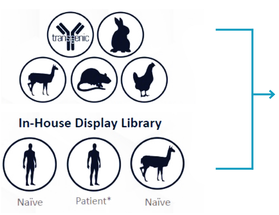
Bispecific Antibody Discovery and Characterization

Erik van Buijtenen, Bianca Boers, Milou Smits, Debby Kruijsen, Sander van Duijnhoven
IPA (ImmunoPrecise Antibodies) (Europe), B.V. Pivot Park, Kloosterstraat 9, 5349 AB Oss, The Netherlands

Bispecific antibodies

Bispecific antibodies (bsAbs) enable the engagement of multiple epitopes thereby opening new possibilities for therapeutic use, including dimerization of receptors, bridging of two different cell types, and targeting dual-positive tumor cells. IPA has a long-standing history in custom Ab discovery and Ab engineering allowing the generation of large target-specific antibody panels with broad characteristics (epitope bins, functionality, kinetics). These monoclonal Ab panels can serve as input for the generation of novel bsAbs. IPA offers a comprehensive portfolio of services for bsAb design, purification and polishing, and evaluation of bsAb integrity and purity by means of analytical methods such as HIC-HPLC, CIEX-HPLC, CE-SDS or SDS-PAGE. Furthermore, validation of bifunctional bsAb properties can be assessed in a high throughput mode on recombinant protein level or cellular level by cellular bridging and/or bsAb mediated cellular activation assays and Octet-based label-free interaction analysis. In this poster we exemplify our services for an in-house discovered Fab x scFv-Fc bsAb, employing the knob-into-hole technology for heterodimerization.

Complete antibody discovery technology portfolio drives selection of large panels of functional antibodies



Hybridoma
- Proprietary hybridoma-electrofusion technology
- Semi-solid media fusion and clone picking
- Ability to screen every clone generated from fusion

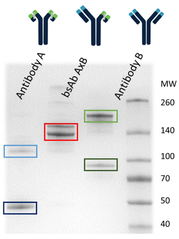
B cell Select®
- Proprietary B cell selection technology
- Maintains native heavy and light chain pairings
- Applicable for all antigens including low-immunogenic or difficult targets

Phage Display
1. In-house human patient and naïve libraries (scFv)
2. Naïve llama libraries (VHH)
3. DeepDisplay™ - custom immune libraries (multiple species, including transgenic animals)

- Applicable for all antigens, including low-immunogenic or complex targets

Large panel of sequence and functionally diverse antibodies funnel into tailored development and characterization assays for further lead candidate selection.

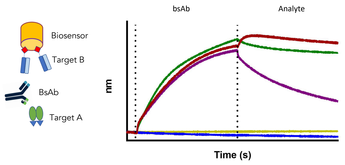
Non-reducing SDS-PAGE shows bsAb integrity



Antibody A: scFv-Fc homodimer
Antibody A: scFv-Fc half antibody
bsAb AxB*
Antibody B: Fab-Fc homodimer
Antibody B: Fab-Fc half antibody

* Multiple bands visible due to non-polished nature of Abs

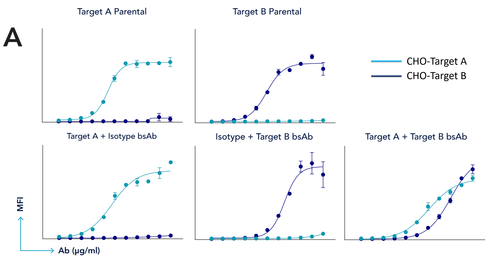
BsAb binds recombinant targets simultaneously



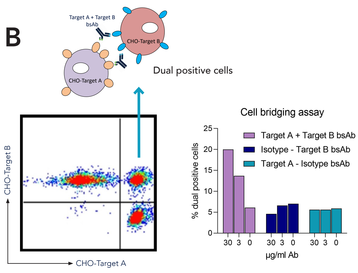
Sensor	Loading	BsAb	Analyte
	Target B	Target A + Target B bsAb	Target A
	Target B	Target A + Target B bsAb	-
	Target B	Isotype + Target B bsAb	Target A
	-	Target A + Target B bsAb	Target A
	Target B	Target A + Isotype bsAb	Target A

BLI (Octet) was used for data acquisition
Representative data for analysis of larger panel of generated bsAb

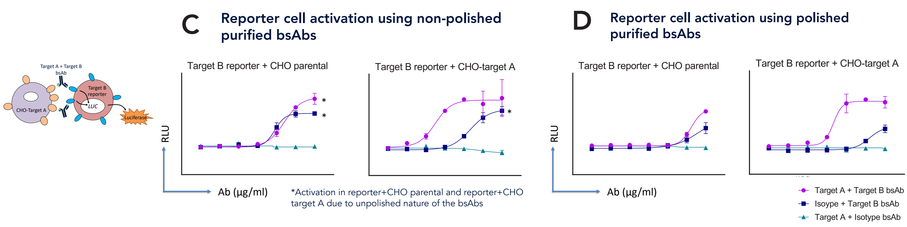
BsAb arms bind cell-expressed targets



BsAb mediates target-specific cellular bridging



BsAb initiates Target B receptor signaling upon cellular clustering



Functional analysis of bsAbs: A. Parental bivalent, single-arm isotype control and bsAb binding to target-expressing cells. B. Bridging assay of target-expressing cells using bsAbs and related bsAb isotype controls. C. Reporter cell activation using bsAbs before polishing of purified bsAbs showing unfavorable background activation due to presence of monovalent/homodimer parental Ab and/or aggregates. D. Reporter cell activation using purified plus polished bsAbs showing reduced single arm activation and specific bsAb-mediated cellular activation, representing the importance of applying appropriate purification technologies for delegate functional screening assays. (Representative data for analysis of larger panel of generated bsAb.)

Conclusions

- IPA's multi-species Ab technology platforms have demonstrated successful discovery of functionally diverse, genetically distinct panels of lead candidate antibodies.
- Early integration of molecular optimization and antibody engineering allows for the generation of highly diverse bsAb panels.
- High-throughput characterization of designed, produced and polished bsAbs enables selection of clinically relevant bsAb combinations validated for developability, kinetics, and functional activity.