

Novel Genetic Immunization for the Development of Therapeutic Antibodies Targeting Conformational and Native Antigens

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Abstract

The recent COVID-19 pandemic shows the limitation of the conventional vaccination which uses peptides, recombinant proteins or attenuated viruses as antigens... The genetic immunization based on *in vivo* transfection of RNA or DNA gave a strong and consistent response. The concept was first advanced in the early 1990s, but was not developed because of an initial lack of efficiency. Recent technical advances in plasmid design, adjuvants, gene delivery techniques and route of administration allowed renewed interest in the field.

The main advantage of such an approach, the antigen is displayed conformationally to the immune system due to the *in-vivo* expression of native antigens.

For its therapeutic projects, Covalab has developed its own and proprietary DNA immunization technology using an optimized plasmid allowing for highest antigen expression as well as simple and efficient DNA delivery.

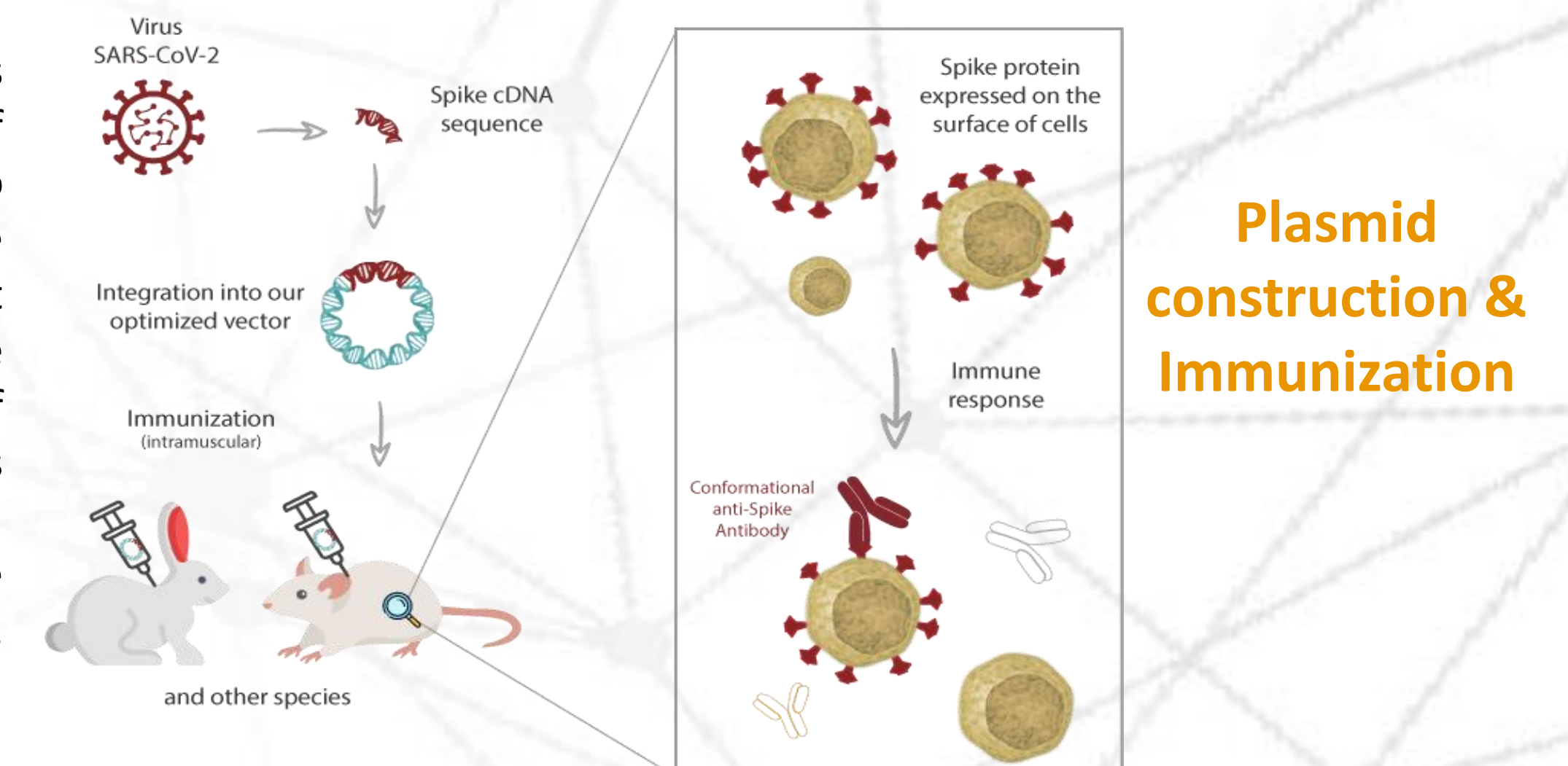
This platform is applied to tumor antigens with the aim of developing "First in class" therapeutic antibodies and challenging the existing antibodies to improve the therapeutic window. In this work, a proof of concept was made to develop successfully neutralizing antibodies for SARS-CoV-2 Spike protein. And compared with immunization using recombinant protein (the standard method), our DNA immunization process has shown a faster immune response, a better ratio IgG/IgM, as well as higher antibody affinity and specificity.

