Challenges in pre-clinical development of biologics

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Acknowledgements:
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Biologics vs Small MW NCEs
What’s the difference?
Protein therapeutics “biologics”

- **Current generation**
  - recombinant proteins, binding proteins, cytokines; pegylated proteins: antibodies (mAbs), antibody fragments (e.g. Fabs), antibody drug conjugates (ADCs)

- **Next generation?**
  - above proteins with non-natural amino-acids / post-translational modifications (e.g. altered glycosylation pattern), nanobodies, bi-specific antibodies, new scaffolds ... ...
Monoclonal antibodies vs NCEs:

<table>
<thead>
<tr>
<th>mAb</th>
<th>New Chemical Entity</th>
</tr>
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<tbody>
<tr>
<td>150,000 dalton</td>
<td>200-500 dalton</td>
</tr>
<tr>
<td>Biological production process – heterogeneous (post-translational modifications)</td>
<td>Chemical production process - homogeneous</td>
</tr>
<tr>
<td>High species selectivity (affinity / potency)</td>
<td>Generally less selective</td>
</tr>
<tr>
<td>Multi-functional – target binding, Fc effector function, FcRn binding</td>
<td>Single target</td>
</tr>
<tr>
<td>Toxicity – largely “on target” mediated “exaggerated pharmacology”</td>
<td>Toxicity – often “off target” mediated</td>
</tr>
<tr>
<td>Slow clearance; long half-life (days) – infrequent dosing (weekly / monthly) ?</td>
<td>Rapid clearance; short half-life (hours) – frequent dosing (daily)</td>
</tr>
<tr>
<td>Target can affect PK behaviour (Target Mediated Drug Disposition)</td>
<td>Mostly linear PK; non-linearity from saturation of metabolic pathways</td>
</tr>
<tr>
<td>Drug-Drug Interaction – few examples and mostly PD related</td>
<td>DDI – many examples and metabolic and/or PD related</td>
</tr>
<tr>
<td>Immunogenicity sometimes observed</td>
<td>Immunogenicity rarely observed</td>
</tr>
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Outline of the presentation:

- **PK-PD behaviour of mAbs**
  - inherent IgG characteristics
  - ligand binding models and Target Mediated Drug Disposition

- **Developing an integrated bio-analytical strategy**
  - PharmacoKinetics
  - PharmacoDynamics (target ligand)
  - Immunogenicity

- **Selection of appropriate starting doses for early clinical testing**
  - FDA guidance 2005
  - NOAEL / (PAD) MABEL

- **Summary**
A simple mAb PK model

limited volume of distribution \( \sim 7\text{L} \)
slow clearance \( t_{1/2} \sim 20\text{ days (human)} \)

FcRn (tight binding at pH6) protects IgG from degradation & explains long serum half-life

Roopenian and Akilesh.
Nature Reviews Immunology 2007; 7: 715
Mouse, rat and monkey FcRn recognise human IgG

NB - binding affinity of human IgG to mouse FcRn is 10x higher than to human FcRn

- “inherent” human IgG kinetics scales reasonably well across species
- NB when target mediated disposition is absent
Engineered proteins may have altered FcRn binding

Ligand binding model (closed system)

At equilibrium: \[ K_d = \frac{[\text{mAb}] \cdot [\text{ligand}]}{[\text{mAb- ligand complex}]} \]

Assumptions:
- static system; no turnover of ligand, no loss of mAb to distribution and elimination, instantaneous equilibrium
Ligand binding model (closed system) TGN1412

- static system; no turnover of ligand, no loss of mAb to distribution and elimination, instantaneous equilibrium

Dose (mg/kg) 0.0001 0.001 0.01 0.1 1 10
Receptor occupancy (%) 0 20 40 60 80 100

TGN1412 = 18.7 nM (immediately post-dose)

mAb + ligand → mAb – ligand complex

K_d = 1.88 nM

Dose 0.1 mg/kg
MW 150,000
plasma volume 2.5L

Tcell 1.9 x 10^6 mL^{-1}
CD28 / cell 150,000

CD28 = 0.95 nM at baseline
A simple mAb PK-PD model

Soluble target:
- mAb-ligand complex tends to take on the elimination characteristics of the mAb

Cellular target:
- mAb-ligand complex tends to take on the elimination characteristics of the ligand (TMDD apparent)
Lowe PJ et al: On setting the first dose in man: Quantitating biotherapeutic drug-target binding through PK and PD models
Basic & Clin Pharmacology & Toxicology 2009; 106: 195-209

