Monoclonal Antibody (mAb) Disposition & Pharmacokinetics

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OBJECTIVES

I. **<u>Brief</u> introduction to pharmacokinetic parameters important in the discovery and development of monoclonal antibody (mAb) therapeutics**
II. **Overview of the properties and processes that govern mAb disposition**
III. **Discuss and provide examples of the role biophysical characterization has in understanding mAb disposition and engineering of molecules**

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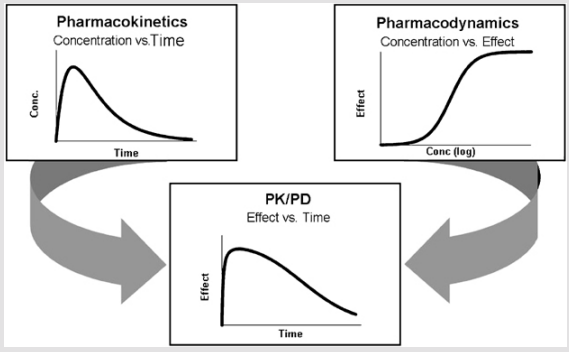
INTEGRATIVE PHARMACOLOGY

PK – what the body does to the drug



PD – what the drug does to the body

- **Pharmacokinetics (PK): the study of the time course of drug concentration *in different body spaces* & the relationship between concentration and the time course of drug action.**
- **Pharmacodynamics (PD): the study of the time course of the biological effects of drugs.**

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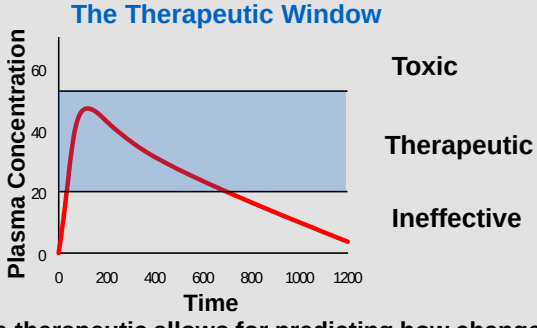
PK DESCRIBES THE KINETICS OF DRUG ABSORPTION AND DISPOSITION

Assumption: the safety (adverse effects) and efficacy (therapeutic effects) of a drug are related to its concentration



- **Understanding the PK of a therapeutic allows for predicting how changes in dosing variables (e.g. dose amount, route, frequency, etc.) or how altering the molecular characteristics of a therapeutic will impact the concentration versus time profile and therefore the therapeutic profile.**

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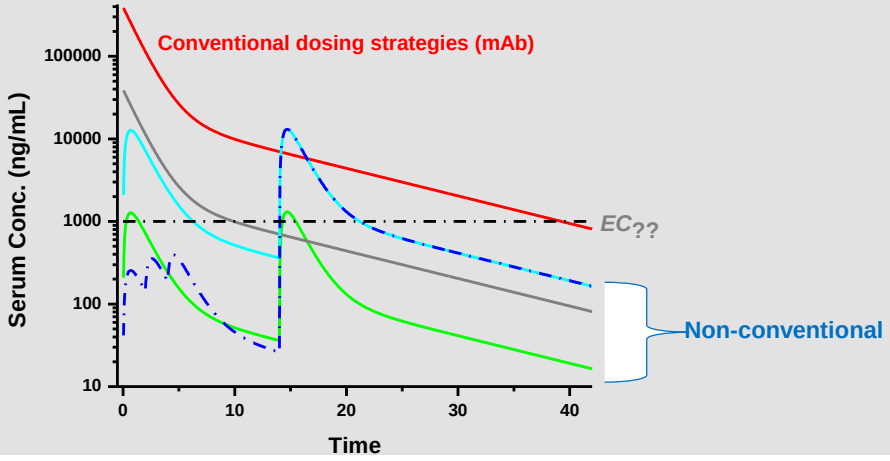
PK, PHARMACOLOGY, & BIOLOGY GUIDE DOSING



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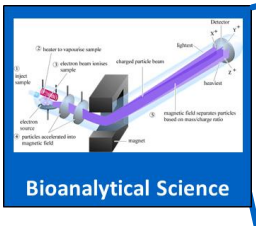
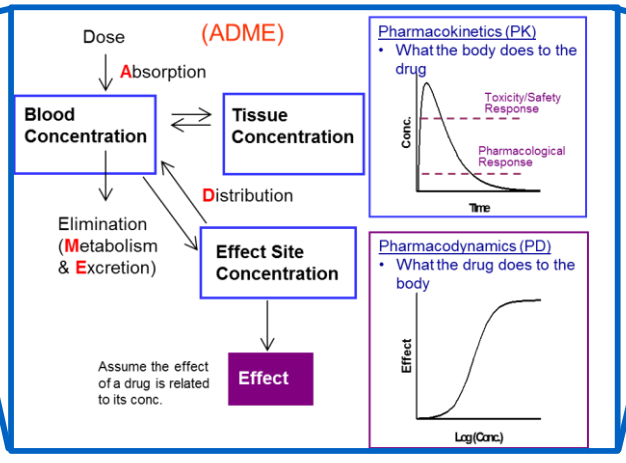
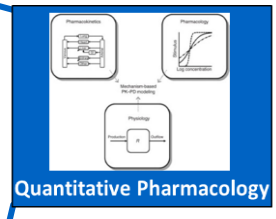
CHARACTERIZATION OF DRUG DISPOSITION & PK/PD



Combine BA and QP to combine to establish core deliverables





Apply across therapeutic areas, modality in discovery and development

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PRIMARY PK PARAMETERS: CLEARANCE & VOLUME

Clearance (CL):

The volume of blood that is cleared of its content of drug per unit time (mL/min, L/hr, mL/day, etc.)

- **Describes the capacity of irreversible elimination of a compound from blood and is a consequence of perfusion, diffusion, filtration, metabolism and transport**

Volume of distribution (V):

The apparent volume into which drug distributes to account for measured compartment concentration at a given dose

- **Describes the relationship between the drug concentration in the accessible body fluid (blood) and the drug in the tissues of the body at the site of action(s)**

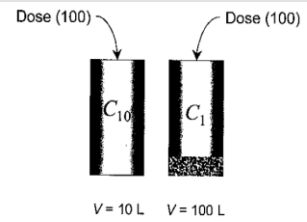

Figure 2.3 Schematic illustration of the volume of distribution, V. A dose of 100 units of drug is dissolved in each of the buckets, resulting in a concentration C of 10 and 1 unit·L^{-1} since the apparent bucket volumes are 10 and 100 L, respectively. The greyish area at the bottom of the right hand bucket represents active charcoal.

Gabrielsson & Weiner *Pharmacokinetic & Pharmacodynamic Data Analysis 4th Ed.*

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SECONDARY PARAMETERS

- **Elimination rate constant (k_{el} or K) :**
  - **Fractional rate constant relating amount of drug in the body which is eliminated per unit time**
- **Half-life ($t_{1/2}$):**
  - **Expresses the period of time required for the amount or concentration of drug to decrease by one-half.**
  - **$t_{1/2} = \ln 2/k_{el}$**
- **Area under the concentration-time curve (AUC):**
  - **Relates to the amount of drug absorbed systemically**
  - **Units: unit material * volume^{-1} * time; e.g. µg*hr/L**

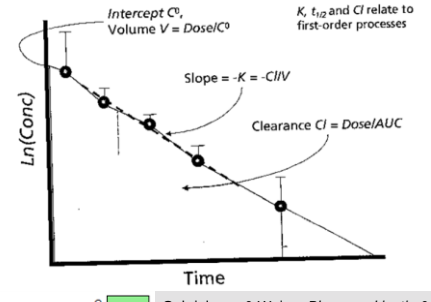
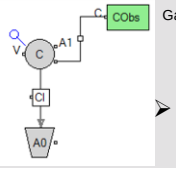


Figure 2.6 Semi-logarithmic plot of plasma concentration-time data. The data show mono-exponential decline described by Equation 2:2. The intercept of the concentration axis is C⁰, slope is (-1 times) the elimination rate constant K, volume V is the ratio of Dose-to-C⁰, and clearance Cl is obtained from the ratio of Dose-to-area under the curve AUC.

Gabrielsson & Weiner *Pharmacokinetic & Pharmacodynamic Data Analysis 4th Ed.*



➢ **Representation of 1 compartment, i.v. dosing model with 1st order elimination**

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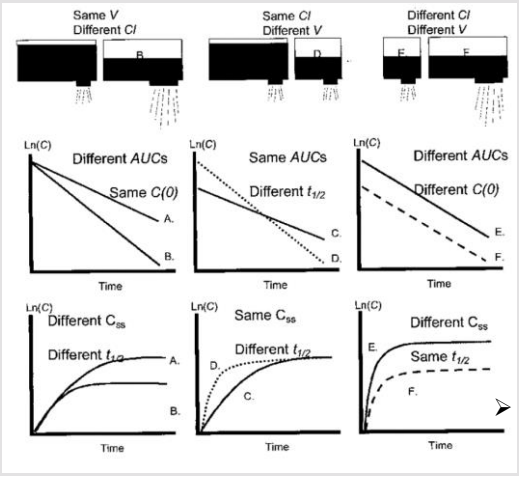
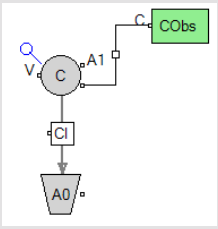
INTEGRATION OF VOLUME & CLEARANCE





➢ **The *relationship* between CL and V parameters determines the observed pharmacokinetic time course of a drug.**

➢ **Repeat dosing; same frequency**

Gabrielsson & Weiner *Pharmacokinetic & Pharmacodynamic Data Analysis 4th Ed.*

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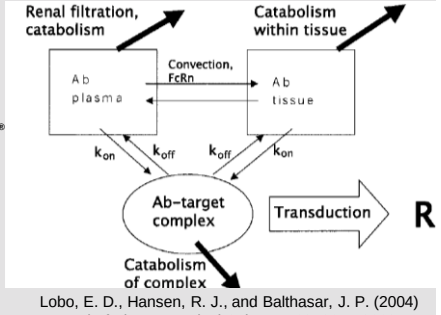
FACTORS INFLUENCING DISPOSITION & PK OF PROTEIN THERAPEUTICS

- **Administration route and target access**
  - **Parenteral dosage forms: Intravenous (i.v.), sub-cutaneous (s.c.), intramuscular (i.m.)**
  - **Extent of biodistribution limited by size and/or physicochemical properties**
- **Size of molecule/composition/charge**
  - **Addition of Fc units – Enbrel®, Orencia® (CTLA4-Ig)**
  - **Addition of carbohydrate moieties - Epogen® vs. Aranesp®**
  - **PEGylation/other modifications -Neupogen® vs. Neulasta®, Cimzia®**
- **Target**
  - **Regulation/Distribution of target protein**
  - **Circulating versus cell surface bound**
- **Immunogenic Response**
  - **Neutralizing vs. Non-neutralizing responses**



Lobo, E. D., Hansen, R. J., and Balthasar, J. P. (2004) Journal of Pharmaceutical Sciences 93, 2645-2668.