The easy-to-use method does not require any specific material such as gene gun or electroporator allowing its application to various animals (mouse, rat, rabbit, sheep, goat, lama, ...).

Introduction

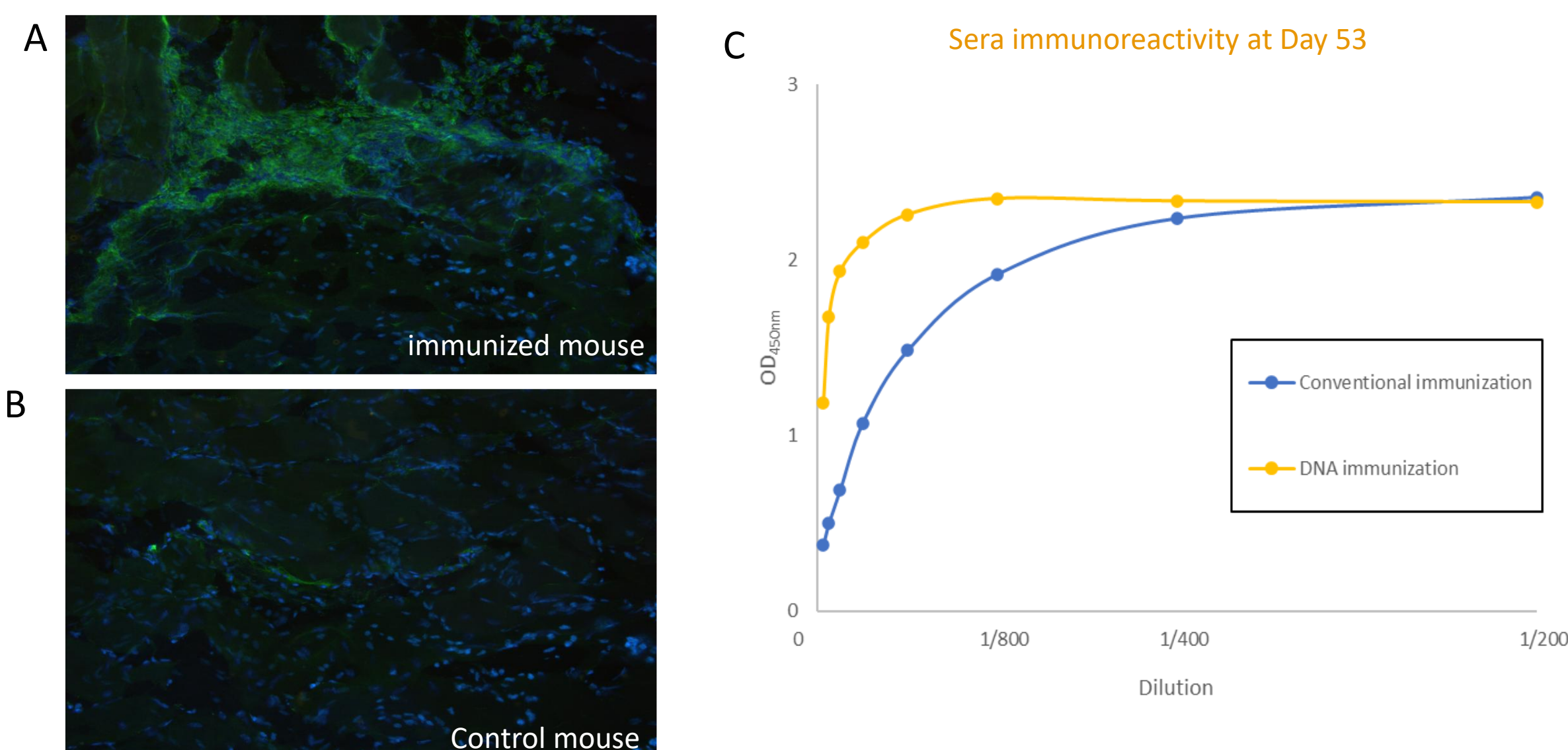
The generation of antibodies against recombinant proteins and membrane receptors in particular, is often associated with several problems stemming from the lack of purity, homogeneity and conformational similarity with the native form. Low protein expression and instability of purified protein also affect the quality of the antigen and are problematical for raising good antibodies. Several approaches have been used to overcome these problems such as adding ligand stabilizers during the purification or detergents to maintain protein folding. However, the generated antibodies are more often reacting with different forms of the antigens or with linear epitopes exposed by the detergent treatments. The DNA immunization approach circumvents these problems as full-length proteins can be expressed *in vivo* when they are delivered in the form of DNA vaccines. Furthermore, it is well known that the structural integrity of proteins is critical for the induction of functional mAbs. Expression of well-folded immunogens *in vivo* by DNA immunization offers the most promising prospect for inducing mAbs with the desired biological activities.

