Slide 1

Non-Linear Elimination

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Slide 2

Revision
Clearance and Elimination

- Elimination describes irreversible loss of the drug from the body
- Clearance describes the relationship between the rate of elimination and concentration.

$$CL = \frac{Rate\ Out}{Concentration}$$

- This can also be thought of as a measure of an organ's efficiency at eliminating drug.

$$CL = Q \cdot \frac{C_{arterial} - C_{venous}}{C_{arterial}}$$

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Elimination describes the irreversible loss of drug from the body. This is predominately via metabolism or excretion; irreversible drug binding (e.g. to protein or another drug) also constitutes elimination, as the bound drug molecule cannot return to act on target receptors.

Clearance (CL) describes the rate of elimination of drug from the body to concentration. Another way of thinking about clearance is that it is a measure of an organs efficiency at eliminating drug. This is determined by organ blood flow (Q) and the extraction ratio $\left(\frac{C_{arterial}-C_{venous}}{C_{arterial}}\right)$ which is the difference between the venous and arterial concentration. Total clearance is thus the sum of clearances by each organ.

Clearance is a key pharmacokinetic parameter because it helps quantify the rate of elimination. Understanding the rate of elimination, and by association CL, is crucial for rational dosing. To maintain a target concentration drug should be replaced at the rate at which it is lost (Maintenance Dose Rate = Target Concentration · Clearance).

In model fitting, clearance can be estimated, though the true value will remain unknown. When estimated the modeller will get some idea of how it varies between individuals (between subject variability) and how precise the estimate is (Standard Error of the estimate).

Slide 3

Revision
First and Zero Order Kinetics

- We can describe absorption processes as being dependent or independent of concentration.
  - Zero Order: Constant amount is absorbed per unit time.

  - First Order: Constant proportion of drug is absorbed per unit time.

- We have previously described elimination as first-order.
  - A constant proportion of drug is eliminated per unit time

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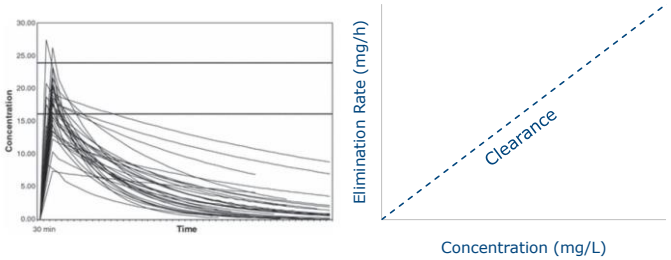
Absorption processes can be described as first-order (dependent on concentration) or zero-order (independent of concentration).

For compartment models which describe the rate of elimination as a first-order process ($Rate\ Out = CL{\cdot}C$), a constant proportion of the concentration of drug is eliminated per unit time.

