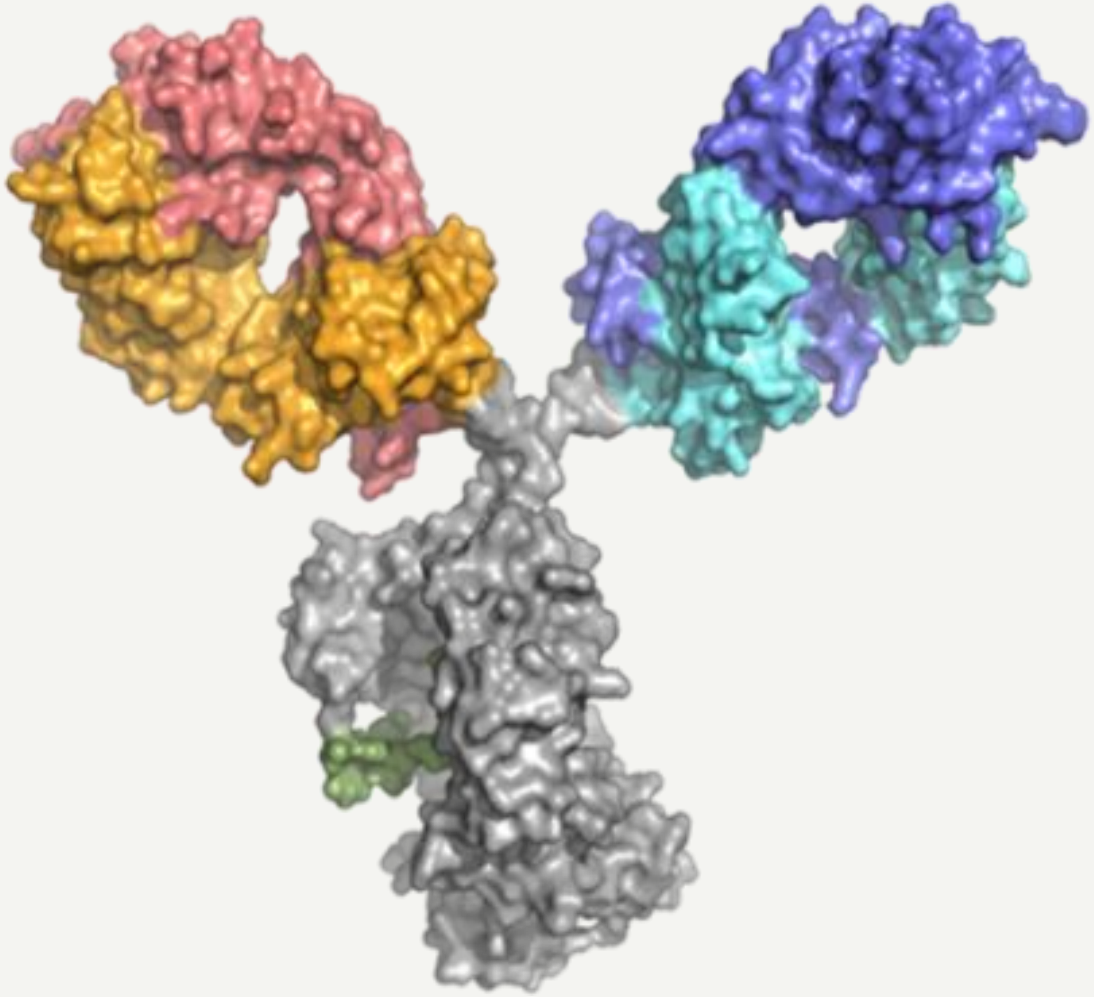

From research to market: the evolving assay strategy of a bispecific antibody

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April 2023 | public use

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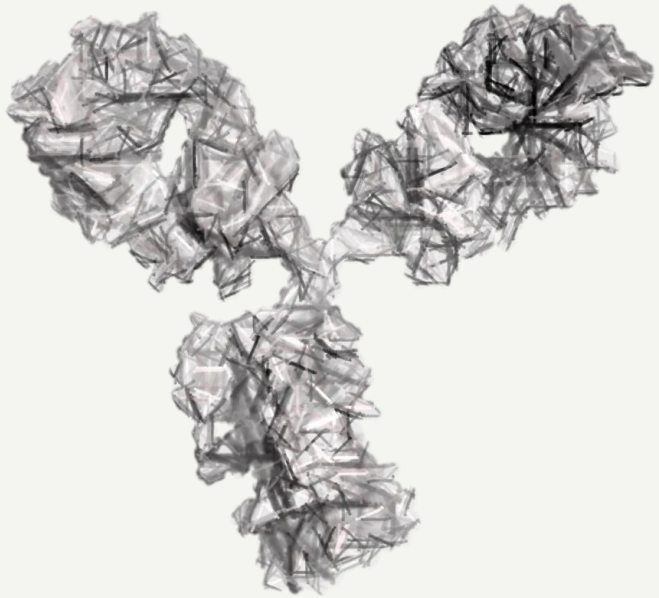
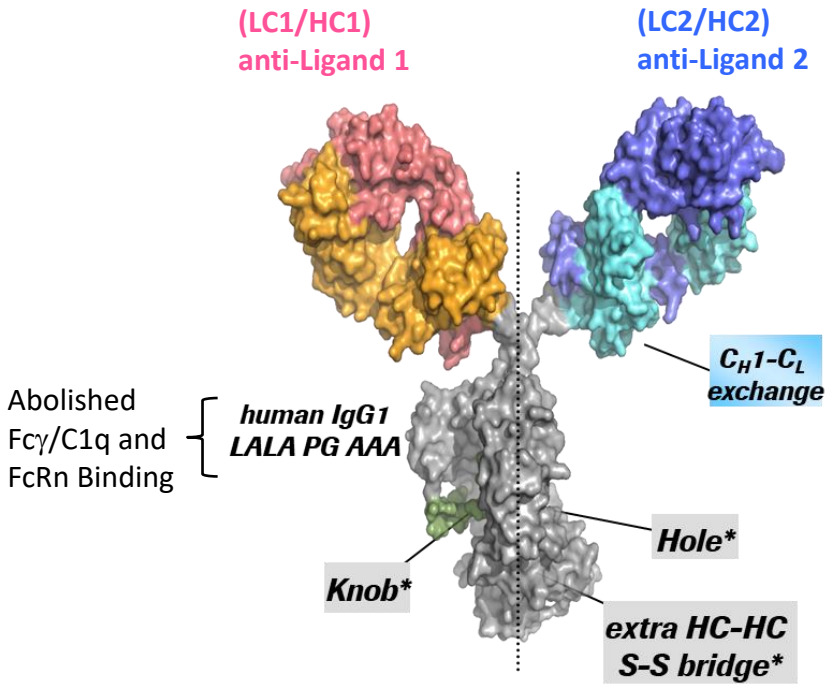
Introduction



