)	Predicted PK Parameter Estimate	Observed PK Parameter in Cancer Patients
$V_d$ (L/kg)	0.01	0.04
CL (mL/hr/kg)	0.22	0.98



## PHARMACOKINETIC CHARACTERISTIC OF MACROMOLECULES

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- Endogenous concentrations
- Absorption
- Distribution
- Metabolism
- Elimination

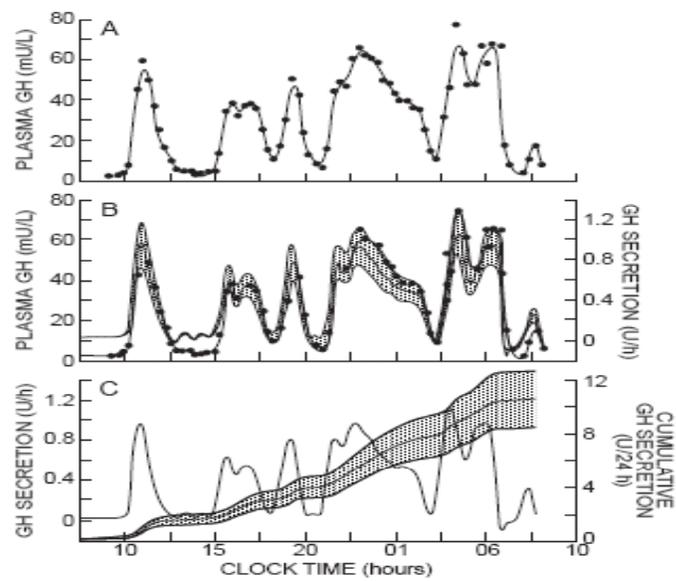


## THE PROBLEM OF ENDOGENOUS CONCENTRATIONS OF MACROMOLECULES

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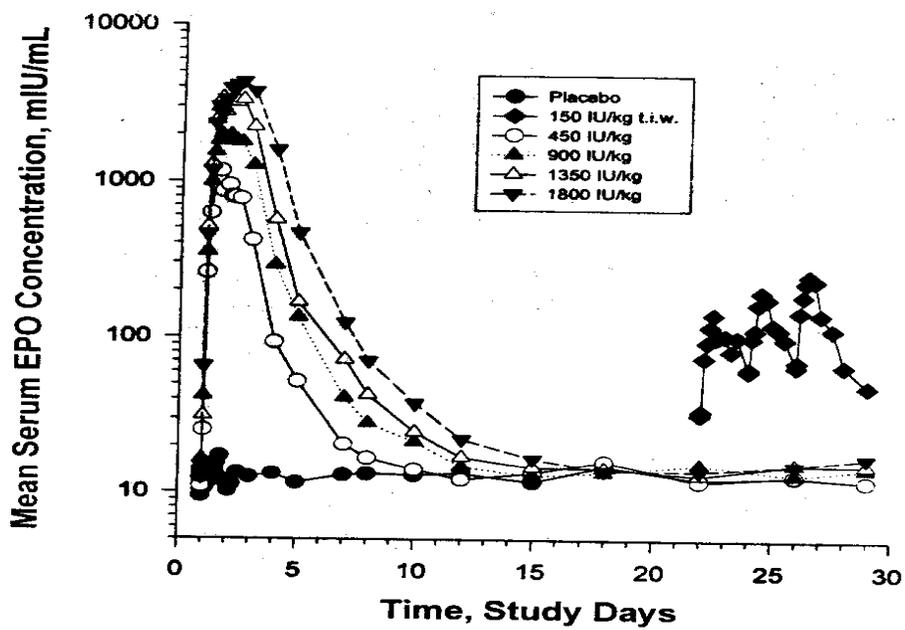
- Endogenous concentrations - What do you do with them?
- Two examples
  - Growth Hormone
  - Erythropoietin

# Growth Hormone

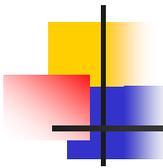


Albertsson-Wikland K, et al. Am J Physiol 1989;257:E809-14.)

# ERYTHROPOIETIN



Cheung et al CPT 1998; 64:412-423

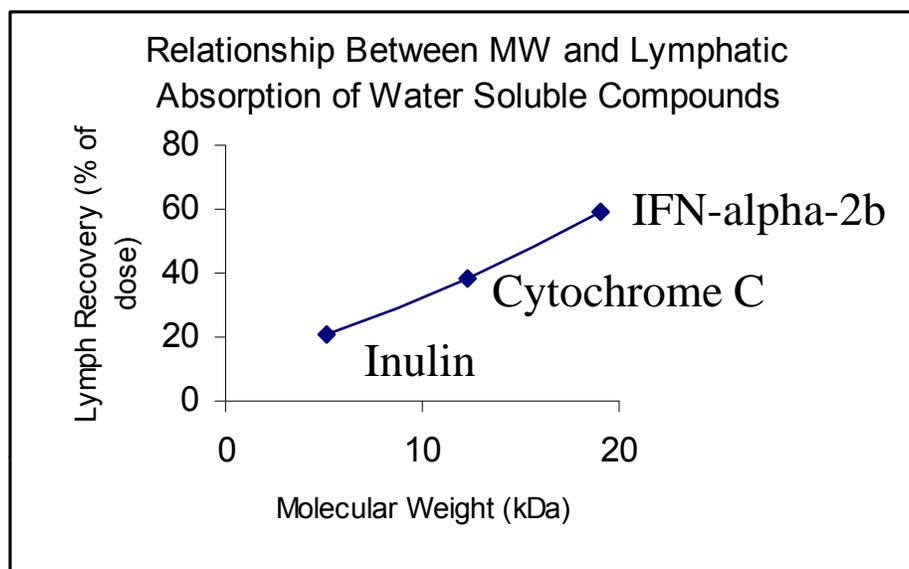


## ABSORPTION OF MACROMOLECULES

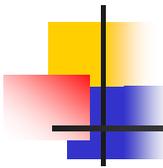
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- Flip-flop model
- Site of administration

