L5 & 6 What makes biologics & nanomedicines successful?

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Lecture Series at the
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for Siberian Branch of Russian Academy of Sciences

What makes biologics & nanomedicines successful?

Intended learning outcomes:

- To be aware of reasons for failure of the pharmaceutical industry (Pharma) paradigm for small molecule drugs
- To be aware of how biologics and nanomedicines develop despite Pharma
- To understand the main differences driving success:
  - pharmacokinetics – very long circulation times
  - similar structures for different diseases
  - selectivity for diseased tissue (L6 & 7)
  - selectivity to cross particular biological barriers (L6 &7)
  - selectivity for disease expression (RNA - Moscow)

Why makes the Pharma drug paradigm fail?

For high oral bioavailability *via* transcellular absorption across the small intestine by passive diffusion, Pharma selects small molecule drugs *in vitro* with hydrophobic properties (Log P):

- to partition into lipid bilayer membranes (to diffuse through cells) and also
- convenient to medicinal chemists to increase binding to the protein target site and block activity *in vitro*.

Leads to failure of safe & efficacious responses in man:

- few 'clean' drug targets, needed for small molecules (most targets expressed in diseased & healthy tissue → off target)
- penetrate diseased & healthy tissue → side effects
- selects structures that our bodies are evolved to exclude by drug efflux and metabolism, with many different genotypes (polymorphisms) - so one drug does not suit all
- ignores selectivity for transporters, tissue and disease state

Why: Lipinski's "Rule of 5" (Ro5)

To alert medicinal chemists to potentially poor oral absorption characteristics (solubility and permeability):

- not more than 5 hydrogen bond donors (OH and NH groups)
- not more than 10 hydrogen bond acceptors (notably N & O)
- a molecular weight < 500 (160 – 480)
- a partition coefficient or log *P* < 5 (-0.4 to 5.6)

Combinatorial chemistry tends to produce higher molecular weight and robotic screening has selected more lipophilic and less water-soluble compounds.

Drugs failing 'Ro5' in particular therapeutic classes, mainly **natural** products (*eg* antibiotics, antifungals, vitamins, cardiac glycosides) and **biologics**, both often ligands for absorption by biological transport.

Lipinski characterisation of drugs

Drug name	MLogP	OH+NH[c]	MWT	N+O[d]	Alert[e]
Aciclovir[a,b]	−0.09	4	225.21	8	0
Alprazolam[a]	4.74	0	308.77	4	0
Aspirin[b]	1.70	1	180.16	4	0
Atenolol[a,b]	0.92	4	266.34	5	0
Azithromycin[b]	0.14	5	749.00	14	2
AZT[a]	−4.38	2	267.25	9	0
Benzyl-penicillin[b]	1.82	2	334.40	6	0
Caffeine[b]	0.20	0	194.19	6	0
Candoxatril[b]	3.03	2	515.65	8	0
Captopril[a]	0.64	1	217.29	4	0
Carbamazepine[a]	3.53	2	236.28	3	0
Chloramphenicol[b]	1.23	3	323.14	7	0
Cimetidine[a,b]	0.82	3	252.34	6	0
Clonidine[b]	3.47	2	230.10	3	0
Cyclosporine[a]	−0.32	5	1202.64	23	2
Desipramine[a,b]	3.64	1	266.39	2	0
Dexamethasone[b]	1.85	3	392.47	5	0
Diazepam[b]	3.36	0	284.75	3	0
Diclofenac[a]	3.99	2	296.15	3	0
Diltiazem-HCl[a]	2.67	0	414.53	6	0
Doxorubicin[b]	−1.33	7	543.53	12	2
Enalapril-maleate[a]	1.64	2	376.46	7	0
Erythromycin[b]	−0.14	5	733.95	14	1
Famotidine[a]	−0.18	8	337.45	9	0
Felodipine[a,b]	3.22	1	384.26	5	0

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Biopharmaceutics Classification System (BCS)

Reliability of predicting bioavailability from dissolution studies

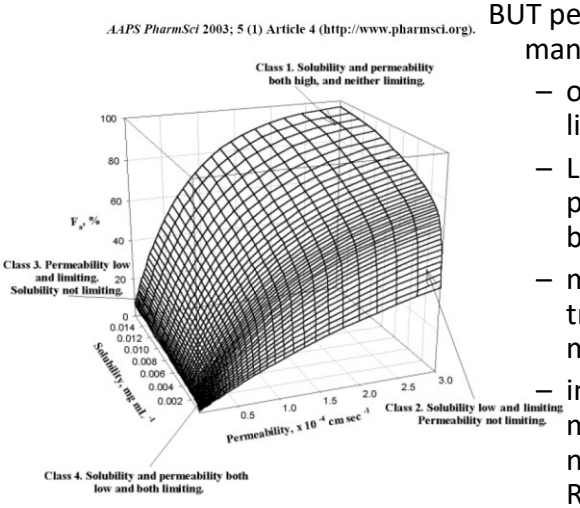
	High solubility	Low Solubility
High permeability	**Class I** High solubility High permeability **Eligible for Biowaiver**	**Class II** Low solubility High permeability **Eligible for Biowaiver only if weak acids, highly soluble at pH6.8, plus**
Low Permeability	**Class III** High solubility Low permeability **Eligible for Biowaiver if very rapidly dissolving**	**Class IV** Low solubility Low permeability **Not eligible for Biowaiver**