Introduction
Mode of Action

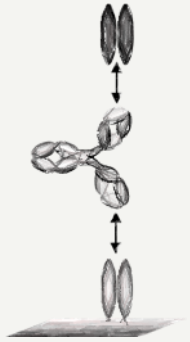


bispecific antibody, 1+1 format

Mode of Action

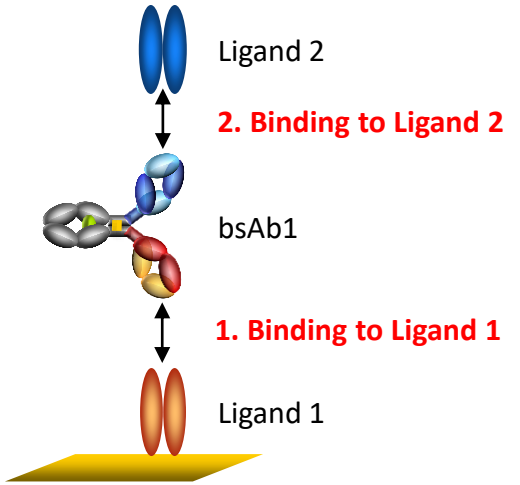
- **binds** to the **receptor binding** domains of two **soluble** ligands: **Ligand 1** and **Ligand 2**
- **neutralization of these ligands** & modulation of the respective downstream signaling pathways.
- both functionalities are considered to have a relevant biological effect related to clinical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

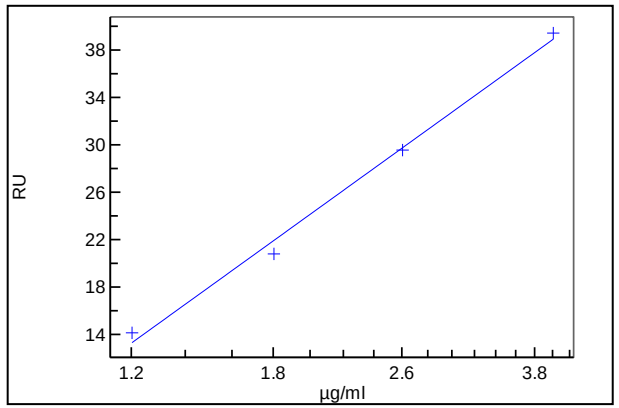
Potency assay for early clinical development



