carterra®

APPLICATION NOTE

Understanding Epitope Coverage Can Guide the Discovery of Therapeutic Antibody Cocktails for Infectious Diseases

The Carterra® LSA™ can be used to screen and characterize large panels of monoclonal antibodies (mAbs) to better understand their epitope coverage. In the case of infectious diseases, a greater understanding of an individual's antibody repertoire in response to infection can help guide the triage of antibody candidates towards an effective treatment. Using the Carterra LSA, the screening process is high throughput and consumes very little sample, allowing researchers to quickly generate an information-rich data set to help guide the selection of antibody combinations to serve as therapeutic antibody cocktails.

Introduction

Monoclonal antibodies (mAbs) are highly attractive as therapeutic agents due to their naturally high affinities and exquisite target specificities. With the biopharmaceutical market forecast to reach sales of almost USD 390 billion in 2024, and with the clinical pipeline of therapeutic antibodies being at record number[1], therapeutic antibodies are projected to experience further commercial and clinical success.

While most currently marketed antibody products treat cancer or inflammatory and autoimmune disorders, antibody therapeutics are providing transformative medicines for infectious diseases. This essentially exploits the role that antibodies evolved in nature to achieve – fighting invading pathogens and foreign molecules – and contrasts with traditional therapeutic antibodies already on the market, which target human proteins with the goal of interfering with a natural biological pathway.

Antibodies offer some key advantages in the treatment of both viral and bacterial infections. Due to their high specificity, antibodies can selectively target pathogens for neutralization without disturbing the natural microbiota, unlike many antibiotics. They can also function by multiple mechanisms of action, and when used in combination with antibiotics or other complementary antibodies, can help reduce the growing problem of resistance and provide additive and/or synergistic effects. This provides a way of compensating for biological redundancy that exists naturally – when one pathway fails, another often compensates for it. Reinforcing this, the WHO has acknowledged that antimicrobial resistance to mAbs is a potential threat and recommended that "this threat may be overcome by targeting highly conserved epitopes or by using antibody cocktails containing more than one mAb."[2]

In this application note, we demonstrate how high throughput surface plasmon resonance (SPR) technology enabled the characterization of a panel of human mAbs recovered by B cell cloning from two patients infected with human immunodeficiency virus type 1 (HIV-1). Taking advantage of the high throughput capabilities of Carterra's SPR technology, researchers could quickly map the epitope coverage of antibodies known to neutralize the virus and identify a subset of antibodies that target distinct, complementary epitopes. The targeting of distinct epitopes infers their use of different mechanisms of action in achieving the functional phenotype of "neutralization."

The Carterra LSA's expanded throughput compared with other label-free methods makes it uniquely suited to performing epitope binning or mapping assays on large panels of mAbs, producing comprehensive epitope coverage data early in the drug discovery process. When merged with orthogonal data, such as cell-based neutralization data, knowledge of the epitope landscape - as provided by epitope binning experiments - can guide a triaging process that identifies promising candidates for a broadly neutralizing antibody cocktail therapy.

Antibody cocktails against infectious agents

With the success of therapeutic antibodies over recent years, the pharmaceutical industry is seeking innovative approaches to increase potency and extend their applications. Targeting infectious diseases is becoming a more serious unmet medical need as bacterial resistance to antibiotics spreads and viruses evolve causing pandemics such as the recent Ebola outbreaks in Western Africa in 2014 and 2019. New therapies that can be developed quickly in response to urgent needs and that target multiple unique mechanisms to combat resistance and improve potency are always required.

The use of antibody cocktails as therapeutic products, pioneered by Symphogen and

others, is producing promising results in the clinic. Notable examples for infectious diseases are ZMapp and ZMAb, which are cocktails of inhibitory mAbs against the Ebola virus that were used to treat 15 patients under compassionate-use protocols in 2014[3].

These cocktails of recombinant human mAbs contain multiple antibodies, each targeting a different epitope, that collectively provide broad neutralization of an invading pathogen **(Figure 1)**. Although the mixture usually contains only two or three mAbs, the result is a polyclonal-like response whereby multiple epitopes on the same antigen are recognized to provide a more potent effect[4]. Ideally, the mAbs in the cocktail should be carefully selected to deliver a similar potency and therefore comparable neutralization and protection as an entire immune response that would naturally comprise hundreds of mAbs against the invading pathogen. The three or so chosen mAbs, are then manufactured and administered as a cocktail for prophylactic or therapeutic use against the viral infection.