"Brick dust"

Combinatorial chemistry tends to produce higher MW and robotic screening tends to select less soluble (Class II) or less permeable (Class III) compounds, or both – Class IV 'brick dust'

Biopharmaceutics Classification System

Aim is Class 1 molecule because absorbed fraction F_a is a function of permeability & solubility (dissolution rate, transit time *etc.*)

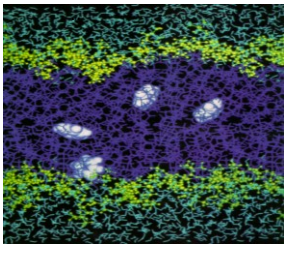


BUT permeability depends on many other mechanisms too:

- only LogP - related for lipophilic compounds
- Log P relates to solvent partition, not biomembranes
- many other mechanisms of transport through bio-membranes
- including large, hydrophilic macromolecules & nanoparticles, which fail Ro5

Lipid bilayer does not behave like simple water / solvent partition (LogP)

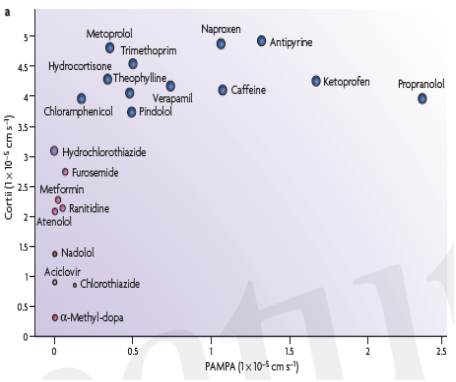
- Diffusion coefficients inside the bilayer depend on solute size less than in water or in solvent
- Size dependence of permeability instead depends on partitioning into the lipid bilayer, which is lower than partitioning at bulk water/solvent interfaces.
- Lipid head group interactions and constrained motion of hydrocarbon tails near head groups restrict partitioning.

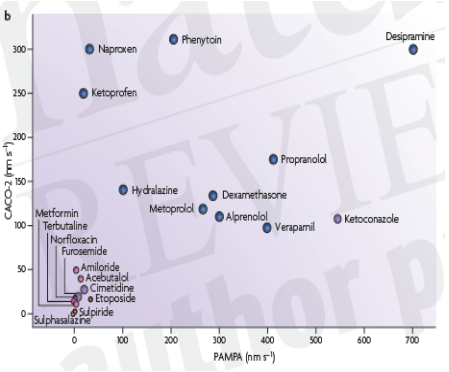


MD simulation of membrane diffusion:

- motions differ in center & near surface, both differ from bulk phase
- rotational isomerizations (gauche/trans) gate channels between voids
- differing motions available to different drugs.

Poor correlations of permeability between different lipid bilayers & epithelial cell monolayers





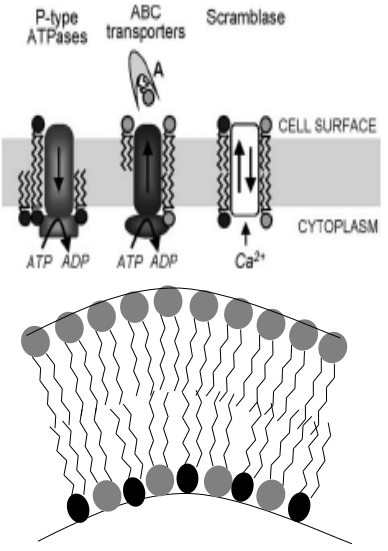
eg artificial lipid (Corti) and parallel artificial membrane permeability assay (PAMPA)

eg CaCo2 cell monolayer epithelial model and PAMPA

Where does the transcelluar absorption paradigm come from and why does it make drugs unspecific?

- Drugs evolved in natural products to be non-selective *eg* plant metabolites (bad taste, poisons) to avoid being eaten by animals
- Non-selective penetration :
  - only advantage where highly-selective against target tissue or localised effect, bound or eliminated elsewhere
  - otherwise healthy organ toxicity because drug target often expressed in both healthy and diseased tissue.
- Effective protective systems to avoid toxicity:
  - serum protein binding of lipophilic molecules
  - efflux transporters (*eg* P glycoprotein)
  - defensive metabolisms, with signalling to switch on
- Natural bioactive compounds often selective when hydrophilic, because do not penetrate everywhere and specific transporters expressed in particular tissues.

Drug efflux, transport & endocytosis

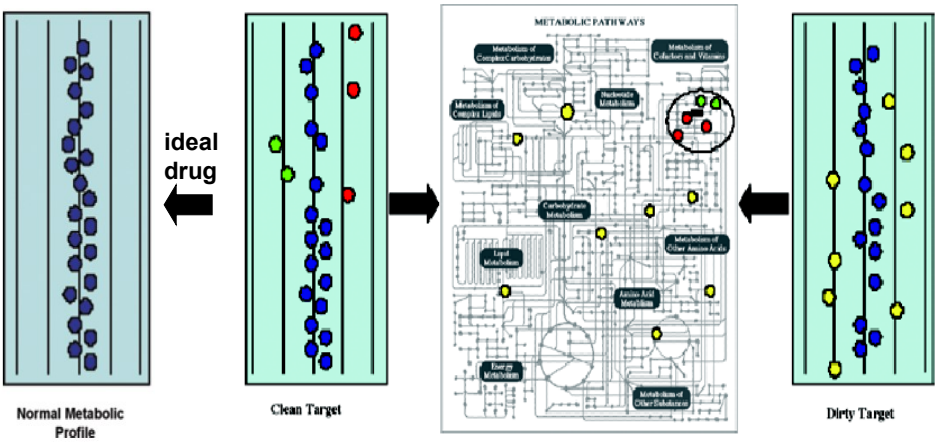


ATP-dependent flip/flop-ases maintain asymmetric lipid distribution by moving specific lipids towards (P4-ATPase family members) or away from the cytosolic leaflet (ABC transporters). Lipid asymmetry collapsed by the transient activity of ATP-independent scramblases.