Slide 4	**First-Order Elimination** • When elimination is described as first-order clearance is assumed to be independent of concentration (or organ blood flow). • Clearance is constant  Gonçalves-Pereira J et al. Clin Microbiol Infect. 2010 Aug;16(8):1258-63.	When elimination is described as first-order process, clearance is assumed to be constant, thus independent of concentration or organ blood flow. The rate of elimination is however concentration dependent. The figure on the right illustrates the increase in the rate of elimination with increasing concentration as well as the constant nature of clearance. Glomerular filtration is an example of a first-order process. Gentamicin is mainly eliminated by glomerular filtration, the figure on the left illustrates the time course of gentamicin concentration.
Slide 5	**Non-Linear Elimination** • The assumption that clearance is constant may not hold under certain conditions • Processes that rely on proteins are saturable at high concentrations (e.g. eliminating enzymes, transporters, protein binding) • Activity of proteins can be induced or inhibited • Non-linear elimination occurs when clearance is dependent on concentration (variable rather than not constant). 5 	The assumption that clearance is constant (independent of concentration) ignores the possibility that processes involved in elimination may become saturated (capacity limited). Elimination processes which require proteins such as transporters or enzymes may be taken up by substrates so there are few free transporters, or enzymes available, furthermore cofactor may be depleted. Protein amount and/or activity may also be inhibited or induced. Therefore, when concentration is high and the elimination pathway becomes saturated, an increase in concentration no longer increases the rate of elimination. Clearance is no longer constant as processes such as metabolism or renal secretion are capacity limited. The implications of non-linear elimination for dosing and drug therapy means that small increases in dose can result in large increases in concentration, and that the time to elimination will differ with the rate of input. Note non-linear kinetics is not exclusive to elimination. Non-linear kinetics mainly occurs in elimination processes as the enzymes and co-factors involved in drug metabolism (e.g. CYP450, n-acetyltransferase) may become saturated or depleted. Absorption and distribution processes may also exhibit non-linear kinetics (e.g. saturation of transport in gut wall, blood brain barrier transport).
Slide 6	**Causes of Non-Linear Elimination** 1. Saturation of elimination pathways 2. Induction of elimination pathways 3. Large Molecule Pharmacokinetics 6 	There are many causes of nonlinear elimination. Three causes will be discussed in this lecture. Metabolic clearance is the most common. This is because it is a capacity limited reaction in which the enzyme or its cofactor is not in limitless supply.

Slide 7

Saturation of Metabolic Clearance

- Substrate (S) binds reversibly to an enzyme (E) forming an substrate-enzyme complex (SE) which then reacts irreversibly to form metabolite (M) and enzyme

$$S + E \leftrightharpoons SE \rightarrow M + E$$

- Michaelis and Menten describe the rate of enzymatic reaction (reaction velocity) to be related to
  - S: substrate concentration
  - V_{max}: the maximum velocity of the system
  - K_m: concentration at which the reaction velocity is 50% of V_{max}

$$Reaction\ velocity = \frac{V_{max} \cdot S}{K_m + S}$$

- Considering reaction velocity as the rate of elimination (or metabolism):

$$Rate\ of\ Elimination = \frac{V_{max}}{K_m + C} \cdot C$$

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Substrate (drug) will bind reversibly to an enzyme to form a substrate-enzyme complex: $S + E \leftrightharpoons SE$.The substrate-enzyme complex reacts irreversibly to form a metabolite, subsequently the enzyme becomes free to bind free substrate: $SE \rightarrow M + E$

The Michaelis-Menten model of enzyme kinetics describes the relationship between concentration and rate of enzyme-mediated reaction. Reaction velocity can increase until 100% of enzymes are saturated. Indeed, the equation for reaction velocity is a hyperbolic curve with an asymptote at V_{max} (as concentration tends towards infinity, reaction velocity plateaus near V_{max}).

The rate of reaction can be considered to be equivalent to the rate of elimination (i.e. the rate of drug metabolism is the same as the rate of elimination), then clearance can be thought of as $\frac{V_{max}}{K_m + C_{central}}$.

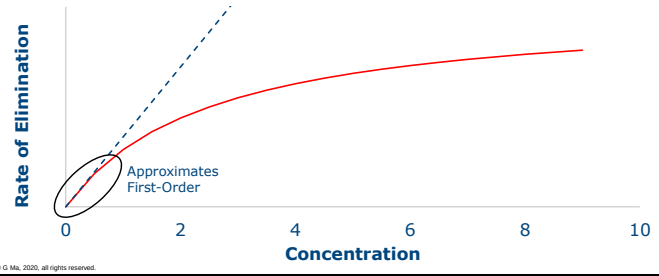
S describes the concentration of drug. V_{max} can be thought of as the maximum rate of elimination, thus is a function of the number of enzymes available. K_m (the Michaelis-Menton constant) is a function of the eliminating enzyme, it is inversely related to binding affinity for the substrate and enzyme; an enzyme with high affinity for the substrate will have a low K_m and will reach 50% of V_{max} at lower concentrations compared to an enzyme with a higher K_m.

