Polyclonal solution for next generation multispecific antibody therapeutics:

Examples of anti-idiotypic antibody development for ligand binding assay

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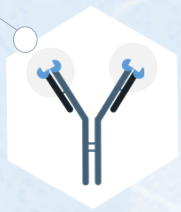
INTRODUCTION The field of therapeutic antibody drugs has experienced explosive growth with now more than 120 molecules approved for treating various human diseases, including many cancers, autoimmune, metabolic and infectious diseases. Thanks to recombinant methods their development is still evolving creating a next generation that can take different shapes such as bispecific, trispecific, multispecific, ScFv (Single-chain variable Fragment) or ADC (Antibody Drug Conjugate). In the context of these next generation antibodies therapeutics, the challenge echoes on preclinical and clinical immunoassay development to build antibody tools, like Anti-Drug Antibodies (ADA), Neutralizing ADA (NADA), anti-idiotype (anti-ID), for bioanalysis methods such as immunogenicity (positive control) and Pharmacokinetics (PK).

1 Example of Next-gen Ab formats

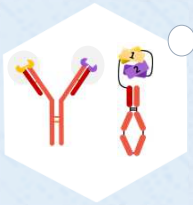
Monospecific Antibody (MsAb)

Most common format of therapeutic Ab

- Contain two antigen-binding sites targeted on the same target.



Bispecific Antibody (BsAb)



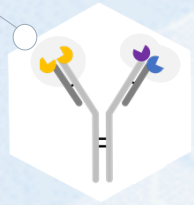
Contain two different antigen-binding sites

- 2 epitopes targeted on a single antigen.
- 2 antigens targeted on the same tumor cell.
- 2 antigens targeted on different cells.

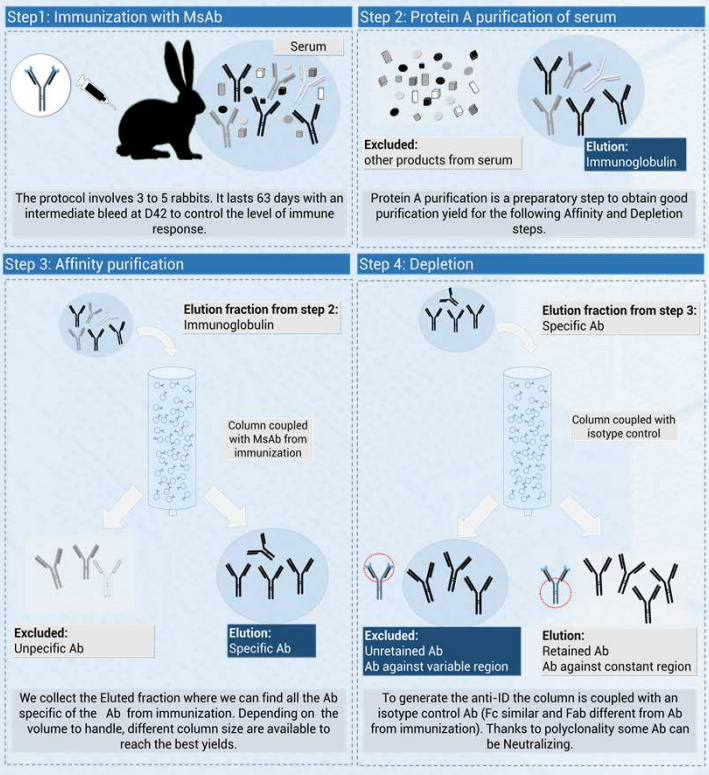
Trispecific Antibody (TsAb)

Engineered to bind three different targets

- Trispecific antibodies allows to close the gap between cancer and cytotoxic T cells, promoting cancer cell lysis



2 Global strategy for anti-ID generation: MsAb



3 Developments of anti-ID from rabbit polyclonal immunization

Bispecific Antibody (BsAb) → CLASSICAL SHAPE

Objective : Selection anti-ID of the first binding site

- Immunization of 3 rabbits.
- Same protocol as described in global strategy (2).
- Depletion with BsAb specific to the second binding site (irrelevant Ig).

- Validation by ELISA control on Excluded fraction and Elution fraction of interest with irrelevant Ig specific with irrelevant Ig.

→ATYPICAL SHAPE

Objective : Selection anti-ID of the first binding site

- Immunization of 3 rabbits.
- Same protocol as described in global strategy (2) without depletion step.
- Affinity with BsAb specific to the second binding site (irrelevant Ig).

CONCLUSION Polyclonal strategy stands as a powerful tool for complex anti-ID development, It allows to obtain a large amount of anti-ID with neutralizing effect. The success of this strategy depends on major key points :

- No control of the immune response of the animals especially with atypical shapes that involves Antigene presentation.
- Necessity of irrelevant Ab in enough quantity to build the purification strategy of the different specific fractions.

After development, Biacore™ analysis is performed to determine affinity constant. The neutralizing activity of the Ab generated can also been evaluated by the following study (right panel).