Asymmetric distribution of different-sized lipids result in curvature and assists endosome vesicle formation. Lipophilic drug molecules also distributed across the membrane bilayer.

Why un-selective tissue penetration is bad with 'dirty' drug targets

ONLY optimal when 'clean' drug-able target, specific for diseased tissue/organ:



Ideal treatment metabolic profile

Diseased metabolic profile

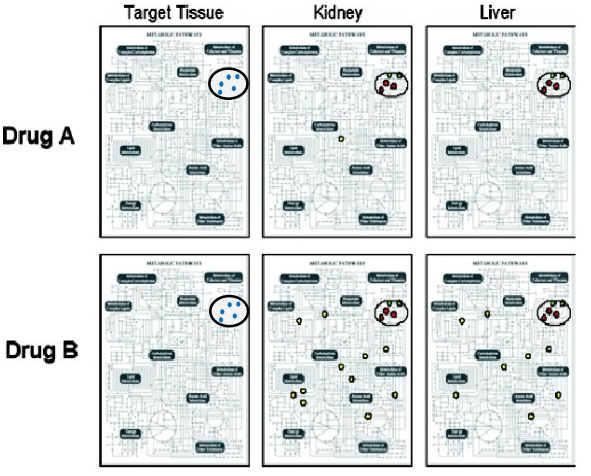
Poor treatment metabolic profile

Metabolomic pre-clinical studies

Metabolomic profiling to identify possible side effects:

Drug A causing few undesired biochemical effects in target and non-target tissues (ideal but unusual)

Drug B causing desired effects in target tissue but undesired biochemical effects in non-target tissue (too common).



Penetration & transport through barriers

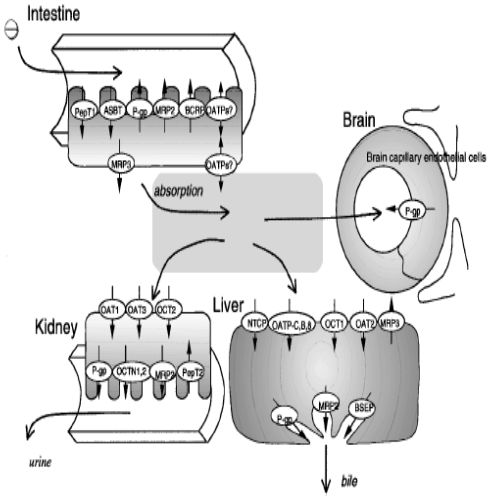
Very different ~~when we look beyond small drug 'Ro5' paradigm~~

- Passive – 'equilibrative':
  - paracellular diffusion - hydrophilic, low MW
  - transcellular diffusion
    - lipophilicity, LogP, low MW … Lipinski 'rule of 5'
    - ~~pore-mediated diffusion~~
    - carrier-mediated diffusion
    - facilitated diffusion

medicinal chemistry focus

- Active transcellular – 'concentrative' energy-requiring
  - transporters
    - some broad spectrum, including drug efflux
    - many selective
  - transcytosis
    - non-selective cell penetration
    - selective receptor-mediated transcytosis

Homeostasis only by selective control over transport - different transporter distributions at epithelia & endothelia



with different effects:

- drug-nutrient & drug-drug interactions
- drug bioavailability increase, decrease or variability
- transporter polymorphisms - variability between people

Pgp	P glycoprotein
BSEP	bile salt export pump
MRP1-4	multidrug resistance associated prot.
BCRP	breast cancer resistance protein
NTCP	Na taurocholate cotransport protein
ASBT	apical sodium-dependent bile acid tr.
PEPT1-2	oligpeptide transporters
OCT1-3	organic cation transporters
OCTN1-2	novel organic cation transporters
OAT1-3	organic anion transporters
OATP	organic anion transporting peptide

Transporters exploited to increase bioavailability & targeting *eg:*

- **Acyclovir** (anti-viral) - amino acid ester prodrug → valacyclovir:
  - oral biovailability (AUC) increased in humans 3-5x.
  - rationale applied by Roche to design valgancylcovir.
- **Amoxicillin** (antibiotic)- 70% increased absorption rate and 25% increase in bioavailability upon co-admin with nifedipine in humans
  - nifedipine increases proton concentration at the apical surface of epithelial cells, increasing driving force for amoxicillin transport *via* hPEPT
  - change in surface pH by nifedipine may be a consequence of decreased concentration of intracellular Ca^{++}.
- **Pravastatin** (anti-cholesterol) - transporters involved in oral absorption, hepatic uptake, and biliary excretion