## RELATIONSHIP BETWEEN MW AND LYMPHATIC ABSORPTION OF WATER SOLUBLE COMPOUNDS



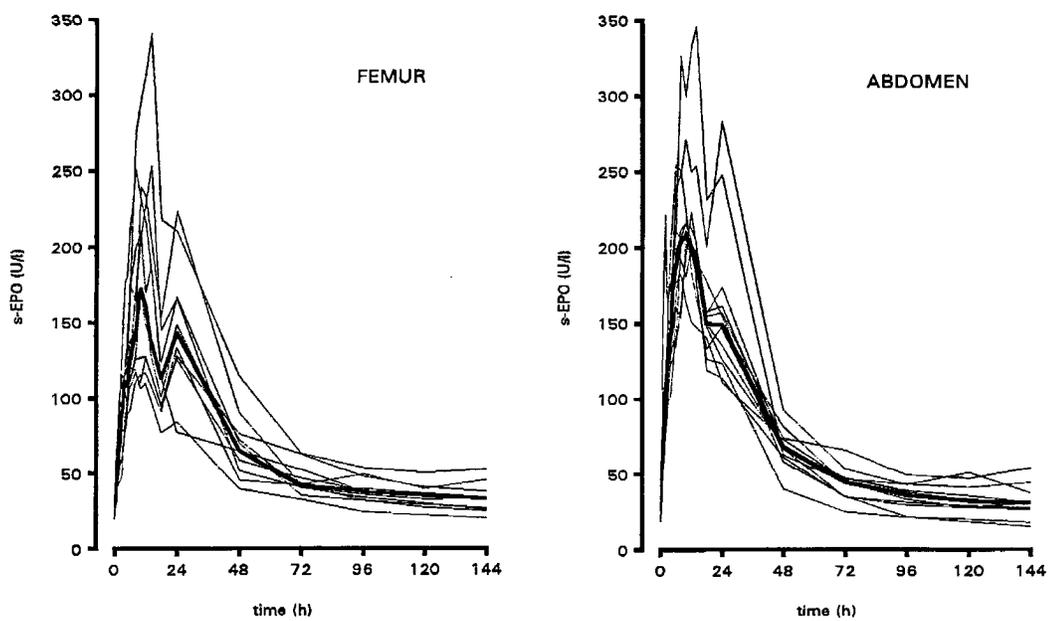
Supersaxo A et al. Pharm Res 1990; 7:167-169



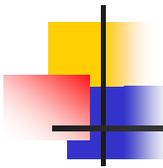
## COMPARISON OF ABSORPTION AND ELIMINATION RATE CONSTANTS

Macromolecule	Route of Administration	$K_a$ (hr <sup>-1</sup> )	$K_e$ (hr <sup>-1</sup> )
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



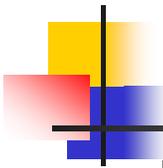
Jensen JD et al Eur J Clin Pharmacol 1994; 46:333-337



## DISTRIBUTION OF MACROMOLECULES

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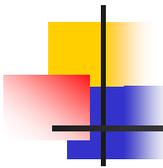
- Volume of Distribution
- Binding Proteins



## DISTRIBUTION VOLUMES OF REPRESENTATIVE MACROMOLECULES

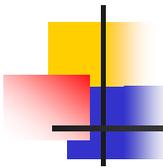
Macromolecule	MW (kDa)	$V_1$ (mL/kg)	$V_{ss}$ (mL/kg)
Inulin	5.2	55	164
Factor IX	57	136*	271*
IL-2	15.5	60	112
IL-12	53	52	59
G-CSF	20	44	60
rt-PA	65	59	106

\* Calculated from literature



## PHARMACOKINETICS of MARKETED MONOCLONAL ANTIBODIES

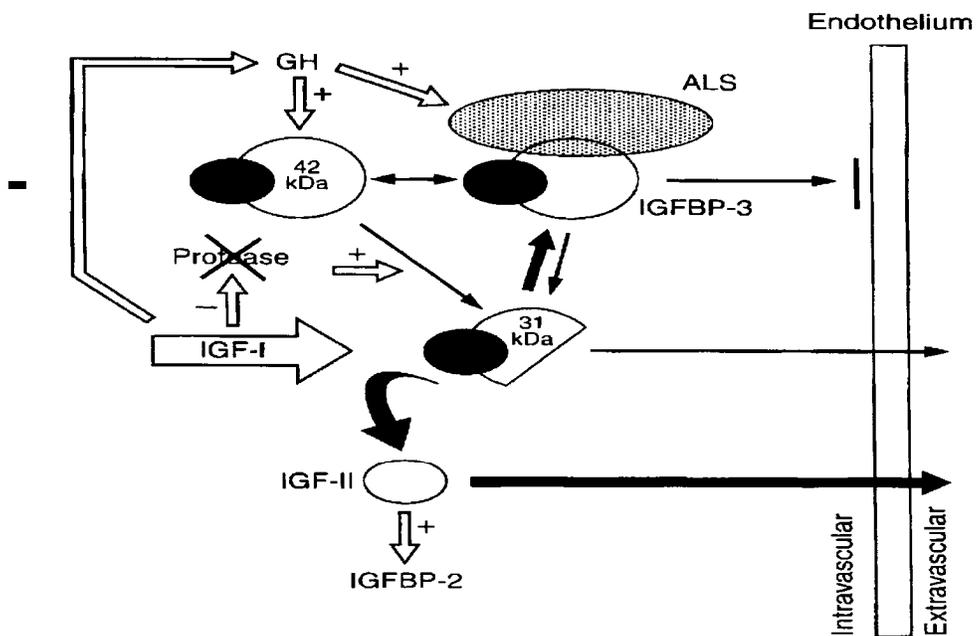
<b>Mabs</b>	<b>Molecular Weight (kD)</b>	<b><math>T_{1/2}^a</math> (Days)</b>	<b><math>V_1^a</math> (L)</b>	<b><math>V_{ss}^a</math></b>
Avastin	149	13-15	3	3.5-4.5 L
Erbix	152	ND <sup>b</sup>	2.7-3.4	2-3 L/m <sup>2</sup>
Raptiva	150	6-7.5 <sup>c</sup>	NR <sup>d</sup>	9 L <sup>e</sup>
Humira	148	12-18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