Determining which antibodies will work together in an antibody cocktail to generate this full-scale immune response is a complex process. Drug discovery researchers need to identify a small set of mAbs with different but complementary functions, which in turn is the result of them targeting different epitopes on the antigen. An analytical technique such as epitope binning using high throughput SPR helps researchers better understand the epitope repertoire of an antibody panel[5]. This is immensely valuable in guiding the triage process that selects the optimum set of clones for an antibody cocktail. Of course, a caveat to this type of analysis is first identifying, isolating, and producing the relevant antigen in recombinant form.

Using B cell cloning to recover the antibody repertoire of an infected individual

Generating an antibody library that is relevant to a particular strain of an infectious agent is the first step in developing a targeted antibody cocktail. Identifying and isolating the specific antigen and ensuring that the library represents the entire antibody repertoire responsible for targeting and neutralizing the infection is key.

Before the advent of antibiotics and vaccines, serum therapy was widely used to treat or prevent infectious diseases through a process of passive immunization[2]. This transfer of neutralizing antibodies helps to raise an immune response against a specific pathogen to treat infection. Similar principles can be employed using a process termed B cell cloning to recover antibodies from an infected individual which often represent the numerous mechanisms for pathogen neutralization. Characterization of these mAbs can then ultimately determine those that should comprise a therapeutic antibody cocktail. This approach has recently been successful in recovering diverse HIV-1 neutralizing antibody clones from two patients infected with the virus[8].

Analyzing epitope coverage with high throughput SPR

Following recovery of a panel of human mAbs from infected patients, screening and detailed characterization is necessary to distil to a subset of clones for use as the therapeutic cocktail. High throughput SPR is uniquely advantageous in this regard because it enables both screening and characterization to be performed in the same step at the start of the drug discovery process, and with minimal sample volume requirements. The

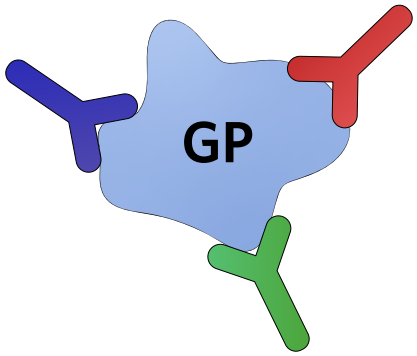

Figure 1: Simple cartoon depicting how multiple different antibodies, targeting distinct epitopes on a glycoprotein antigen, such as Ebola or HIV-1 envelope, can be combined as a therapeutic cocktail to achieve broad neutralization[6,7].

Carterra LSA can perform this functional screening method - based on comprehensive binning experiments - quickly and accurately to provide detailed information on the epitope landscape of a large antibody library, such as that generated from B cell cloning.

The use of antibody cocktails to target multiple epitopes is analogous to the way acupuncture simultaneously targets multiple specific points to achieve pain relief.

Epitope binning to guide the selection of an antibody cocktail that broadly neutralizes HIV-1

High throughput SPR was used to assess the epitope repertoire of an antibody panel targeting HIV-1 glycoprotein that was recovered from infected individuals by B cell cloning[8]. By merging epitope binning data with structural data, researchers were able to identify clusters of neutralizing mAbs that mapped mostly to epitopes in the Env V3 loop region and the CD4 binding sites on the HIV-1 Env glycoprotein, which is a major target for antiviral therapies and vaccines against HIV-1.

The network plot in **Figure 2** shows how the antibodies cluster according to their epitope specificity, providing several choices per group to take forward into an experimental drug cocktail approach. Based on the premise that antibodies targeting a similar epitope likely share a similar mechanism of action, selecting an antibody from each of the three determined epitope clusters would likely generate a combined cocktail with a broadly neutralizing effect, recapitulating the potency of the entire panel. Other characteristics of the antibodies, such as affinity and developability properties would be additional criteria for whittling the selection to an appropriate therapeutically-viable cocktail.

Taken together with a recent study on Ebola virus, where non-neutralizing antibodies were able to cooperatively neutralize the virus[9], this provides further support for the use of antibody cocktails as a promising treatment option for infectious diseases.

Using epitope diversity as a surrogate for functional diversity

This work clearly demonstrates the insight that can be achieved when adopting an epitope-centric approach to identify antibodies with diverse neutralization capabilities. It also shows how knowledge of epitope diversity can act as a surrogate for functional diversity.

Epitope binning works in a combinatorial and pairwise manner to organize mAbs into epitope families according to their specificities[5]. Knowing the diversity of the epitopes available in the panel provides the ability to select for functionally diverse mAbs and achieve the desired phenotype of broad neutralization. Some recent studies[10,11] demonstrate this relationship between epitope and neutralization, and, in turn, protection.