Over-simplified use of 'Ro5' in discovery & BCS in development

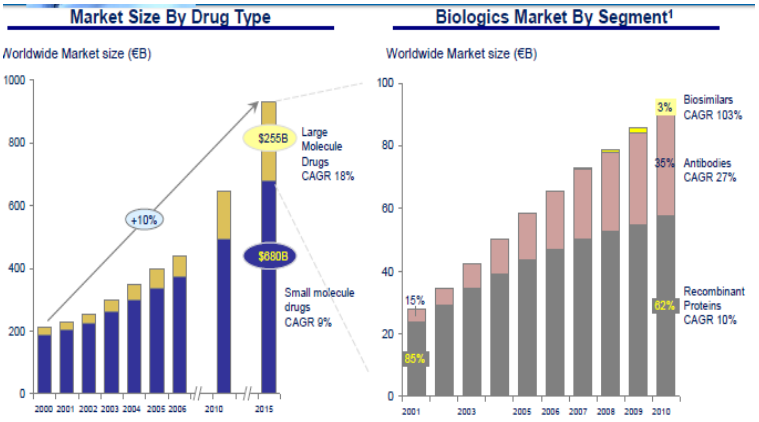
- rejection of many naturally-bioactive compounds of higher selectivity, because they fail the 'Ro5';
- Log P – based design promotes xenobiotic defences (efflux pumps, drug metabolism)
- non-selective penetration only optimal for 'clean' drug targets, increased side effects for dirty targets, increasingly complex drug targets even more challenging
- transporters effect influx and efflux at polarised epithelial and endothelial barriers, so transporter distribution, kinetics, interactions *etc.* important
- large hydrophilic protein assemblies cross barriers (including brain) by transcytosis whereas most combinatorial/HTS compounds do not (*viz.* L7-8).

Introduction to biologics

- Strong growth over the last 2-3 decades of large molecule drugs, particularly proteins:
  - >35% new drugs in clinical trials & marketed
  - some now 'blockbuster' drugs (sales > $ billions)
  - shift of large Pharma to include large molecules
  - growth in 'Bio-similars' (bio-generics) as innovator biologics come off patent, and 'Bio-betters'.
- Resulting influence on routes of administration (RoA) - development of alternatives to oral dose forms (lung, nose, skin by needle-free injectors & skin patches)
- Need to address different medicines design issues (penetration of barriers, heterogeneity, instability & immuno-genicity of large molecule drugs).
- Human monoclonal antibodies developed as the major group of biologics currently (DNA/RNA/epigenetics next).

Biologics high growth

Biologics ~37% of drug market with higher compound annual growth rate (CAGR): overall 18% (cf 9% small molecule drugs) of which antibodies 27%, biosimilars 103% CAGR



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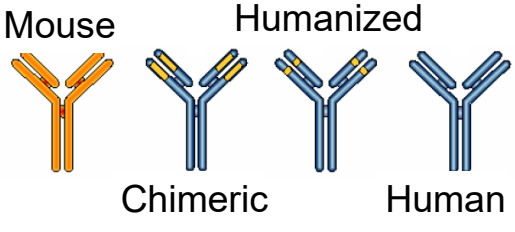
What's driven the growth of biologics?

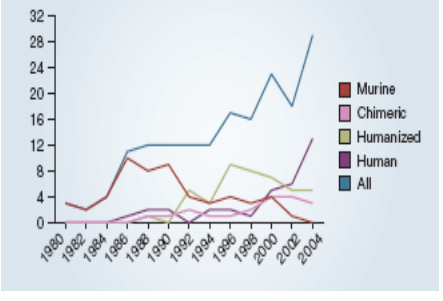
- Traditional 'one drug suits all' blockbuster paradigm → higher failure rates in discovery pipelines, in drug development, in clinical trials & post-marketing approval.
- Discovery technologies (genomics, robotic high throughput screening) not returned on large investment.
- Biologics developed in earlier decades by biotech companies filling gaps in large pharma pipelines.
- Advances in parenteral RoAs driven by biologics.
- Replace diseased tissue functionality (*eg* protein hormones & blood factors, gene therapy, tissue engineering)
- Highly specific binding to modify or block function of target.

Biologics *v.* small molecule drugs: *reasons for higher success rate*

- **Versatility -** replace diseased tissue as well as modify.
- **Unspecific binding** to molecular structures other than the desired molecular target can cause toxicity including tumorogenicity - not applicable to therapeutic proteins.
- **Blood levels of drug and duration of action** in man not appropriate (*eg* elimination half-life too long/too short; bad metabolite spectrum) – not applicable to mAbs.
- **Less frequent dosing** – long circulation times compared to small molecule drugs (weeks cf hours)
- Risk of **drug-drug interactions –** lower or not applicable.
- **Different structures** for each indication – not applicable to the similarly–structured mAbs, nucleic acids.
- **Inappropriate molecular target** applies to both
- **Immunogenic effects** – higher risk for biologics, addressed by stealthing, humanization.