In the present study, an optimized plasmid containing the cDNA encoding the SARS-Cov-2 spike protein was constructed and used to immunize host animals (mouse & rabbit). Critical parameters such as plasmid design, route administration, use of immune modulators (adjuvants) ..., have been optimized to improve our technology and offer a unique and powerful approach. Based on this technology, we are developing novel and challenging monoclonal antibodies against several clinically validated and commercially successful targets (see our product portfolio).



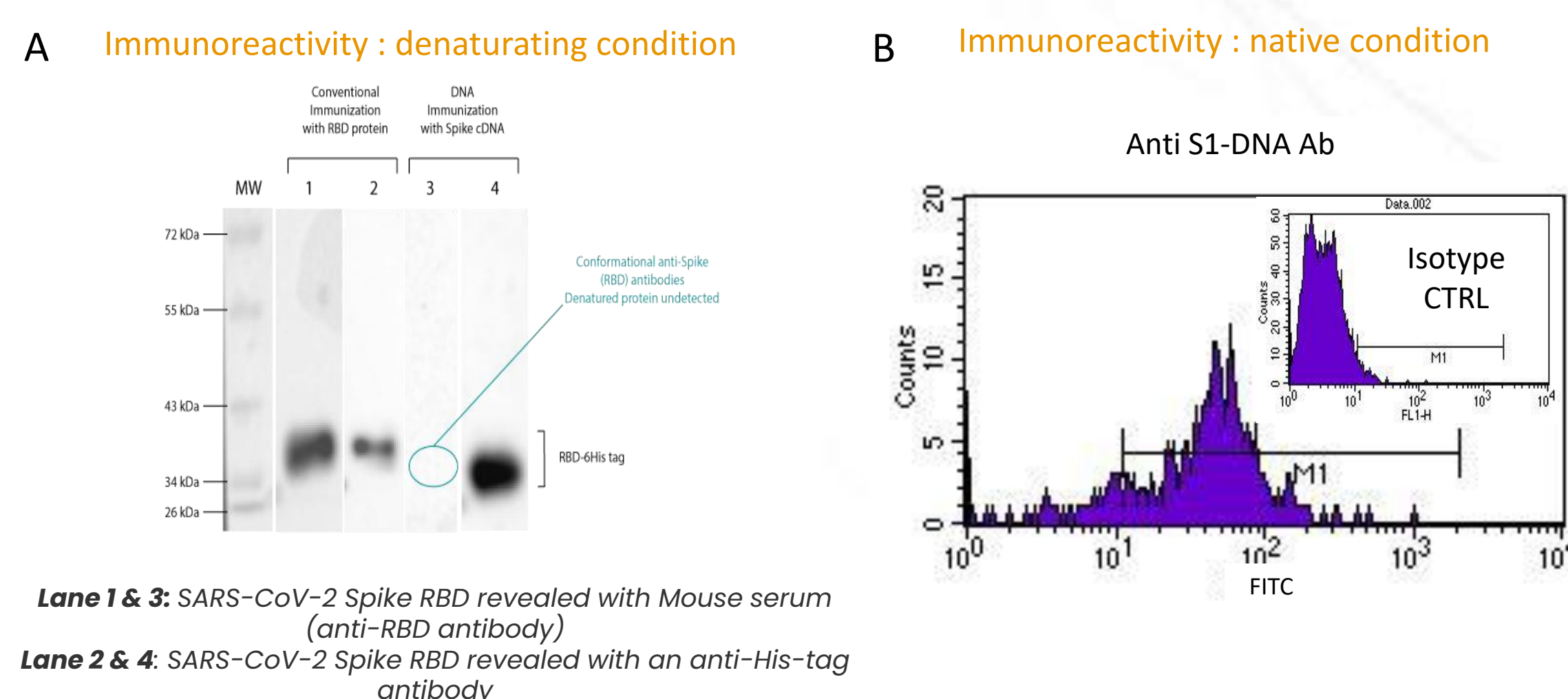
IHC-F and ELISA

IHC-F analysis of SARS-Cov-2 full length Spike protein expression *in vivo* using anti SARS-CoV-2 Spike antibody: (A) Immunized mouse (intramusculaire). (B) control mouse. (C) Mice immune-response followed by ELISA, comparing conventional immunization (recombinant protein, blue curve) and DNA immunization (yellow curve), 53 days post primo injection.



WB & Flow Cytometry

Comparative study of the immunoreactivity of the antibodies developed by DNA and conventional immunizations: (A) Western-Blot analysis using RBD protein in denaturation conditions. (B) FACS analysis of the reactivity of the antibody anti-S protein using hACE2 expressed in CHO cells and S1-subunit to form the natural complex.



Affinity Study

The affinity of mAbs from DNA immunisation to SARS-Cov-2 RBD protein was evaluated by bilayer interferometry (BLI) (n=3).

Antibody	Kd (nM)
mAb-1	0.411±0.179
mAb-2	0.266±0.064
mAb-3	5.54±0.97
mAb-4	0.048±0.037

Oncology targets: Focus on anti TROP-2 antibody

Trophoblast surface antigen 2 (TROP-2) is a type I transmembrane glycoprotein involved in Ca²⁺ signaling. TROP-2 is overexpressed in most human cancers and drives malignant progression. TROP-2 based ADC is already FDA approved for TNBC and urothelial cancer. **Here we developed our own clone library using our DNA immunization technology :**