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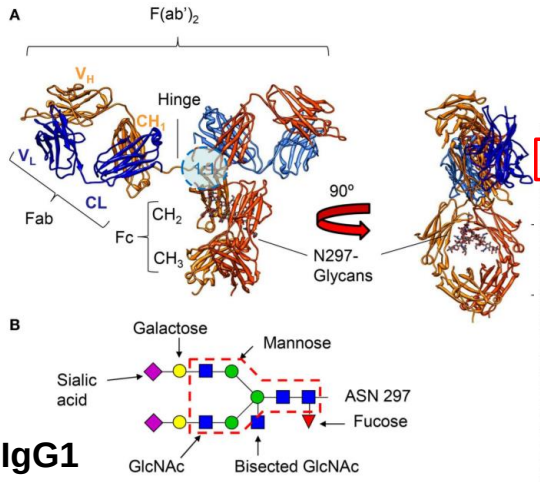
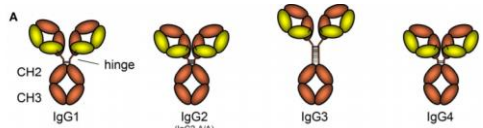
Variable IgG Structure and Effector Function





	IgG1		IgG2 (IgG2-A/A)		IgG3		IgG4	
General								
Molecular mass (kD)	146		146		170		146	
Amino acids in hinge region	15		12		62[a]		12	
Inter-heavy chain disulfide bonds	2		4[b]		11[a]		2	
Mean adult serum level (g/l)	6.98		3.8		0.51		0.56	
Relative abundance (%)	60		32		4		4	
Half-life (days)	21		21		7/~21[a]		21	
Placental transfer	++++		++		++/++++[a]		+++	
Antibody response to:								
Proteins	++		+/–		++		++[c]	
Polysaccharides	+		+++		+/–		+/–	
Allergens	+		(–)		(–)		++	
Complement activation								
C1q binding	++		+		+++		–	
Fc receptors								
FcγRI	+++[c]	65[d]	–	–	++++	61	++	34
$FcγRIIa_{H131}$	+++	5.2	++	0.45	++++	0.89	++	0.17
$FcγRIIa_{R131}$	+++	3.5	+	0.10	++++	0.91	++	0.21
FcγRIIb/c	+	0.12	–	0.02	++	0.17	+	0.20
$FcγRIIIa_{F158}$	++	1.2	–	0.03	++++	77	–	0.20
$FcγRIIIa_{V158}$	+++	2.0	+	0.07	++++	9.8	++	0.25
FcγRIIIb	+++	0.2	–	–	++++	1.1	–	–
FcRn (at pH < 6.5)	+++		+++		++/+++[a]		+++	

[a] Depends on allotype.
[b] For A/A isomer.
[c] Multivalent binding to transfected cells. Adapted from Bruhns et al. (2).
[d] Association constant ($\times 10^8\ M^{-1}$) for monovalent binding (2).
[e] After repeated encounters with protein antigens, often allergens.

Vidarsson, G., Dekkers, G., and Rispens, T. (2014) IgG Subclasses and Allotypes: From Structure to Effector Functions, Frontiers in Immunology 5, 520.

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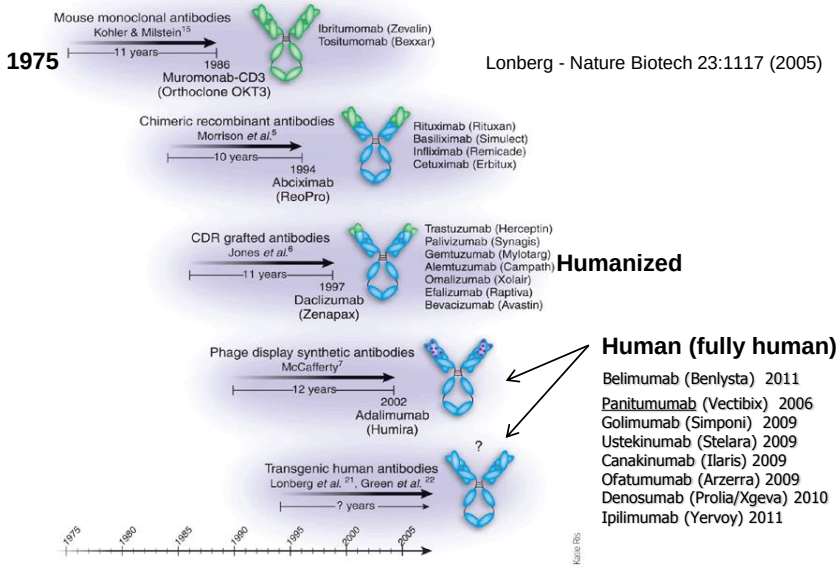
ANTIBODY ENGINEERING – TECHNOLOGIES



Lonberg - Nature Biotech 23:1117 (2005)

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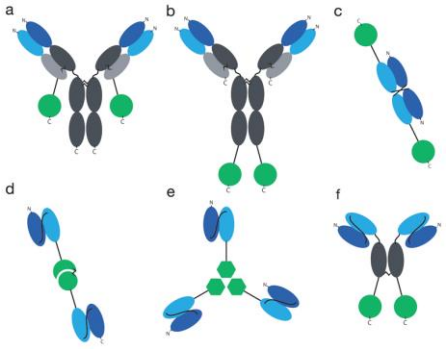
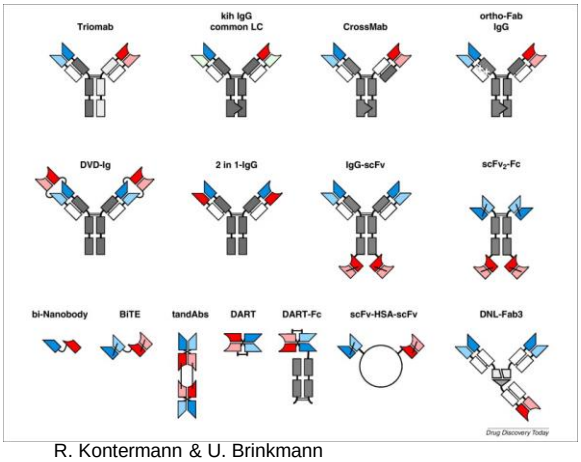
IMMUNOCYTOKINES, BISPECIFICS AND FUSION PROTEINS: EXAMPLES OF NEXT GENERATION SCAFFOLDS

Examples of immunocytokines & fusions



J. Kiefer. & D. Neri
Immunol Rev. 2016 Mar; 270(1): 178–192.
doi: 10.1111/imr.12391

Examples of bispecifics



R. Kontermann & U. Brinkmann
Drug Discovery Today, Volume 20, Issue 7, 2015, 838–847
http://dx.doi.org/10.1016/j.drudis.2015.02.008

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ELIMINATION OF MONOCLONAL ANTIBODIES (mAbs)

Two primary elimination routes :

1. **'Nonspecific' elimination via phagocytic and endothelial cells of the reticuloendothelial system (RES)**
   - **RES clears both antigen-bound and free (unbound) mAbs, is <u>not saturable</u> and thus follows linear kinetics**
2. **Antigen specific target-mediated disposition (TMD)**
   - **Because target-mediated disposition is dependent on binding to antigen (target) this is usually a saturable process for membrane associated antigens and therefore may follow nonlinear kinetics**

➢ ***Depending on the antibody isoform and target, the relative contribution to mAb clearance from these elimination routes will vary***

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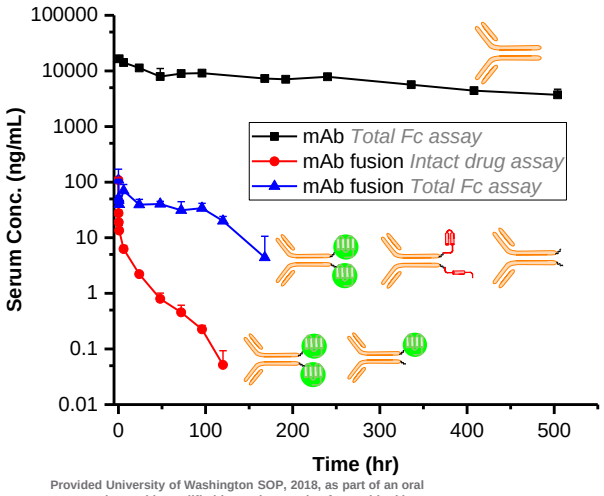
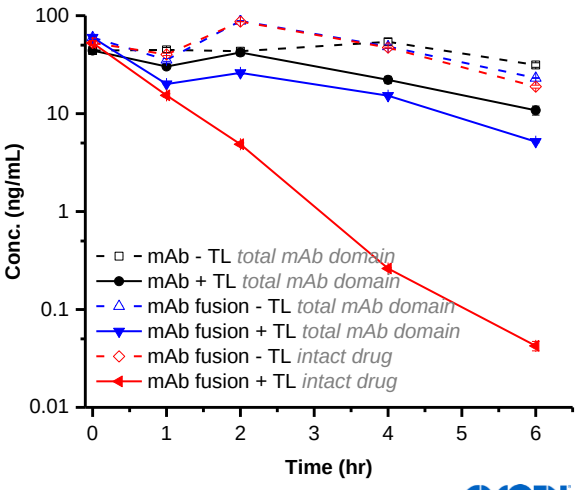
CATABOLISM OF MULTI-SPECIFIC AND FUSIONS IS OF PRIMARY CONCERN FOR ACCURATE PKPD

In vivo mouse PK



In vitro thermolysin stability (37°C)



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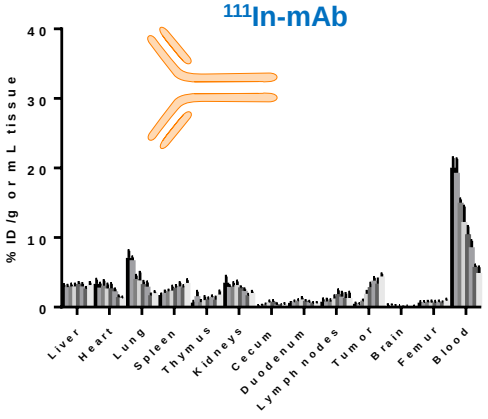
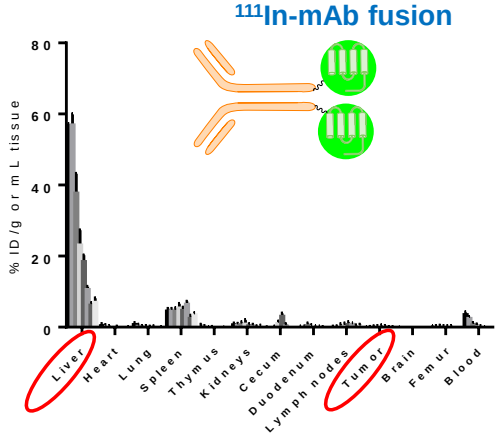
ENGINEERING CAN ALTER THE PRIMARY SITES OF UPTAKE & CATABOLISM

^{111}In-mAb



^{111}In-mAb fusion



- **mAb fusion demonstrates minimal to no tumor accumulation.**
- **We must characterize the biodistribution to guide engineering strategies and rationalize pharmacology of antibody-derived drugs**