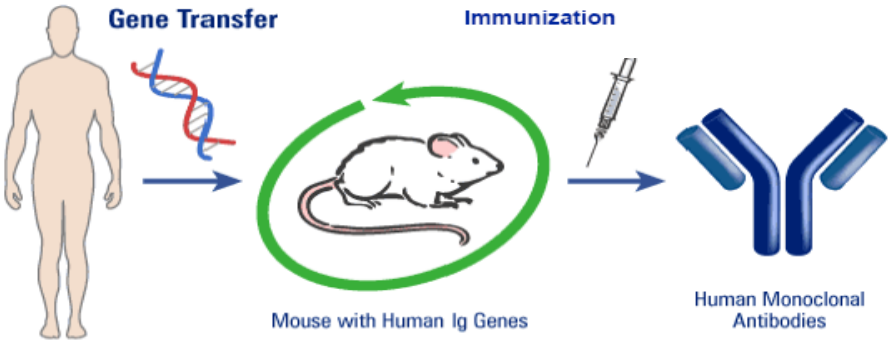
Human antibody development





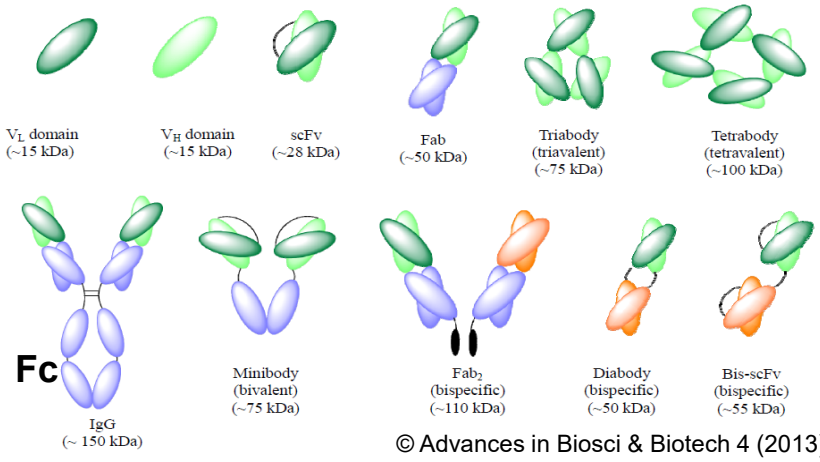
- Human hybridomas difficult, mouse used
  - immunogenic anti-mouse reactions & rapid clearance
  - lack human Fc functions and recycling into circulation.
- Recombinant mAb engineering with human Fc
  - chimeric - mouse variable region (Fv, antigen binding)
  - humanized – mouse antigen binding loops (CDRs)
  - fully human antibodies produced *via* mouse

Fully human recombinant antibodies



- 4 mouse IgG gene loci coding for 4 protein subunits replaced with human transgenes in transgenic mouse
- mouse immunized to raise immune response
- B cell selection, hybridoma production, bioreactor cell culture to produce human antibodies
- antibody Fc effector functions also engineered

Antibody fragments



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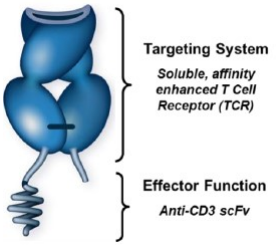
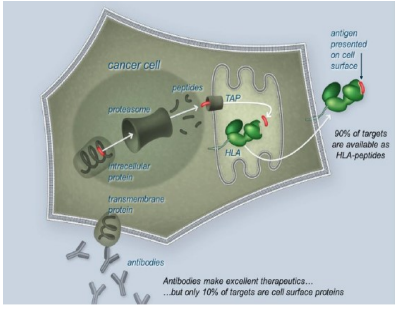
Variable region antibody fragments used mainly for imaging:

- lack Fc interaction with immune system
- lack long circulation times *via* FcRn recycling receptor

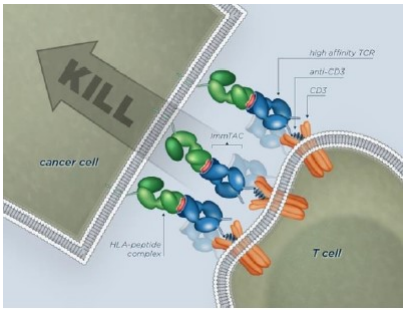
T Cell Receptor (TCR)

Many more targets are accessible to TCRs, which bind to the antigenic peptides presented on the surface of cells by MHC / HLA proteins to re-direct cytotoxic T cells against intracellular infections and cancer (in Phase II clinical trials)





www.immunocore.com



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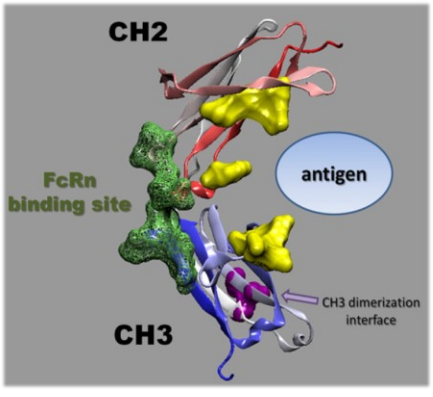
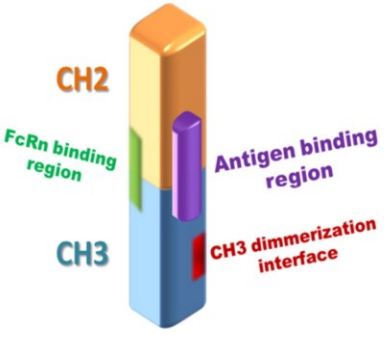
Fc fragment engineering





