Biomarkers for the response to immunotherapy in patients with non-small cell lung cancer

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English summary

Lung cancer is the leading cause of cancer death in the Netherlands, with 14.000 diagnoses and over 10.000 deaths per year in 2019. For years chemotherapy was the only (palliative) treatment, with a short survival of only months. Since the introduction of immunotherapy, this survival has increased significantly, with the first results showing a survival of even a few years.

Immunotherapy reactivates the immune system to recognize and attack the tumor, leading to tumor shrinkage. This form of immunotherapy has a low percentage of side effects compared to chemotherapy. However, if a side effect occurs, it is often more severe. Inflammation of the gut (colitis), liver (hepatitis) or lung (pneumonitis) are common. In addition, when a patient suffers from an autoimmune disease, such as rheumatoid arthritis, there is a chance of a flare of this disease. Most side effects are treatable with corticosteroids, a drug that inhibits this auto immune response.

In the first two chapters, two frequently used immunotherapies are described. In **chapter 1** and **chapter 2,** nivolumab and pembrolizumab are introduced and described in terms of mechanism of action, efficacy, toxicity and quality of life of patients.

As described in **chapter 1 and 2**, only 20% shows a response in this form of therapy. Immunotherapy is expensive, with a few thousand euros per cure. A good selection of patients is important for limiting the development of severe side effect and cost reduction. Also, patients could be offered another therapy in time that may be effective. A biomarker could help. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes, or the response to a therapeutic intervention, as described by Lee et al [26]. Examples are C-reactive protein (CRP) in the diagnostics of an infection, a pregnancy test for the presence of a pregnancy and prostate specific antigen (PSA) for the detection of prostate cancer. A frequently used biomarker for the prediction of response of immunotherapy is PD-L1, the target for immunotherapy. For this biomarker a biopsy is needed, which is often hard to acquire. Besides, PD-L1 is not very accurate.

This thesis is about the search for a new biomarker. Criteria for a perfect biomarker are an understandable rationale, accurately predicts or monitors a responder before treatment, is minimally invasive, is easy to collect and perform, is reproducible, robust, repeatable, fast and cost-effective.

The first investigated biomarker is the use of platelets in blood. These platelets contain different fragments of genetic material (RNA). Earlier research showed that RNA could be used for detecting lung cancer. Because platelets are involved in the immune response, we expected that this would allow us to predict whether someone would respond or not. In **chapter 3** we analyzed the blood of 286 patients, all drawn before start of immunotherapy treatment. Our results show that the used of platelets is a little bit better than to select patients randomly, however, this is not sufficient for clinical practice.

The second investigated biomarker is the use of proteins for the development of a classifier. Proteins are made and used for different processes in the human body, such as CRP in inflammation processes. In **chapter 4** blood of 289 patients were analyzed, all samples were taken before start of treatment. All blood samples were analyzed with two different tests; the result of the first test determined the second test. This resulted in three groups: A group that would respond, that probably would respond and would not respond. Duration of response and survival were examined, which appeared to differ significantly between the three groups. Further analysis showed that proteins used for this classifier were associated with different processes of inflammation and wound healing cascades.

The third investigated biomarker is the use of exhaled breath. Exhaled breath contains for a small part of volatile organic compounds. These gasses arise from various processes in the body, such as inflammatory processes or the metabolism. These gases could therefore also be used to predict response. In our research, an electronic nose (eNose) was used for this purpose. In **chapter 5** exhaled breath from 143 patients were analyzed, all samples taken before start of treatment. Here we hypothesized that we could identify a group that would not respond, so that these patients could start another treatment. With this test we show that we could save treatment in 24% of the patients. In **chapter 6**, data of patients who were sampled both before and during treatment were added. Here we show that adding the results after the start of treatment improves the results, with only a few false-negative and false-positive results. This could be used for the follow-up of patients who just started immunotherapy.

The electronic nose shows great results, however, nature beats that. In **chapter 7** is described what would happen if a dog is used as a biomarker. A Spanish research group shows that, with the use of a dog in the detecting of lung cancer, there was a low number of false-positive and false-negative results. However, disadvantages include the difficulty of getting a dog registered as a biomarker and the stability of results in dogs over time.

The fourth biomarker investigated is the use of tumor markers. These markers were introduces years ago in different laboratories cross the world, but so far it has not been possible to use these markers in practice for predicting response. In **chapter 8**, tumor markers of 376 patients who received immunotherapy were used. Five different markers were used, namely NSE, Cyfra21.1, CEA, CA125 and SCC. Our aim was whether we could predict if someone would not respond based on a relatively easy tests based on two conditions (a 50% increase and above a minimal value), preferably using multiple biomarkers. This turned out to be quite accurate, using cyfra21.1, CEA with/without NSE. The advantage of this test is that it is already available in different laboratories, it cheap and quit easy to measure. The downside it that the rate of false-negatives, with a positive test defined as some who would not respond, is still quite high. In addition, this test is only useful for the follow-up of patients, which makes it less cost-effective.

In summary, there are four biomarkers investigated for the prediction on response to immunotherapy, some of which are promising. Next to these four biomarkers, many other biomarkers are in development, beside the once who are already used in clinical

practice. With this large amount of data, it is sometimes difficult to see which biomarker you will use when, at what time and in what combination. To be able to study this without involving real patients, it could be useful to use a model. This way you can analyze thousands of patients with different test combinations and sensitivities and specificities through simulations. In **chapter 9** an example of a model I shown. Using data from 248 patients in combination with the literature, a model was built that was representive of reality with regard to duration of treatment and survival. Then, this model as used to see how often we should run tests to detect side effects. Here we show that with least testing we were also able to detect a side effect on time.

In the discussion the different markers are discussed and compared. Future perspective shows us that most markers are further developed. Maybe combinding tumor markers will lead to better results.