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Neo-antigens in cancer immunotherapy

Since the initial success of anti-CTLA-4 and anti–PD-1/PD-L1 treatment, the field has obtained a better understanding of the antigenic determinants that can be recognized by T cells in clinically effective cancer immunotherapies, something that I have discussed in chapters 2 and 3. In chapters 4, 5 and 7, I have described a set of tools that can be used to identify and monitor antigen specific T cell responses, and in particular T cell responses directed towards neo-antigens. In chapters 6 and 7, I show that in patients with metastatic melanoma, T cells frequently recognize neo-antigens, making this antigen class a particularly interesting target in cancer immunotherapy. Finally, I have discussed the importance of biomarkers to predict response to immunotherapy in chapter 8, and have analyzed the potential occurrence of immunoediting in patients with melanoma in chapter 9. The results of the latter analysis do not support the idea of large-scale immunoediting, but are at best consistent with the possibility of a partial sculpting of the neo-antigen repertoire. In this final part of the thesis, I will discuss the challenges and opportunities to implement neo-antigen specific immunotherapy as a means to treat cancer patients.

Diversity as a key characteristic of neo-antigens

Neo-antigen specific immunotherapy can be defined as immunotherapy that specifically aims to steer immune reactivity towards neo-antigens. The identification, or at least sufficiently accurate prediction, of the spectrum of neo-antigens that is present on a tumor is essential for this type of immunotherapy. This process is in my view best started by the analysis of its mutational landscape – the combination of the type and the number of genomic variants present within each tumor that may lead to the production of a novel epitope repertoire. For prediction of the patient-specific neo-antigen repertoires, standard next generation sequencing of tumor material can be performed. In addition, for projects that aim to describe properties of the neo-antigen repertoire across tumors, The Cancer Genome Atlas (TCGA) and COSMIC contain very large amounts of data that can be accessed (Forbes et al., 2015; Weinstein et al., 2013).

Central to the concept of targeting neo-antigens is the diversity that characterizes this class of antigens. In the next sections, I will discuss three aspects of neo-antigens that are key to consider when aiming to target this class of antigens; i) the diversity in the number of mutations, ii) the unique mutational spectrum of each tumor and, iii) the genomic heterogeneity within individual tumors.
One of the most striking observations made when analyzing the mutational landscapes of human tumors is that human tumors can vary tremendously in their number of mutations, not only between malignancies but also within individual tumor types. For example, the mutational load of patients with melanoma varies from approximately 0.5 – 100 (median of approximately 20) somatic mutations per megabase of coding genome. While mutational load alone does not dictate the size of the available repertoire of neo-antigens, a roughly linear relationship can be expected, especially at higher mutational loads. Indirect support for this notion is also provided by the observation that lung cancer patients with a higher mutational load have a higher likelihood of response to immune checkpoint therapy than lung cancer patients with a lower mutational load (Rizvi et al., 2015a). By the same token, patients diagnosed with mismatch repair deficient colorectal cancer (CRC), which often results in large numbers of mutations, are shown to respond significantly better to anti-PD-1 therapy as compared to mismatch repair proficient CRC patients (40% versus 0% of patients, respectively showed an objective clinical response, (Le et al., 2015)). As a second variable, not only the number of mutations, but also the type of somatic variants – the mutational spectrum – differs significantly between tumor types. For instance, whereas a profound UV-signature is present in cutaneous melanomas; a completely different (carcinogen induced) signature is present in the majority of lung cancers (Alexandrov et al., 2013; Vogelstein et al., 2013). At this point it is unclear whether these differences in mutational signatures significantly influence the available neo-antigen repertoire, but I predict the effect will not be substantial. Finally, the use of next generation sequencing has shown that the mutational landscape within human tumors can not only change over time, but is also highly complex, with different regions within one tumor showing partially distinct sets of genomic variants (as reviewed in (Jamal-Hanjani et al., 2015)).

As each single tumor harbors its own unique mutational landscape – both in size and in type, which can even vary within a single tumor – it is crucial to incorporate this state of diversity in the decision-making process when targets are considered for neo-antigen specific immunotherapy. An incomplete overview of the mutational spectrum will likely be obtained if one would for example rely on a single biopsy only as the identified somatic mutations will not necessarily be present in 100% of all tumor cells. Practically this means that there is a need to analyze a number of tumor biopsies from the same patient and carefully review what the most appropriate targets are, for which I will provide some guidelines below.