Slide 8

$C \ll K_m$

- If concentration is low relative to K_m ($C \ll Km$), there are plenty of enzymes and elimination appears to follow first order kinetics.

$$Rate\ of\ Elimination = \frac{V_{max}}{K_m + C} \cdot C \approx CL \cdot C$$



When concentration is low, C is negligible relative to K_m.

$CL \approx \frac{Vmax}{Km}$ and elimination is limited by concentration rather can metabolic capacity. Thus, the rate of elimination can be approximated by first-order kinetics ($Rate = CL \cdot C$).

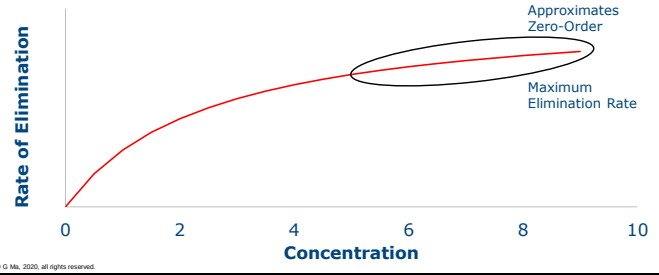
In the figure first-order elimination (concentration-dependent) is illustrated by the blue dashed line. The red line illustrates the rate of elimination as a function of concentration $\left(\text{Rate} = \frac{V_{max} \cdot C}{K_m + C}\right)$, as concentration increases the assumption of linear clearance (independent of concentration) no longer holds.

Slide 9

$C \gg K_m$

- If concentration is high relative to K_m ($C \gg K_m$), enzymes become saturated, and elimination rate becomes constant (pseudo-zero-order)

$$Rate\ of\ Elimination = \frac{V_{max}}{K_m + C} \cdot C \approx V_{max}$$



Further Reading Chapter 9 (Nonlinear Pharmacokinetics) in Applied Biopharmaceutics & Pharmacokinetics, Sixth Edition (Shargel L, Wu-Pong S, & Yu A)

When concentration is much greater than K_m, elimination processes (e.g. metabolism, renal secretion) approach the capacity limit (V_{max}) as enzymes become saturated. Since K_m is negligible relative to C, the rate of elimination occurs at a constant rate (≈V_{max}) that is close to the maximum elimination rate. The rate of elimination is thus approximately zero-order (independent of concentration), however, in reality concentrations are never high enough to produce zero-order elimination (this requires concentrations to be infinite). The use of pseudo-zero order elimination is an approximation that is sometimes seen in the literature but is often misleading (Holford NH. Clinical pharmacokinetics of ethanol. Clin Pharmacokinet. 1987;13(5):273-92.).

Concentrations that are neither small nor large in relation to K_m will give rise to a mixed-order reaction. The mixed-order reaction should be considered as the general case for all drugs eliminated by metabolism. The first-order approximation is very common. True zero-order elimination does not occur in reality but may be approximated at very high concentrations.

For drugs which follow linear kinetics, the elimination half-life is constant (independent of with dose or concentration). For drugs which follow non-linear kinetics, elimination half-life and clearance will not be constant, but rather dependent upon dose and concentration.

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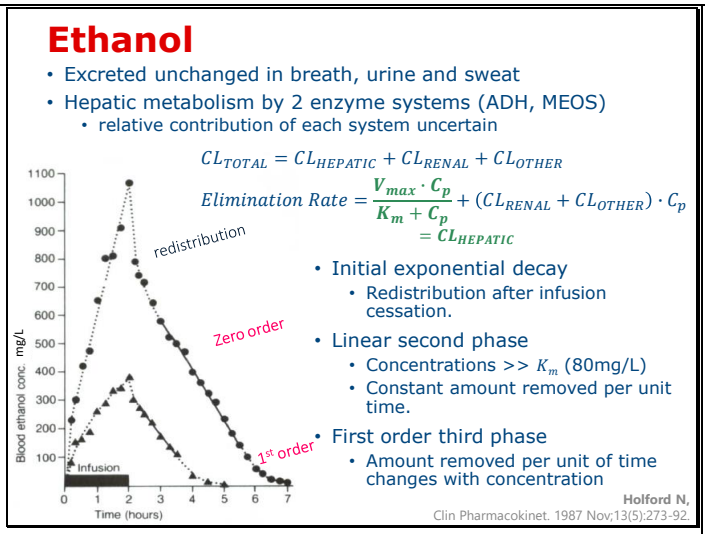