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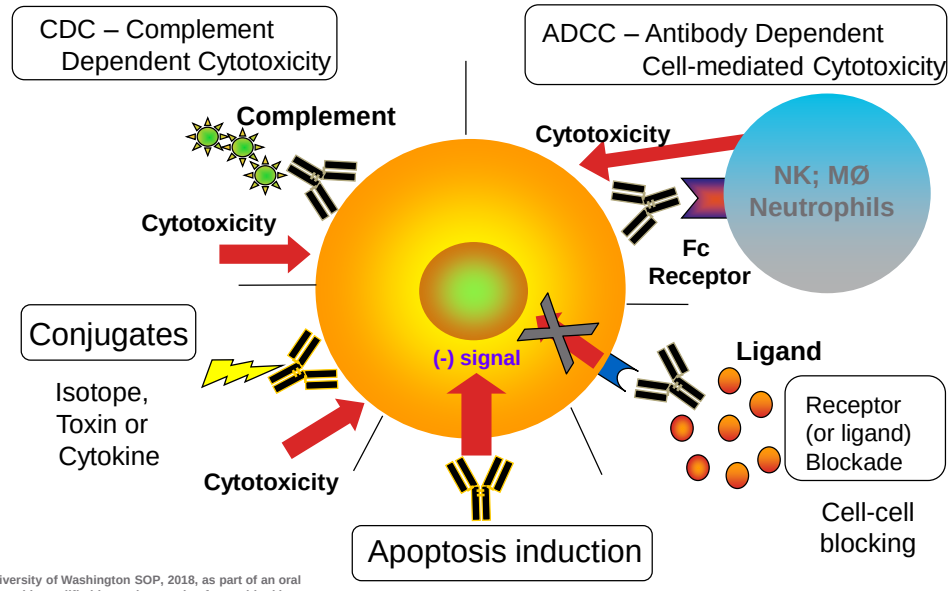
mAb therapeutic functions



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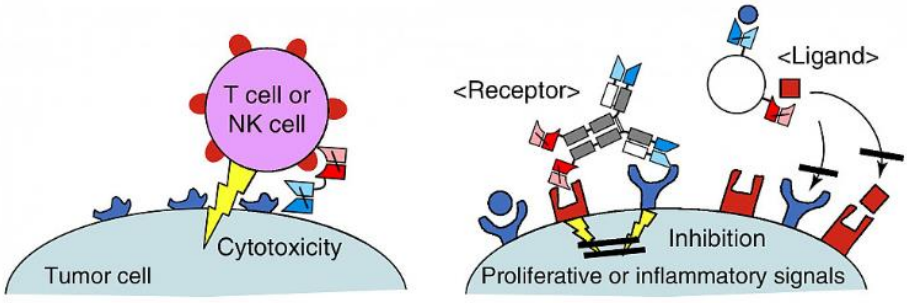
NEXT GENERATION PROTEIN THERAPEUTICS:

ADDING FUNCTIONALITY FOR IMPROVED SELECTIVITY THROUGH TARGETING OR FOR NOVEL MECHANISM OF ACTION



Kontermann and Brinkmann. Bispecific Antibodies. *Drug Discovery Today;* Volume 20, Number 7, July 2015

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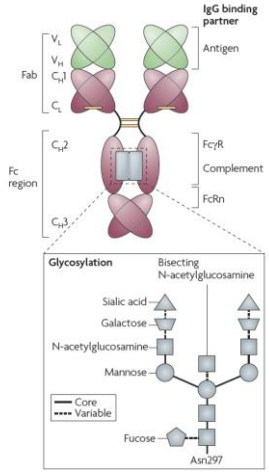
SELECT IGG ISOFORM AND MODIFICATIONS BASED ON THERAPEUTIC NEED; IMPACTS MAB DISPOSITION



IgG binding partner	Protein strategies for modifying interactions	Potential impact of modifying interaction
Antigen	Mutate V domain sequences using display libraries and/or rationale design	Altered binding affinity or specificity
FcγR, Complement	Mutate Fc sequence using display libraries and/or rationale design; select IgG isotype	↑ or ↓ ADCC ↑ or ↓ ADCP ↑ or ↓ CDC
FcRn	Mutate Fc sequence using display libraries and/or rationale design	↑ or ↓ half-life
	Antibody fragment lacking Fc	↓ Half-life, ↓ CDC, ↓ ADCC and ↓ ADCP
	Glycosylation strategies for modifying FcγR and complement interactions	
	Aglycosylation	↓ ADCC, ↓ ADCP and ↓ CDC
	Bisecting N-acetylglucosamine	↑ ADCC
	Non-fucosylation	↑ ADCC

Cahn & Carter (2010) *Nat. Rev.* Immunol. 10: 301-16

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- ***Antigen non-specific elimination and salvage of mAb therapeutics through RES and other receptor mediated processes***

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ELIMINATION OF THERAPEUTIC PROTEINS

- **Same catabolic pathways as endogenous or dietetic peptides and proteins**
- **Negligible non-metabolic elimination**

Molecular weight	Elimination site	Predominant elimination mechanisms	Major determinant
<500	Blood, liver	Extracellular hydrolysis Passive lipoid diffusion	Structure, lipophilicity
500–1,000	Liver	Carrier-mediated uptake Passive lipoid diffusion	Structure, lipophilicity
1,000–50,000	Kidney	Glomerular filtration and subsequent degradation processes (see Fig. 4)	Molecular weight
50,000–200,000	Kidney, liver	Receptor-mediated endocytosis	Sugar, charge
200,000–400,000		Opsonization	α_2-macroglobulin, IgG
>400,000		Phagocytosis	Particle aggregation

Note: Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability for proteases, aggregation to particles, formation of complexes with opsonization factors, etc. Mechanisms may overlap and endocytosis may occur at any molecular weight range.

Meibohm & Braeckman, In: Crommelin, Sindelar, Meibohm (eds.), Pharmaceutical Biotechnology, 3rd ed, Informa Healthcare 2007

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HEPATIC UPTAKE AND DEGRADATION OF PROTEINS

- **Major site of protein metabolism for larger molecular weight proteins**
  - **Usually initiated by endopeptidases with subsequent proteolytic degradation by exopeptidases**
- **Intracellular uptake as a prerequisite**
  - **Small peptides**
    - **Passive diffusion or carrier-mediated uptake and subsequent degradation**
  - **Large peptides, proteins, and antibodies**
    - **Pinocytosis (fluid-phase endocytosis)**
    - **Receptor-mediated endocytosis**

    Examples:
      - **Low density lipoprotein receptor (LDLR)**
      - **Low density lipoprotein-related protein (LPR)**
      - **'Scavenger' Receptors: mannose/fucose-binding C-type lectin receptors; β-GalNAc**
    - **Target-mediated endocytosis**

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HEPATIC UPTAKE MECHANISMS & CATABOLISM

Cell type	Uptake mechanism	Proteins/peptides transported
Hepatocytes	Anionic passive diffusion Carrier-mediated transport	Cyclic and linear hydrophobic peptides (<1.4 kDa; e.g., cyclosporins, CCK-8)
	RME: Gal/GalNAc receptor (asialoglycoprotein receptor)	N-acetylgalactosamine-terminated glycoproteins, galactose-terminated glycoproteins (e.g., desialylated EPO)
	RME: Low density lipo-protein receptor (LDLR)	LDL, apoE- and apoB-containing lipoproteins
	RME: LDLR-related protein (LRP receptor)	α_2-macroglobulin, apo-E-enriched lipoproteins, lipoprotein lipase (LpL), lactoferrin, t-PA, u-PA, complexes of t-PA and u-PA with plasminogen activator inhibitor type 1 (PAI-1), TFPI, thrombospondin (TSP), TGF-β and IL-1β bound to α_2-macroglobulin
	RME: Other receptors	IgA, glycoproteins, lipoproteins, immunoglobulins intestinal and pancreatic peptides, metallo- and hemoproteins, transferrin, insulin, glucagon, GH, EGF
	Nonselective pinocytosis (non-receptor-mediated)	Albumin, antigen–antibody complexes, some pancreatic proteins, some glycoproteins
Kupffer cells	Endocytosis	Particulates with galactose groups
Kupffer and endothelial cells	RME	IgG, N-acetylgalactosamine-terminated glycoproteins
	RME: Mannose receptor	Mannose-terminated glycoproteins (e.g., t-PA, renin)
	RME: Fucose receptor	Fucose-terminated glycoproteins
Endothelial cells	RME: Scavenger receptor	Negatively charged proteins
	RME: Other receptors	VEGF, FGF (?)
Fat-storing cells	RME: Mannose-6-phosphate receptor	Mannose-6-phosphate-terminated proteins (e.g., IGF-II)

Abbreviation: RME, receptor-mediated endocytosis.

Meibohm & Braeckman, In: Crommelin, Sindelar, Meibohm (eds.), Pharmaceutical Biotechnology, 3rd ed, Informa Healthcare 2007

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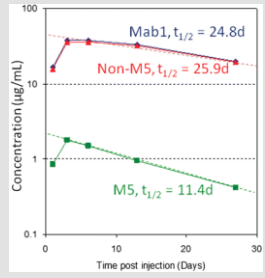
MANNOSE RECEPTOR IMPACT ON THE PK OF MABS

High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans

Table VI.
Calculated decrease in total PK AUC attributable to faster clearance of therapeutic Mabs containing at least one Fc M5 glycan at different dosing regimens (see text for details)

Mab	Dose	% decrease in AUC due to faster M5 clearance, average of 2 (range)	PK time range (h)
Mab1	1000 mg intravenously	1.06 (0.84–1.27)	1–816
	300 mg intravenously	0.90 (0.85–0.94)	2–816
	100 mg intravenously	1.09 (0.95–1.22)	1–312
	300 mg subcutaneously	1.70 (1.63–1.77)	24–648
Mab2	1000 mg intravenously	2.77 (2.58–2.96)	1–168
Mab3	20 mg/kg intravenously	1.31 (1.02–1.59)	1–696
Mab4	20 mg/kg intravenously	5.83 (5.73–5.92)	0.5–336



Goetze *Glycobiology* 2011 21(7): 949-959

- **Secondary and tertiary structure predicted to prevent uptake through mannose receptor**
- **Likely antibody specific due to conformational dynamics**
- **Mannose content tracked as a "quality attribute" for drug product**

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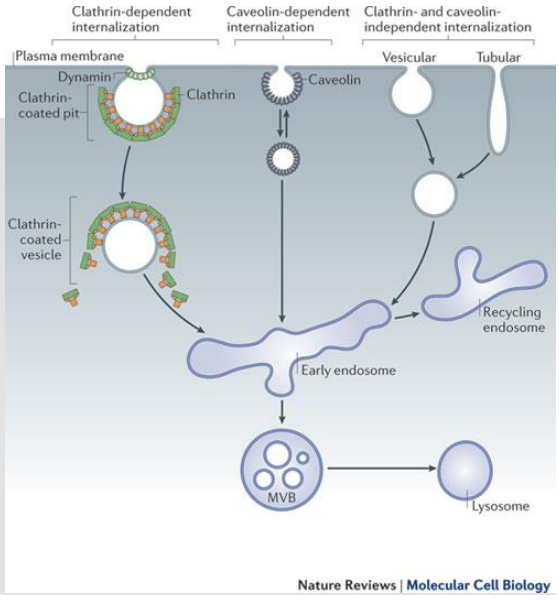
MOLECULAR UPTAKE

- **Wide array of internalization mechanisms on eukaryotic cells**
- **Clathrin-independent**
  - **Phagocytosis**
  - **Macropinocytosis**
  - **Caveolin-dependent endocytosis**
- **Clathrin-dependent**
  - **Receptor-mediated endocytosis**

➢ **mAbs serve as opsonins and enhance binding for phagocytosis through Fc receptors**



McMahon, H. & Boucrot, E. (2011) *Nature Rev. Mol. Cell.* 12: 517-533

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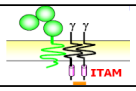
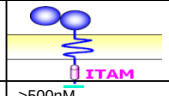
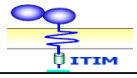
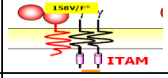
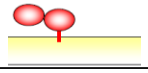
HUMAN FC GAMMA R PROPERTIES