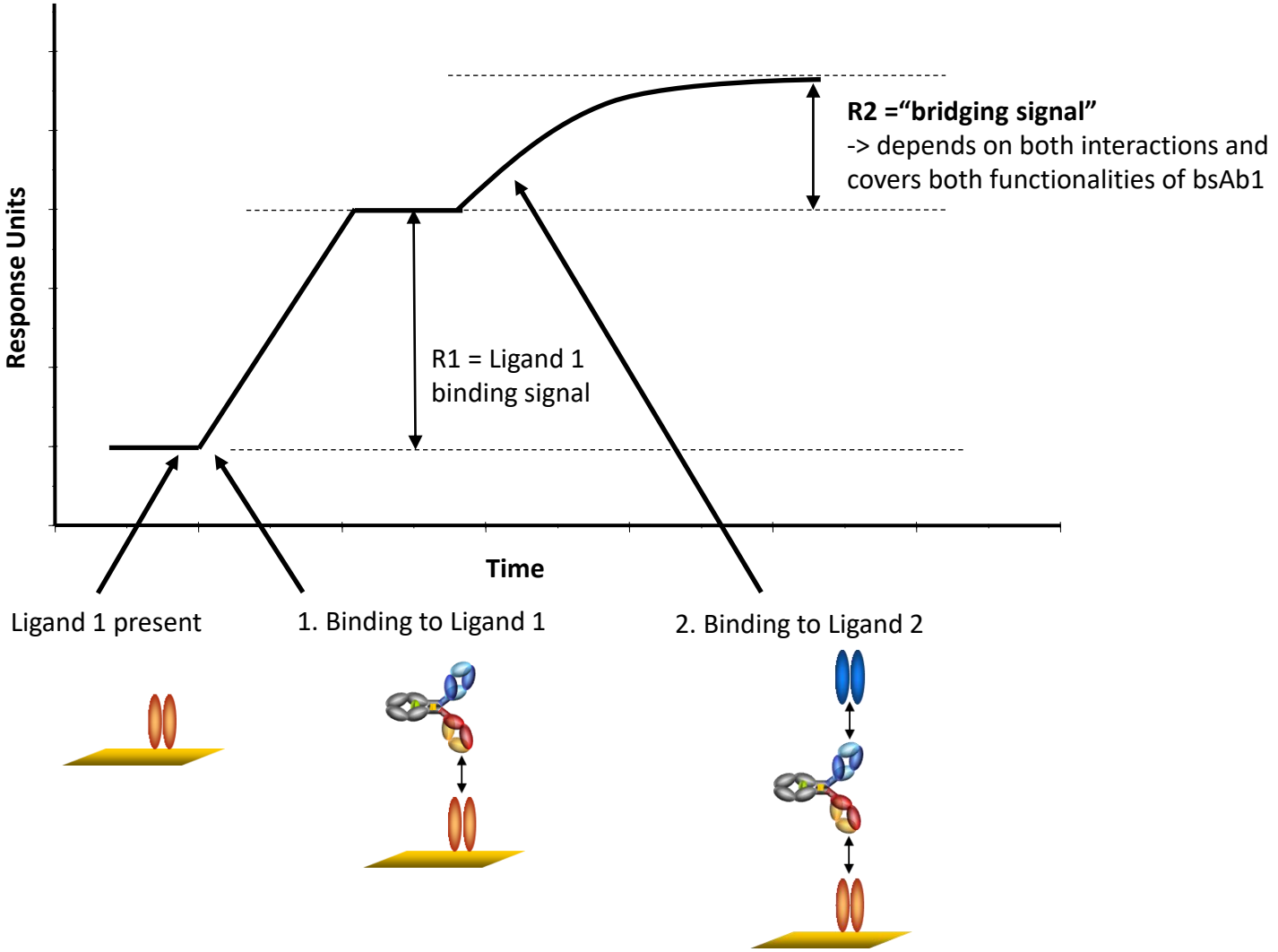
Bridging SPR assay

Release assay for phase 1-2





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



Potency assay for late clinical development

Considerations for pivotal phase Potency assays selection

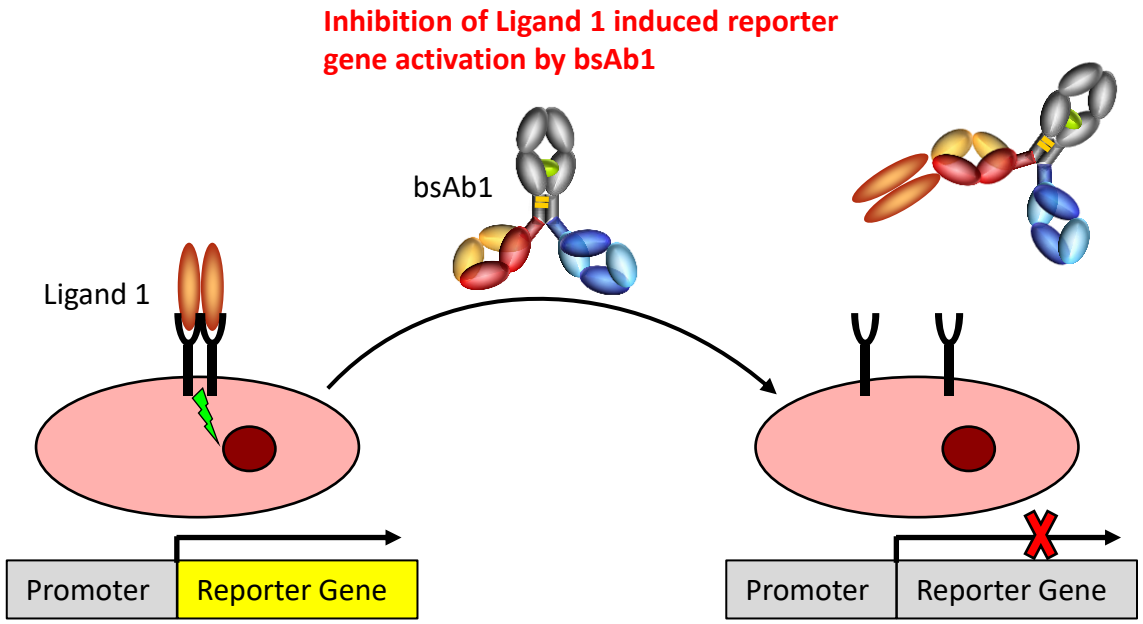
- Per regulatory guidances, MoA-reflective **cell-based assays** are required to support Ph3 pivotal clinical studies.

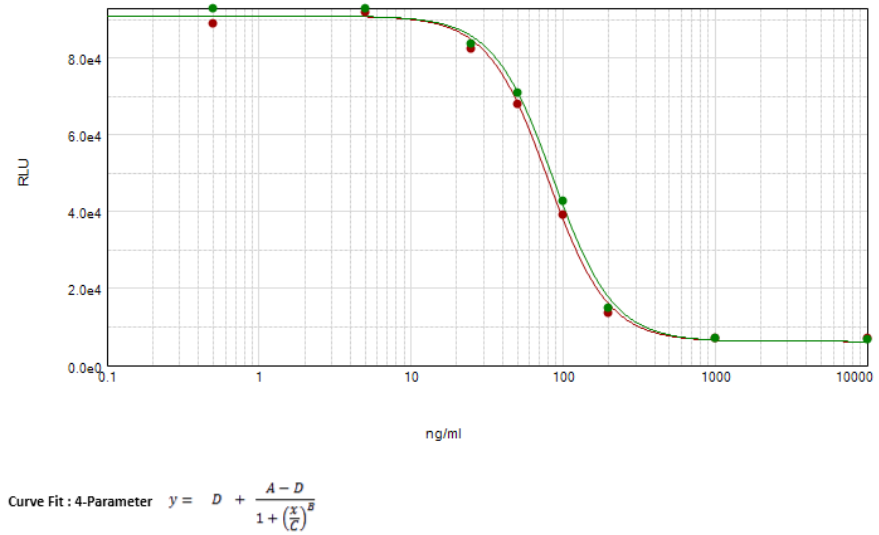
- Based on the MoA of bsAb1 to sequester Ligand 1 and 2 from their respective receptor, simultaneous binding of the two ligand does not appear to be required. Therefore the **"bridging" of Ligand 1 and 2 does not need to be reflected** in the potency assay.

