Early safety assessment of biologicals

Risks identified from nonclinical development: AGAH Annual Meeting, Munich 2014

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Outline

• Mechanisms of toxicity
  – examples
• Predicting clinical effects from preclinical data
  – PK-PD considerations
  – Species selection
  – Use of in vitro data
• “Off target” binding and adverse effects
Protein therapeutics “biologics”

Current generation
Recombinant proteins (hormones, cytokines, growth factors, clotting factors…) pegylated proteins, antibodies (mAbs), antibody fragments (e.g. Fabs), antibody drug conjugates (ADCs)

Next generation
Engineered antibodies, bispecifics, ADCs, antibody fragments and related products, above proteins with non-natural amino-acids / post-translational modifications (e.g. altered glycosylation pattern), new protein scaffolds (e.g. fibronectins, anticalins, fynomers, ……)

Mechanisms of Toxicity
• Chemical-based toxicity of drug and/or metabolites
• Mechanism of action-related “On-target toxicity”
  – Exaggerated and adverse pharmacologic effects at the target of interest
• “Off-target toxicity”
  – Due to modulation of other target(s) – may be related targets or or totally unrelated to target of interest
  – Rare for biologics – based on experience to date
  – But impact of more protein engineering and novel scaffolds?
• Adverse consequences of immunogenicity / anti-drug-antibodies
  – Immunogenicity in animals does not generally predict immunogenicity in humans
    • Impact of more protein engineering and novel formats?
  – Translatability of ADA-related adverse effects for humans?
    • PRCA and thrombocytopenia predicted from animals which developed ADAs to erythropoietin and thrombopoietin
    • Hypersensitivity reactions, immune complex pathologies?
### Examples of adverse findings leading to termination / delays in clinical development

- **“On-target toxicity”**
  - Anti-DLL4 mAb - proliferative vascular lesions in skin, heart, lung in rats; liver sinusoidal dilation and centrilobular hepatocyte atrophy with increased transaminases in rats & monkeys
  - Anti-CD40L – thromboembolism in patients
  - TGN1412 – cytokine storm in healthy volunteers
- **“Off target toxicity”**
  - Platelet activation and hypotension in monkeys
  - Unexpected “off target” binding resulting in death of monkeys
- **ADA-related**
  - GSK anti-TNFR product – in vitro and in healthy volunteers
  - Thrombocytopenia – PEG-Hu-MDGF – ADAs cross-reacted with endogenous TPO
  - IC related pathologies in animal species: hypersensitivity reactions, thrombocytopenia, vasculitis, IC adverse effects in e.g. kidney, liver, lung, eye, heart........

Safety-related regulatory actions for biologics approved in the US and EU: Giezen et al. 2008 JAMA 300 (16): 1887-1896

### New clinical trial with TGN1412

Human regulatory T cells areselectively activated by low dose application of the CD28 superagonist TGN1412/TAB08, Tabares et al, 2014, Eur J Immunol

- Low doses (0.1 μg/kg to 7 μg/kg) of TAB08 given over a 4 to 12h IV infusion to healthy volunteers
- Starting dose 1000-fold less than 0.1 mg/kg in 2006 trial
- Dose-dependent systemic release of Treg-cell signature cytokine IL-10 in absence of pro-inflammatory factors associated with the CRS of the 2006 TGN1412 study
- “Results demonstrate the potential of appropriately dosed CD28SA and corticosteroid co-medication to mobilize human Treg cells for the treatment of autoimmune and inflammatory conditions”
Anti-CD40L mAb

Biogen Halts Anti-CD40 Ligand Monoclonal Antibody Trials

On October 21, 1999, Biogen Inc. (Cambridge, MA) announced that it had halted several trials of its anti-CD40 ligand monoclonal antibody compound until the company completes addressing issues relating to thromboembolic adverse events. Biogen said it was working closely with the FDA on reviewing data and determining when trials could be resumed.

