2nd EMA Workshop on Biosimilar Monoclonal Antibodies, 24 October 2011

Session 1.1 Non-Clinical Issues:
«Off-target toxicity – does it occur and how should we detect it?»

Innovator Industry Presentation

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Unexpected in-vivo findings

- Cases of “off-target” findings with mAbs have been published. Since cause may involve MoA, CDR/Fc characteristics and/or product attributes, the broader term “**unexpected in-vivo findings**” is used here

- **In-vivo assessment for potential unpredicted effects is an important part of non-clinical safety testing**
  - Unexpected binding, **not detected by in-vitro testing,** can significantly affect the **in-vivo behaviour and toxicity** of a mAb
  - **In-vitro assays will only address the known, but not the unknown**

- For addressing unexpected in-vivo findings, the following should be considered
  - Reference to **ICH S6 R(1)** (slide 3)
  - **Case example** (slide 4)

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In-vivo studies to be considered to address potential for unexpected effects as per ICH S6 R(1): Section 2.1

- The text below was included in **ICH S6 R(1) to address the potential for unexpected in-vivo effects** with products directed at exogenous targets
  - This approach is considered **appropriate to support entry of biosimilar products into the clinic**
  - The choice of relevant species should be **justified by the sponsor**

> For monoclonal antibodies and other related antibody products directed at foreign targets (i.e., bacterial, viral targets etc.), a short-term safety study (see ICH S6 Guideline) in one species (choice of species to be justified by the sponsor) can be considered; no additional toxicity studies, including reproductive toxicity studies, are appropriate. Alternatively, when animal models of disease are used to evaluate proof of principle, a safety assessment can be included to provide information on potential target-associated safety aspects. Where this is not feasible, appropriate risk mitigation strategies should be adopted for clinical trials.

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Unexpected in-vivo findings – Case mAb Y

- **Antibody mAb Y –** Target expression is highly **restricted**
  - Finding: **rapid profound thrombocytopenia**; mild to marked decreases in red cell mass, after a single dose at ≥50 mg/kg via SC or IV route. After 40hrs, platelet decreased 92-99% from pretest (absolute counts 4-50x10e3/ul).
  - At 72 hours, increased numbers of **activated macrophages**, some with mitotic figures, and macrophages with phagocytized erythrocytes in the spleen.
  - **No** detectable **binding of mAb-Y to cynomolgus peripheral blood or bone marrow cells** in-vitro. **In-vitro, mAb-Y induced cynomolgus** peripheral blood monocytes (**PBMs**), but not human PBMs, to phagocytize platelets.
  - The **F(ab)'2** portion of the molecule did **not induce phagocytosis** of platelets.
  - Three mAbs sharing the same Fc framework as mAb-Y competitively bound to and had similar biological activity against the intended target. **None of these antibodies had the haematologic liability in-vitro or in-vivo.**
  - **Modification** of the **Fc portion** of mAb-Y to decrease FcR binding **attenuated** the **in-vivo** and **eliminated the in-vitro** hematologic responses.
  - Together, data demonstrate that the haematologic effects of mAb-Y in cynomolgus monkeys occurred through an mechanism involving **both the Fc and CDR** portions of this specific monoclonal Ab.

Example provided by Ian Pyrah, Amgen

Unpredicted in-vivo findings - Summary

- **Binding/interaction** of mAbs **to a single blood cell type** (eg, platelets, monocyte/ macrophage) has been shown
- Unexpected findings may, however, occur in **any** organ or tissue
- May be driven by **Fc** and **CDR**
- **In-vivo nonclinical studies were required** to reveal these findings – not clear why one mAb shows an effect whilst others do not.
- **Post-translational modifications can affect structure of mAbs and thus Fc interactions and function. Therefore, it is prudent to conduct limited in-vivo animal studies to provide assurance of PK/PD/safety for biosmilar molecules.**
- Findings are considered **uncertain** whether **translatable** to human in-vivo
- **In-vitro assays will only address the known, but not the unknown**

- BACKUP

Unexpected in-vivo findings – Case AMGX

- **AMGX** (IgG2), used to treat autoimmune and inflammatory diseases
  - Finding: After 1st dose, "rapid **profound thrombocytopenia** with decreased platelet granularity, lowered mean arterial pressure, and transient loss of consciousness and flushing" after a single dose in monkeys. **Not** seen **with 3 other mAbs** against **same target**.
  - Platelet counts at 15 minutes after dosing were decreased 90-98% from pretest; less after subsequent doses; no ADAs, complement, nor vasculitis involved.
  - Subsequent *in vitro* tests revealed **activation and aggregation of platelets** in macaque monkey species, but not for baboon or human platelets (not seen for the other 3 mAbs). Intact AMGX with Fc and CDR domains required ($F(ab)_2$ didn't cause it).
  - Preincubation of AMGX with the intended target eliminated platelet activation.
  - Data support that activation of macaque platelets requires binding of AMGX by the **Fc portion to the FcgRIIa** receptor and by the **CDR** to an as yet **undetermined epitope** (off-target). Platelet RNA and protein analysis data indicated target is not expressed on platelets.

Example provided by Ian Pyrah, Amgen