CHAPTER 1

General introduction
ANTIBODY-BASED TREATMENT IN CANCER

The scientific concept of a “magic bullet” to specifically eradicate disease, without harming the body, was developed by Paul Ehrlich (1854-1915) (1). Treatment of cancer with chemotherapy or radiotherapy does not meet these expectations as significant toxicity is caused by destruction of normal healthy cells.

In 1975, George Kohler and Cesar Milstein discovered a way to produce large numbers of identical, monoclonal antibodies (mAbs) directed against specific target antigens (2). This hybridoma technique allowed the development of rituximab, a chimeric mouse/human monoclonal antibody with binding specificity to the target antigen CD20. This target antigen is a transmembrane protein expressed on the surface of normal and malignant B cells. It was considered a suitable target antigen as absence of CD20 expression in B-cell non Hodgkin lymphoma at initial diagnosis is extremely rare (1-2%) (3). Although normal B cells are targeted as well, overall toxicity (e.g. increased infection risk) is limited and treatment with rituximab is usually well tolerated. Depletion of CD20-positive B-cells generally does not cause permanent side effects as mature plasma cells and B-cell progenitor cells do not express CD20.

In 1980, proof-of-principle of efficacy was demonstrated as the first patient receiving an anti-CD20 antibody showed a decrease in circulating tumor cells (4). Rituximab was the first therapeutic mAb in oncology and was initially approved in 1997 for the treatment of follicular lymphoma (5). Subsequently, rituximab was implemented in the treatment for all B-cell malignancies. The clinical impact has been found to be significant. For example, the 2-year overall survival of previously untreated elderly patients with diffuse large B cell lymphoma was 70% vs 57% for rituximab combined with chemotherapy versus chemotherapy alone (6).

“All that glitters is not gold”

Despite the initial response, patients often relapse (7). It remains unclear which patients will benefit from further treatment with rituximab. Various mechanisms of rituximab resistance have been proposed (8).

One mechanism is insufficient cell death after rituximab binds to CD20 (downstream effects). To increase cell death, novel, fully humanized, anti-CD20 mAbs have been developed: ofatumumab for increased complement-dependent cytotoxicity and obinutuzumab for increased antibody-dependent cellular cytotoxicity. Other approaches for increased efficacy include radio-immunotherapy.
(tositumomab and ibritumomab-tiuxetan), antibody-drug conjugates (for example
rituximab conjugated to doxorubicin) and bispecific mAbs (antiCD20xantiCD3
mAb).

When using the same target, in this case CD20, it is important to know
whether the target antigen is still present and whether there is target engagement.
Reduced CD20 expression has been associated with an inferior survival indeed
(9). However, at relapsed/refractory disease, a biopsy is not always performed.
Even when a biopsy is available, there are several limitations. Reduced CD20
expression cannot be reliably quantified by immunohistochemistry (IHC) (9). In
addition, a single biopsy may not be representative due to sampling error and
target expression may be heterogeneous. Therefore, even when CD20 expression is
considered absent by IHC, it is still a matter of debate whether subsequent
treatment with rituximab should be withheld.

This emphasizes the need for a reliable biomarker to predict which patients
will benefit from rituximab treatment. Moreover, this would lead to improved cost
effectiveness of the treatment, as rituximab is an expensive drug (10). For patients
who are not likely to respond, different treatment approaches (e.g. aimed at
different target antigens or other modalities) should be considered. In 2018, 33
mAbs have been approved by the FDA for the treatment of cancer (11). Ideally,
mAbs provide selective treatment by targeting the tumor, without affecting normal
tissues. For mAb-based treatments, the choice of a target antigen which is ‘available’
and tumor-selective is essential to increase chances of success (maximal efficacy,
minimal toxicity). Assessment of target engagement in normal tissues should
provide information which target is suitable for development of antibody-drug
conjugates and help to improve safety. Preclinical studies provide limited
information, as target expression in normal tissues may be different between
animals and humans. Traditionally, in drug development, safety and dose finding
is assessed in a first-in-human phase I dose escalation trial, followed by evaluation
of preliminary efficacy in a phase II trial. If the results are promising late-stage
trials in larger patient cohorts are performed, before the new drug is ready for
regulatory review. Currently, many mAbs are under development for the treatment
of cancer (phase 1 [200 mAbs], phase 2 [110 mAbs], late stage trials [33 mAbs])
(11). This process requires a considerable amount of time, effort, financial resources
and large numbers of patients to participate in clinical trials. In general, the time
from drug discovery to regulatory approval is estimated at 15 years, of which
approximately 7 years in phase I to III trials (12).
Patient and drug selection becomes increasingly important in nowadays societal discussions about affordability of so-called “expensive targeted drugs” (13). Therefore, it is important to answer the following questions:

- Which patient (or subgroup of patients) will benefit from the drug?
- Which drug has high potential (safety and efficacy) for further development?

For antibody-based therapy, target engagement in the tumor is considered a requirement for efficacy, while target engagement in normal tissues considered unfavorable (e.g. for development of antibody-drug conjugates).

A reliable biomarker of target engagement could increase efficacy and reduce unnecessary toxicity and costs. In addition, detection of subgroups of patients benefitting from treatment would omit the need for large randomized clinical trials with heterogeneous patient populations. Intelligent trial design including a biomarker could shorten the time from early clinical development to approval of antibody-based therapy.