Receptor	FcgR1 (CD64)	FcgR IIa (CD32)	FcgR IIb (CD32)	FcgR IIIa (CD16)	FcgR IIIb (CD16)
Structure					
Binding to IgG1 Order of affinity	1-10nM IgG1=G3 IgG4 IgG2	>500nM IgG1=G3 IgG1=G2* IgG4	>500nM IgG1=G3 IgG4 IgG2	>500nM IgG1=G3 IgG4 IgG2	? IgG1=G3 IgG4 IgG2
Cellular expression	MO, DC, eosinophils neutrophils	MO, DC, platelets, eosinophils, neutrophils	B cells, MO, DC, neutrophils	**NK**, activated MO, DC	Neutrophils
Function	Phagocytosis, monomeric mAb and immune complex uptake, stimulation for cytokine secretion	Phagocytosis, immune complex uptake, stimulation for cytokine secretion	Inhibition of immune response	**ADCC**	Activation upon cross-linking?
Allelic prevalence (caucacians)		131H/H: 44% 131H/R hets:31% 131R/R: 25%	I232:: T232:	**158V/V: 11%** 158F/V hets:39% 158F/F:50%	Wt isoform is158V/V

Cell killing: IgG1=IgG3>IgG4>IgG2 Gessner *Ann. Hematol.* 1998, 76:231-48

AMGEN

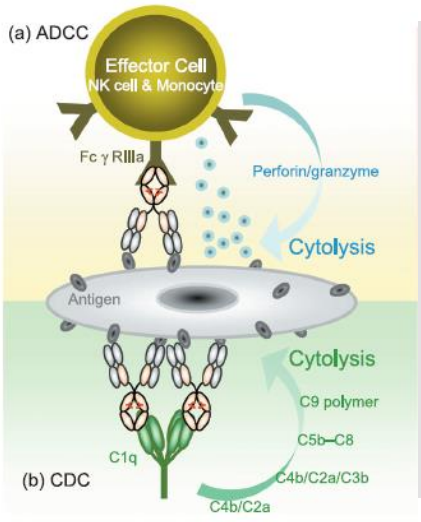
ADCC and CDC: Mechanisms of Action

ADCC
1. Fc receptors on effector cell binds target-engaged mAbs
2. Initiates release of cytolytic enzymes

*Enhanced FcR-Fc interactions lead to improved clinical outcomes

CDC
1. C1q complement protein binds "multimeric" IgG bound to target
2. Initiates complement cascade

Role of FcR in clearance?



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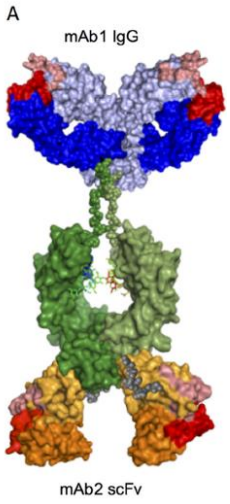
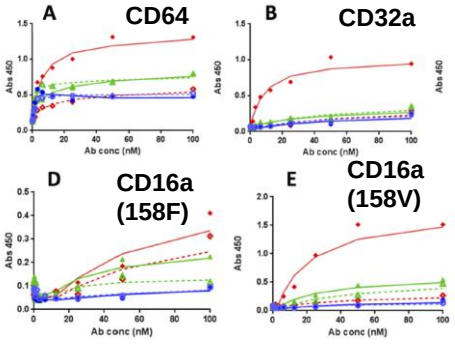
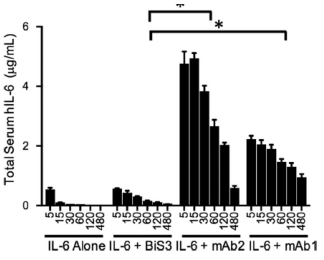
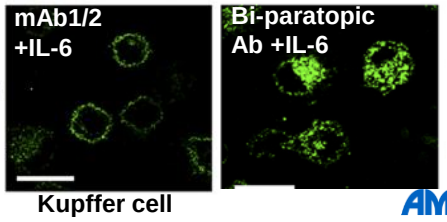
ENGINEERING FC GAMMA INTERACTIONS TO ENHANCE CL OF SOLUBLE ANTIGEN









Kasturirangan, S., et al. (2017) *The Journal of biological chemistry 292*, 4361-4370.

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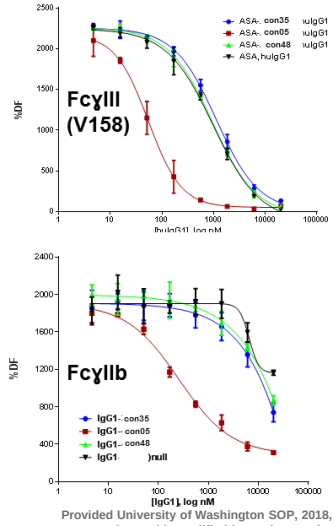
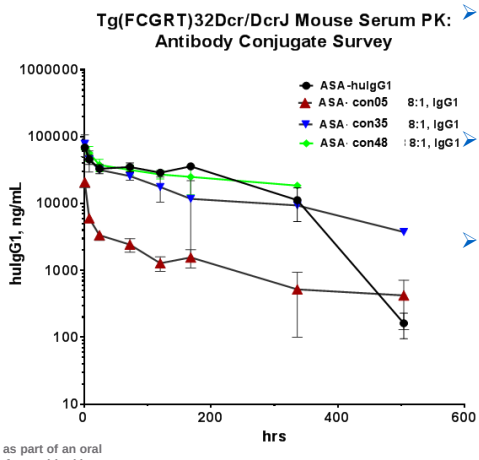
Chemical Modification of IgGs Affects Specific Receptor-Mediated Clearance Mechanisms *in vivo*

Conjugate selectivity *in vitro*:



Conjugate selectivity *in vivo*:

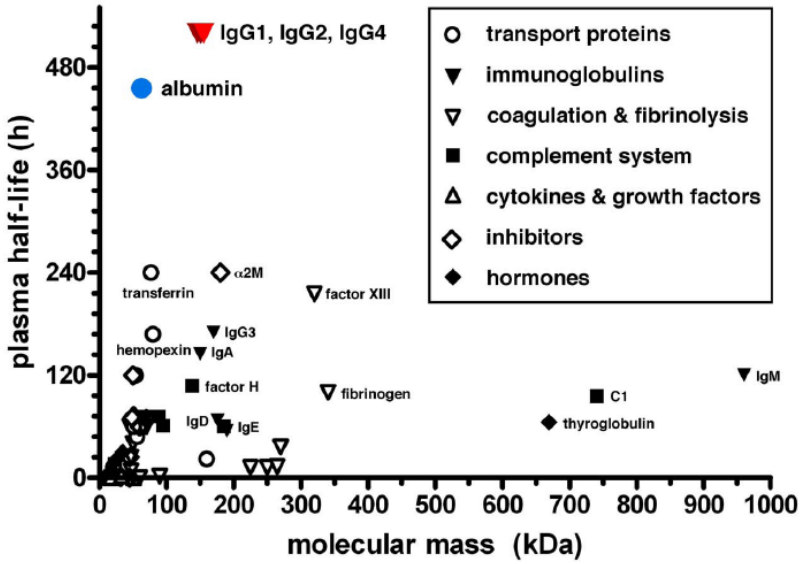


- **Pyrene-based fluor selectivity *in vitro* supported by *in vivo* PK results**
- **Strong correlation between FcγR affinity (IC_{50}) *in vitro* and *in vivo* CL**
- **Suggests chemical conjugation can influence mAb biodisposition via receptor-mediated effects**

AMGEN

Half-lives of plasma proteins

R. Kontermann- Beyond Abs Mtg 2008



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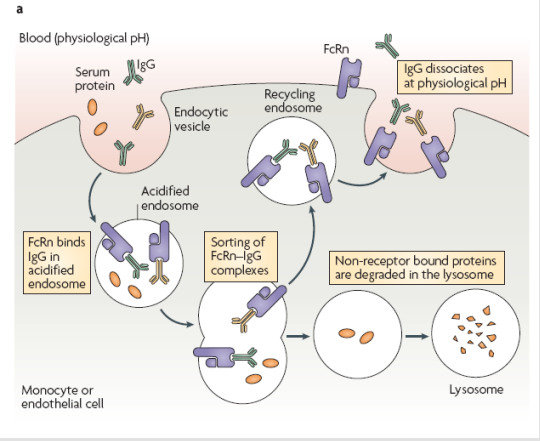
NEONATAL FC RECEPTOR (FCRN) MEDIATES RECYCLING OF IGG & ALBUMIN



Roopenian & Akilesh *Nat Rev Immunol* 7:715

FcRn:

Fc receptor neonatal

- Brambell identified receptor responsible for maternal to fetal IgG transfer
- Heterodimer consisting of the glycosylated class I MHC protein αFcRn and a β_2 microglobulin (m) subunit
- Expressed in endothelial cells of vasculature, APC, Kidney, muscle
- IgG recycling due to pH-dependent binding to FcRn in acidic endosomes

IgG 1, 2, and 4 ~ 21d T ½

IgG3 ~ 7d T ½ (H435R)

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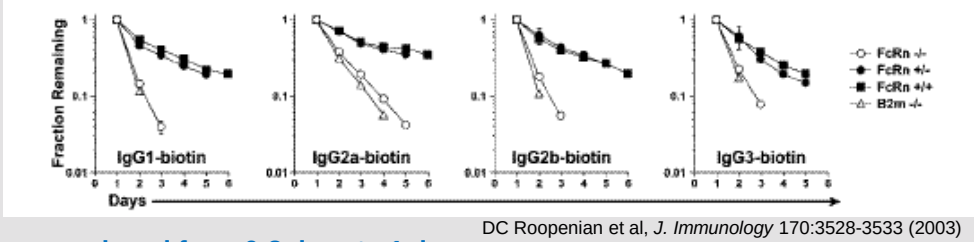
FCRN KNOCKOUT MICE HAVE SIGNIFICANTLY INCREASED IGG ANTIBODY CLEARANCE



DC Roopenian et al, *J. Immunology* 170:3528-3533 (2003)

Half-lives were reduced from 6-8 days to 1 day

- **Consistent with other proteins that are not efficiently cleared by the kidney**

KO mice have 20-30% of the normal IgG levels as wild type mice

- **IgG1 is present at a concentration of 8mg/ml in human serum (therapeutic antibodies are usually between 1-300ug/ml)**
- **90% of serum proteins are IgG**

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➢ *Antigen specific elimination*