- 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

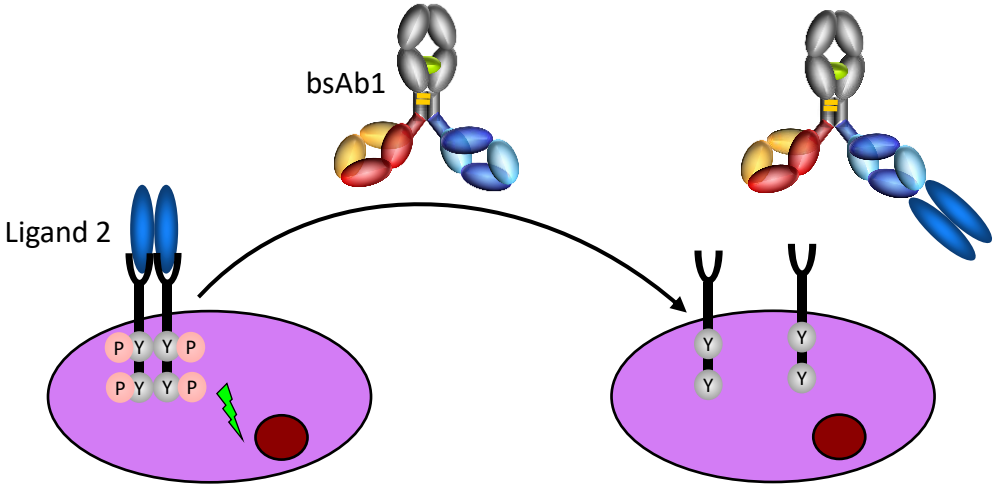


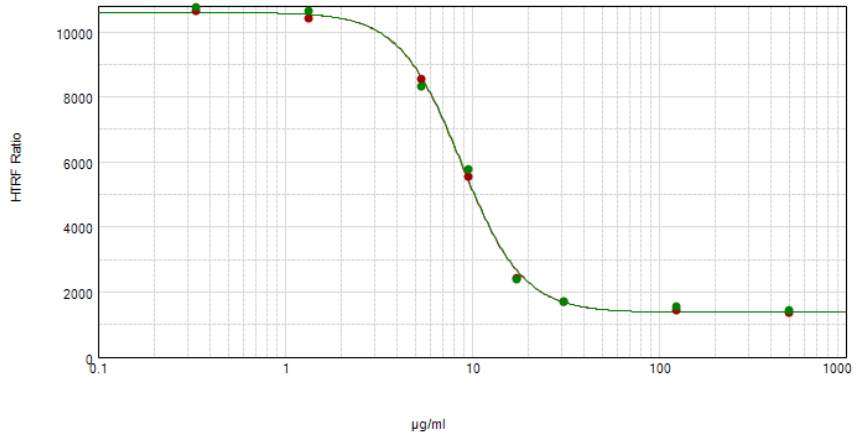


- 4PL calculation model
- Precision: 5%

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

Inhibition of Ligand 2 induced receptor phosphorylation by bsAb1

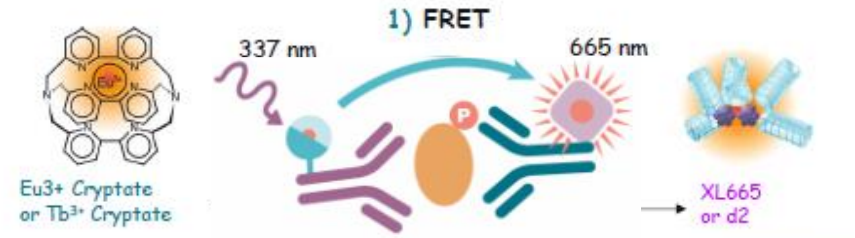




Curve Fit : 4-Parameter $y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$

HTRF: Homogeneous Time-Resolved Fluoresence

cisbio

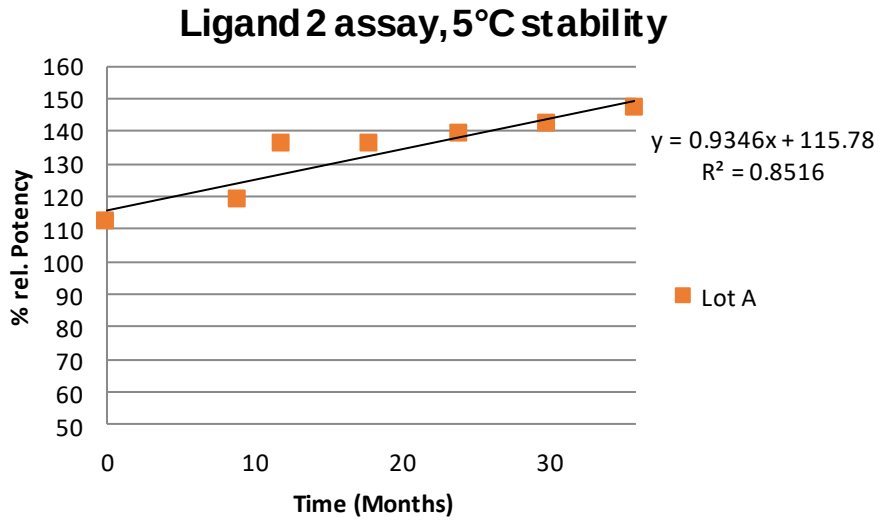


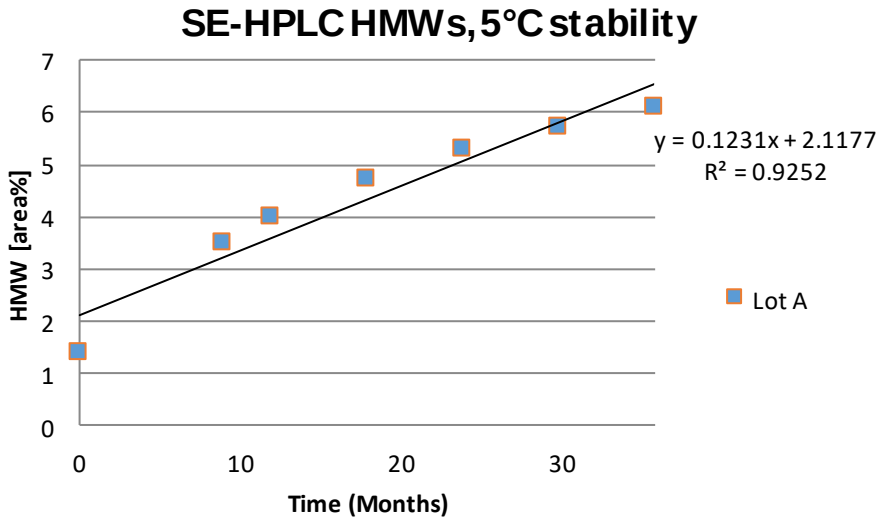