Having discussed key characteristics of neo-antigens I will now review three crucial aspects that need to be considered in order to clinically implement treatment of patients with neo-antigen specific immunotherapy: First of all, what are optimal targets? Secondly, which
patients should we treat? And finally, how should we treat these patients? The diversity of choices and considerations belonging to each step will be discussed and propositions will be done on how to handle these.

What are optimal targets?

The presence of a somatic mutation in the exome of a tumor does not necessarily mean that T cells will recognize the mutated gene product. Whether or not this is the case is a probabilistic process where (among other things) the type of substitution and the ability of the variant sequence to bind to one of the patient’s HLA alleles play a role. As only a small fraction of exonic mutations leads to the formation of a neo-antigen, it is crucial that these few appropriate targets can be identified.

One can take different approaches to identify neo-antigens; firstly, the use of algorithms to predict outcome of different aspects of the antigen presenting process is now general practice when putative epitopes need to be selected (e.g. prediction of MHC affinity and proteasomal cleavage (Kesmir et al., 2002; Lundegaard et al., 2008)). However, it is reasonable to assume that this prediction pipeline can be further improved when additional aspects are taken into account. First, how different a putative neo-antigen is from the self-proteome may be taken into account, as there is evidence that this influences the likelihood of T cell recognition (Calis et al., 2012). In addition, the addition of RNA expression as a filter (described in chapter 5) will help to predict the level of epitope presentation. While these algorithms have proven to be very useful to enrich for true neo-antigens recognized by T cells, there is certainly still an urgent need to improve this pipeline, as the majority of predicted neo-epitopes appear to be false positives. Importantly, algorithms that can accurately predict the immunogenicity of antigens that are presented at the cell surface are presently lacking, as little data has been available to train such algorithms. Moreover, the models that have been developed to date have been trained on data derived from T cell responses towards viral epitopes (e.g.: (Calis et al., 2013)), which could significantly differ from T cell responses towards neo-antigens, as in the latter case the difference between ‘foreign’ and ‘self’ is often restricted to a single amino acid. Experiments to advance our knowledge on this matter could involve vaccination studies in humanized mouse models in which both T cell responses towards mutated and wildtype epitopes are monitored. Results from such studies could be used to further improve the current models of immunogenicity, as described by Calis et al. (Calis et al., 2013). As an additional limitation of the current epitope prediction strategies, a sophisticated way to combine the output of computational algorithms that describe
different aspects of the antigen presentation process is lacking as well. Currently, neural networks, such as netCTL (Larsen et al., 2005) and netTepi (Trolle and Nielsen, 2014) combine some of the relevant processes, but lack immunogenicity and RNA expression (as a surrogate for protein production rate) as components. The development of a model that combines each different parameter with a weight proportional to its importance is necessary to fully exploit the output of every individual algorithm.

An alternative for choosing neo-antigen targets on the basis of genetic data is their identification through immunomonitoring. The rationale behind this is that recognition of those antigens validates their presentation and immunogenicity in the most direct way. A negative consequence of this approach is however that a subset of appropriate targets are overlooked that may not have been immunogenic in the patient but could be important after boosting. Here, I will discuss the advantages and disadvantages of a set of three immunomonitoring platforms that can be used to identify pre-existing responses to guide the choice of neo-antigen specific immunotherapy targets (Table 1). Each platform has different characteristics that could be of importance when considering the use of immunomonitoring as a way to guide the choice of neo-antigenic targets.

One of the most frequently used tools to monitor antigen specific T cell responses in humans is the use of pMHC-multimers (Altman and Davis, 2003). For this purpose first putative epitopes are predicted from whole exome sequencing data, specific for the patients’ autologous HLA alleles. pMHC multimers specific for these predicted epitopes are used as tools to detect neo-antigen specific T cell responses either in the TIL- or peripheral blood compartments of the patient. The advantages of this approach are that it is highly sensitive and that in addition to the antigen-specificity of the T cells, the magnitude of the response is revealed. However, it is not unbiased, but relies on computational algorithms to select epitopes and only CD8+ T cell responses can be monitored. Additionally, MHC multimers are not always available for all the HLA class I alleles of a patient, and this approach does not provide information with respect to the functional capacities of the neo-antigen specific T cells.
Table 1. Advantages and disadvantages of different immunomonitoring platforms to select appropriate neo-antigenic targets.

