



Universiteit  
Leiden  
The Netherlands

## CD20 as target for immunotherapy

Engelberts, P.J.

### Citation

Engelberts, P. J. (2019, January 10). *CD20 as target for immunotherapy*. Retrieved from <https://hdl.handle.net/1887/68231>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/68231>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/68231> holds various files of this Leiden University dissertation.

**Author:** Engelberts, P.J.

**Title:** CD20 as target for immunotherapy

**Issue Date:** 2019-01-10

# Summary

Most lymphomas and leukemias are neoplasms of B cells. Due to the many different B cell development stages from which these neoplasms arise, the resulting diseases are quite heterogeneous, which amongst other things is manifested in different tumor growth location, proliferation potential and surface antigen repertoire. Nevertheless, some population characteristics are found in almost all B cell malignancies as the cell-of-origin is identical. One of these is the cell surface antigen CD20. Originally used as a marker to distinguishing B cells from other lymphocytes, it quickly became a target for immunotherapy. Immunotherapy is a treatment that makes use of immune system components to fight cancer, in this case by the injection of a monoclonal antibody specifically targeting one protein: CD20. The addition of CD20-targeting antibodies to an anti-tumor treatment allows your immune system to recognize CD20-expressing B cells (diseased and healthy), and dispose of them.

In **chapter 2**, I reviewed the CD20 targeting antibodies used for immunotherapy. The lessons learned from immunotherapy and some of the attempts done to increase the therapeutic efficacy of antibodies, including incorporating them into novel therapeutic strategies as bispecific antibodies as well as CAR-T cells have been addressed and it is concluded there is still room to further improve CD20 targeting molecules for the treatment of B cell neoplasms.

Although CD20 expression is found on more than 90% of B cells and malignancies thereof, the expression itself is variable amongst tumor types (from low to high, ALL<CLL<M-ZL<MCL<FL<DLBCL). As CD20 is a slowly internalizing target and expression levels are associated with differentiation stages of B cells we postulated that CD20 expression could not only be used a diagnostic or therapeutic target but also as a biomarker to address therapeutic activity of CD20-targeting drugs in personalized medicine approaches. In **chapter 3** we show that using quantitative flow cytometry CD20 mAb occupancy on CD20<sup>+</sup> cells can be monitored. This method can be used to study both antigen and antibody characteristics which can be applied as a pharmacodynamic tool to specifically tailor CD20 antibody therapy for individual patients.

Complement-mediated cytotoxicity (CDC) is an important Fc-mediated effector function employed by CD20 antibodies such as rituximab and ofatumumab (type I CD20 antibodies) to kill tumor cells. In **Chapter 4**, we studied the unexpected observation that type I CD20 antibodies were able to induce CDC even in absence of a functional IgG Fc domain. IgG Fc-independent CDC, so-called accessory CDC, was found to dependent on C1q and on expression of the B cell receptor. It was hypothesized that accessory CDC occurs through CD20-antibody dependent clustering of CD20 and the BCR, thereby allowing the BCR to bind C1q and initiate complement activation. This accessory CDC

probably aides to the potent CDC found in certain CD20 engaging therapeutics.

The therapeutic landscape in B cell neoplasms is continuously changing due to the approval of new drugs. Ibrutinib is one of the newest drugs approved for B cell malignancies such as CLL and MCL. Ibrutinib is a small molecule inhibitor of BTK (Bruton's tyrosine kinase), an intracellular kinase downstream of the BCR pathway that is vital for B cells. The mechanism of action of ibrutinib is different from that of CD20 targeting antibodies, and we therefore studied the effect of combining ibrutinib and CD20 antibodies in *in vitro* and *ex vivo* in **Chapter 5**. We demonstrated that the Fc-mediated cellular effector functions of CD20 targeting antibodies, antibody-dependent cellular toxicity (ADCC) and antibody-dependent phagocytosis (ADCP), were greatly diminished in the presence of ibrutinib. Similar observations were obtained with the Phosphoinositide 3-kinase (PI3K) inhibitor idelalisib, indicating the drug interference between antibody Fc-mediated effector functions and small molecule inhibitors occurs more broadly and should be considered when studying drug combinations.

In **Chapter 6** we describe the preclinical development of a new bispecific antibody targeting CD20 and CD3 for the treatment of B cell malignancies. Comparison with alternative B cell targets demonstrated that CD20 was the most optimal target for a B cell targeting CD3 bispecific in terms of potency. By modifying the IgG Fc domain of the CD3xCD20 bsAb, T cell cytotoxic activity was ensured to be strictly dependent on crosslinking of CD3-positive T cells

with CD20-positive B cells. This has led to Clinical Trial and Investigational New Drug applications for this molecule and a first-in-human study in Relapsed, Progressive or Refractory B-Cell Lymphoma was initiated in the first half of 2018 (NCT03625037).

In **chapter 7**, I revisited the role of CD20 in normal B cell development based on a review of the literature. The function of CD20 in B cell ontogeny may, at least in part, explain the potency of CD20 targeting antibodies. Also in Chapter 7, each the studies described in this thesis are revisited and placed into context with current day view on B cell development and therapeutic insight.

Overall, after several decades of research and therapeutic experience with antibodies targeting CD20, new functional discoveries as well as therapeutic advances are still being made, and CD20 therefore remains a highly attractive and fruitful target for the therapy of B cell malignancies as well as certain B-cell mediated autoimmune diseases.