Further Reading:
Holford N. Clinical pharmacokinetics of ethanol. Clin Pharmacokinet. 1987 Nov;13(5):273-92.

An understanding of mixed-order kinetics can help avoid dosing a drug at a concentration near enzyme saturation.

Ethanol is excreted unchanged in breath, urine and sweat, but undergoes hepatic metabolism, thus clearance from each pathway is additive to give CL_{TOTAL}.

Models can be used to describe the clearance pathways at each organ. These may or may not be linear. Ethanol elimination can thus be described by a system which describes elimination at the kidneys (CL_{RENAL}) and through the sweat and breath (CL_{OTHER}) using first-order processes and clearance at the liver ($CL_{HEPATIC}$) as a saturable process (since K_m is often surpassed due to the large doses taken).

The concentration-time curve shows three obvious phases. The top of the curve has an exponential decay; this is due to rapid redistribution of ethanol following cessation of the infusion. In the second phase, (pseudo-zero order) concentrations are well in excess of the K_m, and the amount removed per unit of time is approximately constant. As concentrations get lower we see first-order kinetics emerge where the amount removed per unit of time changes with concentration (a linear relationship between concentration and rate of elimination).

Note that mixed order elimination occurs at all concentrations, the K_m (80 mg/L) is not a switch at which point the kinetics change but rather we see a gradual slowing of the rate of elimination with increasing concentration.

Note that it is difficult to estimate individual K_m and V_{max} for the two hepatic metabolism enzyme systems; alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS). A shared K_m and V_{max} seem to work just as well when describing ethanol elimination.

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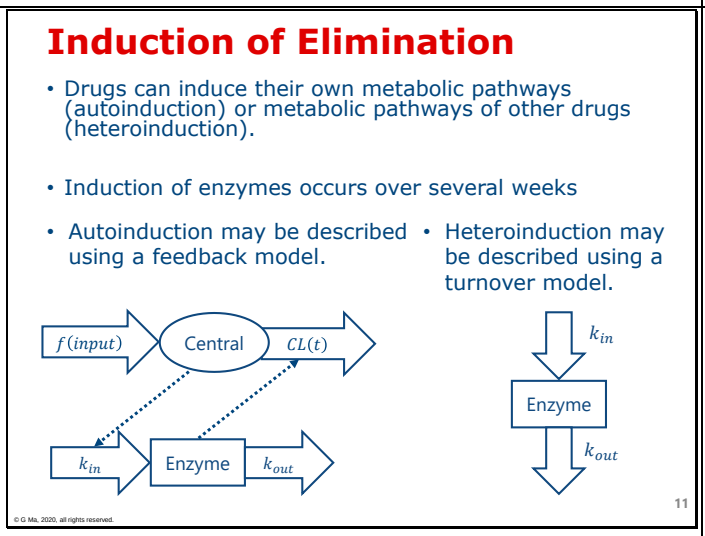


Further Reading:
M Hassan et al. A mechanism-based pharmacokinetic-enzyme model for cyclophosphamide autoinduction in breast cancer patients. Br J Clin Pharmacol. 1999 Nov; 48(5): 669–677.

C von Bahr, E Steiner, Y Koike, J Gabrielsson. Time course of enzyme induction in humans: effect of pentobarbital on nortriptyline metabolism. Clin Pharmacol Ther . 1998 Jul;64(1):18-26.

