Novel Human-Relevant Preclinical Safety Testing Strategy for Recombinant Human Monoclonal Antibodies Directed Against Foreign Targets

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PURPOSE

International Council of Harmonization (ICH) guidance[1] recommends a significant reduction in animal-based testing for a therapeutic monoclonal antibody (mAb) directed against a foreign antigen when evaluating preclinical safety. This drug class-specific testing approach is in line with legislation[2] and policy[3] guiding regulatory agencies to prioritize modern testing approaches that safeguard human health while replacing animal-based experiments, but at present it is unclear how regulatory agencies will respond to data from an exclusively non-animal approach.

We propose a non-animal preclinical safety testing strategy that supports a single-dose, first-in-human (FIH) clinical study for a well-characterized human recombinant monoclonal antibody directed against a foreign antigen, and we present a case study therapeutic antitoxin to be discussed with regulators.

METHODS

A detailed assessment of the potential toxicity risks specific to the target drug class was performed using ICH guidance and the growing body of data from pharmaceutical mAbs. As shown in Table 1, **the safety profile for a purified human recombinant mAb therapeutic directed against a foreign antigen indicates low risk to humans, and the non-animal safety testing strategy was developed to fully mitigate the two "low" risks.**

Table 1. Potential human toxicity risks specific to the target drug class

Potential Risk	Degree of Risk for Target Drug Class	Rationale
Excessive on-target activity (exaggerated pharmacology)	Negligible	• Drug within the target class has high specificity / affinity for an antigen foreign to humans
Off-target binding	Low (to be fully mitigated by preclinical strategy)	• Low risk for antibody with high specificity / affinity for non-human target
Genotoxicity, Carcinogenicity, Reproductive Toxicity	Negligible	• Testing for these is not recommended by ICH S6(R1) for the target drug class[1]
Toxic metabolite or reactive intermediate	Negligible	• Metabolism of therapeutic proteins is well-understood and gives no safety risk[1]
Immunogenicity	Low (to be fully mitigated by preclinical strategy)	• Unwanted human immune response is unlikely for a fully human antibody with a non-human target

RESULTS

Attributes of the Target Drug Class

The case study therapeutic antitoxin is a candidate treatment for diphtheria consisting of two human recombinant mAbs[4]. Table 2 details this product and shows the type of well-characterized, purified, and highly specific product to which the non-animal strategy may be applied.

Risk to Mitigate: Off-Target Binding (Figure 1)

Immunohistochemical (IHC) Study
- Screen for mAb binding to a standard panel of human tissues
- Recommended by ICH guidance[1]

Cell-Based Array Technology
- Screen for mAb binding to thousands of human proteins in a native environment
- Cover a high percentage of the human proteome

Risk to Mitigate: Immunogenicity (Figure 2)

Animal data is not predictive of human immunogenicity[5], so the preclinical strategy includes a battery of well-established *in vitro* assays using different human cell types:

Human cellular assays with Peripheral Blood Mononuclear Cells (PBMCs), Dendritic Cells (DCs), and Monocytes
- Assess immunogenicity by exposing mAb to human cells related to immune response and measuring immune response data
- Use three cell types to best encompass possible mechanisms of immunogenicity
- Source cells from diverse human donors to understand immune response in different populations

Table 2. Attributes of the preclinical testing strategy target drug class along with the characteristics of the case study therapeutic

Product	Sequence Source	Antibody Type	Sequence-Defined	Target	Specificity Data	Affinity Data	Purity Data
Preclinical Strategy Target Drug	Fully Human	Recombinant Monoclonal	Yes	Antigen foreign to humans	• Characterization data proving mAb is highly specific for intended antigen	• Data proving that mAb binds strongly to intended antigen and is therefore unlikely to bind other sites	• Data showing the absence of impurities that may cause human health or safety risks
New Therapy for Diphtheria (Combination of 2 mAbs)[4]	Fully Human: selected through phage display using a human immune antibody library	Recombinant Monoclonal: IgG1 format	Yes	Diphtheria Toxin (exotoxin responsible for diphtheria morbidity and mortality)	• Minimal Epitope Regions Defined • Domain Mapping Performed: • Antibody 1 binds to Receptor Binding Domain of toxin • Antibody 2 binds to Catalytic Domain of toxin	• Toxin Binding with Titration Elisa: • Antibody 1 EC50[a] = 0.027 µg/mL • Antibody 2 EC50 = 0.022 µg/mL • Toxin Affinity with Microscale Thermophoresis: • Antibody 1 EC50 = 13 nM • Antibody 2 EC50 = 14 nM • *in vitro* Neutralization Potency: • Combined Antibodies = 160 IU/mg	• To be gathered