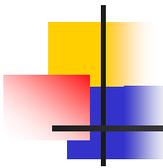


## EFFECTS & RELEVANCE OF MACROMOLECULE BINDING TO $\alpha_2$ -MACROGLOBULIN

<b>Macromolecule</b>	<b>Effect</b>	<b>Relevance</b>
<b>NGF</b>		<b>Assay interference</b>
<b>IL-1</b>	<b>Regulation of proliferation of thymocytes</b>	<b>Regulatory protein</b>
<b>IL-2</b>	<b>Impaired proliferation of T-cells</b>	<b>Inactivation</b>
<b>TGF<math>_{\beta}</math></b>	<b>Growth of kidney fibroblasts</b>	<b>Clearance</b>

# HYPOTHETICAL MODEL of the BINDING EFFECTS of IGF-1

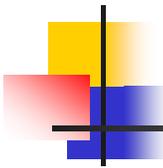




## METABOLIC EFFECTS OF MACROMOLECULES

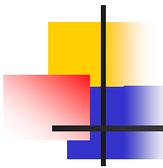
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- Effects on P450s



## EFFECTS OF MACROMOLECULES ON P450 CYP ENZYMES

Macromolecule	Isoenzyme	Effects
IFN- $\gamma$	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

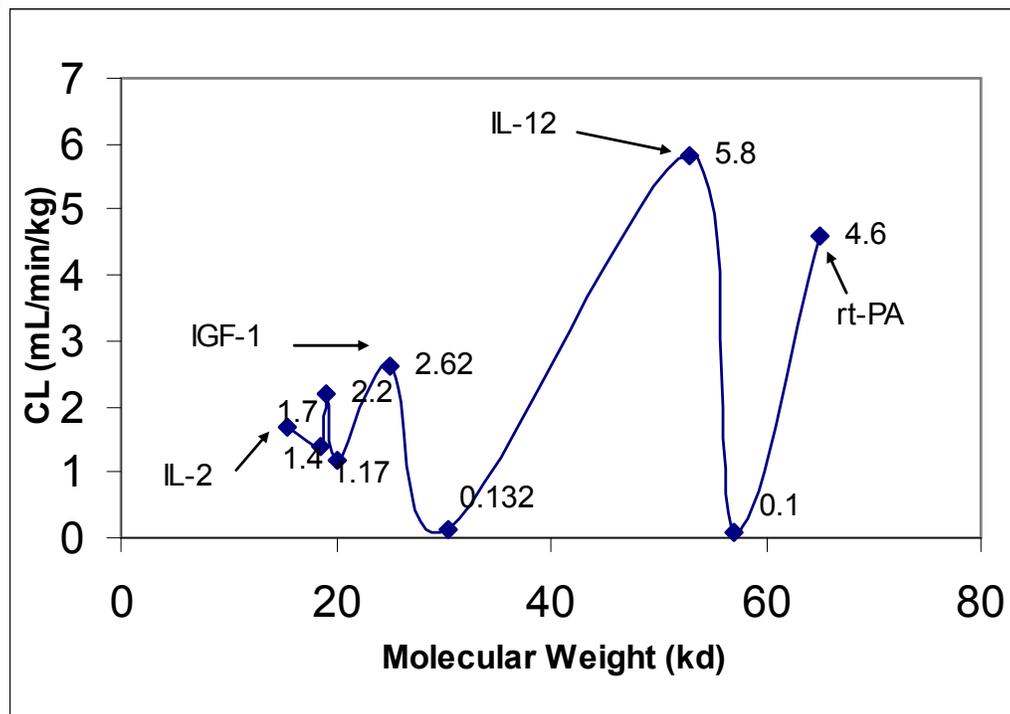


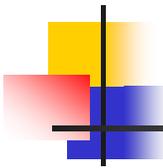
## EXCRETION OF MACROMOLECULES

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

## RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND ELIMINATION CLEARANCE

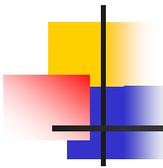




## LIVER CELL SURFACE RECEPTORS FOR CLEARANCE OF CARBOHYDRATES & MONOSACCHARIDES

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<b>Specificity</b>	<b>Cell Type</b>
<b>Gal/Gal/NAc</b>	<b>Liver parenchymal cells</b>
<b>Gal/GalNAc</b>	<b>Liver Kupffer and endothelial cells Peritoneal macrophages</b>
<b>Man/GlcNAc</b>	<b>Liver Kupffer and endothelial cells Peritoneal macrophages</b>
<b>Fuc</b>	<b>Liver Kupffer cells</b>



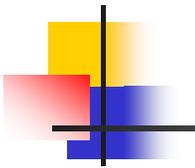
## DIFFERENCES BETWEEN rhEPO AND NESP (NOVEL ERYTHROPOIESIS-STIMULATING PROTEIN)