On turnover models: Chapter 21 (Time Course of Drug Response) in Principles of Clinical Pharmacology. ISBN: 9780123854711

Some drugs may induce enzymes or transporters involved in their own elimination (autoinduction) or induce enzymes involved in the elimination of other drugs (heteroinduction). This induction may increase enzyme amount and/or activity.

Induction of metabolic enzymes is time-dependent so requires repeated dosing to be observed and for the changes to reach steady-state.

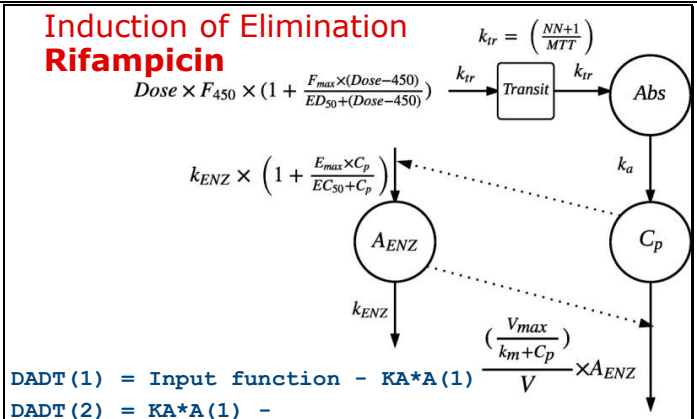
Autoinduction can be described using a model which involves feedback between concentration and clearance; a higher concentration of drug will result in an increase to the enzyme pool and increase it's clearance. Therefore, the level of enzyme should change with respect to concentration.

The figure on the left uses an one-compartment model to describe drug disposition, with input function, $f(input)$, and inducible clearance described as a function of time, $CL(t)$. The amount of enzyme in the body is described using a turnover model. The concentration of drug in the central compartment increases the production rate of enzyme (k_{in}), thus changes in the pool (amount) of enzyme in the body affects the clearance function, $CL(t)$. Note, drug concentration can drive an increase in enzyme transcription and translation (i.e. expression or synthesis), and/or reduction in the enzyme loss.

In contrast to autoinduction, where the concentration of drug induces a change in the pool of enzyme responsible for its own metabolism leading to a change in it's own clearance, heteroinduction is less elaborate. A feedback model is not required as the inducing drug (e.g. phenobarbital) alters the amount of enzyme which is responsible for the metabolism of another drug (e.g. nortriptyline). The figure on the right illustrates such a turnover model, phenobarbital will stimulate the enzyme production rate (k_{in}), thus at steady-state, the amount of nortriptyline metabolising enzyme will have increased.

Slide 12

Induction of Elimination
Rifampicin

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DADT(1) = Input function - KA*A(1)
DADT(2) = KA*A(1) -
     ((VMAX/(KM+CP))*FSIZECL)/V2)*A(2)*A(3)
DADT(3) = KENZ*(1 + EFF) - KENZ*A(3)
EFF = (EMAX*CP)/(EC50 + CP)
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Svensson et al.
Clin Pharmacol Ther. 2018;103(4):674–683.

Further Reading:
Svensson et al. A Population Pharmacokinetic Model Incorporating Saturable Pharmacokinetics and Autoinduction for High Rifampicin Doses. Clin Pharmacol Ther. 2018;103(4):674–683.

Smythe et al. A Semimechanistic Pharmacokinetic-Enzyme Turnover Model for Rifampin Autoinduction in Adult Tuberculosis Patients. Antimicrob Agents Chemother . 2012 Apr;56(4):2091-8.

