From research to market: the evolving assay strategy of a bispecific antibody

Dr. Alexandre Briguet, Senior Principal Scientist – Analytics Bioassay
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Introduction
Introduction

Mode of Action

- **binds** to the receptor binding domains of two soluble ligands: **Ligand 1** and **Ligand 2**
- **neutralization of these ligands** & modulation of the respective downstream signaling pathways.
- both functionalities are considered to have a relevant biological effect related to clinical efficacy and to be **independent** from the respective other functionality.
- effector function silent
Potency assay for early clinical development
Bridging SPR assay
Release assay for phase 1-2

- Measures bridging function (R2)
- Linear PLA calculation model
- Precision: 2%

R2 = "bridging signal" -> depends on both interactions and covers both functionalities of bsAb1

R1 = Ligand 1 binding signal
Potency assay for late clinical development
Considerations for pivotal phase Potency assays selection

• Per regulatory guidances, MoA-reflective cell-based assays are required to support Ph3 pivotal clinical studies.

• Based on the MoA of bsAb1 to sequester Ligand 1 and 2 from their respective receptor, simultaneous binding of the two ligand does not appear to be required. Therefore the “bridging” of Ligand 1 and 2 does not need to be reflected in the potency assay.

• Considering that both bsAb1 functionalities have independent biological effects related to clinical efficacy, two independent cell-based assays, each addressing one of the two bsAb1 functionalities are appropriate to reflect the MoA.
Ligand 1 specific cell-based potency assay:
Ligand 1 reporter gene assay

- Inhibition of Ligand 1 induced reporter gene activation by bsAb1
- bsAb1
- Promoter
- Reporter Gene

- 4PL calculation model
- Precision: 5%

Graph showing

\[
\text{Log IC}_{50} = \frac{D - L}{1 + \left(\frac{D - L}{C_{50}}\right)^2}
\]
Inhibition of Ligand 2 induced receptor phosphorylation by bsAb1

Ligand 2 specific cell-based potency assay:
Ligand 2-receptor phosphorylation assay

- 4PL calculation model
- Precision: 5%
Methods comparability study
Methods comparability study
HMW-related hyperpotency in the Ligand 2-receptor phosphorylation assay

- Increase in anti-Ligand 2 potency during storage at 5°C
- Correlation between increase in HMWs and anti-Ligand 2 potency
Methods comparability study
HMW-related hyperpotency in the Ligand 2-receptor phosphorylation assay

- increase in potency correlating with an increase in HMW content observed in the Ligand 2-receptor phosphorylation assay
- no change in potency in the Ligand 1 reporter gene assay
- no change in potency in the SPR-based potency assay
Methods comparability study
HMW-related hyperpotency in the Ligand 2 receptor phosphorylation assay

- hyperpotency of the HMWs fraction (mainly dimer) in the Ligand 2-receptor phosphorylation assay
- potency of the monomer fraction unchanged in both assays
- change in potency upon storage at 5°C are due to the formation of HMWs
- increased potency of the HMWs in the Ligand 2-receptor phosphorylation assay is considered to reflect the in vivo situation
- why no hyperpotency of HMWs in the SPR assay?
Molecular mechanism of hyperpotency toward Ligand 2?
Explanation: avidity driven binding of bsAb1 dimers to Ligand-2 multimers

Monomer
Dimer

cell-based assay

SPR assay

high density of bsAb1 on the SPR chip favors an avid binding mode to Ligand 2
Methods comparability study
Reduced potency of aspartate isomers in the Ligand 2-receptor phosphorylation assay

- decrease in potency in the SPR based assay
- no potency loss in the Ligand 1 reporter assay
- no potency loss in the Ligand 2-receptor phosphorylation assay
- HMWs hyperpotency in the Ligand 2 assay may have compensated for potency loss towards Ligand 2 caused by other molecule damage (e.g. CDR isomerization)
Methods comparability study
Reduced potency of aspartate isomers in the Ligand 2-receptor phosphorylation assay

- Hyperpotency of HMWs in the Ligand 2-receptor phosphorylation assay
- Decreased potency of monomer correlating with the amount of aspartate isomerization measured in the anti-Ligand 2 CDR region of bsAb1
- Unaffected potency in the Ligand 1 reporter assay congruent with absence of CDR modification in the anti-Ligand 1 arm of bsAb1
Methods comparability study
Effects of light stress