### ■ rhEPO

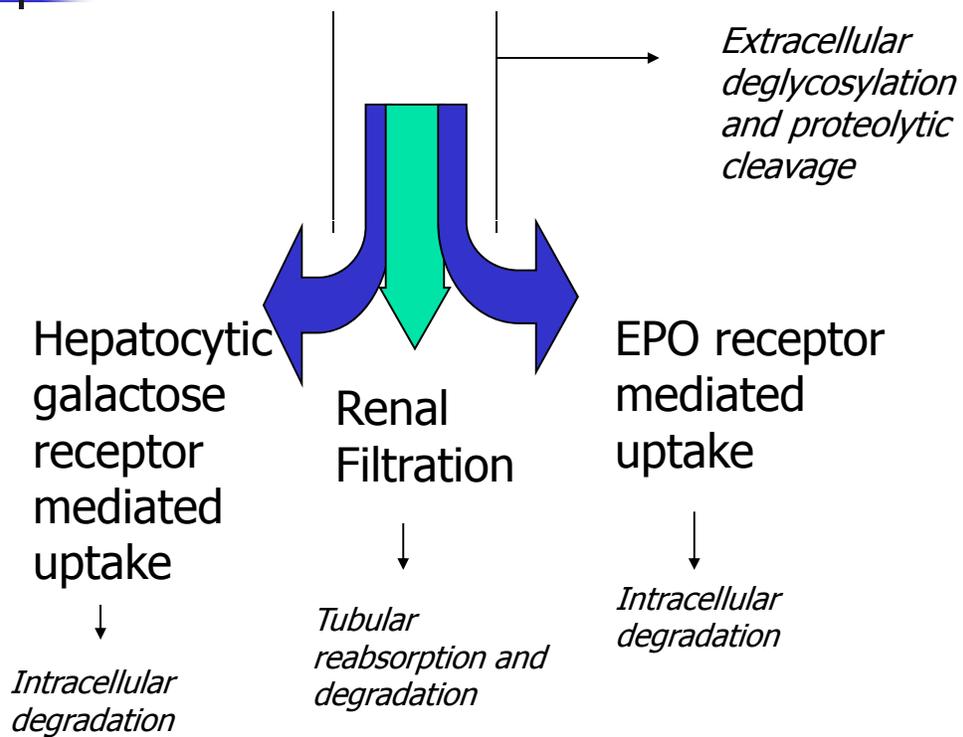
- 165 normal amino acid sequence
- Up to 40% carbohydrate
- 3 N-linked sugar chains
- Up to 14 sialic acids
- 30.4 Kd
- Plasma  $T_{1/2}$  = 4-8 hrs

### ■ NESP

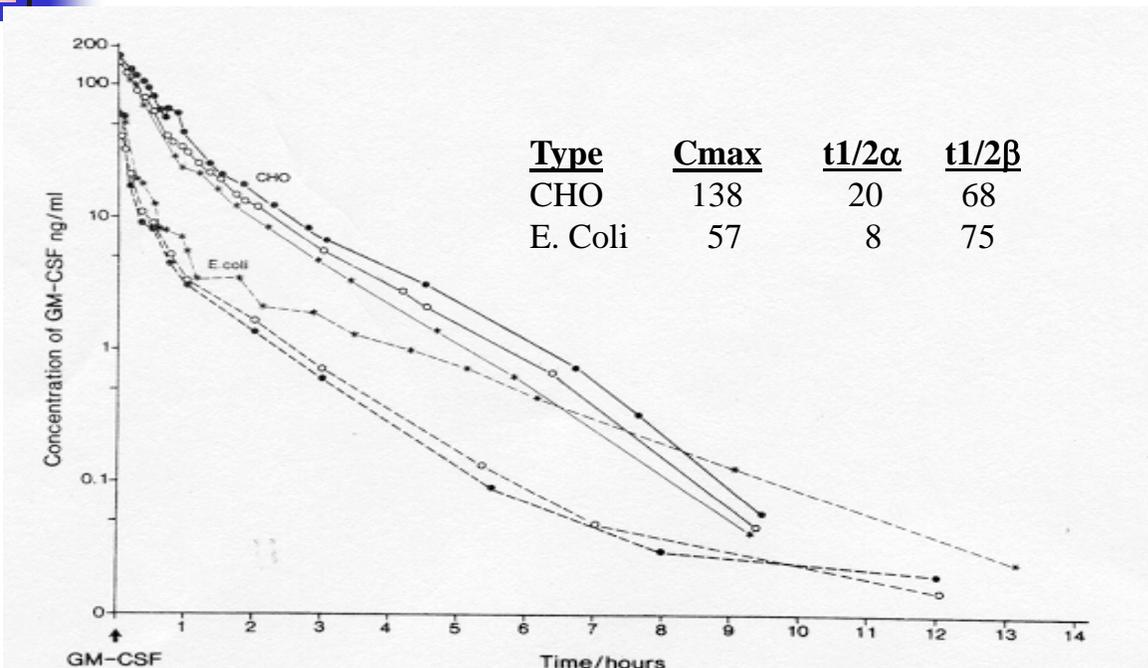
- 5 amino acid exchanges
- Up to 52% carbohydrate
- 5 N-linked sugar chains
- Up to 22 sialic acids
- 38.5 Kd
- Plasma  $T_{1/2}$  = 24 hrs