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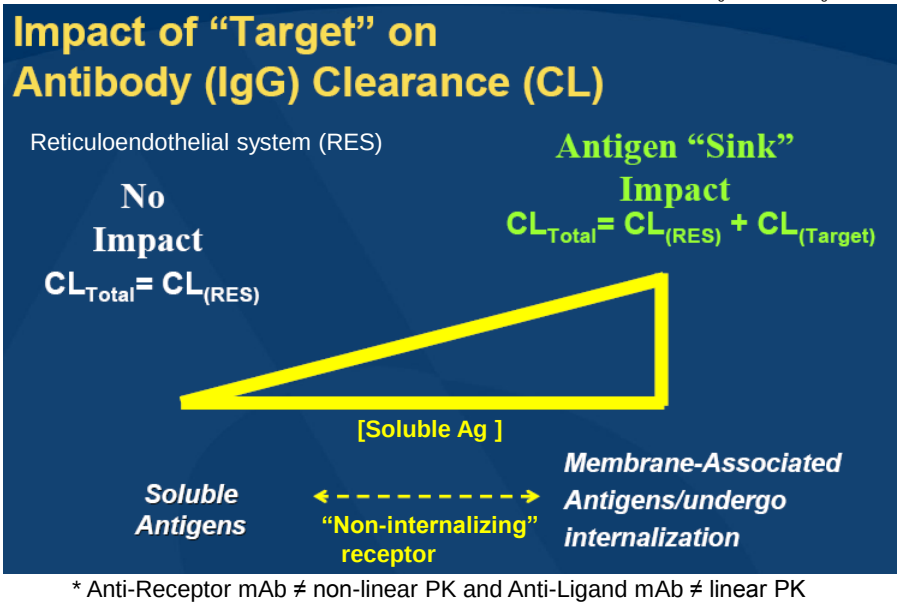
2006 IBC mAb meeting: M. Tabrizi – Abgenix



- **High concentrations of soluble Ag can increase clearance**

* Anti-Receptor mAb ≠ non-linear PK and Anti-Ligand mAb ≠ linear PK

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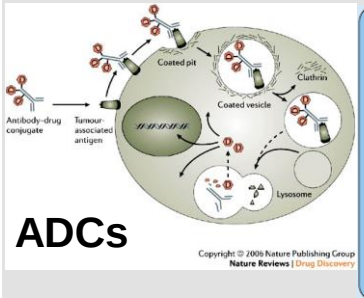
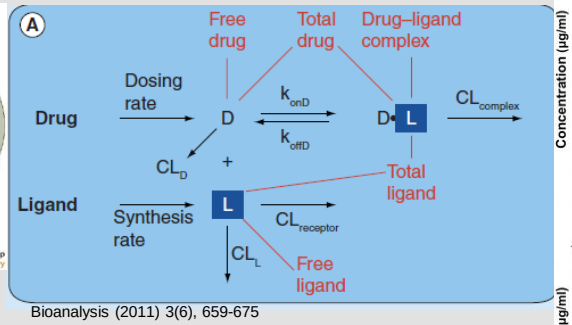
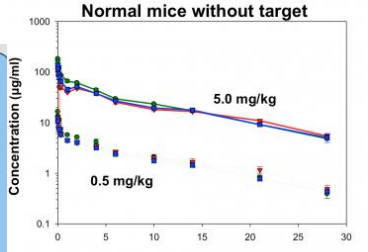
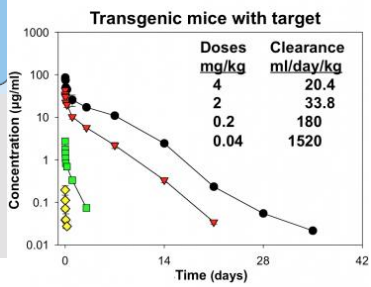
TARGET-MEDIATED DISPOSITION (CLEARANCE)





Bioanalysis (2011) 3(6), 659-675

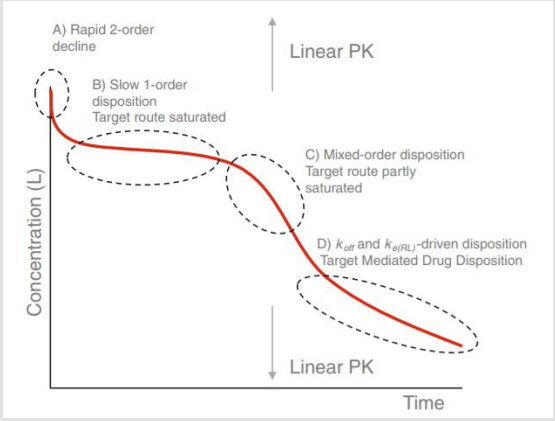




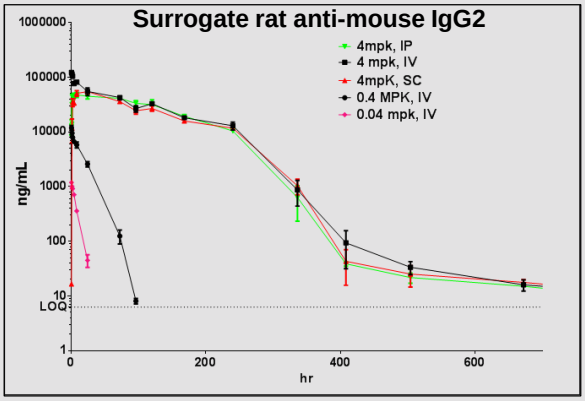
- **TMDD can be a desired attribute in certain cases (e.g. ADCs)**
- **Typically, it calls for thorough assessment of receptor biology across species**
- **Do we have a therapeutic window amenable to overcoming TMDD?**

AMGEN

UNDERSTANDING TMDD SERUM PK PROFILES



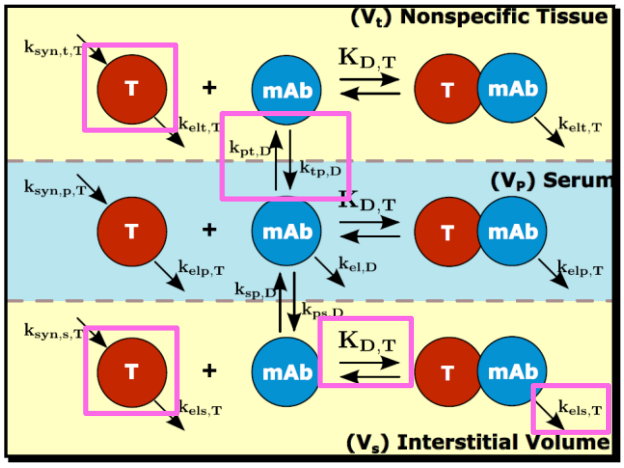
Peletier, L. A., and Gabrielsson, J. (2012)
J Pharmacokinet Pharmacodyn 39, 429-451.



- **Cassette dosing of competing mAb blocks TMDD of therapeutic**
- **14C-NSP Biodistribution demonstrated clearance of therapeutic predominantly in skin**

AMGEN

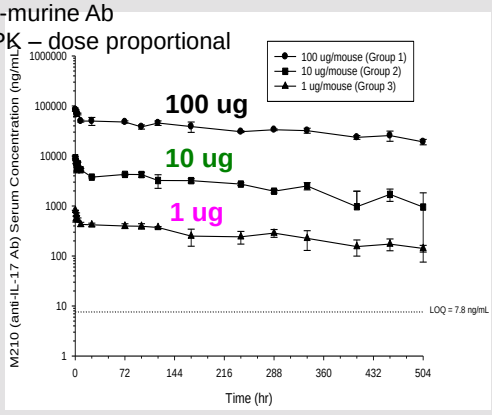
De-risking Requires Knowledge of Target Disposition



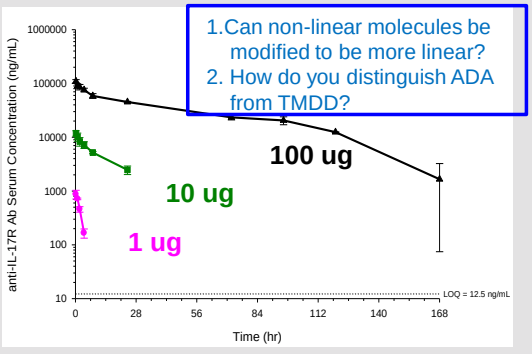
- Analysis at the cell/target level to inform the site of action minimal PBPK model
  - Drug/Target/Complex turnover, disposition, and elimination
- **K_D, k_{on} –** can be measured via analysis of binding to whole cells
- **$k_{el,s}$** – Receptor/drug internalization rate measured by flow cytometry or imaging
- **k_{pt}/k_{tp}** – partition ratio via tissue extravasation measurement
- **[T] – target concentration in tissue(s) of interest requires knowledge of receptor density and cell type distribution (IHC) in the organ**

TARGET INFLUENCES LINEAR VS. NON-LINEAR PK PROFILE
EXAMPLE: ANTI-SOLUBLE LIGAND VS. ANTI-MEMBRANE RECEPTOR

mAb 1:
Target: Circulating Ligand X
Rat anti-murine Ab
Linear PK – dose proportional



mAb 2:
Target: Cell Surface receptor to Ligand X
Rat anti-murine Ab
Non-linear PK – not dose proportional



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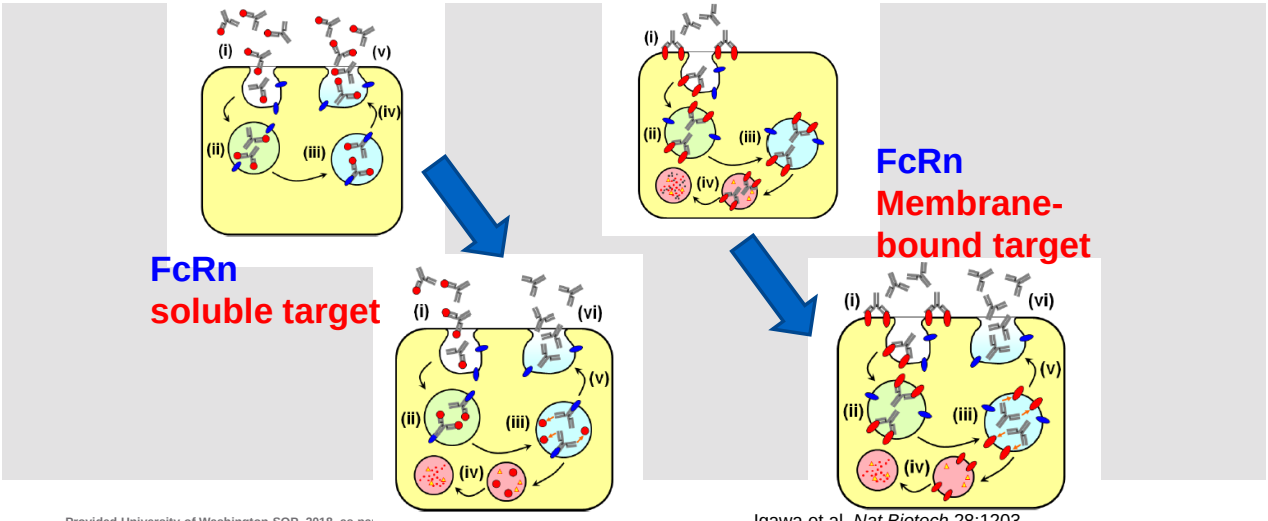
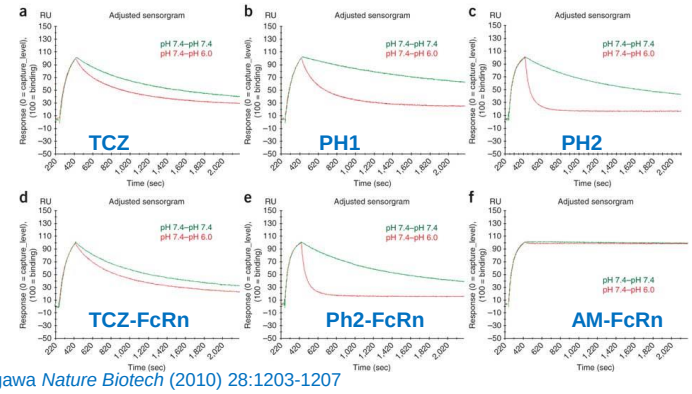
FcRn can salvage mAb bound to either soluble or membrane associated antigen/target