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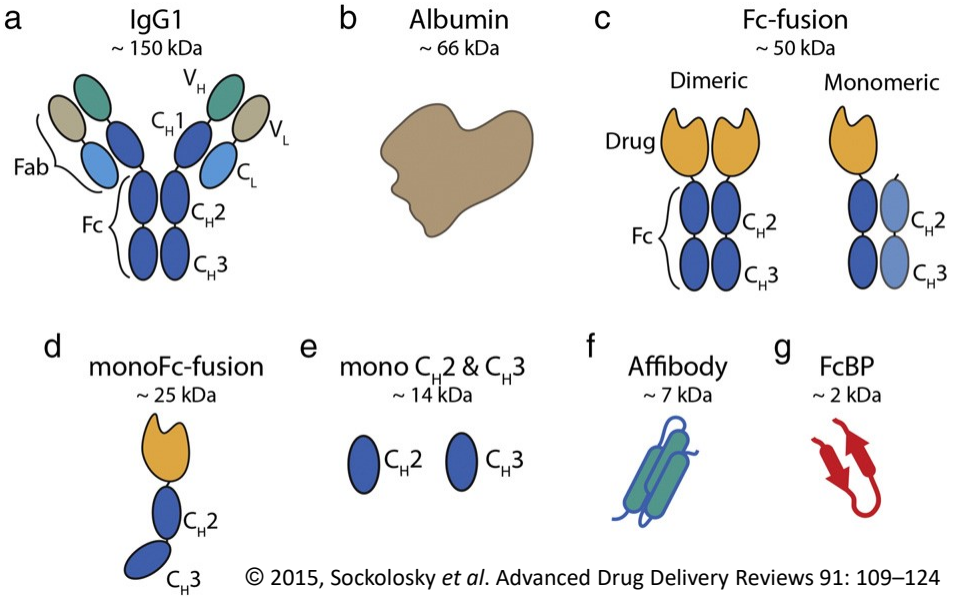
Fc engineering maintains interaction with immune system & binding site for antibody recycling receptor FcRn to enable long circulation times

Recycling Fc & fusions



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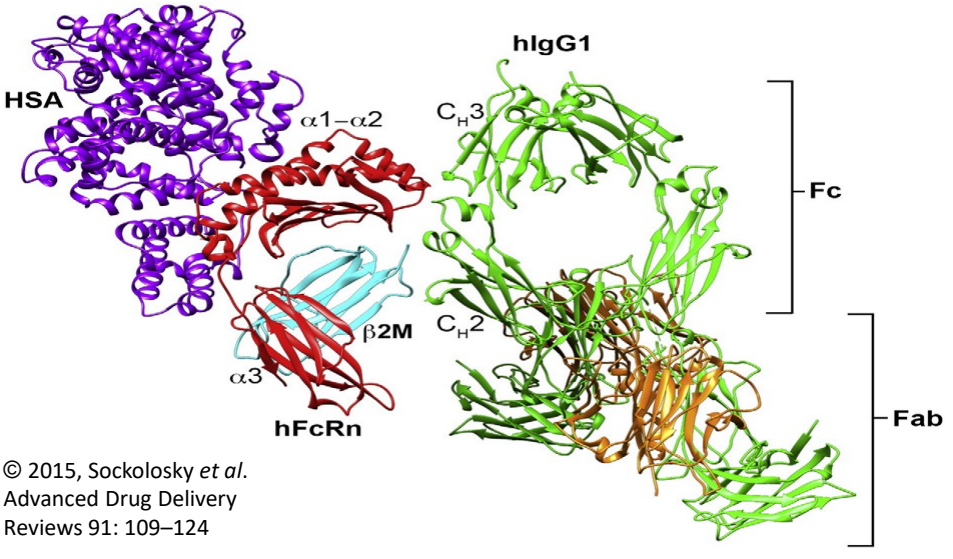
Albumin & IgG bind to FcRn at different sites



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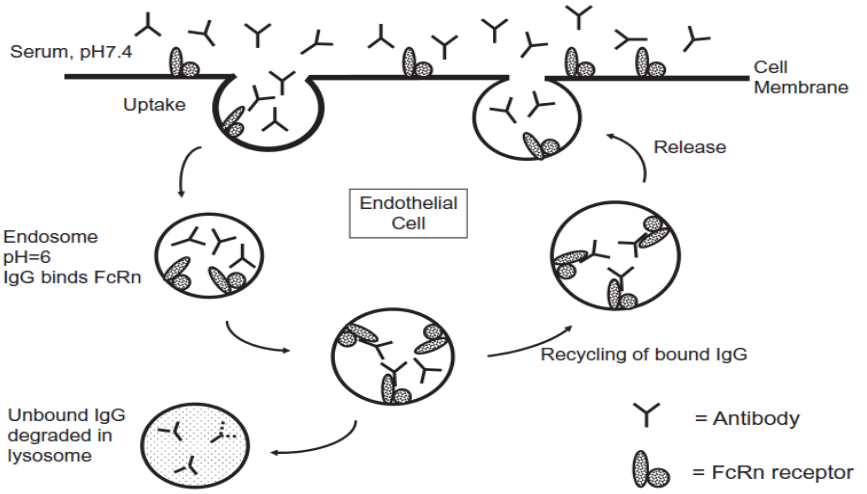
Human IgG recycling