<table>
<thead>
<tr>
<th>Immumonitoring platform</th>
<th>Short description</th>
<th>Essential materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>1. pMHC multimers</td>
<td>pMHC multimers are used to analyze reactivity towards putative mutated short epitopes. These epitopes are selected based on computational algorithms.</td>
<td>1. TIL/blood; 2. MHC multimers matching the patient’s HLA genotype; 3. Short peptides.</td>
<td>1. Highly sensitive approach; 2. Magnitude of the T cell response is known; 3. Possibility of significant multiplexing</td>
<td>1. Not an unbiased approach, but based on epitope selection; 2. Monitors CD8+ T cell responses only; 3. MHC multimers are not available for each HLA allele; 4. Does not give information on the potential to produce cytokines in response to epitopes.</td>
</tr>
<tr>
<td>2. Long peptides on autologous B cells</td>
<td>Long peptides covering all non-synonymous mutations are loaded on autologous B cells. Reactivity towards neo-antigens is measured by cytokine production.</td>
<td>1. TIL/blood; 2. Autologous immortalized B cells; 3. Long peptides.</td>
<td>1. Unbiased approach; 2. Considers both CD4+ and CD8+ T cell responses; 3. Gives information on the ability to produce cytokines.</td>
<td>1. Responses of T cells that are (no longer) functional are missed; 2. Lower sensitivity as compared to pMHC multimer analysis; 3. It is unclear whether the processing of epitopes resembles the in vivo situation.</td>
</tr>
<tr>
<td>3. Mini-genes transduced into MHC matched cells</td>
<td>Mini genes covering the non-synonymous mutations are used to transfect cells that express the autologous MHC alleles. Reactivity towards neo-antigens is measured by cytokine production.</td>
<td>1. TIL/blood; 2. Cells expressing the HLA alleles of the patient; 3. Mini-genes.</td>
<td>1. Unbiased approach; 2. Considers both CD4+ and CD8+ T cell responses; 3. Considers epitopes which are processed in vivo.</td>
<td>1. Responses of T cells that are (no longer) functional are missed; 2. Sensitivity of approach is unknown.</td>
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To provide an alternative to the use of MHC multimers, we have developed a platform to monitor neo-antigen specific T cell responses in which autologous B cells are immortalized and subsequently loaded with a library of long peptides that cover all non-synonymous mutations within a patient’s tumor. In this strategy, neo-antigen specific T cell responses are identified by cytokine production in co-culture assays (described in chapter 7). The most important advantages of this approach are that it is unbiased (it can cover potentially all non-synonymous mutations) and it is suitable to follow both CD4+ and CD8+ neo-antigen specific T cell responses. Also, it gives an indication on the ability of the antigen specific T cells to produce (a variety of) cytokines. This is at the same time also a disadvantage of the platform, since neo-antigen specific T cells that are (no longer) functional will not be detected. Moreover, this platform has a lower sensitivity as compared to the pMHC multimer approach, and at this point it is unclear whether the long peptides are both efficiently loaded on MHC class I and MHC class II; an aspect that is also likely to vary between epitopes (van Buuren et al. unpublished results).

As a second alternative, colleagues at the NIH have developed a platform in which mini-genes encoding for every possible non-synonymous mutation are transfected into fibroblasts expressing the autologous HLA alleles (Tran et al., 2014). Cytokine production of T cells from TIL cultures or peripheral blood in co-culture with the transfected fibroblasts is used as a read-out. While the advantages and objectives of the mini-gene and the long-peptide platform are largely overlapping, an advantage of the mini-gene-based strategy is that processing of the encoded peptides is likely to mirror that of the mutant sequence within the tumor cells. As a downside, the currently used cytosolic expression strategy might limit entry of epitopes into the MHC class II pathway, and thus diminish the ability to detect MHC class II-restricted T cell responses.