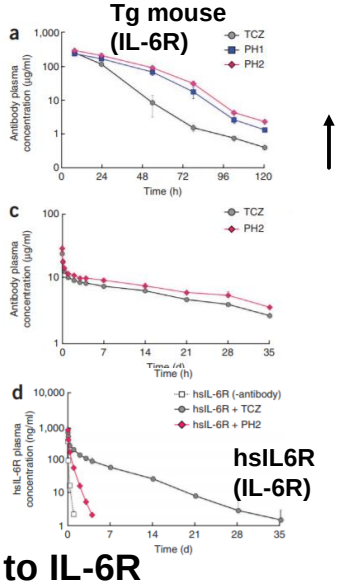
Igawa et al, *Nat Biotech* 28:1203

AMGEN

ENGINEER IN PH-DEPENDENT ANTIGEN BINDING TO IMPROVE FCRN RECYCLING



Igawa *Nature Biotech* (2010) 28:1203-1207



- **TCZ parent mAb targeting huIL-6R**
- **PH1 & PH2 pH dependent binding mutants**
- **AM is affinity matured TCZ with greater affinity to IL-6R**

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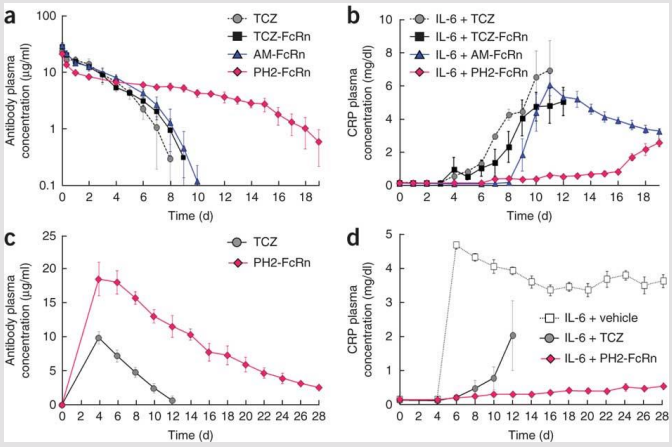
PH-DEPENDENT BINDING CAN IMPROVE THE PK/PD PROFILE OF A MEMBRANE RECEPTOR TARGETING MAB



- **Anti-human IL-6R mAbs observe cross reactivity to nonhuman primate IL-6R**

Igawa *Nature Biotech* (2010) 28:1203-1207

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- ***Other factors impacting mAb disposition & PK***

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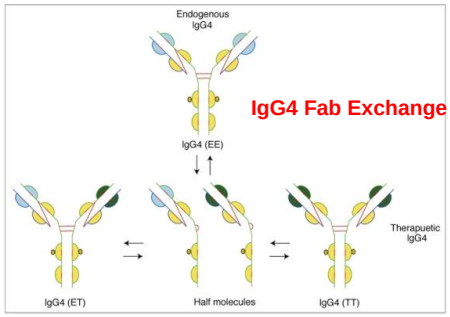
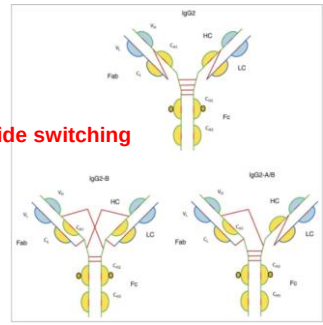
LOSS OF MOLECULAR INTEGRITY IMPACTS BOTH PK AND PD

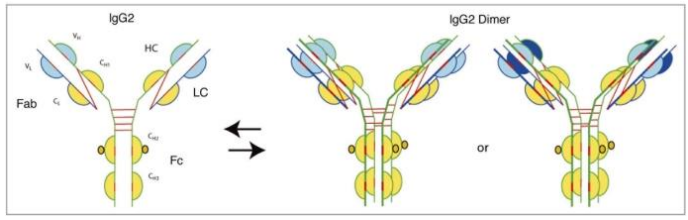


IgG4 Fab Exchange

IgG2 disulfide switching



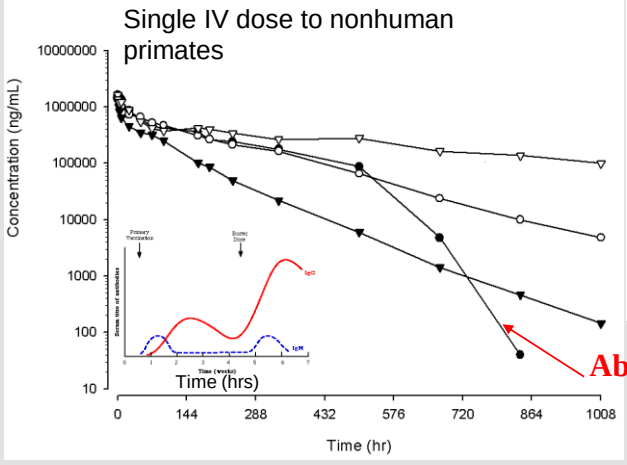
IgG2 homo- and heterodimer formation

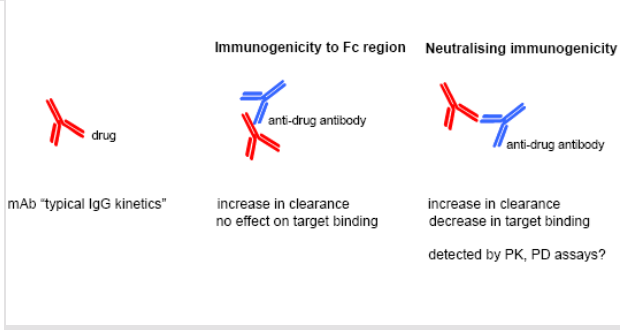


Correia *mAbs* (2010) 2(3):221-232

AMGEN

ANTI-DRUG ANTIBODIES (ADA) CAN AFFECT PK & EFFICACY





- **Preclinical ADA does not predict second species ADA,nor does it predict clinical ADA**
- **Kinetics of 1° immune response vs. ADA**
  - **pre-existing titers?**
  - **molecule attribute?**

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- ***Molecular ADME:***
- ***Case study of role of biochemistry & biophysics in understanding IgG disposition***

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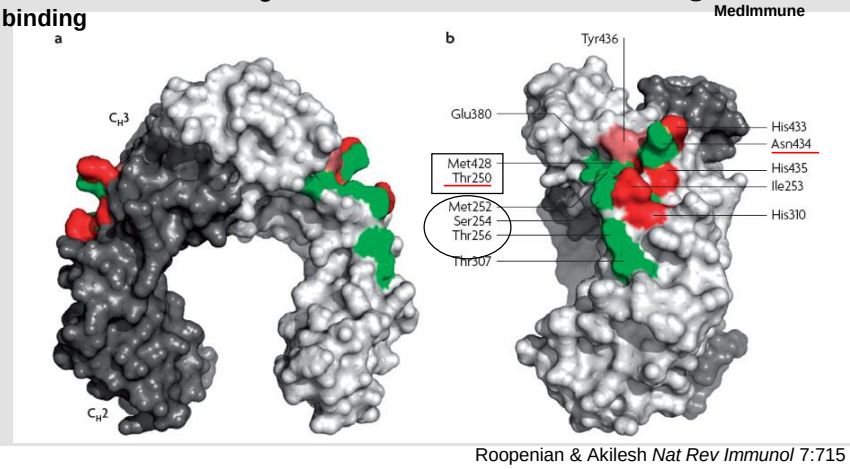
IGG CH2/CH3 RESIDUE INTERACTIONS WITH FCRN

ALTER FC REGION RESIDUES TO PROMOTE HIGHER AFFINITY INTERACTIONS WITH FCRN

Red/pink – abrogate/reduce FcRn binding
Green – enhance binding

◯ **YTE** (MedImmune) ▭ **PDL** — **LS-Xencor**



Roopenian & Akilesh *Nat Rev Immunol* 7:715

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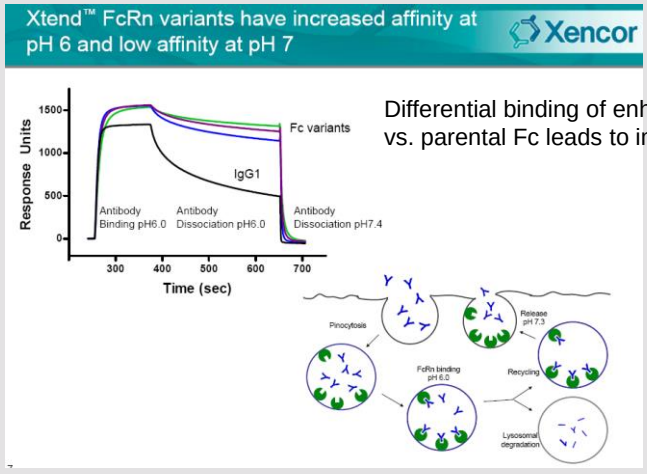
IBC Ab Eng mtg 2008



Differential binding of enhanced Fc vs. parental Fc leads to improved PK

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NOT AS SIMPLE AS JUST FC/FCRN INTERACTIONS…

- **Altering the interaction between the Fc region and FcRn at pH 6.0 has been shown to improve the t1/2 of mAbs in vivo.**
  - **Gurbaxani, B. *Molecular Immun.* (2013) 56(4): 660-674 & references therein**
- **A clear correlation between affinity at 6.0 and t1/2 has not been observed with conflicting results published**
  - **Gurbaxani, B. *Molecular Immun.* (2013) 56(4): 660-674 and references therein**
- **Studies suggesting net charge of antibody impacts electrostatic interactions on cell surface and alters pinocytosis rates**
  - **Igawa, T. *PEDS* (2010) 23(5): 385-392**
  - **Boswell, A. *Bioconjug. Chem.* (2010) 21(12): 2153-2163**
- **Studies suggesting influence of Fab region on FcRn binding and PK**
  - **Wang, W. *Drug. Metab. Disp.* (2011) 39(9): 1469-1477**
  - **Suzuki, T. *J. Immunol.* (2010) 184(4): 1968-1976**

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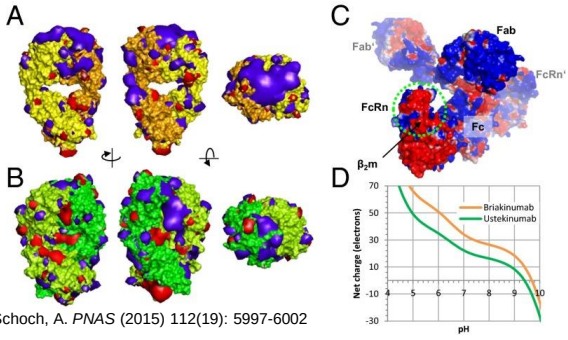
CHARGE-MEDIATED INFLUENCE OF THE ANTIBODY VARIABLE DOMAIN ON FCRN-DEPENDENT PHARMACOKINETICS

- **Investigate the influencing factors of the Fab region to FcRn-mediated IgG homeostasis**
- **Briakinumab and ustekinumab both fully human IgG1 to human p40-subunit of IL-12 & IL-23 with no X-reactivity to mouse**
- **Nearly identical constant domains with minor differences in several allotype-specific amino acids all of which are outside the cognate FcRn-binding region**



Schoch, A. *PNAS* (2015) 112(19): 5997-6002

Table 1.