- 4PL calculation model
- Precision: 5%

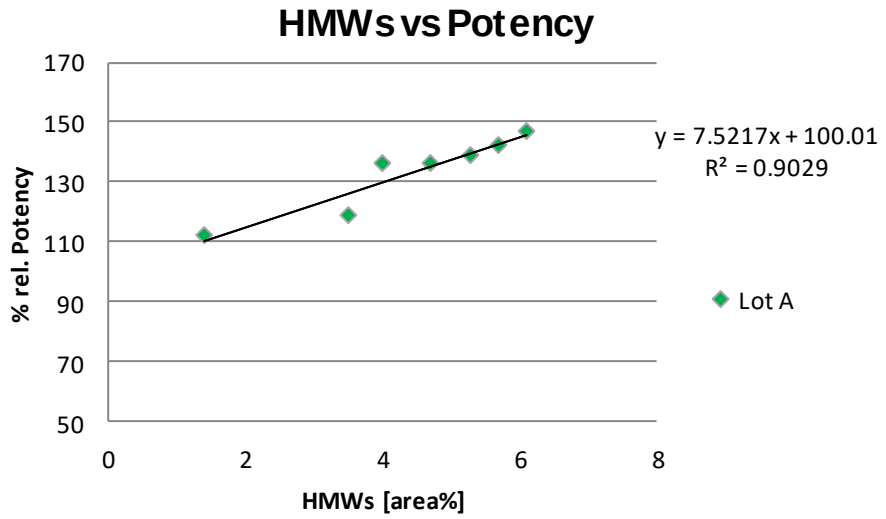
Methods comparability study

Methods comparability study

HMW-related hyperpotency in the Ligand 2-receptor phosphorylation assay



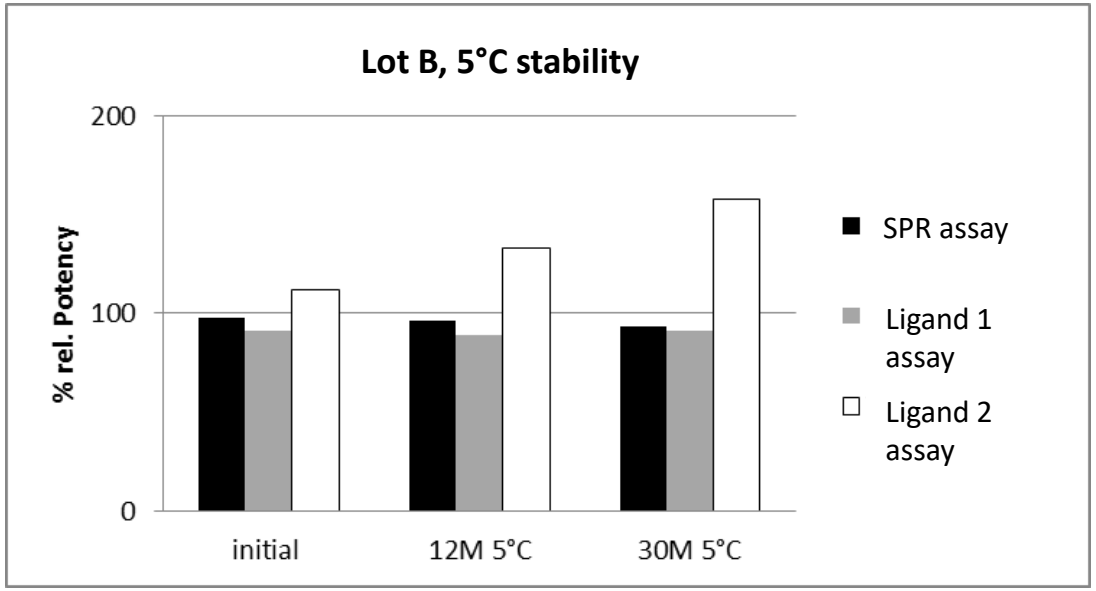




- increase in anti-Ligand 2 potency during storage at 5°C
- correlation between increase in HMWs and anti-Ligand 2 potency

Methods comparability study

HMW-related hyperpotency in the Ligand 2-receptor phosphorylation assay

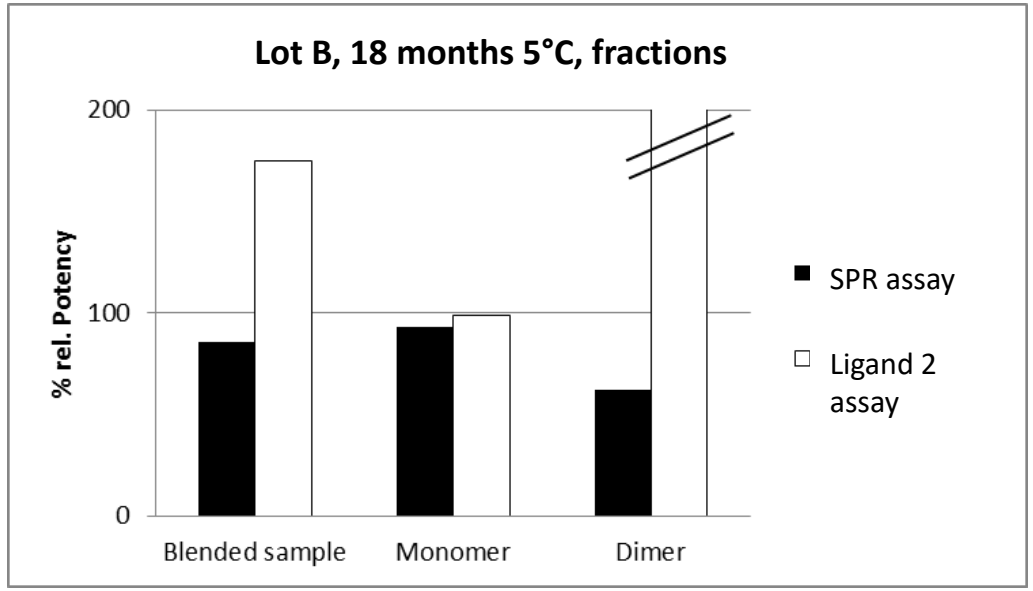


HMW (Dimer) [area%]	1.8 (1.7)	4.8 (4.5)	6.5 (6.0)

