Disruptive In Vivo Rational Antibody Discovery

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ABSTRACT

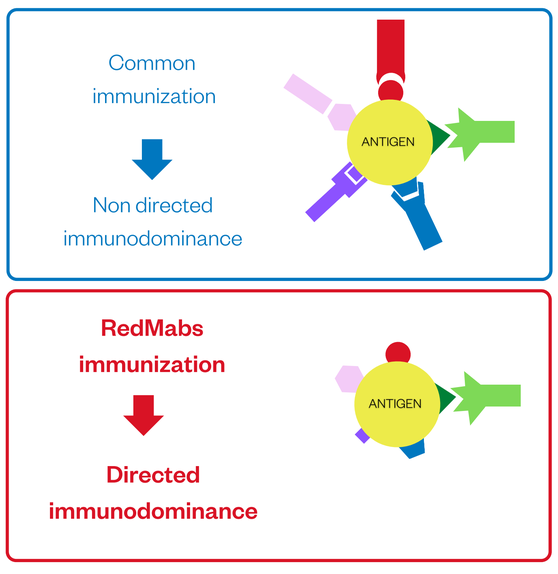
IIn vivo immunization has been an enormous technological advantage since 1975 when Milstein y Köhler invented the production of monoclonal antibodies. Since then, hundreds of monoclonal antibodies have been developed for diagnosis and therapeutical purposes. However, immunization protocols and adjuvants, a fundamental part of the success, had not been developed further despite being tremendously important. Here we present a novel and disruptive technological platform for In vivo rational antibody discovery. In this platform, both design, protocol optimization, and proper adjuvants and immunomodulators were used. We present the first results of this platform applied to an undisclosed antigen immunization of BALBC mice. It can be observed that the number of specific hybridomas obtained against desired epitope have increased by a factor of fourteen. We believe that these results are really promising. RedMabs platform breaks the technological barriers of undruggable targets, opening huge opportunities for the development of new drugs.

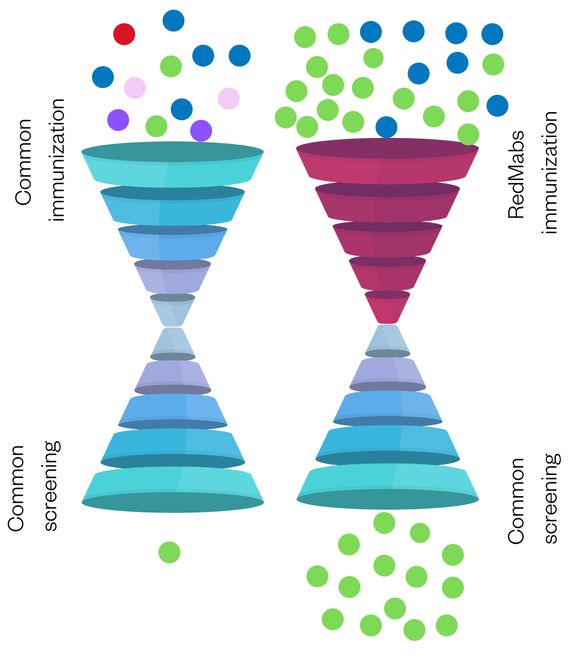
Introduction

RedMabs in vivo antibody discovery platform is a disruptive tool in the immunization field. A combination of antigen design, immunization protocols, and BioClonal own adjuvants and immunomodulators originates this novel platform. Application of this platform fundamentally gives rise to two phenomena: An expansion of the germ line and the directionality of immunodominance. As a consequence, a greater number of antigen-specific B cells can be obtained, as well as a greater percentage of these towards the desired epitope. Of special interest is the use of this platform in immunogens against which antibodies cannot be obtained with common protocols. This would be the case, for example, of very small antigens such as carbohydrates or low molecular weight proteins, poorly immunogenic epitopic areas, or immunogens whose receptor ligand system is unknown. Here we present results of the application of RedMabs platform to an undisclosed antigen, being compared with common immunization protocols.

Results

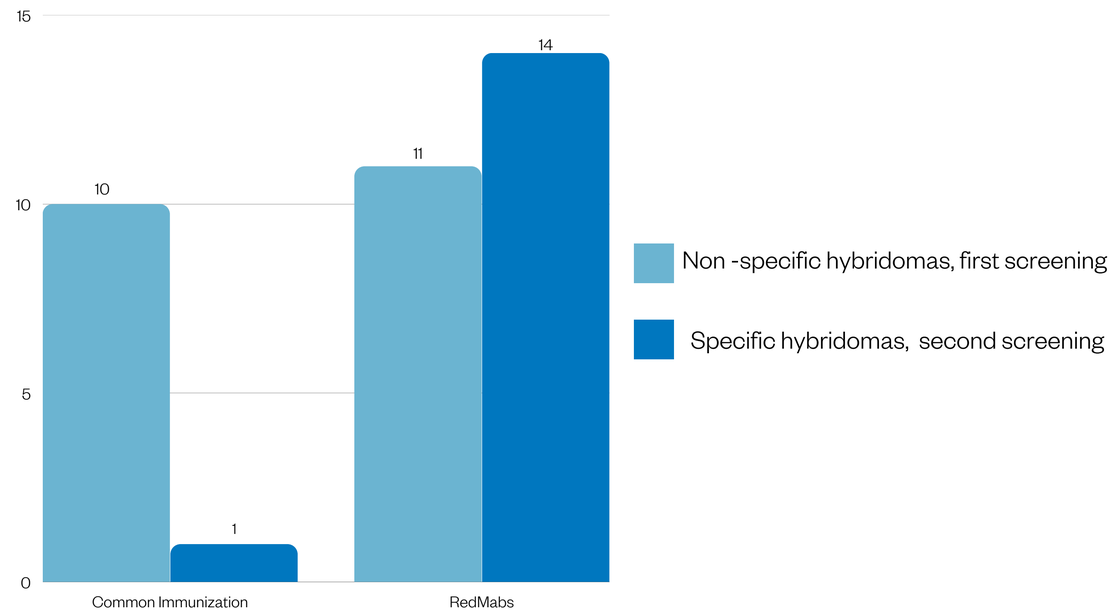
Positive wells in first screening were cloned and re tested using same conditions. A total of eleven clones were positive using common immunization protocols, and twenty-four were obtained using RedMabs platform. Those clones were evaluated against native protein of undisclosed target in second screening. In this case, just one clone was detected as positive against the desired epitope in common immunization, while fourteen of twenty-four clones in RedMabs showed positiveness in same conditions. These data correspond to 58% of RedMabs platform success against 9% of common immunization.