The feedback model used to describe autoinduction in the previous slide has been used to model rifampicin, an antibiotic used in the treatment of tuberculosis. It is metabolised at intestinal wall and actively excreted into the bile at liver. Rifampicin is one of the most potent induces of the cytochrome P450 system and will cause its own metabolising enzymes to become induced during the first few weeks of therapy. A decrease in rifampicin exposure with time with repeated dosing has been reported. The dose dependent absorption of rifampicin, coupled with the capacity limited elimination means patients receiving high doses are at risk of overdosing. Contrasting that is the auto-induction of metabolism that could lead to sub-therapeutic concentrations and treatment failure should it not be accounted for in the first weeks of therapy.

DADT(1) describes concentrations in absorption compartment (Abs). Rifampicin absorption is modelled using a series of transit compartments before arriving at the absorption compartment (Abs) and absorbed into the central compartment (C_p) via a first-order process. To account for dose dependent absorption the relationship between dose and F was described using an E_{max} relationship where bioavailability (F) was described as a function of bioavailability for 450 mg (F_{450}; assumed to be 1), the maximal increase in bioavailability (F_{max}) and the dose above 450 mg that corresponds to half the F_{max} (ED_{50}). The transfer rate between transit compartments is described by the transit rate constant (k_{tr}) which is 1+NN (number of transit compartments) divided by the mean transit time (MTT).

DADT(2) describes cconcentration in central compartment (C_p). The rate of elimination is described by a mixed-order process, $\left(\frac{V_{max}}{k_m+C_p} \cdot A_{ENZ}\right)/V$, that depends upon the amount of enzyme (A_{ENZ}). In the model code clearance $\left(\frac{V_{max}}{k_m+C_p}\right)$ is scaled to body size (FSIZECL), and the CL model is multiplied by A(2) the amount in the central compartment (C_p) as well as A(3), the amount of enzyme (this scales CL for autoinduction).

DADT(3) describes enzyme kinetics. The turnover model for enzyme production describes zero-order formation of enzyme as well as first-order enzyme degradation using the same rate constant (k_{ENZ}) for simplicity. The stimulation of enzyme formation rate by plasma concentration of drug (C_p) is described by an E_{max} model (EFF in the model code).

Slide 13

Large Molecule Pharmacokinetics

- 'Biologics' are a group of drugs that include growth factors, cytokines, hormones and monoclonal antibodies (mAb's)
- mAb's in particular often have strongly nonlinear PK
  - Bind IgG targets with very high specificity
  - This binding often dominants kinetic profile
  - Termed 'Target Mediated Drug Disposition' (TMDD)
- Clearance may be through
  - metabolism in cells after pinocytosis or receptor-mediated endocytosis, renal filtration (small molecules) etc = all contribute to linear elimination
  - via the targets themselves ('target mediated clearance')

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More than 40 monoclonal antibodies now approved for use. They mainly target immunoglobin G1, with a few towards IgG2 and IgG4. mAbs are used to treat several autoimmune disorders, lupus, familial hypercholesteriemia, some cancer and tumor types.

mAbs are typically large molecules designed to bind to a specific target with high affinity. This contributes to the disposition of biologics and result in non-linear pharmacokinetics. Generally, at high concentrations linear elimination pathways dominate, but at low concentrations mAb elimination is dominated by saturation of irreversible binding to the target (due to finite number of targets on the cell surface), thus non-linear elimination occur.

mAb's undergo metabolism (proteolysis) and other nonspecific CL (including antibody salvage) which follows linear elimination.

Most mAb's are too large for glomerular filtration (linear; first-order) although antibody fragments and other smaller biologics may be eliminated this way.

Receptor-mediated endocytosis is one mechanism by which mAbs get taken up into the cell and can then subsequently be broken down. The receptor it binds may or may not be the target, but when it is the target, this is called target mediated clearance.