- 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

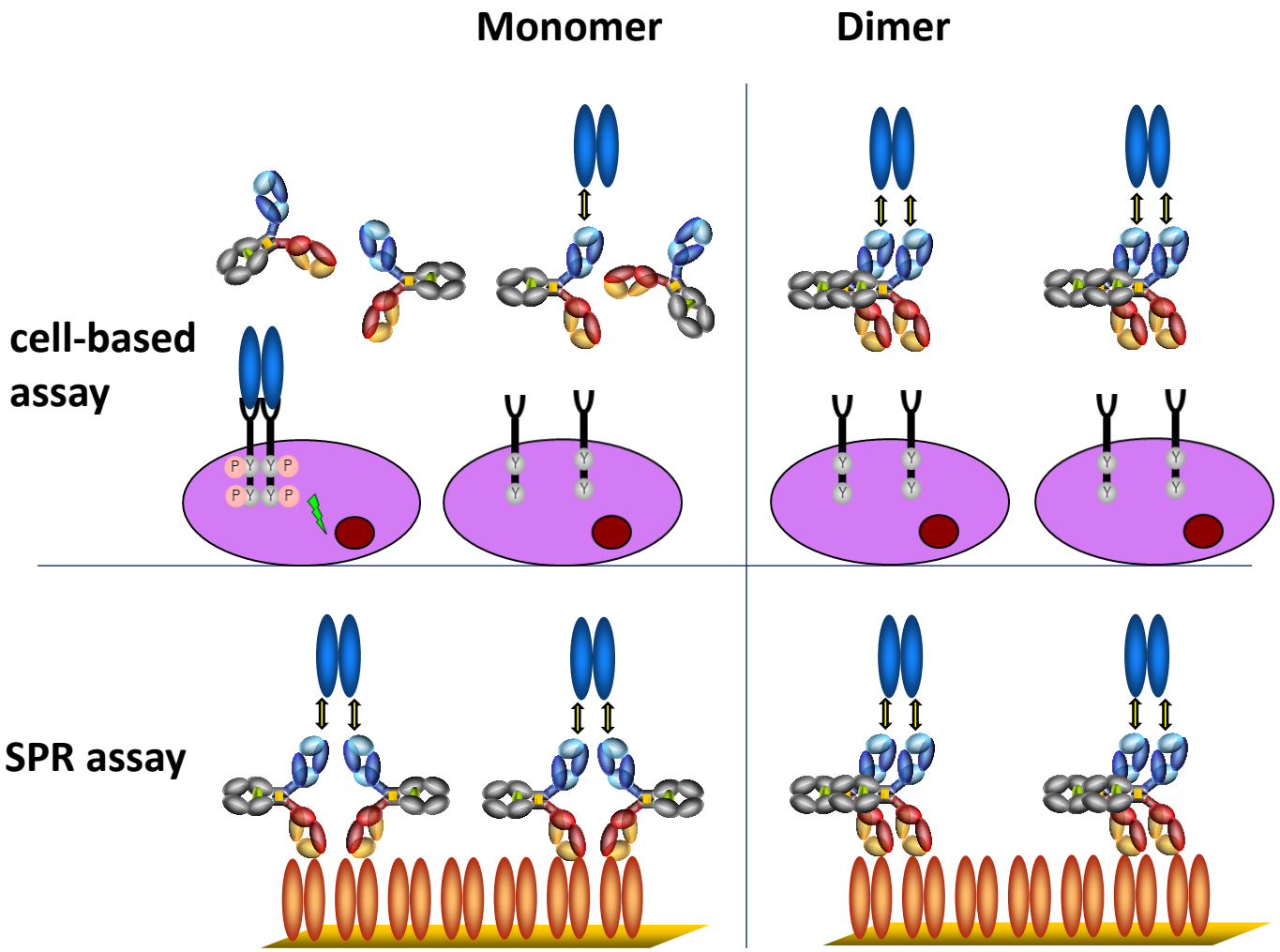


HMW (Dimer) [area%]	10.5 (9.9)	0.2 (0.2)	92.7 (87.6)

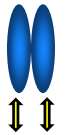
- hyperpotency of the HMWs fraction (mainly dimer) in the Ligand 2-receptor phosphorylation assay
- potency of the monomer fraction unchanged in both assays
- change in potency upon storage at 5°C are due to the formation of HMWs
- increased potency of the HMWs in the Ligand 2-receptor phosphorylation 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



One binding event =
Weak binding
(Affinity)

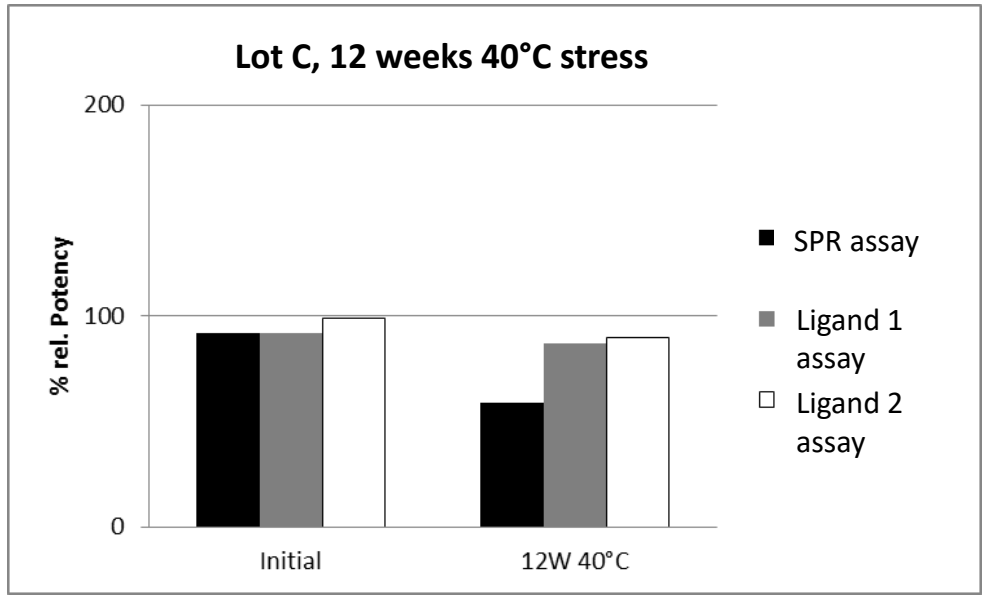


Two binding events =
Strong binding
(Avidity)