Affinity Study

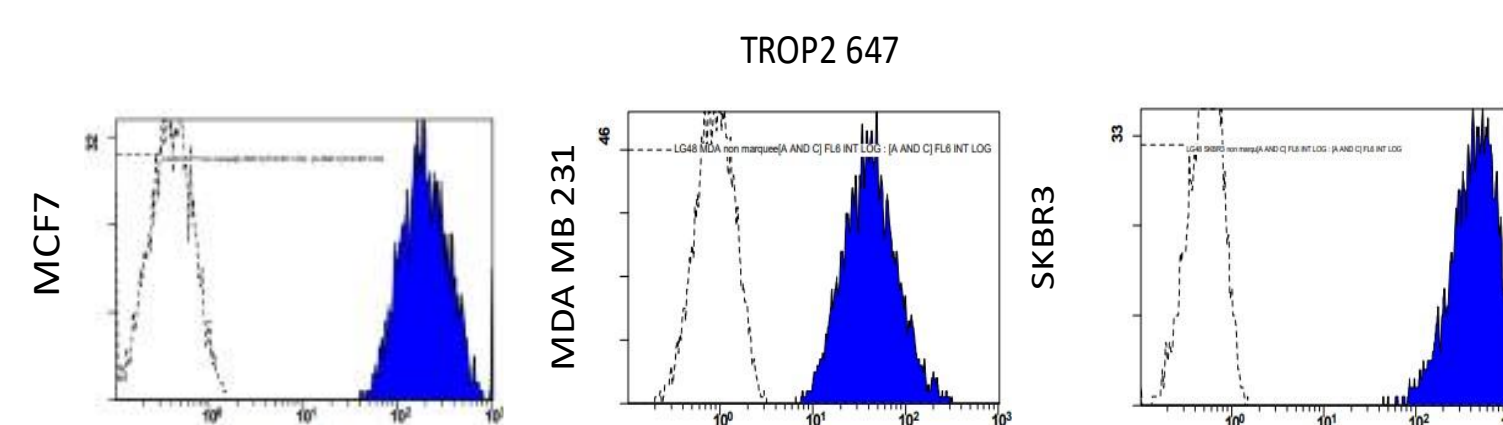
The affinity of mAbs generated from DNA immunisation to TROP-2 protein was evaluated by bilayer interferometry (BLI) (n=3).

Antibodies affinity

Antibody	Kd (nM)
mAb-1	0.12 ± 0.03
mAb-2	0.42 ± 0.02
mAb-3	0.57 ± 0.02
mAb-4	0.20 ± 0.06
Sacituzumab	0.79 ± 0.02
Datopotamab	0.76 ± 0.03