Slide 14	**Target Mediated Drug Disposition**
	• Target receptor binding often happens quickly and at low concentrations
	  • Targets are in high supply and mAb binds with high specificity and high affinity
	• Once bound, receptor and mAb complex are internalised for cell membrane bound receptors, and broken down for cytosolic receptors
	• Target Mediated Drug Disposition (TMDD) tends to be
	  • visible at low concentrations (at high concentrations linear elimination dominants, target binding is saturated)
	  • results in faster than linear elimination alone
	  • Nonlinear elimination will increase when mAb targets are increased
	  • Targets will saturate
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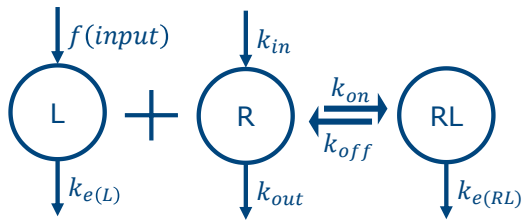
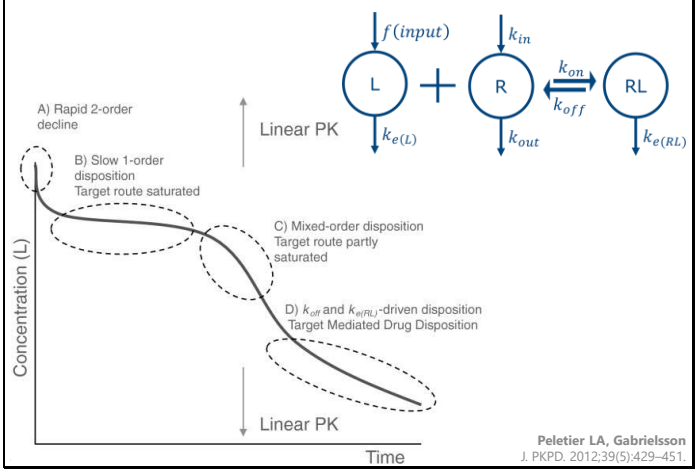
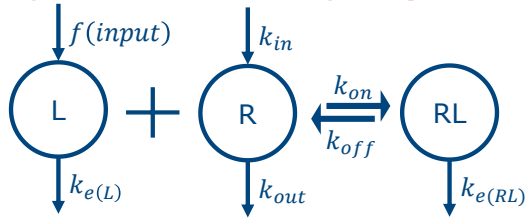
		Most mAbs bind with high affinity (they have a low K_d, the equilibrium dissociation constant: $K_d=K_{off}/K_{on}$). This is done by designing the mAbs to dissociate slowly (so K_{off} is small). K_d is inversely related to affinity, i.e. when K_d is low, affinity is high. The half-life for dissociation can range from a matter of minutes through to several days. Further complicating these kinetics is immunogenicity: mAbs are foreign to the body and so may stimulate an immune response from the patient depending on their genetics, disease, etc. This may be large or minor. Response typically includes production of anti-drug antibiotics that can bind the mAb to either inactivate it or clear it. FcRn (or the Brambell receptor) is a pathway designed to protect IgG from phagocytosis and subsequent catabolism and acts to return IgG complexes to the extracellular fluid. This can also alter the internalization (or recycling back out of the cell) for mAbs. These various factors illustrate the complex roundabout of mAbs in the body, making TMDD a difficult pharmacokinetic problem.