While the above-described strategies provide a means to identify potential or actual neo-antigens, they do not provide any information on the safety of inducing or enhancing T cell responses specific for those antigens. It is generally assumed that neo-antigen specific immunotherapy should be safe and this is based on the assumption that T cells are able to discriminate between the wild-type and the mutated epitopes – of which the mutant form is exclusively present at the tumor site. Thus far, pre-clinical studies assessing the efficacy of neo-antigen specific vaccinations have not shown any concerning data in terms of safety (Castle et al., 2012; Gubin et al., 2014; Kreiter et al., 2015). However, as – among other things – antigen expression levels, antigen presentation and T cell immunity is expected to be different in humans we can not exclude that safety concerns arise when actual cancer patients are treated with this type of therapy. An interesting but relatively unexplored field is that of cancer-induced autoimmunity. In rheumatic diseases like dermatomyositis and scleroderma, there is a close temporal relationship between
malignancy and autoimmunity onset (Shah et al., 2015). In scleroderma, this temporal clustering seems to be limited to a subgroup of patients with antibodies that recognize the self-antigen RPC1. An intriguing study showed that the majority of scleroderma patients with cancer and RPC1-specific antibodies had somatic mutations in the gene encoding for RPC1 (POLR3A). Moreover, in two out of three patients tested, unique CD4 T cell clones were present in blood that specifically recognized either the mutant or wild-type RPC1, but antibodies in these patients were reactive towards both (Joseph et al., 2014). Based on these data, the authors hypothesize that a single, strongly immunogenic epitope may generate an autoreactive (cellular and humoral) immune response towards both the mutated as well as the non-mutated RPC1 protein, thereby causing tissue injury and eventually scleroderma. If this scenario would also hold true for other mutated antigens, this would be a serious safety concern, and exclusion of neo-antigens derived from proteins associated with autoimmune syndromes may be prudent. In addition, physicians should keep a close eye on the possible development of scleroderma or other autoimmune diseases in the ongoing or planned clinical studies with neo-antigen vaccines.

Having considered different approaches to identify appropriate neo-antigenic targets and possible safety concerns, the final aspect to consider when choosing targets for neo-antigen specific immunotherapy is the possibility of immune evasion by epitope loss. While my analyses in chapter 9 do not provide evidence for large-scale immunoediting in patients with melanoma, it does not exclude that this could occur in other tumor types; a possibility that is strengthened by an analysis of Hacohen and colleagues (Rooney et al., 2015). In addition, epitope loss may become more prevalent at the moment a stronger selective pressure is exerted as a consequence of therapeutic intervention. A possible way to reduce the likelihood of such epitope loss is the selective targeting of neo-antigens derived from genes that are crucial for tumor survival – ie. oncogenes and essential genes for which only the mutant copy is left. To this end I discuss three different classes of neo-antigens that should be considered in a clinical perspective: i) Driver mutations, ii) essential passenger mutations and iii) true passenger mutations (Heemskerk et al., 2013). An interesting approach is to select epitopes from driver mutations – these mutations confer a selective advantage to the cells affected. It is unlikely that tumor cells are selected that do not carry this mutation, however the limited number of driver mutations present in each tumor type will likely result in a small set of putative epitopes to target, making this an extremely challenging approach. An alternative could be the targeting of neo-antigens from essential passenger mutations, which are mutations present within an essential (household) gene in absence of the wild-type copy. As is the case for neo-antigens that arise as a consequence of driver mutations, it is expected that loss of epitopes from this class will be an infrequent event. However, at present, it is unknown whether the number
of neo-antigens derived from such ‘essential passengers’ is substantial. Finally, it is also likely that immune evasion by epitope loss can simply be prevented by the simultaneous targeting of multiple neo-antigens from true passenger mutations. If the number of neo-antigens derived from such true passengers greatly outnumbers that from the other two categories, this may form the most viable approach.

**Which patients should we treat?**

Within the first part of the discussion, I have described different aspects of the identification of neo-antigens that form good targets for immunotherapy. In this section, I will review the factors that should be taken into account to determine which patients should be treated with neo-antigen specific immunotherapy.

Whether or not a patient has an ongoing anti-tumor response could in the future be used as a consideration for selecting patients for neo-antigen specific immunotherapy. The rationale behind selecting patients with a pre-existing immune response is that it may potentially be easier to steer the response towards selected targets when an immune response is initiated already. On the other hand, treating patients with a neo-antigen specific vaccine might induce T cell responses that were previously non-existing. The identification of a set of markers that can identify patients that should be treated with neo-antigen specific therapy is important. This could help future decision making on whether it is more efficient to treat patients with or without a pre-existing immune response.

