Immunogenicity Testing of Therapeutic Antibodies in Ocular Fluids After Intravitreal Injection

10th Open Scientific EIP Symposium on Immunogenicity of Biopharmaceuticals
Lisbon, February 25-27th, 2019

Afsaneh Abdolzade-Bavil on behalf of Bioanalytical Sciences
Roche Innovation Center Munich, Germany
Diabetic Macular Edema (DME) and Age Related Macular Degeneration (AMD) Are Leading Causes of Vision Loss

- Around 200 millions patients in 2020
- Increasing numbers are expected due to aging of the population and the diabetes epidemic
Growth of Abnormal Blood Vessels in Macula Region

- Current Standard-of-Care (SoC) is intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs (e.g. Lucentis)
Innovative Molecule Modalities in Ophthalmology

New Modalities allow longer duration in the eye with less frequent IVT injections

New Modalities

- Mono-specific IgG Therapeutics MW ~150 kDa e.g. Avastin
- Bi-specific IgG Therapeutics MW ~150 kDa
- Bi-specific Fab Therapeutics MW ~50 kDa e.g. Lucentis

- High affinity
- High concentrations
- Increased durability
- Longer duration of action
- Less frequent dosing

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Challenges in ophthalmology studies

- Multiple ocular sample types with limited volume
  - Aqueous Humor: 20-100 µl
  - Vitreous Humor: ~500 µl
  - Retina (tissue): 1 mg
  - Choroid, etc.
- Difficult to access
  - Aqueous Humor: Low sample volume
  - Vitreous Humor: species specific diversity, usually post mortem samples
  - Retina: standardized tissue preparation, post mortem samples
- High sensitivity demands for soluble targets
- Non-Clinical Studies:
  - Different anatomy of the eye in different species
  - Animal AH can only be sampled under narcotic conditions

IVT administration is a burden for patients
The Interplay Between Drug, Target and ADAs in Ophthalmology Studies

- Analyte abundance, sample volume, assay sensitivity
- Total versus free/bound
- Technology and platform evaluation

- Free/active versus total drug
- Assay sensitivity

- State of the art method
- Drug/Target Interference
- Complex dissociation

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Comparison of Bridging and Immune Complex ADA Assay Principles

**Bridging ADA Assay**

- Required formation for Signal
- Situation in case of free Drug

- Drug-Dig
- ADA
- Drug-Bi

Stereptavidin Microtiterplate

The Bridging ADA assay shows signal interference in samples with high drug level.

**Immune Complex ADA Assay**

- Required formation for Signal
- Situation in case of free Drug

- Anti Animal-Dig
- Drug (Animal IgG)
- ADA (Animal IgG)
- Drug (Human IgG)
- Anti human -Bi

Stereptavidin Microtiterplate

The Immune Complex ADA assay enables reliable ADA detection in samples with high drug levels as aqueous and vitreous humor after IVT injection.

*Stubenrauch K. et al, 2012 Bioanalysis*
Comparison of ADA Results Generated by Bridging and Immune Complex ADA Assays

Vitreous humor samples (high drug level)
- IC ADA assay is superior and enables ADA detection in ocular fluids for the first time.
- Bridging assay strongly influenced by high drug concentration, resulting in false negative ADA response.

Serum samples (low drug level)
- Excellent correlation of bridging assay with immune complex ADA assay.

"Immunogenicity testing of therapeutic antibodies in ocular fluids after intravitreal injection"; Uwe Wessels et al, 2018 Bioanalysis
ADA Signals in Ocular Fluids after IVT Injection

ADA results for Aqueous (AH) and Vitreous Humor (VH)

Good correlation of aqueous humor versus vitreous humor ADA data allows the design of future preclinical ophthalmological studies without the need for vitreous humor sampling.

*Uwe Wessels et al, 2018 Bioanalysis*
The good correlation of ADA results from ocular fluids to serum samples indicate that ADA analysis in serum may be predictive for ocular fluids.

* No VH samples on Day 14

Uwe Wessels et al, 2018 Bioanalysis

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Evaluation of an Appropriate Matrix for Immunogenicity Testing after IVT Injection in Non-Clinical Studies

<table>
<thead>
<tr>
<th>Accessibility</th>
<th>Vitreous Humor</th>
<th>Aqueous Humor</th>
<th>Serum/Plasma</th>
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<tbody>
<tr>
<td>Post mortem</td>
<td>Difficult sampling</td>
<td>Limited time points</td>
<td>Easy to access</td>
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<tr>
<td>No time course</td>
<td>Low sample volume</td>
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<td>Time course possible</td>
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<td></td>
<td></td>
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<td>Higher sample volume</td>
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<tr>
<th>Drug level after IVT injection</th>
<th>Vitreous Humor</th>
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<tbody>
<tr>
<td>Very High</td>
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<td>Low</td>
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<tr>
<th>Correlation to Vitreous Humor ADA</th>
<th>Vitreous Humor</th>
<th>Aqueous Humor</th>
<th>Serum/Plasma</th>
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<td>Given</td>
<td></td>
<td>Given</td>
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<td>Early onset</td>
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<th>ADA Assay</th>
<th>Vitreous Humor</th>
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<tbody>
<tr>
<td>Immune Complex Assay</td>
<td>Immune Complex Assay</td>
<td>Bridging Assay</td>
<td>Immune Complex Assay</td>
</tr>
</tbody>
</table>
Summary and Conclusion

- Sensitive and drug tolerant immune complex assay enabled detection of ADAs in ocular samples for the first time.
- Good correlation of aqueous and vitreous humor ADA data allows immunogenicity monitoring without termination of the animals.
- We conclude that systemic ADA analysis might be sufficient for evaluation of immunogenicity in non-clinical and clinical ophthalmology studies.
Roche Ophthalmology LMBA Team
Supporting Ophthalmology Projects from early non-clinical studies to Post Marketing

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Doing now what patients need next