- No thromboembolism reported in 1-month and 6-month repeated dose toxicity studies in cynomolgus monkeys (pharmacologically relevant species)
- Subsequent studies in rhesus monkeys and baboons also reported thromboembolic findings and/or thrombocytopenia
Examples of adverse findings leading to termination / delays in clinical development

• “On-target toxicity”
  – Anti-DLL4 mAb - proliferative vascular lesions in skin, heart, lung in rats; liver sinusoidal dilation and centrilobular hepatocyte atrophy with increased transaminases in rats & monkeys (Anti-CD40L – thromboembolism in patients)
  – Anti-CD40L – thromboembolism in patients
  – TGN1412 – cytokine storm in healthy volunteers

• “Off target toxicity”
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• ADA-related
  – Thrombocytopenia – PEG-Hu-MDGF – ADAs cross-reacted with endogenous TPO
  – IC related pathologies in animal species: hypersensitivity reactions, thrombocytopenia, and adverse findings in e.g. kidney, liver, lung, eye, heart........
  – Provide a weight of evidence that observed adverse effects are secondary to ADA and IC formation and not MOA related
  – Hypersensitivity of biologics: Leach et al, 2014 Toxicologic Pathology 42:293
  – VH domain antibody TNFR antagonist – cytokine release in vitro and in healthy volunteers
  – Predictive immunogenicity / deimmunisation approaches

Cytokine release in healthy volunteers with novel scaffold

Holland et al, Autoantibodies to variable heavy (VH) chain Ig sequences in humans impact the safety and clinical pharmacology of a VH domain antibody antagonist of TNF-α receptor 1. J Clin Immunol, July 2013
Cytokine data from healthy human subjects in a Phase 1 SAD IV study of GSK1995057
- Dotted lines represent group mean data for HAVH-negative subjects
- Dashed lines represent group mean data for HAVH-positive subjects
- Individual subject data for subject Ab1, Ab2 and Ab3 are represented by solid lines.

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Predicting Clinical Effects from Preclinical Data

*Integrated* translational pharmacology, PK-PD and safety assessment studies

**Pharmacokinetics**

\[
\frac{dC}{dt} = -CL\cdot C
\]

**Pharmacology**

\[
E = \frac{E_{\text{MAX}} \cdot C}{EC_{\text{50}} + C}
\]

**Physiology and Disease**

\[
dR/dt = k_{\text{in}} - k_{\text{out}} \cdot R
\]

Mager, Woo, and Jusko. DMD Vol. 24 (2009), No. 1 pp.16-24

**Key Questions to address during early development**

- Question: Can selected biological target be therapeutically modulated, and manufactured and delivered effectively with reasonable dosing regimen?
- Question: What is the anticipated therapeutic dose / concentration
- Question: Target-related safety liabilities - is it safe?
  - Exposure-response relationship and exposure-toxicity relationship explored in *early development studies* – rodent and non-rodent (if both relevant), animal model of disease if appropriate

**PK-PD considerations in early development (1)**

- Small molecule drugs: receptor interaction negligible for mass action in PK model
- Proteins: target/receptor interaction may contribute substantially to disposition of drug leading to *non-linear pharmacokinetics*

- Non-linear PK in cynomolgus monkeys for mAb vs cell surface target (TMDD - target-mediated drug disposition)
- Can be used to generate target saturation curves based on PK-PD model
- Compare PK-PD model predictions with measured receptor occupancy if possible

Lowe et al 2009, Basic & Clinical Pharmacology & Toxicology 106, 195-209
PK-PD considerations in early development (2)

• Non-linear PK for mAb vs soluble target – clearance at 10 mg/kg was faster than mAb with “typical” IgG behavior
• Target saturation observed at 50 mg/kg, not apparent at 10 mg/kg (see troughs) suggesting very high levels of free target in normal monkeys
• Simulations of the PK/PD model in disease state suggest high, frequent doses of antibody are required to maintain neutralisation of the chemokine.

Modeling of drug-target binding for an antibody targeting a rapid turnover chemokine, Subramanian et al., Novartis Poster 2010,

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Predicting Clinical Effects from Preclinical Data

• For small molecule drugs - high dose in a general toxicity study is generally the Maximally Tolerated Dose (MTD)
• For biologics, adverse effects often due to exaggerated pharmacology - MTD approach not generally used to select high dose in toxicity studies
• Highest dose level may be the NOAEL and all dose levels may maximally modulate the target
• Establish shape and steepness of concentration-response relationships
• And MABEL / PAD
Predicting Clinical Effects from Preclinical Data

MABEL is reliant on:
- Robust measures of translational PD biomarkers:
  - target engagement (e.g., receptor occupancy/ligand binding)
  - mechanism (e.g., downstream signalling)
  - outcome (e.g., pharmacological/clinical response)
- Use of a pharmacologically responsive animal species
- Optimal study designs – use of all in vivo and in vitro data
- Translation biomarker plan available at time of candidate selection

Selection of pharmacologically relevant species for translational PK-PD and safety assessment