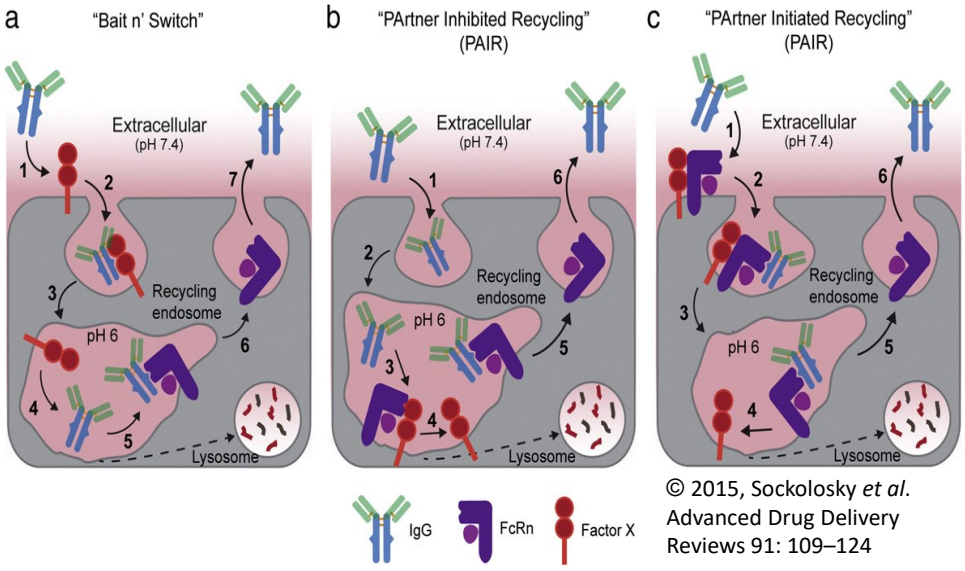
Half life 14-21 days: pH-dependent interaction with FcRn, preventing renal, RES *etc* clearance, allowing infrequent dosing



Antibody recycling

- mAb recirculation by Brambell receptor (FcRn), binding to Fc 'tail', essential for maintaining Ig & albumin levels & homeostasis in blood.
- In adults, FcRn primarily expressed in vascular endothelial cells or RES, with lower levels on monocyte cell surfaces, tissue macrophages, and dendritic cells.
- Fc receptor plays a critical role, but saturates at high IgG concentrations, resulting in an inverse relationship between concentration (dose) and half life:
  - lower half-life for high concentration / dose antibody,
  - OR where high levels of endogenous IgG, as seen in chronic inflammatory diseases

Additional Factor X?



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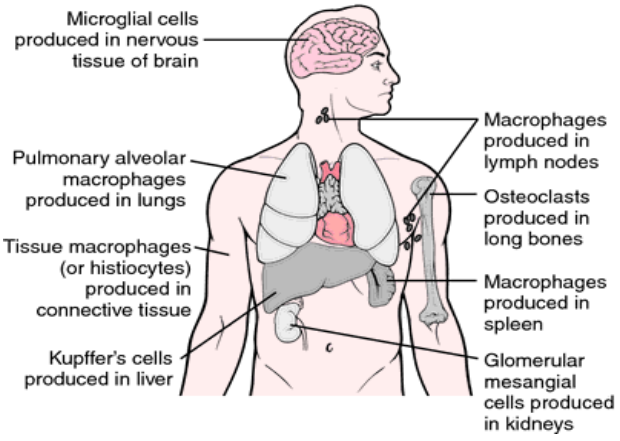
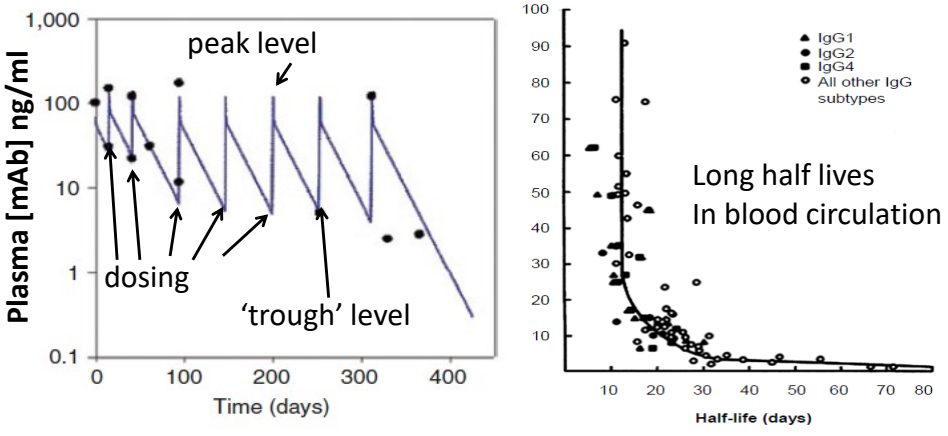
Avoids elimination & degradation

Primary routes are by renal clearance (proteins > nanoparticles) and by proteolytic catabolism after receptor-mediated endocytosis in the cells of the reticulo-endothelial system (RES).



Pharmacokinetics

Where large loading doses and IV RoA (*eg* infliximab left below), concentration – time profiles show very high peak concentrations with low 'trough' drug level monitored before next dose.