Materials and methods

Two lines of immunizations were carried out, in perfect parallel. Hybridomas of anti-undisclosed antigen were generated through five intraperitoneal immunizations of eight week old Balb/c mice for common immunization protocol. Recombinant protein of undisclosed target and Complete Freund´s Adjuvant. The immunization was also boosted three times intraperitonealy with same antigen in Incomplete Freund´s adjuvant.Analogously, same number of mice and conditions of sex and age were used for RedMabs immunization. An emulsion of specific antigen preparation and specific adjuvants and immunomodulators were used. The immunization was boosted three times with other similar preparations, also intraperitoneal.
All spleens were isolated and cells were dispersed under sterile conditions. The spleen cells were mixed with the myeloma cell line SP2/0-Ag14 at 10:1 ratio, while fusion reagent Polyethylene Glycol 6000 solutions After plating the cell mixture to microwell plates, the hybridomas were selected by adding HAT Supplement to the medium. A first screening of antibody secreting clones were carried out using recombinant anti-undisclosed coated ELISA plates. Second screening was carried out using native anti-undisclosed coated ultra-sensitive CLIA plates against desired epitope.



Conclusion

Hybridoma technology had been used in the last century to obtain important drugs for several diseases, improving patients prognosis. However, it is known that efficiency of positiveness in this method is not high. It is established around 10-20% of success in common immunization protocols. Last advantages in high throughput screening or single B cells technologies increased this percentages by factor 4 to 5 the efficiency of common ELISA protocols. However, if total success in terms of positiveness against native targets is fixed, single B cell technologies wont unfold their full potential. RedMabs platforms showed a clear increased in the efficiency of positiveness against native protein in this case, even though common screening method were used.

Combination of RedMabs platform and single B cell technologies would be an enormous advantage in the antibody discovery in the near future.

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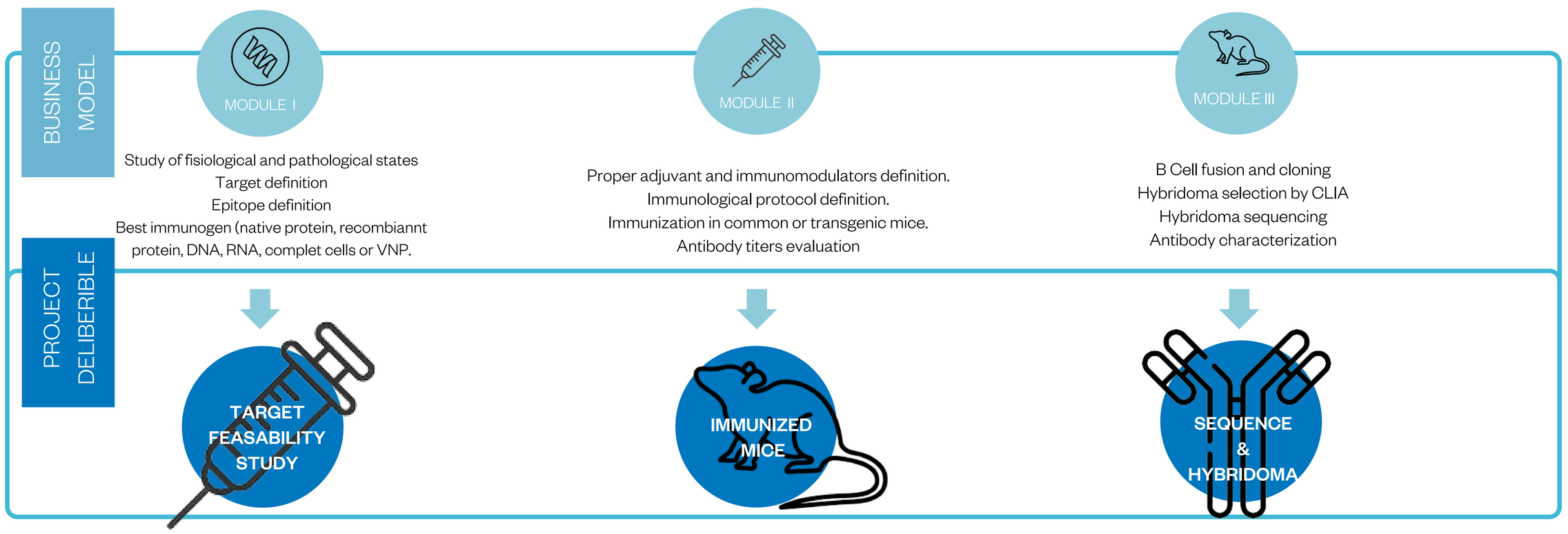

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