One such marker is the presence of (abundant) CD8 T cells at the tumor site, which was found to be a positive predictor in patients treated with anti-PD1 therapy. In the same study it was also shown that a bias in the TCR repertoire of the intratumoral T cell pool toward a limited number of TCR clonotypes was a second marker that had substantial predictive value (Tumeh et al., 2014). Alternatively, expression of the inhibitory molecule PD-L1 on both immune and tumor cells can provide information on an ongoing immune dialog, a marker that was shown to be predictive for bladder cancer and renal cell carcinoma patients treated with anti-PD1/PDL1 (Herbst et al., 2014; Powles et al., 2014). Further (indirect) proof of an ongoing immune response directed towards neo-antigens was obtained in a recently published elegant study. Hacohen and colleagues defined a cytolytic marker “CYT”, which is the sum of the expression of two cytolytic effectors, granzyme A and perforin that are upregulated upon CD8 T cell activation. When they analyzed the relationship of “CYT” with mutational load and with the number of predicted neo-antigens, they found a significant correlation for a large number of tumor types. This indicates the relationship between an ongoing immune response and mutated antigens (Rooney et al., 2015). These data suggest that, in addition to the above markers,
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CYT may potentially be used to identify patients with an ongoing immune response. Detailed information on the above aspects could be obtained for each patient that is eligible for neo-antigen specific immunotherapy and this could guide the decision making process of whether or not patients with or without a pre-existing immune response should be treated with neo-antigen specific therapy.

Another aspect that may be taken into account when considering patients for neo-antigen specific immunotherapy could be the mutational load of the tumor. In a study involving a cohort of lung cancer patients, a significant correlation between mutational load and outcome to anti-PD-1 therapy was observed (Rizvi et al., 2015a). The same trend was observed in a melanoma patient cohort treated with anti-CTLA4 (Snyder et al., 2014). However, also some patients with a low mutational load showed a response to immune checkpoint therapy, making it extremely difficult to use this as a way to include or exclude patients for therapy. This difference in response likely reflects the higher chance of immunogenic neo-antigens present on tumors of patients with a higher mutational load. In line with this, it will likely be more straightforward to select immunogenic neo-antigens to target in patients with a high mutational load. However, despite the fact that it might be a greater challenge to select appropriate targets in patients with a low mutational load, mutational load alone should at this point not yet function as a means to either select or exclude patients for neo-antigen specific immunotherapy.

Looking at the different aspects discussed here and in chapter 8, it becomes clear that the individual parameters by itself will not give enough information to decide which patients would benefit from neo-antigen specific immunotherapy and which would not. Although it seems momentarily unrealistic, the formulation of a function in which different measurable markers can be entered as parameters that would then give a likelihood score describing the “likelihood of response” \( L_{rel} \) to neo-antigen specific therapy seems attractive.

\[
L_{rel} = F[\text{cyt}, T_{ref}, \#Mt, T_{ref} \text{ Expr}_{PD-L1}, T_{clon}]
\]

One could think of cytolytic activity (\( \text{cyt} \)), T cell infiltration (\( T_{inf} \)), TCR clonality (\( T_{clon} \)), mutational load (\( \#Mt \)), expression of PD-L1 (\( \text{Expr}_{PD-L1} \)) (Herbst et al., 2014; Rizvi et al., 2015b; Rooney et al., 2015; Snyder et al., 2014; Tumeh et al., 2014) and possibly pre-existing tumor-specific T cell responses (\( T_{ref} \)) as parameters to include. What is needed to develop such a model is a quantification of each individual parameter in patients treated with neo-antigen specific therapy in which we also know the clinical outcome. Once there is enough data to develop this model, it will become clear which of the parameters is most important and whether this can truly be used to predict outcome to neo-antigen specific therapy.
How should we treat patients with neo-antigen specific immunotherapy?

Based on the recent clinical successes of immunotherapies and the data that strongly support the contribution of neo-antigen recognition in this tumor specific immune response, the clinical application of neo-antigen specific immunotherapy is no longer futuristic. Assuming that the identification of the appropriate neo-antigenic targets will be a streamlined process and that it will become clear in the near future how to select patients that are likely to respond, the next step will be to choose the best way to apply neo-antigen specific immunotherapy.

In this part I will discuss two possible ways to treat patients; i) neo-antigen vaccination and, ii) transfer of neo-antigen specific T cells.