Loss of response (LOR) caused by anti-drug antibodies (ADAs)

LOR leads to interruption of therapy, or replacement with alternative (mAb) therapy:

- **Pharmacokinetic (PK) - binding ADAs** (BAbs) form immune complexes with drug, increase clearance rate & dose required, with indirect pharmacodynamic effect:
  - Reduced effect and patient response
  - BAbs against Fc may reduce antibody recycling - more clearance and lower drug levels
- **Pharmacodynamic (PD) – neutralising ADAs** (NAbs) of higher affinity directly interfere with the activity of the drug
  - binding to epitopes within or proximal to the active site,
  - but above PK effect often greater than PD effect

ADA risk factors affecting efficacy

- Product related – homology to endogenous proteins, non-glycosylated more immunogenic, sequence / peptide affinity for HLA / MHC antigen presenting proteins

Anti-drug antibody (ADA) risks not just antibody product related:

- Genetics – HLA highly polymorphic and combines with product amino acid sequence differences
- Underlying disease - chronic inflammation
- Other medications *eg* immunomodulatory
- Dosage – may affect peripheral tolerance

ADA response: immunological mechanisms

ADA frequency varies with natural immuno-tolerance

- High bio-similarity to proteins encoded by human genome (self proteins) should be well tolerated
- Central & peripheral mechanisms dependent on formation & activation of B and T cell clones

eg Elimination of self-reactive T cell clones

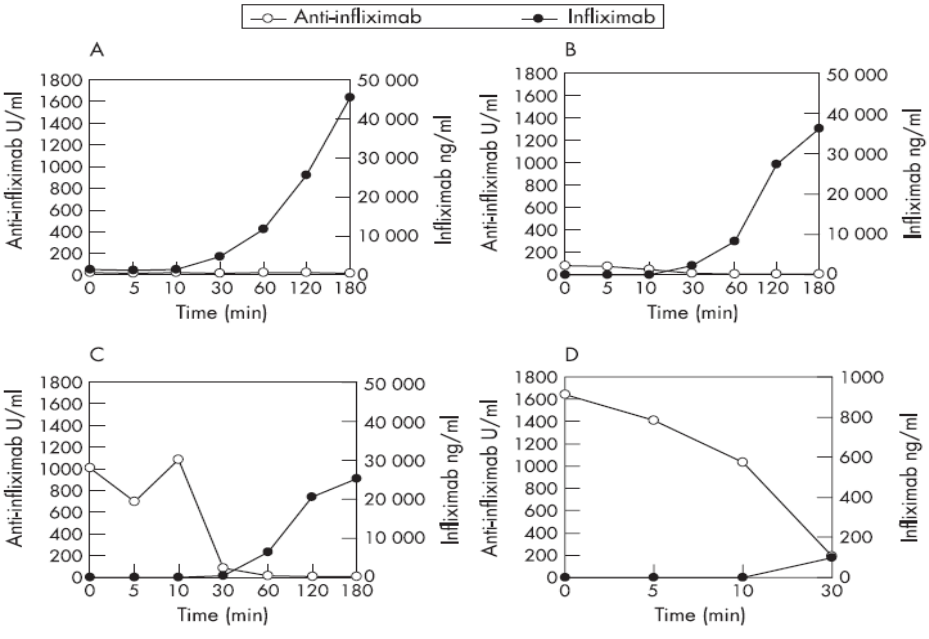
Thymus central tolerance: eliminates immature T cells expressing T cell receptors forming high avidity interactions with self peptides presented in human leukocyte antigen (HLA)

ADA response: loss of peripheral tolerance

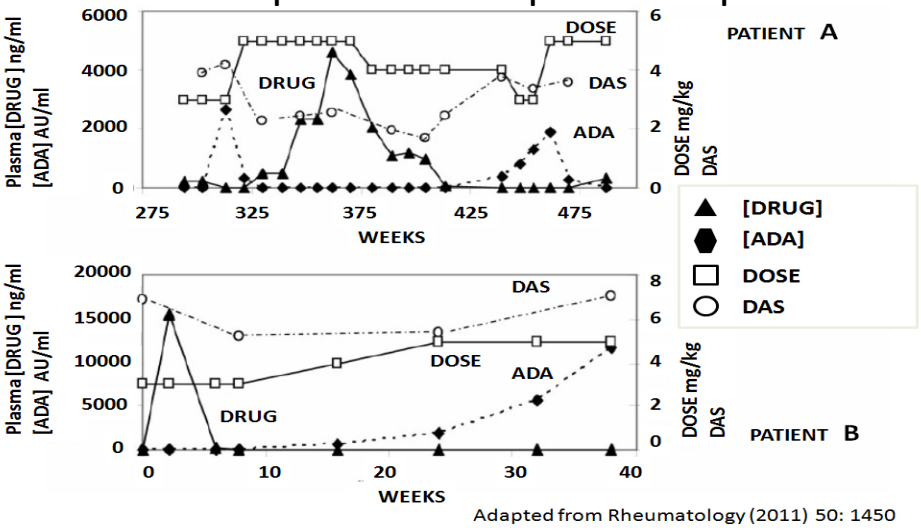
ADAs can be formed against normally tolerated proteins by breaking of peripheral tolerance

- Not all peptides of every self protein expressed in thymus, when a proportion of self-reactive T cells escape to periphery
- Peripheral tolerance ensures that such naive T cells only activated by dendritic cells (DCs) when both antigen specific peptide-HLA complex and co-stimulatory signals
- However, co-stimulation may be activated by pathogens and tissue damage, including by repeated injection, by high levels of inflammation *etc*.

Different patient PK responses



Different patient therapeutic responses



Adapted from Rheumatology (2011) 50: 1450

Drug **'DOSE'**, resulting **'DRUG'** trough levels, anti-infliximab **'ADA'** levels & disease activity score **'DAS'**