## METABOLIC FATE OF EPO

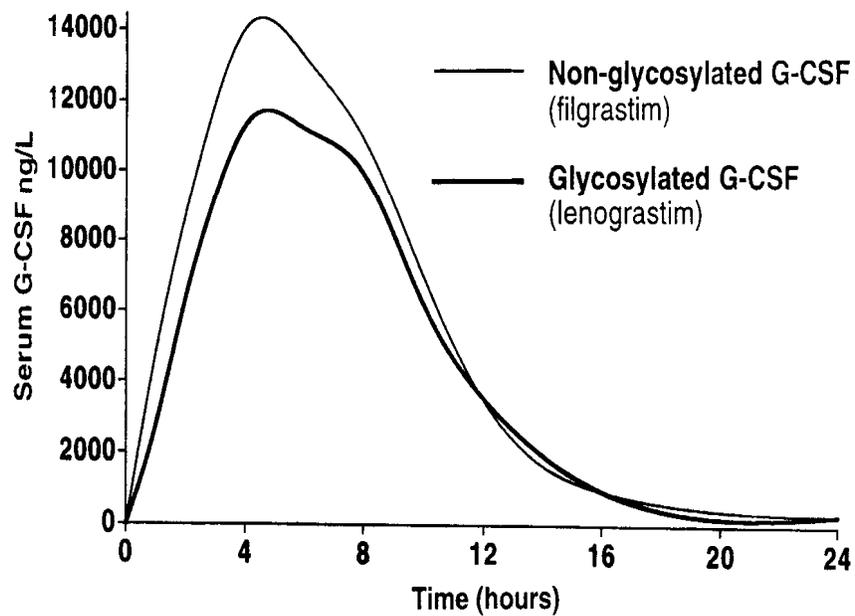


## SERUM CONCENTRATION-TIME PROFILES FOR CHO VS. E. Coli PRODUCED GM-CSF



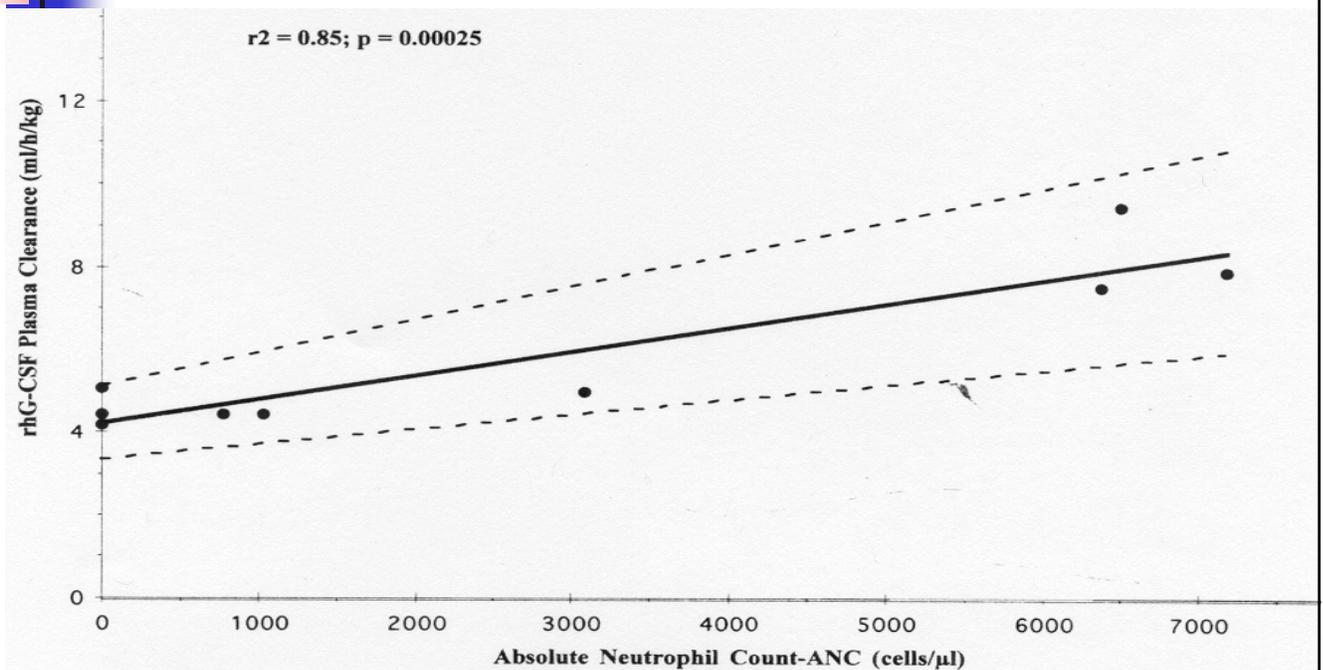
Mortensen HD et al. Eur J Haematol 1993; 50:32-36

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



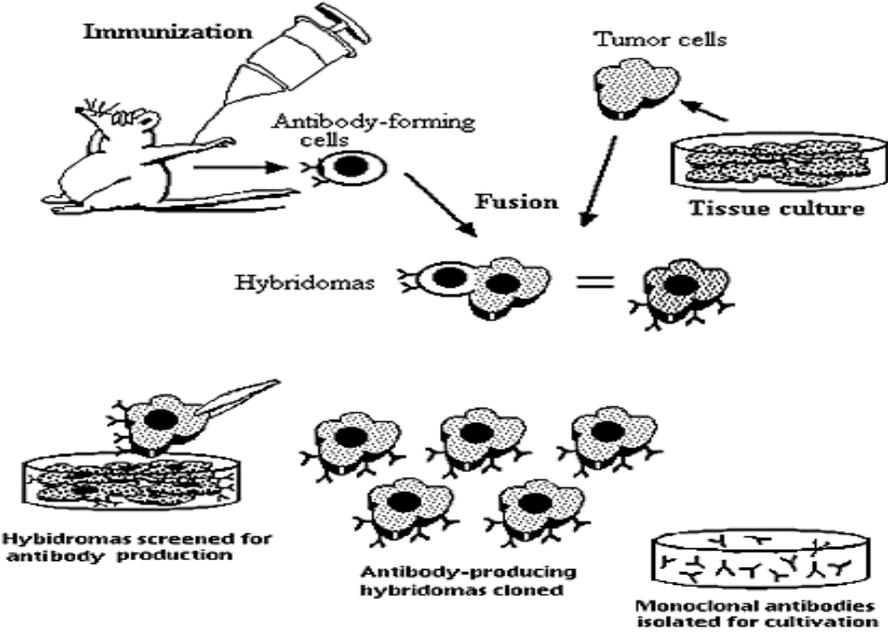
Watts et al. Br J Haematol 1997; 98:474-479