[a]EC50 = Half Maximal Effective Concentration

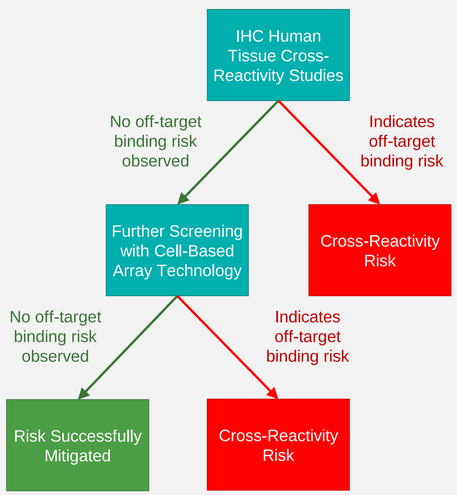

Figure 1. Mitigation strategy for human off-target binding risk

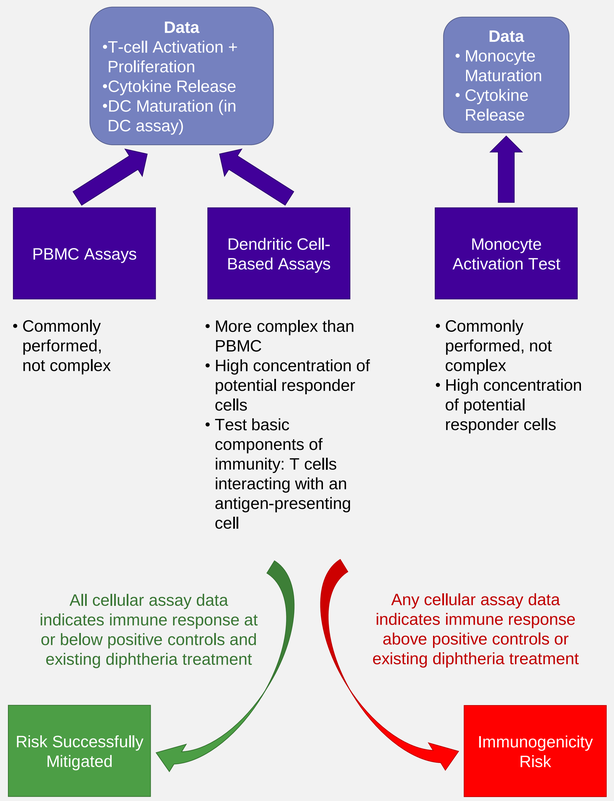

Figure 2. Mitigation strategy for human immunogenicity risk

Risk Assessment

A mAb therapeutic is considered safe for a FIH clinical trial if risks are successfully mitigated (green boxes in the figures) at a dose 100x the proposed FIH dose.
- Appropriate controls (human IgG null control, immune response stimulant such as LPS, product-specific controls, etc.) are included in the assays
- The case study mAb controls include the existing diphtheria treatment as a benchmark

If the studies indicate a risk (red boxes in the figures), further data must be collected before a FIH clinical trial.

Dose Considerations:
- Starting dose for the single-dose FIH trial must be determined outside this strategy
  - Identification of a FIH clinical dose for a mAb using strictly *in vitro* data has been successful[6]
  - FIH dose for the case study mAb was found using *in vitro* data and the established dose of the existing diphtheria treatment

CONCLUSIONS

- **A non-animal preclinical safety testing strategy was developed to address the toxicity risks of a well-characterized human recombinant mAb therapeutic directed against a foreign antigen**
  - The strategy supports a single-dose FIH clinical trial
  - Risks specific to the target drug class are best understood using well-developed human cell and tissue assays
- **The preclinical strategy as it applies to a case study mAb therapeutic product is proceeding through formal discussions with the FDA and EMA**
  - FDA Pre-IND meeting outcomes: Agency is supportive of proposal and does not require additional *in vivo* efficacy testing before reviewing data from the non-animal package
  - EMA early-phase Scientific Advice meeting: pending
- **The regulatory requirement to evaluate new drug candidates using animal-based safety studies prior to a FIH trial may not be necessary given currently-available *in vitro* methods and regulatory guidance[3] that recognize the value of integrating modern science into the review process**

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