Typical mAb PK-PD model(s)
Example 1: mAb against cell surface ligand

anti-CD11a mAb – Raptiva (efalizumab)
- rapid clearance of mAb-receptor complex
- high doses saturate target binding capacity with a return to “normal” IgG kinetics

Example 2: mAb against soluble ligand (MCP-1)

- slow elimination of mAb-ligand complex
- accumulation of total ligand
- rapid turnover of ligand
- rapid saturation of binding capacity
- long half-life total mAb short duration of effect

Accumulation inactive complex

1 x 0.1 mg/kg

2 x 0.3 mg/kg
Example 3: mAb against soluble ligand (IL1-β)

- slow elimination of mAb-ligand complex
- saturable accumulation of total ligand
- long half-life total mAb; long duration of effect

Measured ACZ885 (2 x 10mg/kg)

Measured total IL-1β
Example 3: mAb against soluble ligand (IL1-β)

Accumulation inactive complex

Measured total ACZ885

Simulated free IL1-β

Increasing dose: no change in IgG kinetics

Increasing dose: increases duration of effect
Scalable components of the PK-PD model:

- Inherent pharmacokinetics of the mAb and clearance of the mAb-ligand complex:
  - PK of monoclonal antibodies will generally follow “typical IgG behaviour” and scale reasonably well to man and/or exhibit Target Mediated Disposition and be dependent on the amount of target present and its rate of turnover

- Binding affinity and potency against the target ligand:
  - species differences understood during characterisation of the mAb

- Expression and turnover of the ligand:
  - key drivers of the extent and duration of response; can affect PK behaviour
  - once “maximum” ligand binding is achieved then increasing the dose will primarily increase the duration of response:
    - are SAD/MAD designs appropriate for early clinical development?
  - species differences and differences between healthy individuals vs disease often not well understood
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- **Developing an integrated bio-analytical strategy**
  - PharmacoKinetics
  - PharmacoDynamics (target ligand)
  - Immunogenicity

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- **Summary**
Bioanalytical strategy - PK

**Free-”bioactive” mAb**
- anti-human IgG
- mAb (drug)
- ligand

**sandwich ELISA**

**Total mAb**
- anti-human IgG
- mAb (drug)
- anti-human IgG

**sandwich ELISA** (pre-clinical only)

**competitive ELISA**
- mAb (drug)
- ligand

**bridging ELISA**
- ligand
- mAb (drug)
- ligand

**protein digestion**

**protein LCMS**

**unique “signature” peptide**

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Slide 18 | Early Pre-Clin Dev of Biologics | Peter Lloyd | Berlin March 2011
Bioanalytical strategy – PD (soluble ligand)

**total ligand (target capture)**

- Anti ligand Ab2
- mAb (drug)
- Sandwich ELISA
- Ligand – endogenous binding protein

Technically challenging:
- Detection of total ligand (increasing) in the presence of drug and endogenous binding protein (if relevant)
- Careful selection of capture and detection reagents (Ab1 and Ab3)

**free ligand**

- Anti ligand Ab4
- mAb (drug)
- Sandwich ELISA
- Ligand – endogenous binding protein

Technically very challenging:
- Detection of free ligand (decreasing) in the presence of increasing concentrations of total ligand
- Specificity and sensitivity (LLOQ)
Consequences of immunogenicity:
- increase in clearance of mAb (immune complexes)
- decrease in capacity for target binding (neutralising immunogenicity)
- influence on PK and/or PD assays – integrated assessment?
Example 4: mAb against cell surface ligand (receptor)

Single dose 10 mg/kg i.v. n=3 animals

PK profile

Immunogenicity

Serum concentration vs Time [day]

Rel. Response Unit [RU] vs Time [day]

mAb (drug) and ligand

competitive ELISA

Biacore

ADA

mAb (drug)
Example 4: mAb against cell surface ligand (receptor)

Three doses 100 mg/kg i.v. days 0, 7, 14 (n=3 animals)

- bridging ELISA overcomes limitations of competitive ELISA to measure exposure to drug in presence of ADA response

- rapid “clearance” of drug due to nAbs and/or rapid clearance of immune complexes
Outline of the presentation:

- **PK-PD behaviour of mAbs**
  - inherent IgG characteristics
  - ligand binding models and Target Mediated Drug Disposition

- **Developing an integrated bio-analytical strategy**
  - Pharmacokinetics
  - Pharmacodynamics (target ligand)
  - Immunogenicity

- **Selection of appropriate starting doses for early clinical testing**
  - FDA guidance 2005
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- **Summary**
FDA guidance: 2005

Step 1  Determine “No Observable Adverse Effect Level” (NOAEL)