## RELATIONSHIP BETWEEN G-CSF CLEARANCE AND ABSOLUTE NEUTROPHIL COUNT

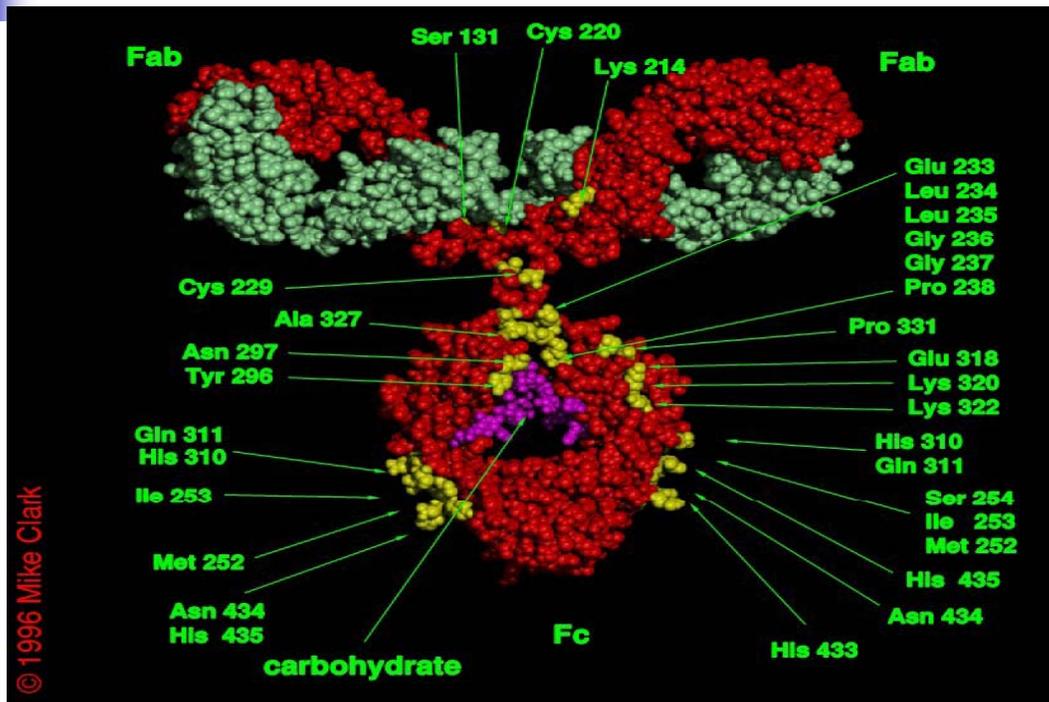


Ericson SG et al. *Exper Hematol* 1997; 25:1313-1325

# MONOCLONAL ANTIBODY PRODUCTION

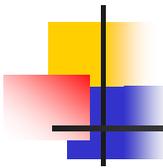


# HUMAN IgG



© 1996 Mike Clark





## CONCEPT OF ANTIBODIES



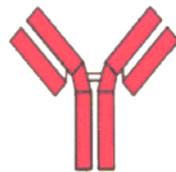
**Murine**



**Chimaeric**



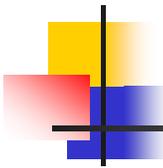
**Humanised**



**Human**

## PROPOSED HUMAN PLASMA CLEARANCE of DIFFERENT ANTIBODY MOLECULES

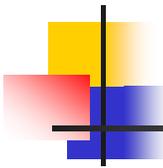
Antibody Molecule	Molecular Weight (kD)	Relative Plasma Clearance (Cl)
Native intact human IgG	150	≈ 21 days
Fully human/humanized	150	↓
Chimeric human-mouse IgG	150	
Whole mouse IgG	150	
F (ab) <sub>2</sub>	110	
Fab'	50	
Single chain FV (scFV)	25	≈ 1 day



## Advantages of mAbs

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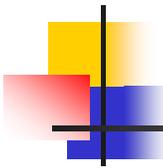
- High specificity
- Long half-life
- Improved benefit-risk ratio (in most therapeutic areas)



## Risks of mAbs

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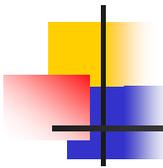
- Immune reactions
  - Signs and symptoms
    - Infusion site reactions
    - Fever
    - Influenza syndrome
    - Acute anaphylaxis
    - Systemic inflammatory responses
- Infection
  - Reactivation of latent bacteria or virus



## Risks of mAbs (continued)

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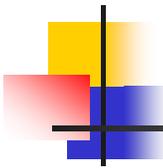
- Platelet and thrombotic disorders
  - Thrombo- and hematopoietic toxicity
- Auto-immune disease
  - Cutaneous or systemic vasculitis
  - Nephritis
  - Colitis
- Cancer



## Safety Related Regulatory Actions for Biologics<sup>1</sup>

- Between 1995 and June 2007, 174 biological products were approved
  - 67 obtained approval in both US and EU
- 82 safety related regulatory actions were issued for 41/174
  - 46 Dear Health Care Professional letter
  - 17 Direct Health Care Professional Communication
  - 19 Black Box warning