High target selectivity

High species specificity
Important “Monkey Business”
Protein Sequence Differences between Species / Subspecies

10 subspecies of cynomolgus:
- Crab-eating Macaque, Macaca fascicularis fascicularis, synonym Macaca irus
- Burmese Long-tailed Macaque, Macaca fascicularis aurea
- Nicobar Long-tailed Macaque, Macaca fascicularis umbrosa
- Dark-crowned Long-tailed Macaque, Macaca fascicularis atriceps
- Con Song Long-tailed Macaque, Macaca fascicularis condorensis
- Simeulue Long-tailed Macaque, Macaca fascicularis fusca
- Lasia Long-tailed Macaque, Macaca fascicularis lasiae
- Maratua Long-tailed Macaque, Macaca fascicularis tua
- Kemujan Long-tailed Macaque, Macaca fascicularis karimondjawae
- Philippine Long-tailed Macaque, Macaca fascicularis philippinensis

21 subspecies of marmosets:
- Genus Callithrix – Atlantic marmosets
  - Common Marmoset, Callithrix jacchus
  - Black-tufted Marmoset, Callithrix penicillata
  - Wied’s Marmoset, Callithrix kuhlii
  - White-headed Marmoset, Callithrix geoffroyi
  - Buffy-headed Marmoset, Callithrix flaviceps
  - Buffy-tufted Marmoset, Callithrix aurita
- Genus Mico – Amazonian marmosets
  - Rio Acari Marmoset, Mico acariensis
  - Manicore Marmoset, Mico manicorensis
  - Silvery Marmoset, Mico argentata
  - White Marmoset, Mico leucippe
  - Emilia’s Marmoset, Mico emiliae
  - Black-headed Marmoset, Mico nigriceps
  - Marca’s Marmoset, Mico marcai
  - Black-tailed Marmoset, Mico melanura
  - Santarem Marmoset, Mico humeralifera
  - Maués Marmoset, Mico mauesi
  - Gold-and-white Marmoset, Mico chrysoleuca
  - Hershkovitz’s Marmoset, Mico intermedia
  - Satéré Marmoset, Mico saterei
- Genus Callibella – Roosmalens’ Dwarf Marmoset
- Genus Cebuella – Pygmy Marmoset
  - Pygmy Marmoset, Cebuella pygmaea

While the differences between species are intuitive, there are also variations between subspecies but even between races or breeds as a result of geographical distribution or isolation.

Predicting Clinical Effects from Preclinical Data:
Determinants of species relevance

- Healthy animals
- Healthy subjects
- Diseased patients

Pharmacologically relevant animal species

- Target sequence homology and polymorphisms across species and strains
- Cell / tissue expression of target, target turnover
- Target binding affinity across species

Molecules do not act if they do not bind...but binding does not mean they act!
- Consider other differences e.g. Fc effector function – FcRn, FcyRs, C1q binding, ADCC, CDC
- Comparative biology, signaling pathways and downstream pharmacology
- In vitro functional assays for evaluating functional equivalence across species
- In vivo PD markers / physiological / pharmacological outcome measure

- Define safety assessment strategy – rodent+non-rodent, non-rodent only, alternative approaches – surrogate product, KI/KO transgenic mouse model, animal model of disease, in vitro approaches ....
- Sufficient information for scaling of animal PK data to predict human PK?
Predicting Clinical Effects from Preclinical Data: Determinants of species relevance

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Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells


Target engagement marker (RO) available for studies in cynomolgus monkeys, but no *in vivo* pharmacological function biomarker and no comparative *in vitro* potency/functionality assay

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**Importance of appropriate *in vitro* data - examples**

- Relevant functional assays to understand relative species differences
- Species differences in target expression
  - E.g. platelet binding, platelet activation & aggregation studies,
- Receptor occupancy in human systems: theoretical, in *vitro*/*ex vivo*
- Cytokine release in human systems
- FcγR binding and ADCC/CDC/apoptosis assays
- Haemocompatibility test
- Tissue cross reactivity / potential for unexpected binding
Theoretical receptor occupancy using a ligand binding model (closed system): as applied to TGN1412

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

TGN1412 = 18.7 nM

(immediately post-dose)

K_d = 1.88 nM

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

• Using human blood samples, the median of TGN1412–Alexa 488 staining gated on CD3+ cells was determined, and the percentage of specific binding was calculated
• Red bar represents range of TGN1412 concentrations theoretically present in the blood of a 70-kg human at a dose of 0.1 mg/kg TGN1412
• Receptor occupancy between 45% and 80% was obtained