Step 2  Convert NOAEL to a “Human Equivalent Dose” (HED)
- generally normalised to body surface area (low MW NCEs)
- mg/kg normalisation recommended for proteins >100K daltons

Step 3  Select HED from the most appropriate species
- additional factors: metabolism, receptors, binding epitopes …
- default: most sensitive species (lowest HED)

Step 4  Apply a safety factor (≥10-fold) to give a: “Maximum Recommended Starting Dose” (MRSD)

Step 5  Adjust MRSD based on the pharmacologically active dose (PAD)
Pro

- simple to use
- supported by historical evidence (mainly conventional NCEs)

Con

- primary focus: NOAEL (steps 1-4)
- secondary focus: pharmacologically active dose (PAD)
- over simplified scaling to man
- focus on dose not exposure
- one algorithm fits all
- step 5 often ignored

FDA guidance: 2005
NOAEL and MABEL (PAD)

NOAEL – No Observable Adverse Effect Level

FDA – “highest dose level that does not produce a significant increase in adverse effects”

“an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase I clinical trial conducted in adult healthy volunteers”

MABEL - Minimal Anticipated (Acceptable) Biological Effect Level

minimal exposure / dose level that is anticipated to produce an acceptable biological effect

“an effect that would be considered acceptable if produced by the initial dose of a therapeutic in a phase I clinical trial”
Example 5: target suppression in safety assessment

- Humanised mAb; high affinity against soluble target
- $K_d$ man < cynomolgus monkey (~10-fold)
- "typical IgG kinetics"
- Target ligand can be measured in the systemic circulation at baseline and is increased in disease
- mAb acts as a "capture system": mAb-ligand complex (detected in serum) is a biomarker for suppression of free ligand via a PK/PD model
- Previous experience with a similar molecule (lower affinity) against the same target
Example 5: 4wk GLP toxicology study

PK-PD model: exposure and total ligand

- pre-clin exposure data conform to a 2-compartment model
- increase in total ligand (measured) fitted to ligand binding model
- PK-PD model allows estimation of free ligand (target suppression)
  NB: free ligand (green) not measured
Example 5: predicted exposure (PK) in man

Cynomolgus monkey PK-PD model scaled to human:
- IgG PK behaviour
- increased target binding affinity (10-fold)

Accumulation inactive complex

Cynomolgus monkey 120mg/kg NOAEL (observed mean data)

>100-fold exposure safety margin ($C_{\text{max}}$)
Example 5: predicted PD (effect) in man

Simulation: predicted target suppression in man
- pre-clin exposure data adjusted to man
- binding affinity in PK-PD model adjusted to man

Monte-Carlo simulation
Example 5: Justification of starting dose in man

Toxicology (exposure)

[1] NOAEL 120 mg/kg

[2/3] HED 120 mg/kg
- adjust for anticipated exposure in man?

[4] Apply ≥10-fold safety factor 12 mg/kg

Pharmacology (response)

[5] PAD / MABEL
- justify based on pharmacology
- max pharmacology achieved at 20mg/kg (6-fold lower than NOAEL)
- adjust for anticipated exposure in man (0.1 mg/kg projected to give >100-fold margin)
- include anticipated duration of effect
- adjust for inter-species differences in affinity / potency (>50% ligand suppression for <48h)

0.1 mg/kg

“Maximum Recommended Starting Dose”
- define anticipated safety window based on NOAEL and MABEL *
0.1 mg/kg

* - NB an additional factor may be added based on uncertainty of data / prediction and relative risk
Benefits of an Integrated Approach

- “Simple” mathematical representation of known biology also represents components of the model which cannot be measured (e.g. low circulating level of free ligand)

- Hypothesis testing
  the ability to test assumptions prior to experimental design, leading to better pre-clinical and clinical studies

- Sensitivity analysis
  elements of the model which are key drivers of the desired outcome (e.g. target expression / target turnover / affinity / potency)
Summary: Challenges in pre-clinical development of biologics

- Inherent IgG characteristics make mAbs attractive drug targets; however; unlike small MW new chemical entities (NCEs):
  - target biology can affect PK behaviour of mAbs / duration of action
    - target expression (how much and where?) and target turnover (how quickly is it replaced?)
    - normal vs disease, gender, species differences should be understood
    - other “sinks” (e.g. shed or decoy receptors), “off target” binding, endogenous binding proteins

- A bioanalytical strategy and appropriate tools needs to be developed
  - integrated interpretation of PK, target binding and immunogenicity

- An integrated PK/PD-(IG) approach can quickly identify appropriate dose and dosage regimen to achieve desired target suppression
  - influence and impact on traditional study design
Thank you for your attention