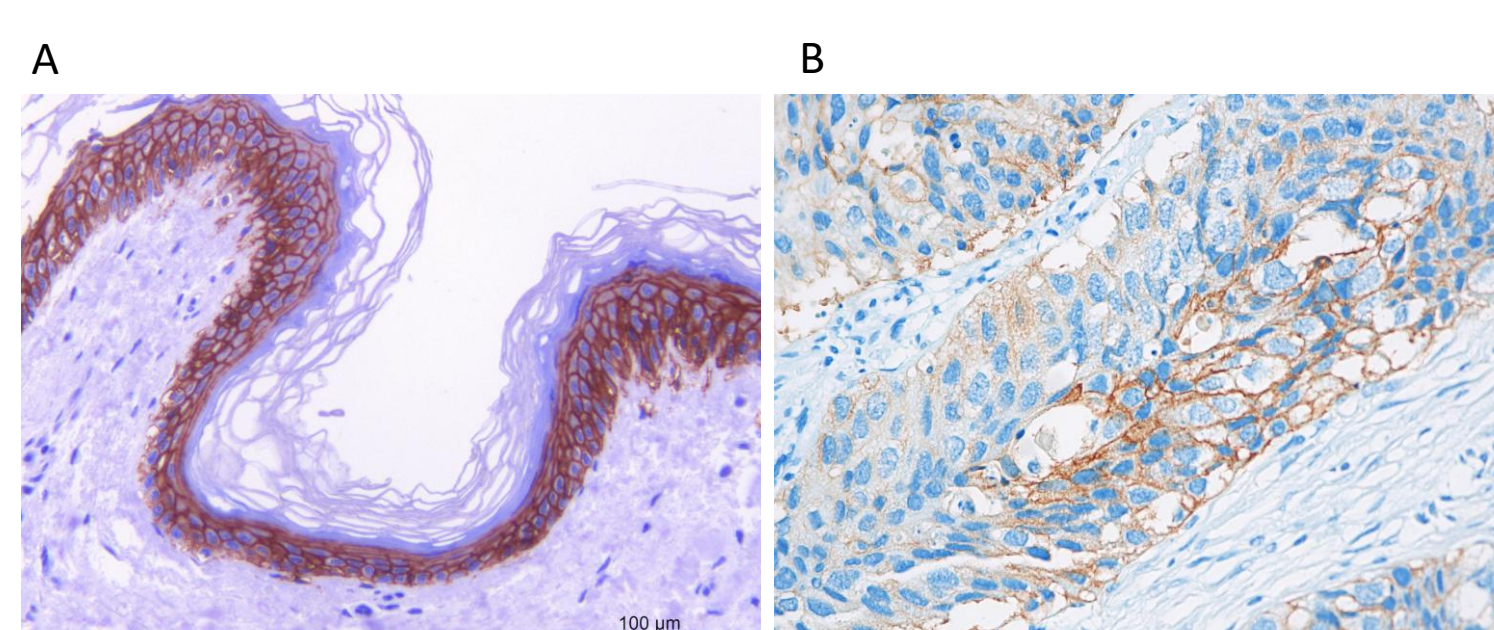
Flow Cytometry analysis

Example of flow cytometry analysis of various cell lines overexpressing TROP-2.



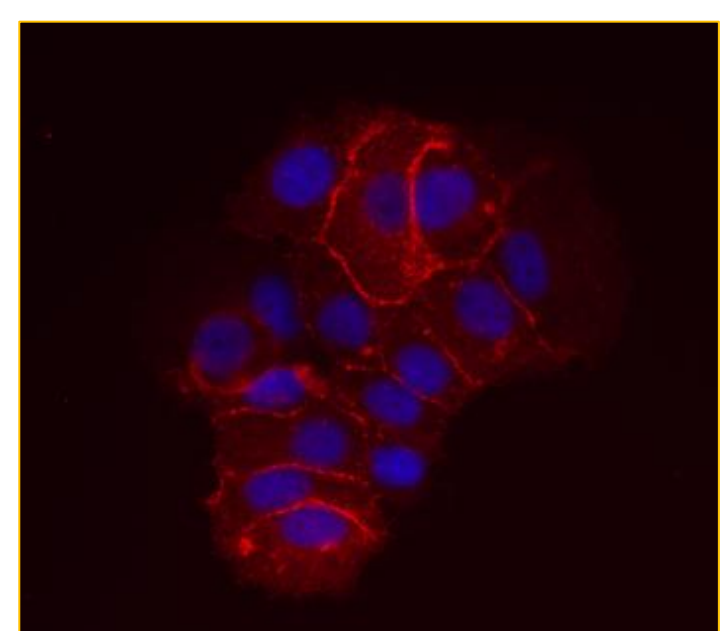
Immuno Histo Chemistry

IHC analysis of TROP-2 protein expression in human tissues using anti TROP-2 antibody: (A) Human skin. (B) Bladder Cancer Tissue Micro Array.