Systematically engineered variants of briakinumab and ustekinumab

Name	Description
Briakinumab	Briakinumab
Ustekinumab	Ustekinumab
mAb 1	Ustekinumab Fv + briakinumab constant domains
mAb 2	Briakinumab Fv + ustekinumab constant domains
mAb 3	Ustekinumab HC + briakinumab LC
mAb 4	Briakinumab HC + ustekinumab LC
mAb 5	Ustekinumab CDRs on briakinumab
mAb 6	Briakinumab CDRs on ustekinumab
mAb 7	Briakinumab R19HCA, K64HCA, R83HCA*
mAb 8	Briakinumab R16HCA, R19HCA, K57HCA, K64HCA R83HCA*
mAb 9	Briakinumab R27LCA, R55LCA, R94LCA*

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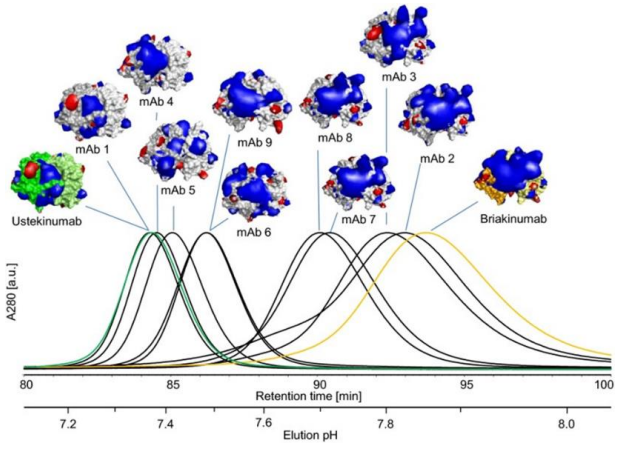
CHARGE-MEDIATED INFLUENCE OF THE ANTIBODY VARIABLE DOMAIN ON FCRN-DEPENDENT PHARMACOKINETICS (CONTINUED)

Systematically engineered variants of briakinumab and ustekinumab

Name	Description
Briakinumab	Briakinumab
Ustekinumab	Ustekinumab
mAb 1	Ustekinumab Fv + briakinumab constant domains
mAb 2	Briakinumab Fv + ustekinumab constant domains
mAb 3	Ustekinumab HC + briakinumab LC
mAb 4	Briakinumab HC + ustekinumab LC
mAb 5	Ustekinumab CDRs on briakinumab
mAb 6	Briakinumab CDRs on ustekinumab
mAb 7	Briakinumab R19HCA, K64HCA, R83HCA*
mAb 8	Briakinumab R16HCA, R19HCA, K57HCA, K64HCA R83HCA*
mAb 9	Briakinumab R27LCA, R55LCA, R94LCA*

FcRn affinities and calculated net charge of all tested antibodies

Name	Ret. time (min)	Rel. K_D	pI (IgG)	q(V_L) pH 6	q(V_L) pH 7.4	q(V_H) pH 6	q(V_H) pH 7.4	q(Fv) pH 6.0	q(Fv) pH 7.4
Ustekinumab	84.3	1	9.3	2.1	1.9	3.1	2.9	5.2	4.9
mAb 1	84.3	1.0 ± 0.22	9.5	2.1	1.9	3.1	2.9	5.2	4.9
mAb 4	84.5	0.5 ± 0.08	9.6	2.1	1.9	6.4	4.3	8.4	6.2
mAb 5	85.1	0.9 ± 0.16	9.9	2.1	1.9	4.1	3.9	6.1	5.9
mAb 6	86.2	0.4 ± 0.17	9.0	3.9	3.0	5.4	3.3	9.2	6.3
mAb 9	86.2	0.4 ± 0.04	9.1	0.8	0.0	6.4	4.3	7.2	4.3
mAb 8	90.1	0.4 ± 0.07	8.8	3.8	3.0	1.4	−0.7	5.2	2.3
mAb 7	90.4	0.2 ± 0.03	9.2	3.8	3.0	3.4	1.3	7.2	4.3
mAb 3	92.4	0.2 ± 0.06	9.3	3.8	3.0	3.1	2.9	6.9	6.0
mAb 2	93.0	0.3 ± 0.19	9.3	3.8	3.0	6.4	4.3	10.2	7.3
Briakinumab	93.7	0.2 ± 0.07	9.6	3.8	3.0	6.4	4.3	10.2	7.3



Schoch, A. *PNAS* (2015) 112(19): 5997-6002

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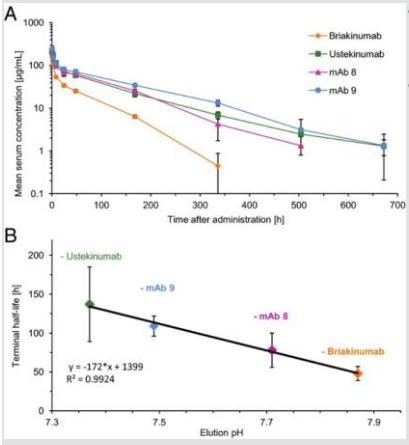
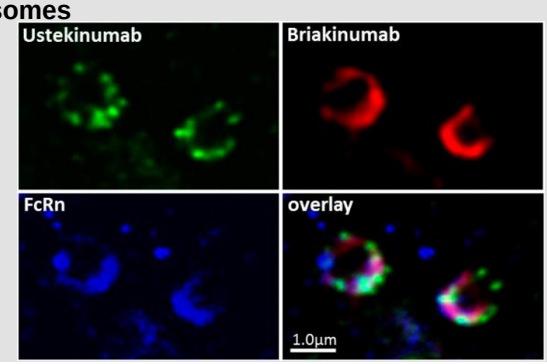
CHARGE-MEDIATED INFLUENCE OF THE ANTIBODY VARIABLE DOMAIN ON FCRN-DEPENDENT PHARMACOKINETICS (CONTINUED)



- **Correlation observed between in vivo PK parameters and FcRn column elution pHs.**
- **Differential sorting of briakinumab and ustekinumab in FcRn positive sorting endosomes**



Schoch, A. *PNAS* (2015) 112(19): 5997-6002

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- ***Image-based strategies for characterization of biodistribution and mechanism of action***

Provided University of Washington SOP, 2018, as part of an oral presentation and is qualified by such, contains forward-looking statements, actual results may vary materially; Amgen disclaims any duty to update.

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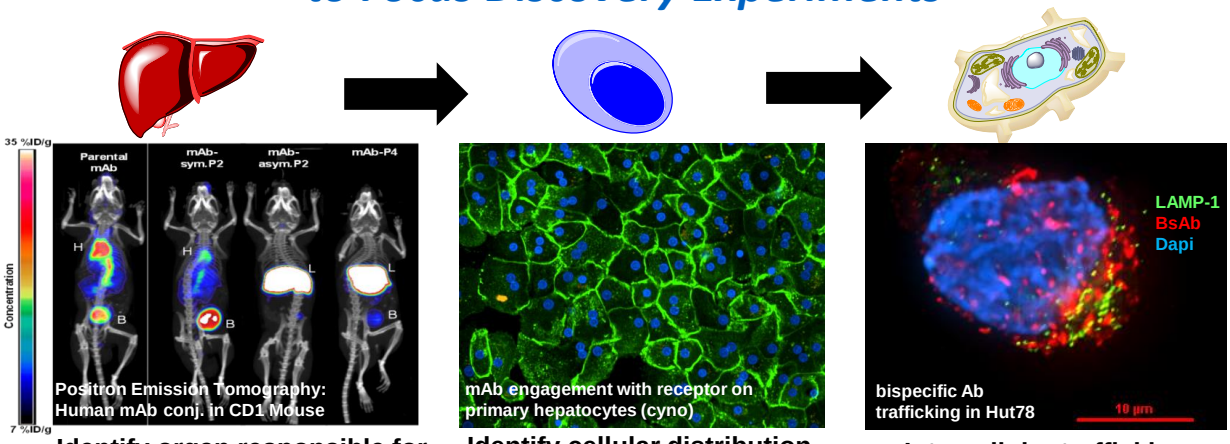
Defining Drug Disposition at Increasing Resolution is Critical to Focus Discovery Experiments



Identify <u>organ</u> responsible for clearing therapeutic

Identify <u>cellular</u> distribution and drug-receptor kinetics (link to PKPD)

<u>Intracellular</u> trafficking (for protein engineering)

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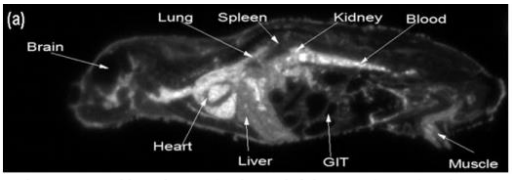
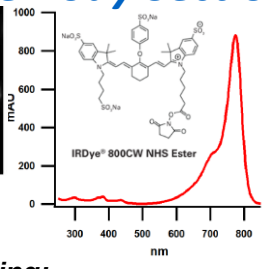
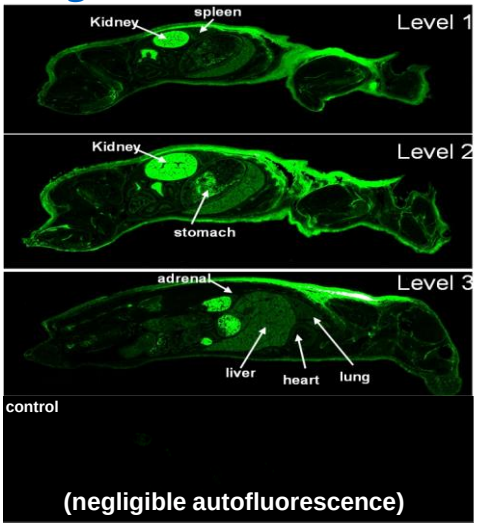
Near IR Fluorescent Probes for Rapid LM Biodistribution with Whole Body Sectioning



L. Abuqayyas, J.P. Balthasar / International Journal of Pharmaceutics 439 (2012) 8–16

(Typical ^{125}I WBAL result in Rodent)





<u>Experimental advantages of nIR imaging:</u>

- Rapid determination of primary CL organs and S.o.A.
- Simple, reliable NHS chemistry used for conjugation
- No lab restrictions as with radiolabels
- near IR spectral region free of interfering tissue autofluorescence
- Minimal light scattering renders ***tissue quantitation*** possible

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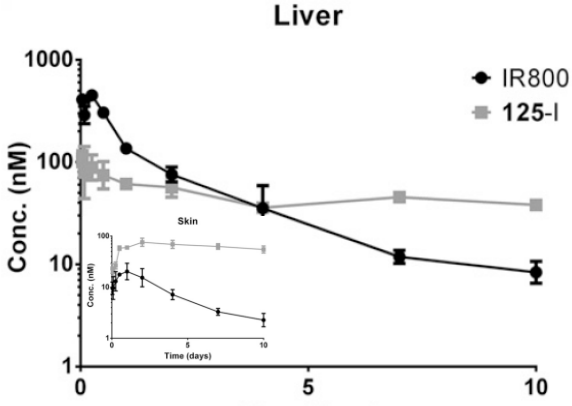
Near-IR Probe Quantitation Reveals Altered mAb-Tissue Exposure Relative to both Unlabled 8C2 & ^{125}I-8C2

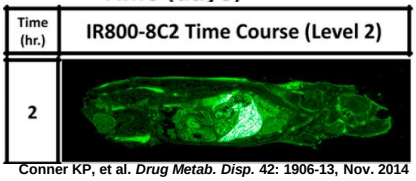
- **Large increase in liver uptake observed**
- Rapid clearance from all tissues is at odds with anticipated residualizing nature of negatively charged probe relative to non-residualizing ^{125}I label
- Suggests IR800 dye affects increased clearance via **specific uptake *and* elimination mechanisms**
  - **e.g. scavenger/salvage receptors**
- **Begs the question: How are conjugated mAbs 'perceived' in vivo?**

Tissue exposure of IR800-8C2 compared with [^{125}I]8C2.