<sup>1</sup>GiezenTJ et al. JAMA 2008; 300:18787-1896



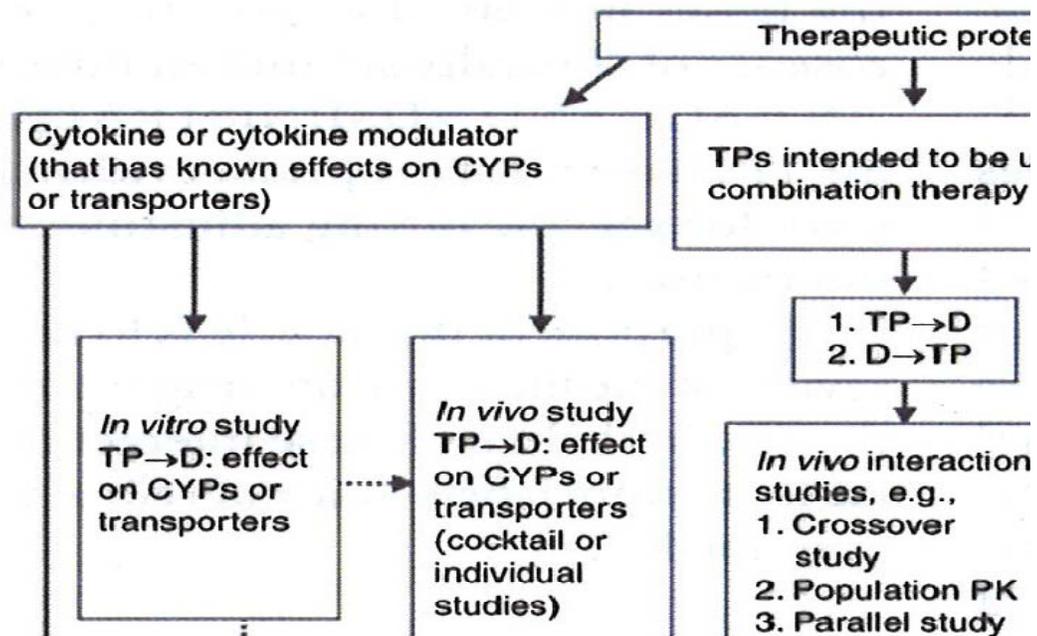
# Drug Interactions

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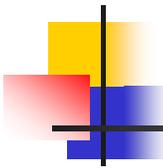
- Some of the principles in the recent draft guidance on drug interactions<sup>1</sup> can apply to biologics

<sup>1</sup>US FDA. Draft Guidance for Industry. Drug Interaction Studies-Study Design, Data Analysis and Implications for Dosing and Labeling.

# Types of DDI Studies Used During Drug Development of Biologics<sup>1</sup>



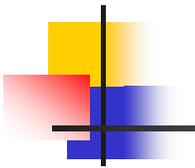
<sup>1</sup>Huang SM, Zhao H, Lee JI et al. CPT 2010;87:497-503



## Points to Consider for DDIs of Biologics

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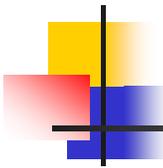
- In vitro or in vivo animal studies have limited value in predicting clinical interactions
- Evaluating drug-drug interactions is particularly important when the therapeutic index is narrow
- Not all interactions between biologics and small molecule drugs are due to CYP or transporter modulation
- If the biologic is a cytokine modulator, there is compelling evidence that cytokine modulation affects the CYP 450 enzyme system



## DESIGN OF ANTIBODIES

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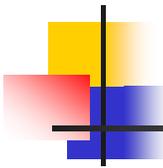
- Molecules that can be attached:
  - Enzymes
  - Toxins
  - Viruses
  - Cationic tails
  - Biosensors



## CHARACTERISTICS THAT AFFECT THE PHARMACOKINETICS OF MACROMOLECULES

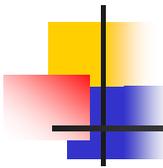
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- Physical characteristics
- Post-translational modification
- Binding
- Route of administration
- Duration of administration
- Frequency of administration



## PATIENT CHARACTERISTICS THAT AFFECT PHARMACOKINETICS OF MACROMOLECULES

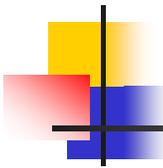
- Age
- Gender
- Disease
- Concurrent drugs



## EFFECTS OF GENDER ON GROWTH HORMONE PK/PD

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- 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

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## The Journal of Clinical Pharmacology

<http://www.jclinpharm.org>

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### **Drug Interaction Studies of Therapeutic Proteins or Monoclonal Antibodies**

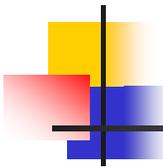
Iftikhar Mahmood and Martin David Green

*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>

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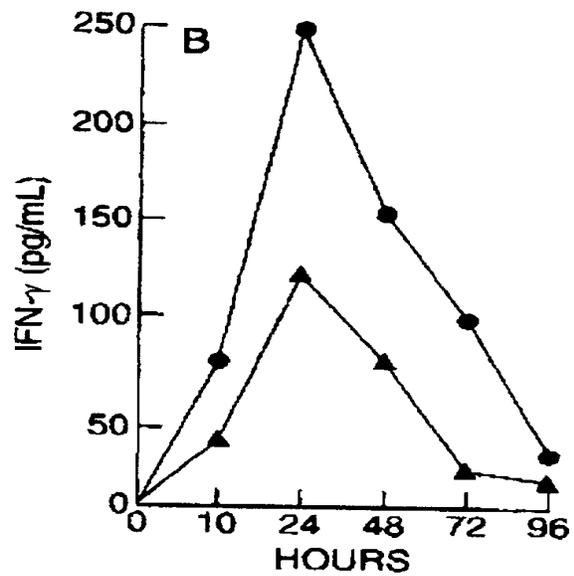
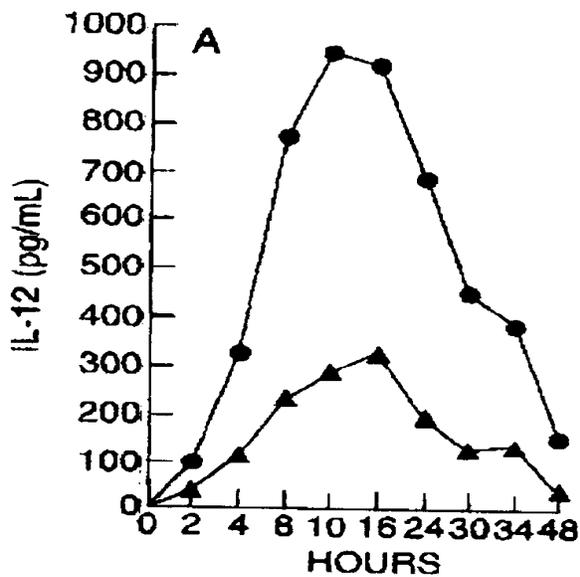