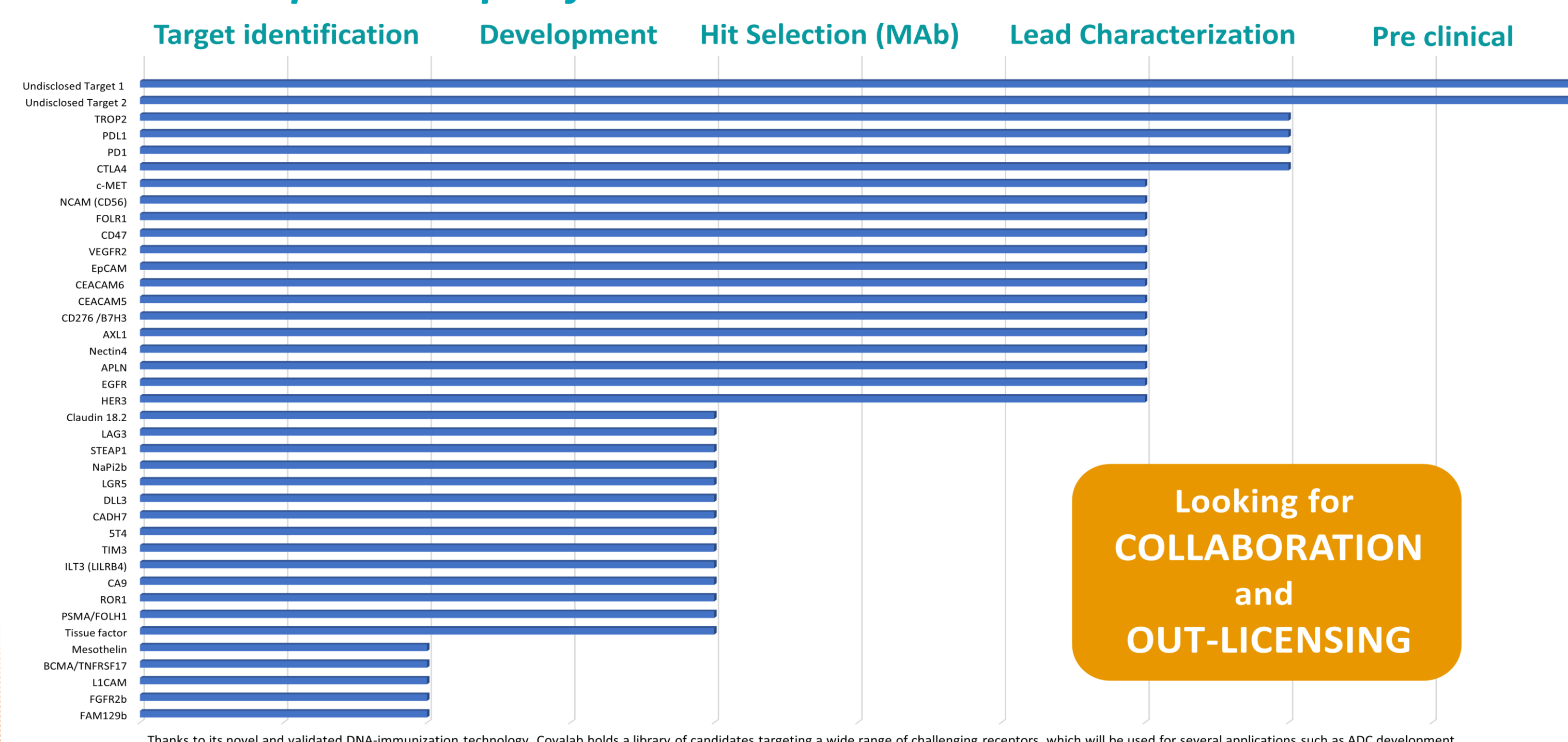


Immunofluorescence

Immunofluorescence staining of MCF7 cells with the anti TROP-2 647 antibody.



Covalab's products portfolio



Conclusion

The "DNA-designed antibodies" method we developed is time- and cost-effective. It does not require protein purification and enables generation of antibodies targeting membrane-anchored and glycosylated proteins in their native form.

The advances in antibody technologies have greatly accelerated the discovery of SARS-Cov-2 neutralizing antibodies (nAb) as well as high affinity antibodies in the field of oncology. Thanks to its novel and validated DNA-immunization technology Covalab holds now a library of candidates targeting a wide range of challenging receptors, which will be used for several applications such as ADC development.