Tissue	AUC (IR800)[a]	AUC (^{125}I)	AUC (IR800)/AUC (^{125}I)	TB (IR800)/TB (^{125}I)[b]
	*nM*day*	*nM*day*		
Liver	636.1 (69)	483.7 (57.1)	1.32	3.24
Spleen	117.1 (15.5)	560.5 (56.9)	0.21	0.52
Lung	213.6 (13.9)	656.4 (65.1)	0.33	0.80
Heart	41.0 (6.6)	406.8 (33.3)	0.10	0.25
Kidney	130.1 (23)	505.5 (45.6)	0.26	0.63
Skin	80.1 (0.5)	630.8 (27.8)	0.13	0.31
Muscle	21.3 (1.4)	1889.9 (7.9)	0.11	0.28
Bone	61.2 (3.3)	324.9 (15.2)	0.19	0.46
GI	56.2 (3.1)	350.3 (101.3)	0.16	0.39

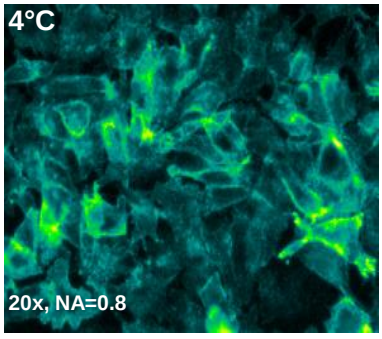
Conner KP, et al. *Drug Metab. Disp.* 42: 1906-13, Nov. 2014

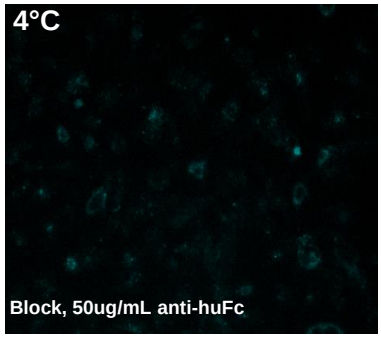


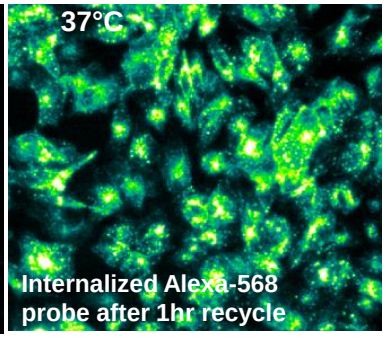


Conner KP, et al. *Drug Metab. Disp.* 42: 1906-13, Nov. 2014

Binding, Uptake, and Recycling by Immunofluorescence

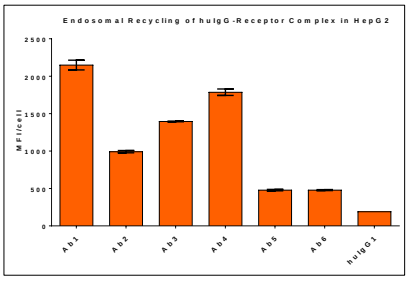






Membrane Accessible Receptor-mAb Complex | **Recycled Fraction**

- **Competancy for target receptor-mediated recycling is verified by immunofluorescence microscopy**
- **Strategy to eliminate recycling-deficient mAbs early in discovery**



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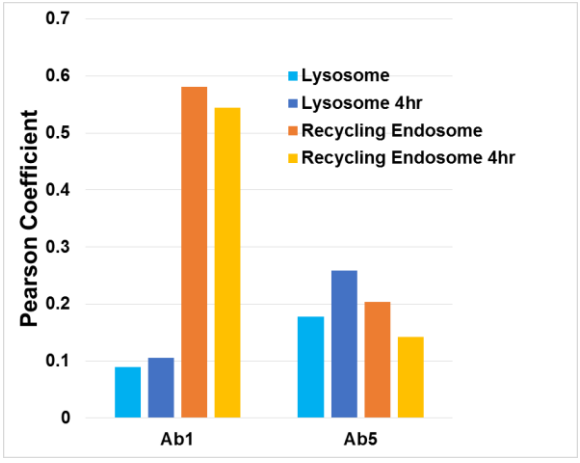
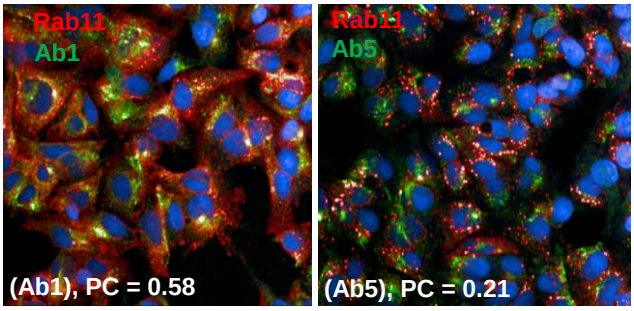
Intracellular Trafficking Analysis Reveals Divergent Endosomal Trajectory for Efficient vs. Poor Recycling mAbs



(Efficient recycler) (Poor recycler)



- **Efficient mAbs maintain high colocalization with Rab11 positive compartments (recycling endosome) within short timescale of assay (2hr)**
- **Poor recyclers concentrate into divergent endosomal compartments toward lysosomal degradation**

57

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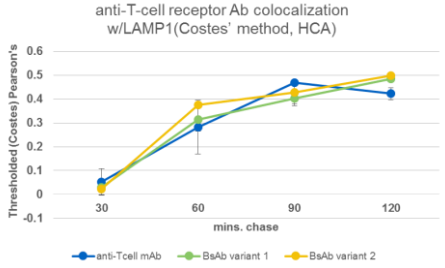
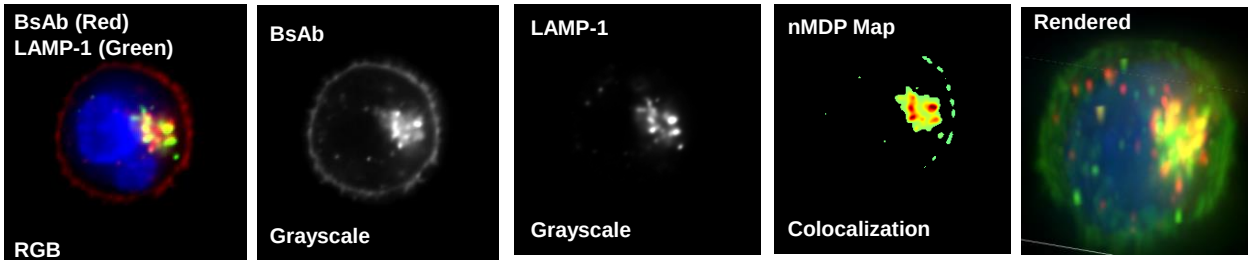
Co-localization Methods for Characterizing Uptake of BsAbs into Lysosomes at 2hr Chase Using Wide-Field Microscopy



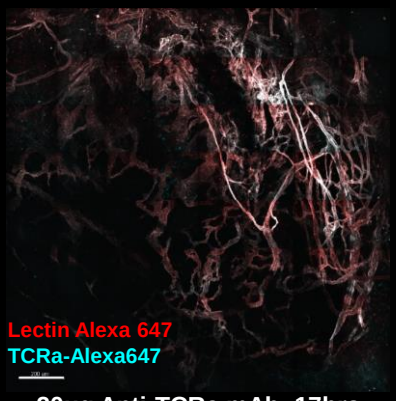
- **BsAb displays fast clearance from central (blood) compartment due to rapid uptake and degradation in T-cells**
- **Trafficking analysis can anticipate PK and catabolite profile differences based on BsAb valency**



Li et al. J. Neuroscience (2004), 24(16) p4070
Jaskolski et al. Journal of Neuroscience Methods 146 (2005) 42–49

INTRA-VITAL IMAGING TO ASSESS TUMOR UPTAKE AND T-CELL ACTIVITY

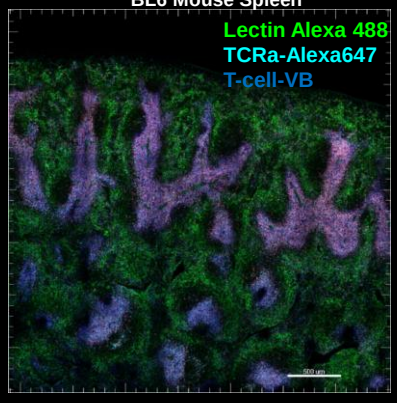
PyMT Tumor in BL6 mouse model



20ug Anti-TCRa mAb, 17hrs

5ug Anti-TCRa mAb, 17hrs

BL6 Mouse Spleen



5ug Anti-TCRa mAb, 2hrs

- Aim is to characterize exposure at the target level in tissue in real time

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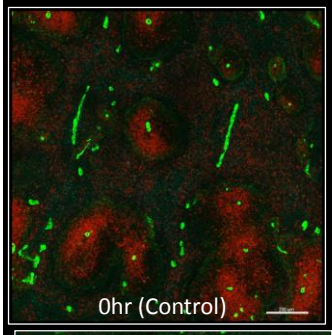
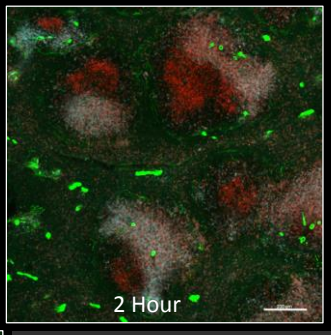
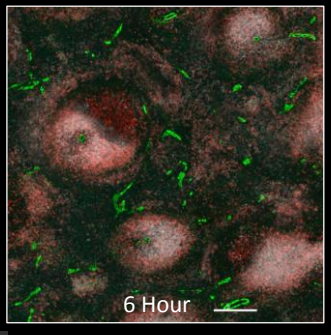
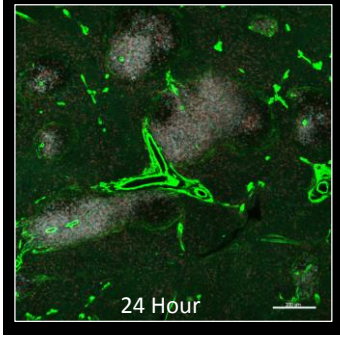
anti-muTCRα (rat IgG2a) Biodistribution (Spleen) Timecourse, 5ug i.v. (0.2mg/kg)

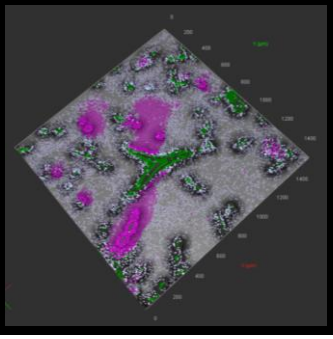






CD2RFP (TILS)
Smooth Muscle Actin
Anti-TCRα Alexa 647





- Pharmacokinetics of tissue uptake can be investigated in real-time
- Quantitation of exposure either by distance from vasculature or measures of PD (e.g. T-cell dynamics/stimulation)