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

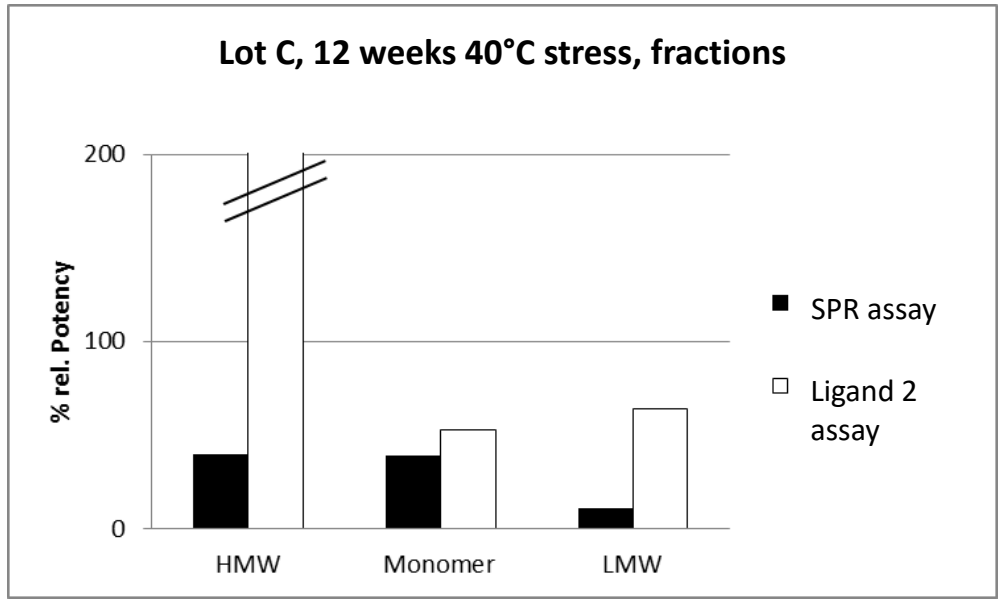


HMW	1.1	5.9
(Dimer) [area%]	(1.1)	(4.9)
% HC_Asp106	95	NT

- 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

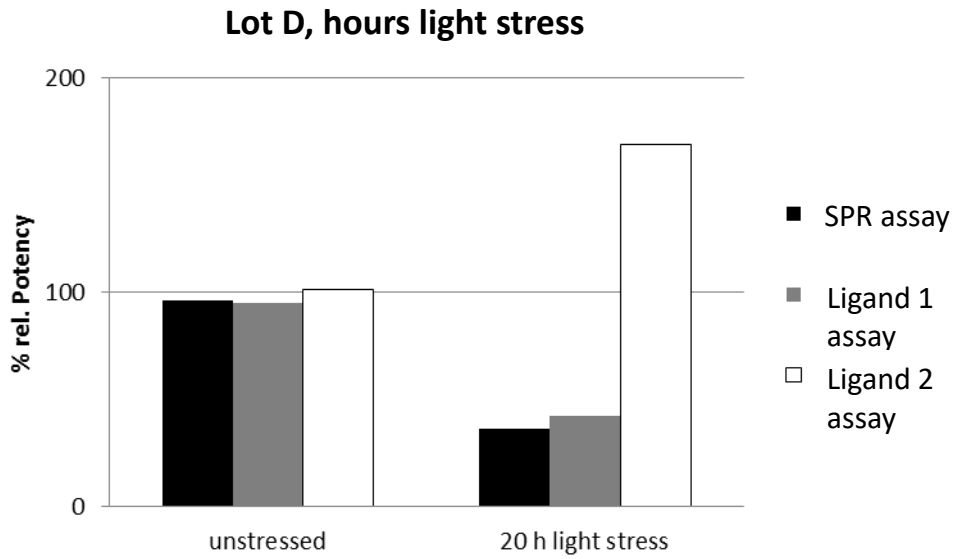


HMW	92.6	0.3	NT
(Dimer) [area%]	(82.6)	(0.3)	
% HC_Asp106	61	60	62

- 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

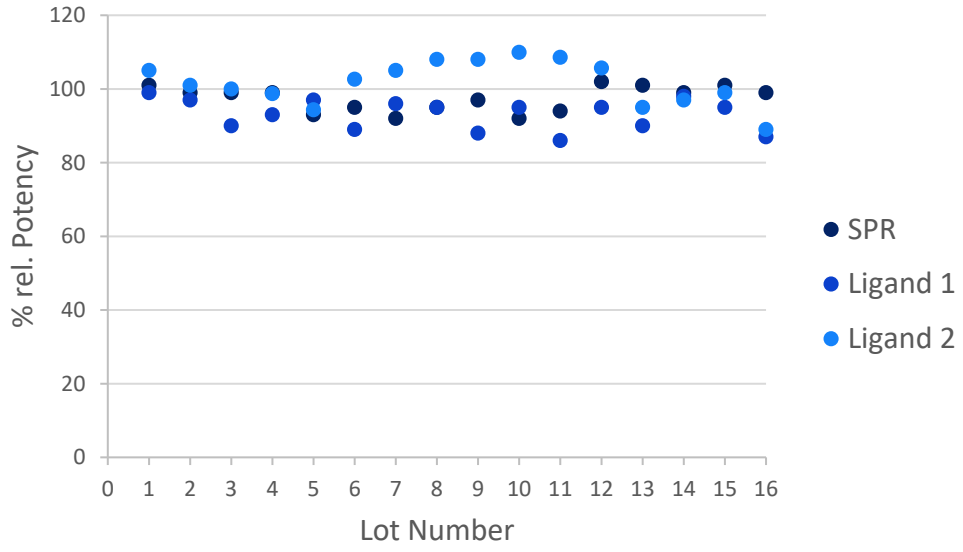


HMW	1.6	24.1
(Dimer) [area%]	(1.5)	(16.5)
% HC_Asp106	95	91

- 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

Methods comparability study

Release data phases 1-3



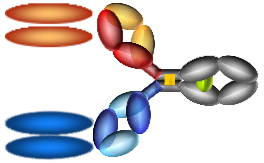
- phase 1-3 lots have comparable potencies in the SPR assay and the two cell-based assays at release