- strong decrease in potency in the SPR based assay
- anti-Ligand 1 functionality affected by the light stress as reflected by the potency loss in observed in the Ligand 1 assay
- increase in potency in the Ligand 2 assay correlating with a strong increase in HMWs

<table>
<thead>
<tr>
<th>Lot D, hours light stress</th>
<th>SPR assay</th>
<th>Ligand 1 assay</th>
<th>Ligand 2 assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>unstressed</td>
<td>100%</td>
<td>95%</td>
<td>95%</td>
</tr>
<tr>
<td>20 h light stress</td>
<td>0%</td>
<td>91%</td>
<td>91%</td>
</tr>
</tbody>
</table>

HMW (Dimer) [area%]
% HC_Asp106

1.6 (1.5)
95

24.1 (16.5)
91
Methods comparability study
Release data phases 1-3

- phase 1-3 lots have comparable potencies in the SPR assay and the two cell-based assays at release
Potency specification of the marketed product
Potency specification
Understanding potency results from SPR vs cell-based assays

**SPR assay**
- 100% potency
- 0% potency
- 0% potency
- 81% potency (90% x 90%)
- 10% inactive CDR

**Cell-based assays**
- 100% potency for anti-Ligand 1
- 100% potency for anti-Ligand 2
- 0% potency for anti-Ligand 1
- 100% potency for anti-Ligand 2
- 100% potency for anti-Ligand 1
- 0% potency for anti-Ligand 2
- 90% potency for anti-Ligand 1
- 90% potency for anti-Ligand 2
Potency specification
Specification and project phases

<table>
<thead>
<tr>
<th>Project Phase</th>
<th>Test</th>
<th>Potency specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 and 2</td>
<td>SPR-based assay</td>
<td>60 – 140%</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Ligand 1 reporter gene assay</td>
<td>60 – 140%</td>
</tr>
<tr>
<td></td>
<td>Ligand 2-receptor phosphorylation assay</td>
<td>60 – 140% (release)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 – 160% (stability)</td>
</tr>
<tr>
<td>Commercial</td>
<td>Ligand 1 reporter gene assay</td>
<td>80 – 120%</td>
</tr>
<tr>
<td></td>
<td>Ligand 2-receptor phosphorylation assay</td>
<td>80 – 120% (release)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 – 135% (stability)</td>
</tr>
</tbody>
</table>
# Potency specification

## Setting commercial specification

<table>
<thead>
<tr>
<th>Ligand 1 assay</th>
<th>Commercial specification</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DP end of shelf-life</strong></td>
<td></td>
<td>covered by clinical exposure no change expected, neither during production nor upon storage</td>
</tr>
<tr>
<td><strong>DP release</strong></td>
<td>80 - 120</td>
<td>TI(0.99/0.95) of all DS and DP batches, release (n=18), from Ph3/PPQ: +/-18 centered around 100% relative potency</td>
</tr>
<tr>
<td><strong>DS release/end of shelf-life</strong></td>
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<table>
<thead>
<tr>
<th>Ligand 2 assay</th>
<th>Commercial specification</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DP end of shelf-life</strong></td>
<td>80 - 135</td>
<td>covered by clinical exposure considering increase in hyperpotent HMW over time</td>
</tr>
<tr>
<td><strong>DP release</strong></td>
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<td>TI(0.99/0.95) of all DS and DP batches, release (n=18), from Ph3/PPQ: +/-17 centered around 100% relative potency</td>
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Summary

- the combination of two cell based assays measuring the neutralization of Ligand 1 and Ligand 2 are suited to assess bsAb1 quality and stability and can detect molecule modifications that are relevant to the MoA
- the use of two independent cell based assays is appropriate since both bsAb1 functionalities have a relevant biological effect related to clinical efficacy independent from the respective other functionality
- differences in results generated by the SPR based assay and the two cell based assays can be explained both by the bridging format of the SPR based assay combining two functionalities in one potency result and by the insensitivity of this assay to the avid binding of bsAb1 dimers to the Ligand 2
- phase 1-3 lots have comparable potencies in the SPR assay and the two cell-based assays at release
- two cell-based assays in conjunction with two independent potency specifications are used for measuring the potency of the marketed product at release and during stability testing
Acknowledgements

Cell-based assays
- Isabelle Haenel
- Rachel Stirchler

SPR assay
- Philipp Metzger
- Joerg Moelleken

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- Hermann Beck
- Adelheid Rohde
Doing now what patients need next