Clustering: a rational design principle for potentiated antibody therapeutics

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SUMMARY

Owing to their high specificity and ability to engage multiple effector functions, combined with their modular and adaptable architecture fit for engineering approaches, antibodies have revolutionized drug development and treatment of human disease. Central to the mechanisms of action of many successful therapeutic antibodies are the affinity and avidity of binding interactions. Affinity can be defined as the strength of a single binding interaction between antibody and antigen, while avidity is determined by the accumulated binding strength of multiple individual non-covalent interactions including 1) Fab-mediated monovalent versus bivalent antigen binding (first order avidity), 2) Fc-mediated clustering and binding of immune effector molecules (higher order avidity) and 3) polyclonal binding interactions (cooperative avidity). Understanding and tuning these multidimensional avidity-based interactions formed the basis of this thesis. Special emphasis was placed on the role of Fc-mediated antibody clustering and how engineering strategies and platforms exploiting antibody clustering can be utilized for the design of novel and improved antibody therapeutics.

Chapter 1 introduces the biology of antibody function including the role of antibody clustering in efficient activation of effector functions such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity and antibody-dependent phagocytosis. I highlighted the pioneering work of Diebolder et al., who showed that CDC efficacy may be improved by introducing single point mutations in the Fc domain that enhance intermolecular Fc-Fc interactions between IgG molecules after cell surface antigen binding, thereby facilitating IgG hexamer formation. In **Chapter 2**, we demonstrated that combinations of antibodies targeting CD20 and CD37 cell surface receptors on malignant B cells, either as wild-type IgG1's or containing a hexamerization-enhancing Fc mutation (E430G), induced enhanced and synergistic CDC compared to the single agents alone. In depth analysis into the mechanism behind this synergy demonstrated that, upon antibody binding, both antibodies co-localized on the cell surface by forming mixed (hetero-) hexameric complexes and substantially enhanced C1q binding and recruitment, indicating more efficient complement activation.

In **Chapter 3**, we describe the preclinical development DuoHexaBody-CD37, a novel bispecific antibody targeting two non-overlapping CD37 epitopes (biparatopic) with an Fc domain engineered to allow enhanced target-dependent antibody hexamerization. Combining two approaches to increase antibody clustering into a single bispecific antibody molecule, including dual

epitope targeting and promoting antibody hexamerization by the E430G Fc mutation, proved to be the best strategy to optimally engage CDC and other Fc-mediated effector functions. In **Chapter 4**, the superior CDC efficacy of DuoHexaBody-CD37 was further demonstrated in primary tumor cells derived from patients with various B-cell malignancies. This research has led to Clinical Trial and Investigational New Drug applications for this molecule, as well as initiation of a first-in-human study in patients with relapsed or refractory B-cell non-Hodgkin lymphomas in the first half of 2020 (NCT04358458).

The observation that IgGs targeting different cell surface antigens can co-engage in mixed hexameric complexes inspired the design of AND-gated antibody pairs in **Chapter 5**, which require a combination of two input signals (dual antigen binding) to license activation of a functional output signal (effector functions such as complement activation or clustering-dependent target signaling). This research illustrated how modulating Fc-Fc-, C1q- and Fc gamma receptor interactions between two different antibody components may allow for precisely tuning and restricting IgG avidity interactions and subsequent complex formation to preferred cell surfaces, thereby potentially also improving the window between efficacy and/or safety.

The fundamental role of avidity as a central trigger for the overall efficacy of antibody functional responses was reviewed in **Chapter 6**. Herein, I comprehensively summarized how avidity interactions orchestrate both natural and therapeutic antibody mechanisms of action and highlighted how tuning avidity interactions may serve as a design principle for improving antibody function or introducing novel properties in next-generation antibody drugs. The detailed studies the biology on ordered antibody clustering provided in this thesis showed that there are multiple strategies to enhance antibody clustering and improve antibody function. The impact of these different approaches is discussed in **Chapter 7.** Overall, these studies emphasize that understanding antibody structure-function relations, as well as antibody interactions with both antigen and effector molecules or cells is crucial for designing novel and more efficacious antibody drugs to treat human disease.