Assessing potential for a cytokine storm
Hazard detection and characterization prior to clinical studies

- Experience has demonstrated that in vivo animal studies (even in NHPs) may not be good predictors of a cytokine storm in humans
- Many companies have developed in vitro cytokine release assays based on human blood or PBMCs to identify and characterize this hazard

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Off target binding / toxicity

- Biologics generally demonstrate high specificity for target
- Assessment of binding to homologous targets – lead selection stage
- Impact of serum on target binding affinity/function
- Off target toxicity due to binding to unrelated targets not common for biologics
- Small but, increasing number of case studies of “unusual PK behavior” and “off-target” effects
  – Greater protein engineering of candidates?

Development of Motavizumab, an Ultra-potent Antibody for the Prevention of Respiratory Syncytial Virus Infection in the Upper and Lower Respiratory Tract

- Several palivizumab variants generated that enhanced the neutralization of RSV in vitro by up to 44-fold
- Unexpectedly, only a small increase of in vivo potency over palivizumab, - poor serum PK and lung bioavailability
- Unexpected broad tissue binding demonstrated
- Changes at three amino acids arising from affinity maturation markedly increased non-specific binding to various tissues
- Reversion of these three residues to the original sequences greatly diminished the tissue binding
Development of Motavizumab, an Ultra-potent Antibody for the Prevention of Respiratory Syncytial Virus Infection in the Upper and Lower Respiratory Tract


Pharmacokinetics of Anti-Abeta Ab2 mAb in Nonclinical Species (1)

Vugmeyster et al, 2011, Pharm Res 28 169601706
Pharmacokinetics of Anti-Abeta Ab2 mAb in Nonclinical Species (2)

- Mean plasma and serum concentrations of anti-Abeta Ab2 and control anti-Abeta mAb following single IV dose to cynomolgus monkeys
- After a single 10 mg/kg IV dose to cynomolgus monkeys, anti-Abeta Ab2 concentrations were lower in serum versus plasma for all time-points tested

Off-Target Platelet Activation in Macaques Unique to a Therapeutic Monoclonal Antibody

- AMG X: mAb vs soluble human protein
- Thrombocytopenia, platelet activation, reduced mean arterial pressure, and transient loss of consciousness in cynomolgus monkeys after first IV dose
- In vitro, AMG X induced activation of platelets from macaque species but not from humans or baboons.
- Other similar mAbs against the same target failed to induce these in vivo and in vitro effects
Off-Target Platelet Activation in Macaques Unique to a Therapeutic Monoclonal Antibody
Santostefano et al, Toxicol Pathol 40: 899, 2012

- The target protein was not expressed on platelets, suggesting that platelet activation occurred through an off-target mechanism.
- AMG X bound directly to cynomolgus platelets and required both the Fab and Fc portion of the mAb for platelet activation.

Mitigation: screening for off target binding

- Assessment of binding to homologous targets included in candidate selection screen
- Protein chips – proprietary, commercial e.g. Protagen
- ELISA-based “polyreactivity” screen (Pfizer)
- Assay based on ELISA detection of non-specific binding to baculovirus particles to identify mAbs with increased risk of fast clearance – Genentech (Hotzel et al, 2012 mAbs 4(6) 753-760).
- Retrogenix platform
  - arrays of expression vectors encoding more than 3500 human plasma membrane proteins – each membrane protein individually over-expressed in the context of human cells.
- Early exploratory tissue cross reactivity studies
- “Regulatory” TCR studies in panel of human (and animal) tissues post candidate selection
- In vivo PK behavior
Triage candidates by assessing PK behavior in rats

- 10 mg/kg IV, n=3 rats/candidate
- Candidate profile (coloured dots) is compared to expected PK behavior in rats (solid line)

Adapted from Steffen Hartmann, Integrated Biologics Profiling, Novartis
8th Annual European Antibody Congress Geneva, November 27th, 2012

Summary

- Define target-related safety questions to be explored
- Avoid “tox-in-a-box” approach
  - i.e. use of GLP toxicity study data (NOAELs etc) alone
- Use an Integrated translational PK-PD-safety assessment approach
  - Aids understanding of shape and steepness of concentration-response relationships
- Requires “relevant” animal species
  - But, also an understanding of the limitations of species for predicting clinical effects
- Supplement limitations/gaps of selected animal species by other means e.g alternative models, *in vitro* approaches...
Thank You!

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