Neo-antigen vaccination, in which a peptide, DNA/RNA or dendritic cell-based vaccine is used with the aim to increase T cell responses against defined (predicted) neo-antigens, has reached the stage of early clinical development. Prior work of Sahin and colleagues in mice models showed that exome guided analysis could be used to identify neo-antigens and that the identified neo-antigens can be used for vaccination and was shown to induce tumor control in a protective as well as a therapeutic setting (Castle et al., 2012). Following this work in mouse models, a set of clinical trials that focus on neo-antigen vaccination in melanoma, glioblastoma, and triple negative breast cancer has been initiated (NCT01970358, NCT02287428, NCT02129075, NCT02035956, NCT02149225, NCT02316457). The first results of a small scale trial in a recent publication by Linette and colleagues showed for the first time the immunogenicity of neo-antigen specific dendritic cell vaccines in three patients with stage III resected melanoma (Carreno et al., 2015). In this study, first the mutational spectrum of the patients was determined. Subsequently, the HLA affinity and RNA expression of a subset of seven epitopes per patient were confirmed. These seven epitopes, plus two non-mutant epitopes from the differentiation antigen gp100 were included in a dendritic cell vaccine that was used to treat these patients. The immune response was followed over time in the blood and the authors showed that they could raise an immune response towards these mutated targets. Since the tumors of these patients were resected, the effect of the vaccine on disease progression could not be judged. Neither was it possible to assess whether this was reactivity towards a true neo-antigen (ie. processed and presented by the tumor) or whether it was merely a reaction towards a foreign piece of protein. This study however does illustrate that it is feasible to generate a patient specific vaccine that is safe to use for treatment and that induces immune responses (Carreno et al., 2015). Questions that remain to be answered are: Should treatment preferentially involve peptide, RNA, DNA or dendritic cell vaccination? Is there a specific subset of patients that will benefit more
from such vaccines over other possible neo-antigen specific therapies (see below)? Do we target both the CD8 as well as the CD4 T cell repertoire? Is neo-antigen vaccination by itself strong enough to induce tumor clearance, or should this be combined with other forms of (immuno)therapy?

An alternative to neo-antigen vaccination is the transfer of neo-antigen specific immune cells, or cell products, which may be generated by (at least) two alternative methods. First of all, the isolation of neo-antigen specific TCRs and the subsequent transfer of these TCRs into autologous PBMCs may be used as a way to generate a neo-antigen specific T cell product. Although the first clinical study with neo-antigen specific TCRs is yet to be initiated, the treatment of patients with TCR transduced T cells specific for other antigen classes (for example the cancer/germline antigen class) has shown to be clinically effective in at least in some cases. In particular, infusion of NY-ESO-1 specific TCR-modified autologous T cells has shown objective clinical responses in patients with both metastatic melanoma and synovial cell carcinoma (Robbins et al., 2015; Robbins et al., 2011). A second way of generating a neo-antigen specific T cell product is by the generation of an autologous T cell product from the peripheral blood or TIL compartments specific for neo-antigens by selection and subsequent expansion ex-vivo. A case study describing the clinical effects of a T cell product biased towards neo-antigen reactivity has provided early proof of principle for this approach (Tran et al., 2014). In this study, a patient with epithelial cancer was treated with a CD4 TIL product that was FACS-sorted to reach a purity of 95% of TIL specific for a mutated gene product. The tumor of this patient showed an impressive tumor regression.

Treatment with TCR transduced T cells has proven to be very powerful, but can also be extremely destructive in those cases where the antigenic target is also expressed on healthy tissue (Parkhurst et al., 2011). The absence of expression of neo-antigens on healthy tissue is expected to limit such toxicity. However, a scenario that is more likely to occur is one of cancer-induced autoimmunity, as I described above. It is therefore crucial to closely monitor the side-effects of neo-antigen specific therapies to not only get a better understanding of this type of treatment, but also to prevent such side effects in the future.

In those patients where a pre-existing response is lacking, an alternative could be to treat patients with a combination of immune checkpoint therapy and neo-antigen specific vaccination or neo-antigen specific T cell product. A treatment schedule where anti-PD-1 /PD-L1 is given initially to activate the T cells in a generic way, followed by neo-antigen specific product to specifically steer this towards the tumor site appears a very attractive format.
Concluding remarks

In this final chapter I have touched upon the different opportunities and challenges with respect to the implementation of neo-antigen specific immune therapy. Although the targeting of the neo-antigen repertoire on human tumors is thought to be safe and potentially effective in terms of tumor control, it is yet to be determined whether this is indeed the case. Whereas the diverse nature of the neo-antigen repertoire raises the important question: “How to select the best targets?”, other open questions are: “How to decide which patients to treat?” and “And how to actually treat them?”.

Answering these questions will guide the development of neo-antigen specific immunotherapy in the future, which is an endeavor to which I hope to contribute further in my future work.
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