## PHARMACODYNAMICS OF MACROMOLECULES

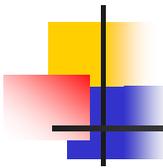
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- Important considerations
  - Regimen dependency
  - Endpoints
  - Models

## REGIMEN DEPENDENCY OF IL-12 PHARMACOKINETICS AND IFN- $\gamma$ STIMULATION



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

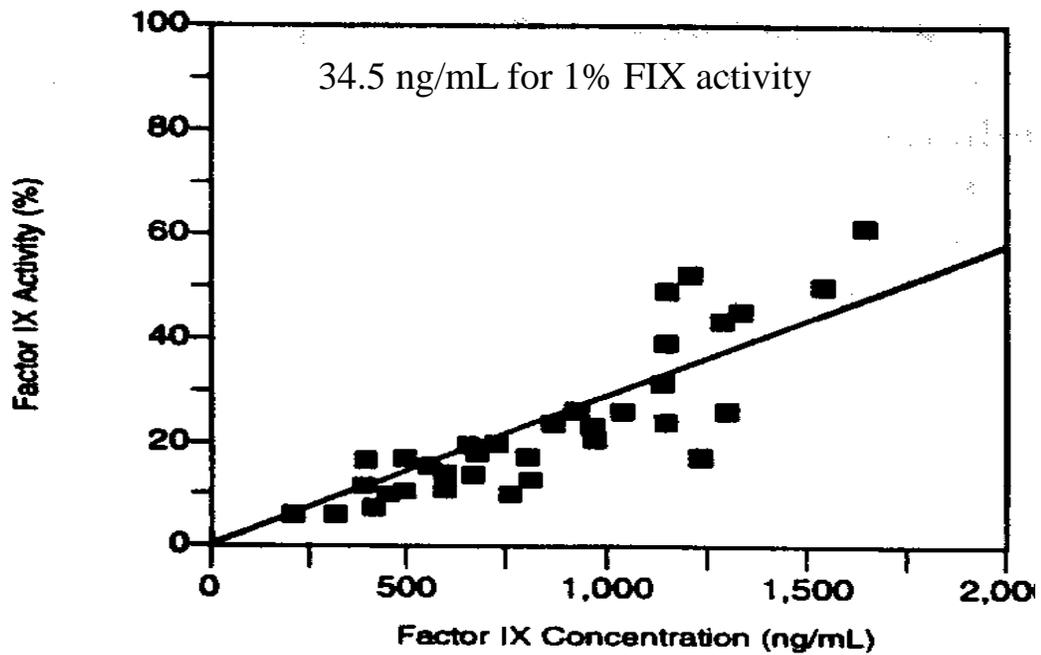


## PHARMACODYNAMIC ENDPOINTS

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- Easy - replacement proteins
  - rFIX
- Difficult- cascade of events
  - IGF-1

## RELATIONSHIP BETWEEN rFIX CONCENTRATION AND ACTIVITY

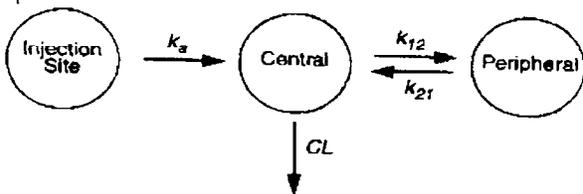


Schaub et al. Seminars in Hematology 1998; 35:28-32

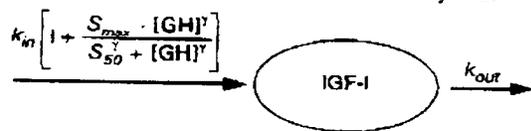
# PK-PD MODEL OF rhGH WITH MEASURED VS. PREDICTED [IGF-1] AFTER SINGLE AND DAILY SC rhGH INJECTIONS

Model of rhGH Pharmacokinetics

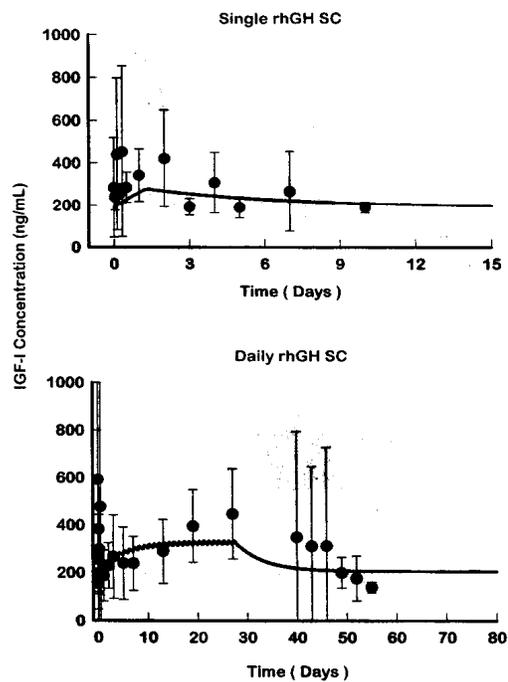
SC Injection

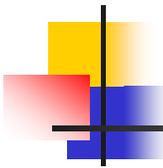


Indirect Response Model of IGF-I Induction by rhGH



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





## PHARMACODYNAMIC ENDPOINTS

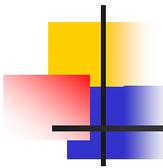
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Omalizumab: Free IgE levels

Clinical outcomes

Basiliximab: Soluble IL-2 receptor

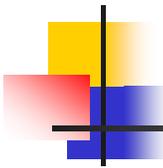
CD25+ T lymphocytes  $\leq 1\%$



## Summary

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- 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

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- Genetic Institute
  - PK/PD Sciences
- Dr. Joyce Mordenti
- Dr. Art Atkinson
- Dr. Juan Lertora