**Antibody imaging with immuno-PET**

Antibody imaging can provide a potential biomarker to predict toxicity and efficacy. Advantages of an imaging biomarker are that it is non-invasive and provides whole body information about normal tissues and tumors. Positron emission tomography (PET) can be used as an imaging modality to obtain functional information on a mAb, after its labeling with a positron emitting radionuclide. A radiolabeled mAb is administered intravenously and subsequently PET-CT scans are acquired to visualize and quantify the distribution over the body. This technique is referred to as antibody-PET or more common as immuno-PET.

Intact mAbs require a relative long period of time (days) to reach the target and accumulate. Therefore, zirconium-89 with a half-life of 78.4 hours is a suitable long-lived positron emitter for immuno-PET (14). Radiolabeling methods were developed and reagents made worldwide available to allow production of stable $^{89}$Zr-labeled mAbs according to Good Manufacturing Practice (GMP) for clinical use (15, 16).

In daily clinical practice, $^{18}$F-fluoro-2-deoxy-glucose (FDG) is the most widely used radiotracer for PET. Increased uptake of $^{18}$F-FDG corresponds with increased glucose metabolism (17). As high glucose uptake is a hallmark of
malignant cells, $^{18}$F-FDG-PET is used for staging and response evaluation for different types of cancer (18, 19). But uptake of $^{18}$F-FDG does not provide information on target engagement of mAbs. For this purpose, we aim to develop $^{89}$Zr-immuno-PET as a clinical tool to guide antibody-based therapy in cancer.

“It is usually a bad idea to develop a new measurement instrument”

Feasibility (including safety), technical validation, biological/clinical validation and cost effectiveness are required for successful implementation of a novel imaging biomarker in clinical cancer research and in daily clinical practice. Only when no existing measurement instrument is suitable for the specific need, and the purpose is of utmost importance, development of a new measurement instrument can be justified. We consider $^{89}$Zr-immuno-PET as a clinical tool to guide antibody-based therapy to meet these criteria, as we described in the previous paragraphs.

Contrary to biospecimen-derived biomarkers (measured from blood or tissue samples), where technical validation usually occurs before biological/clinical validation, these steps are performed in parallel for imaging biomarkers (20).

The following aspects have to be considered to develop $^{89}$Zr-immuno-PET as a clinical tool to guide antibody-based therapy in cancer:

1. Feasibility
   - Are $^{89}$Zr-labeled mAbs safe to administer?
   - What is the radiation exposure due to $^{89}$Zr-immuno-PET?
   - Can $^{89}$Zr-immuno-PET visualize tumor uptake?
   - Can tumor uptake and bloodpool activity be quantified with $^{89}$Zr-immuno-PET?
   - Are the ideas and findings of using $^{89}$Zr-immuno-PET relevant and sustainable?

2. Validation
   - Technical validation
     - What is the measurement variability of $^{89}$Zr-immuno-PET?
       - What is the measurement variability due to noise (e.g. counting statistics)?
       - What is the interobserver reproducibility of tumor quantification?
   - Biological/clinical validation
     - Is $^{89}$Zr-immuno-PET able to measure the underlying biology?
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- Can $^{89}$Zr-immuno-PET be used to assess tumor targeting and is uptake correlated to target expression in biopsies?
- Can $^{89}$Zr-immuno-PET be used to assess target-mediated specific uptake in normal tissues (defined as dose-dependent uptake)?
- Can $^{89}$Zr-immuno-PET be used to assess non-specific uptake in normal tissues (defined as reversible, (e.g. blood volume) or irreversible (due to $^{89}$Zr-residualization after mAb degradation)?

Outline of this thesis

The aim of this thesis was to develop $^{89}$Zr-immuno-PET as a clinical tool to guide antibody-based treatment in cancer. This thesis consists of two parts.

Part I describes the feasibility of the first clinical $^{89}$Zr-immuno-PET study ever. The step from preclinical research to clinical translation of $^{89}$Zr-immuno-PET was performed by the first-in-human study with $^{89}$Zr-labeled chimeric monoclonal antibody (cmAb) U36. Twenty patients with head and neck cancer, scheduled for surgery, received $^{89}$Zr-cmAb U36. Chapter 2 evaluates safety, biodistribution, dosimetry and PET quantification of immuno-PET with $^{89}$Zr-cmAb U36. Chapter 3 reports on the diagnostic imaging performance of $^{89}$Zr-immuno-PET for the detection of primary head and neck tumors and lymph node metastases for the same cohort of patients.

In Chapter 4, a review is provided of the first 15 clinical trials with $^{89}$Zr-immuno-PET in oncology. Lessons learned and technical aspects of study design are discussed.

Part II focuses on validation of $^{89}$Zr-immuno-PET. Chapter 5 describes the measurement of variability due to noise. In Chapter 6, interobserver reproducibility of tumor uptake measures of $^{89}$Zr-immuno-PET was investigated. Chapter 7, deals on the performance of $^{89}$Zr-rituximab-PET as an imaging biomarker to assess CD20 targeting in patients with relapsed/refractory diffuse large B cell lymphoma. Chapter 8 describes the use of immuno-PET in a phase I dose escalation study for detection of specific, target-mediated uptake in normal tissues, using the antiCD44 antibody RG7356 as an example. In Chapter 9, non-specific uptake in normal tissues for therapeutic mAbs (antiCD20, antiEGFR, antiPSMA and antiHER2) was determined as a critical step towards quantification of target engagement. In Chapter 10, the results presented in this thesis are summarized and future perspectives are discussed.
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