Slide 15	**Target Mediated Drug Disposition**  $\frac{dL}{dt} = f(input) - k_{e(L)} \cdot L - k_{on} \cdot L \cdot R + k_{off} \cdot RL$ $\frac{dR}{dt} = k_{in} - k_{out} \cdot R - k_{on} \cdot L \cdot R + k_{off} \cdot RL$ $\frac{dRL}{dt} = k_{on} \cdot L \cdot R - RL(k_{off} + k_{e(RL)})$ 16 	A general model for TMDD includes elimination of drug from the plasma as the combination of first-order elimination from the central compartment, specific target binding (followed by internalization into the cell where it is broken down) and turnover of the target. This figure illustrates a one compartment ligand model coupled to target turnover and formation of ligand-target complex. Input of ligand (drug) into the central compartment is described using an input function, $f(input)$; note that two-compartment models are often used in the literature, however a one compartment model is used here for simplicity. k_{in} and k_{out} are constants which describe turnover of the target (R). Unbound ligand in the central compartment will bind reversibly (k_{on}, k_{off}) to target forming a ligand–target complex (RL). There are therefore two routes by which the ligand may be eliminated, non-target mediated loss of unbound ligand ($k_{e(L)} = \frac{CL}{V}$) and target mediated loss of the ligand-target complex ($k_{(e(RL)}$). The law of mass action allow for derivation of three ordinary differential equations to describe ligand (L), target (R) and target-ligand complex (RL). $f(input)$ describes the input function. $k_{e(L)} \cdot L$ describes irreversible first-order elimination of the ligand (non-specific clearance). $k_{on} \cdot L \cdot R$ describes binding to the target (second order formation of the ligand target complex; dependent upon L and R thus second-order). Target formation is described by a zero-order process (k_{in}) whereas a first-order process ($k_{out} \cdot R$) describes the loss of target. $k_{off} \cdot RL$ describes dissociation from the target. $RL \cdot k_{e(RL)}$ describes the elimination of the ligand-target complex.
Slide 16	**Target Mediated Drug Disposition** 	This model is linear at very low and very high ligand (drug) concentrations. At low ligand concentrations, the target is not saturated so there will be two parallel first-order (linear) routes for ligand removal (non-specific clearance and elimination via the target). At high ligand concentration, elimination via the target becomes saturated so it's contribution to ligand clearance becomes limited. Four phases of TMDD have been observed: 1. Initial distribution phase (drug and target equilibrate) 2. High concentrations so targets are saturated, drug is mainly eliminated by first-order elimination routes (apparent linear phase) 3. Concentrations low enough that the target receptors are not saturated. Drug is eliminated directly as well as in the form of the drug-target complex. Both target mediated CL and nonspecific linear CL dictate the profile

		4. Concentrations are low, target receptors are in in high supply, elimination is a first-order process along with elimination as a drug–target complex. Note ligand concentration in the figure is expressed in the log domain.
Slide 17	**Target Mediated Drug Disposition**  $\frac{dL}{dt} = f(input) - k_{e(L)} \cdot L - (k_{on} \cdot L \cdot R + k_{off} \cdot RL)$ • Assuming • $k_{out} = k_{e(RL)}$ • $k_{e(RL)} \cdot RL = V_{max}$ $\frac{dL}{dt} = f(input) - k_{e(L)} \cdot L - \frac{V_{max} \cdot L}{K'_m + L}$ 	The full model makes several assumptions but can be easily adjusted and extended to reflect underlying physiology. It also has lots of parameters and so a fair amount of data is needed to estimate them all well. Approximations can be used when insufficient data exists to estimate all parameters. One approximation (thus reducing the number of parameters) is to use Michaelis Menten kinetics. This may be suitable when the target concentration is small relative to the free drug concentration and so the target receptors saturate easily at clinical doses and we don't need to capture the profiles at low concentrations relative to target receptors. Here we are not attempting to estimate the irreversible elimination of the ligand-receptor complex, instead we indirectly get a sense of it from the K'_m parameter. K'_m here is not directly comparable to traditional K_m parameters because it does not just represent affinity but both affinity $\left(\frac{K_{off}}{K_{on}}\right)$ as well as irreversible elimination of the ligand-target (RL) complex. Further Reading: Dua et al. A Tutorial on Target-Mediated Drug Disposition (TMDD) Models. CPT Pharmacometrics Syst Pharmacol. 2015 Jun; 4(6): 324–337. Mager DE, Jusko WJ. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J Pharmacokinet Pharmacodyn. 2001; 28: 507–32. Peletier LA. Gabrielsson J. Dynamics of target-mediated drug disposition: characteristic profiles and parameter identification. J. Pharmacokinet. Pharmacodyn. 2012;39:429–451.