Potency specification of the marketed product

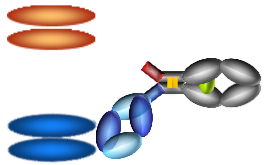
Potency specification

Understanding potency results from SPR vs cell-based assays

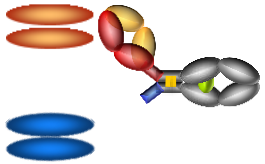
SPR assay



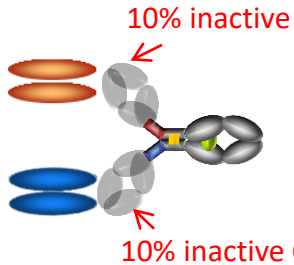
→ 100% potency



→ 0% potency

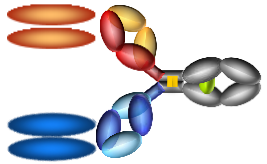


→ 0% potency



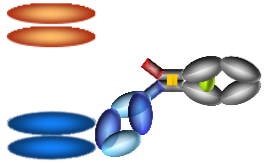
→ 81% potency (90% x 90%)

cell-based assays



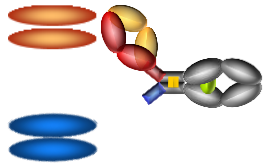
→ 100% potency for anti-Ligand 1

→ 100% potency for anti-Ligand 2



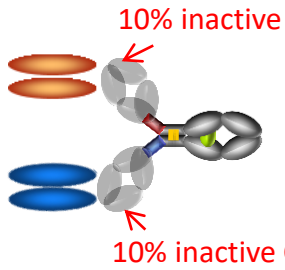
→ 0% potency for anti-Ligand 1

→ 100% potency for anti-Ligand 2



→ 100% potency for anti-Ligand 1

→ 0% potency for anti-Ligand 2

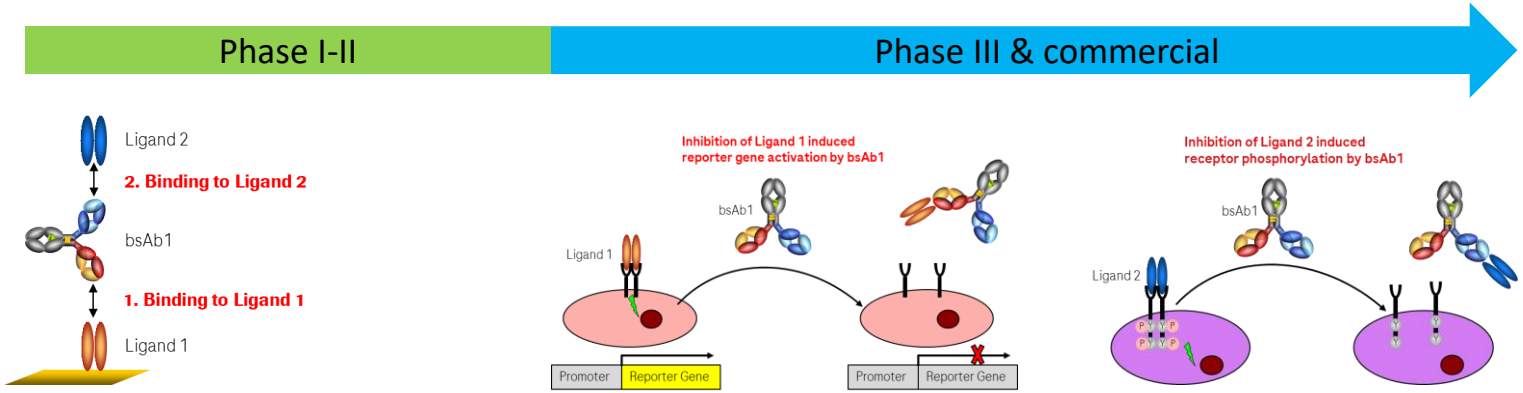


→ 90% potency for anti-Ligand 1

→ 90% potency for anti-Ligand 2

Potency specification

Specification and project phases

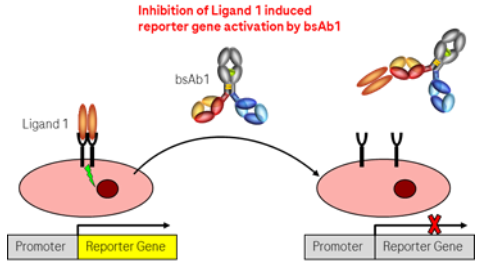


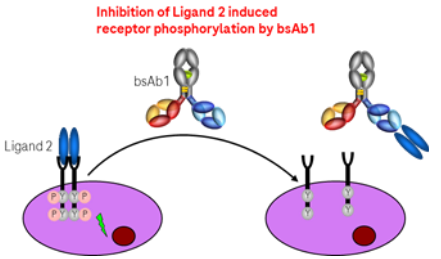
Project Phase	Test	Potency specification
Phase 1 and 2	SPR-based assay	60 – 140%
Phase 3	Ligand 1 reporter gene assay	60 – 140%
	Ligand 2-receptor phosphorylation assay	60 – 140% (release) 60 – 160% (stability)
Commercial	Ligand 1 reporter gene assay	80 – 120%
	Ligand 2-receptor phosphorylation assay	80 – 120% (release) 80 – 135% (stability)

Potency specification

Setting commercial specification

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





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*

Mentors

- *Hermann Beck*
- *Adelheid Rohde*

Doing now what patients need next