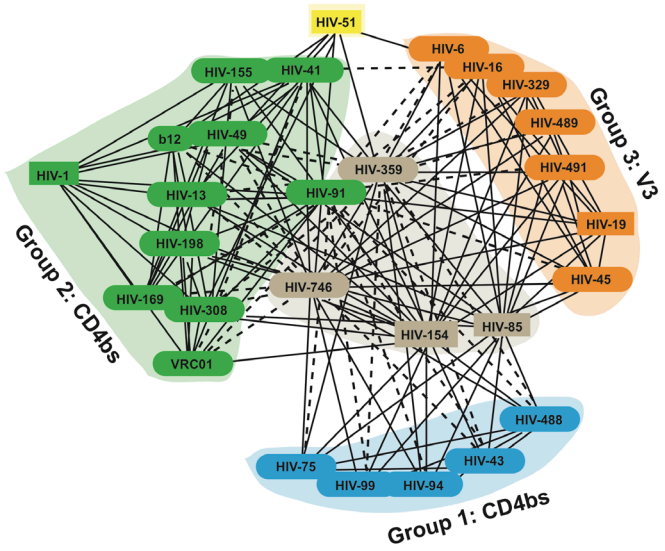

Figure 2: Network plots showing groupings and associations of mAbs from two subjects infected with HIV-1. Each color-coded group indicates mAbs with a competitive relationship: solid lines represent blocking relationships in the network; dashed lines represent asymmetric competition. Group 1 mAbs (blue) and group 2 mAbs (green) are CD4 binding site-specific. Group 3 (orange) are V3 loop region-specific[8].

Summary

The Carterra LSA is a high throughput SPR platform that enables the epitope binning of 384 antibodies in a single run. The ability to perform large epitope binning studies early in discovery with minimal sample requirement provides both a high-throughput and high-resolution way of characterizing an antibody panel's epitope coverage, which is a surrogate for functional diversity. It expands the potential of other, limited throughput label-free platforms, allowing for hundreds of interactions to be monitored in parallel. This means that instead of using low throughput assays to find complementary partners to a benchmark clone, researchers can survey the entire epitope repertoire of mAbs to make better-informed decisions about which mAbs to combine in a cocktail.

In the context of traditional antibody therapeutics, this technology is significantly impacting antibody screening and characterization, enabling higher-information-content assays to be conducted earlier in the research pipeline. This thereby streamlines lead selection and ensures promising antibody clones are not discarded too early due to premature triaging of libraries into subsets. The potential for the Carterra LSA instrument in developing antibody cocktails for infectious diseases is equally momentous.

References

1. *Mordor Intelligence (2019) Biopharmaceuticals Market - Growth, Trends, and Forecast (2019 - 2024) [online]. Available from: https://www.mordorintelligence.com/industry-reports/global-biopharmaceuticals-market-industry [Accessed: 23/04/2019]*
2. *Sparrow, E., et al. Therapeutic antibodies for infectious diseases. Bull World Health Organ. 95(3): 235-237 (2017)*
3. *Davidson, E., et al. Mechanism of binding to Ebola virus glycoprotein by the ZMapp, ZMAb, and MB-003 cocktail antibodies. Journal of Virology. 89(21): 10982-10992 (2015)*
4. *Logtenberg, T. Antibody cocktails: next generation biopharmaceuticals with improved potency. TRENDS in Biotechnology. 25(9): 390-394 (2007)*
5. *Abdiche YN, et al. High-Throughput Epitope Binning Assays on Label-Free Array-Based Biosensors Can Yield Exquisite Epitope Discrimination That Facilitates the Selection of Monoclonal Antibodies with Functional Activity. PLOS ONE 9(3) (2014)*
6. *Saphire, EO., et al. Antibody-mediated protection against Ebola virus. Nature Immunology. 19(11):1169-1178 (2018)*
7. *Caskey, M., et al. Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. Nature Medicine. 25; 547-553 (2019)*
8. *Chukuwama, VU., et al. Increased breadth of HIV-1 neutralization achieved by diverse antibody clones each with limited neutralization breadth. PLOS ONE. (2018)*
9. *Howell, KA., et al. Cooperativity enables non-neutralizing antibodies to neutralize Ebolavirus. Cell Rep. 19(2): 413-424 (2017)*
10. *Wec, AZ, et al. Development of a Human Antibody Cocktail that Deploys Multiple Functions to Confer Pan-Ebolavirus Protection, Cell Host & Microbe. 25: 39-48 (2019)*
11. *Saphire, EO., et al. Systematic analysis of monoclonal antibodies against Ebola virus GP defines features that contribute to protection. Cell. 174(4):938-952 (2018)*

AN112.1-051819

carterra®
info@carterra-bio.com
825 N 300 W Suite C309
Salt Lake City, UT 84103
www.carterra-bio.com