# Exploring and modulating the tumor immune microenvironment: Towards improving patient outcomes of immunotherapy in lung cancer

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# PART III.

## General discussion and summary / samenvatting

# CHAPTER 7

General discussion and future perspectives

**The good news**

During the course of this thesis, the landscape of systemic treatment options concerning non-small cell lung cancer (NSCLC) has kept on changing, which has led to some good news. In the United States, it was reported that the cancer-related death rate had declined over the past two decades. The largest single-year drop of 2.2% was established over the last-measured year, 2016-2017 [1]. This decline was driven by an accelerated drop in lung cancer deaths, which dropped around 5% from 2013-2017. The decrease is attributed at least in part to advances in treatment, like the introduction of immunotherapy. Although these results are encouraging, it must be stressed out that lung cancer is still the leading cause of cancer death even in the United States. Our work here is far from finished.

**Recent developments in the systemic treatment of metastatic non-small cell lung cancer**

After registration of programmed death-ligand 1 (PD-L1)/programmed death 1 (PD-1) blockade in second-line treatment, several phase 3 trials in NSCLC have been performed to evaluate immunotherapy in first-line setting. Firstly, the KEYNOTE-024 study showed a convincing improvement of overall survival (OS) of the PD-1 inhibitor pembrolizumab over platinum-based chemotherapy in patients with previously untreated advanced NSCLC with a PD-L1 expression of ≥50% [2]. Subsequently, the KEYNOTE-189 and KEYNOTE-407 combined platinum-based chemotherapy with pembrolizumab in first-line setting and compared this regimen to platinum-based chemotherapy in all-comers, i.e. irrespective of PD-L1 expression [3, 4]. Progression free survival (PFS) and OS were in favor of the platinum-doublet chemotherapy and pembrolizumab combination, sometimes referred to as 'triple-therapy'. Also, the PD-L1 inhibitor atezolizumab was approved for treatment-naïve advanced NSCLC patients based on two studies that had met their co-primary endpoint of PFS and OS benefit for the addition of atezolizumab to carboplatin and nab-paclitaxel (IMpower 130) and to carboplatin, paclitaxel and bevacizumab (IMpower 150) both in nonsquamous NSCLC [5, 6]. Benefit was irrespective of PD-L1 expression measured as a combined score of expression on tumor cells (TC) as well as on tumor-infiltrating immune cells (IC). However, the Checkmate-026 study failed to show benefit in regard to OS of first-line PD-1 inhibitor nivolumab over platinum-based chemotherapy in advanced NSCLC with PD-L1 ≥5% measured on TC [7]. This discrepancy in benefit compared to the other PD-1 inhibitor pembrolizumab could possibly be attributed to differences in patient selection, but differences in binding ability of the drug to the PD-1 receptor has also been suggested.

Based on previous successes in melanoma patients, blockade of the immune checkpoint cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) has been investigated in NSCLC as well. No randomized trials with monotherapy of the two available anti-CTLA-4 drugs, ipilimumab and tremelimumab, have been performed in NSCLC. Combining PD-1/PD-L1 blockade with CTLA-4 antibodies has led to conflicting results when compared to platinum-doublet chemotherapy in treatment-naïve NSCLC setting. The combination of ipilimumab with nivolumab in the multi-arm CheckMate-227 study improved OS compared to platinum-based chemotherapy for both the PD-L1 positive and negative subgroup [8]. However, the combination of the PD-L1 antibody durvalumab with tremelimumab investigated in the MYSTIC trial showed no PFS or OS benefit over first-line chemotherapy in patients with advanced NSCLC [9]. The role of the addition of CTLA-4 antibodies in advanced NSCLC therefore remains

somewhat unclear, especially now that PD-1/PD-L1 blockade is (part of) the standard of care in metastatic NSCLC first-line setting, and no approval of anti-CTLA-4 treatment in NSCLC has been granted at time of writing. However, results of the CheckMate-227 have led to FDA approval of the ipilimumab plus nivolumab combination in first-line metastatic NSCLC in May 2020.

The recent results have established PD-1/PD-L1 blockade as the new cornerstone in first-line treatment of patients with metastatic NSCLC. However, the unanswered question whether patients with advanced NSCLC with PD-L1 expression ≥50% are better off with triple-therapy or if pembrolizumab monotherapy could suffice still remains. Also, is the addition of chemotherapy to immunotherapy in patients with PD-L1 expression <50% indispensable? The KEYNOTE-042 study compared pembrolizumab monotherapy to platinum-based chemotherapy in first-line setting in patients with metastatic NSCLC and a PD-L1 expression of ≥1% [10]. The authors stated that pembrolizumab monotherapy could be a treatment option for these patients based on the OS benefit in the pembrolizumab group. However, it should be mentioned that the OS benefit was driven by the PD-L1 ≥50% subgroup and that in a subgroup analysis, patients with PD-L1 expression between 1-49% derived no OS benefit from pembrolizumab over chemotherapy; in fact, the OS curves crossed, suggesting that for at least a part of these patient the need for a chemotherapy backbone remains.

As data matures, long-lasting anti-tumor immune responses in advanced NSCLC have been described on PD-1 blockade [11, 12]. PD-L1 expression on tumor cells remains the best and most accessible clinical biomarker for response to immune checkpoint inhibition to date and has been used to guide treatment choices in clinical trials. But based on the issues mentioned above regarding PD-L1 subgroups, PD-L1 expression seems far from perfect to predict the optimal treatment choice for NSCLC patients.

**PART I. Exploring the tumor immune microenvironment**



Because the aim of anti-PD-1/PD-L1 treatment is blocking the interaction between the PD-L1 receptor expressed by tumor cells and the PD-1 receptor on immune cells, or to be more specific on cytotoxic T cells, it seemed the most sensible choice to pick PD-L1 expression in tumors to explore as a biomarker for response. Unfortunately, in advanced NSCLC results were far from clean-cut: objective response rates (ORR) varied from 8% in PD-L1 negative tumors up to approximately 30% in tumors with PD-L1 expression of ≥50% [13-15]. These results prove that other mechanisms of tumor-immune interaction must be present (or are lacking) in order to induce tumor killing by the host immune system through PD-1/PD-L1 blockade. To enable exploration of the tumor immune environment in NSCLC, we built a database of over 600 tumor samples from resected patients and collected patient and tumor characteristics. Immunohistochemical (IHC) staining of PD-L1 on TC and IC separately as well as CD8 infiltration, mutational data, and gene expression mainly of genes related to immunologic function were obtained.

**The role of PD-L1 expression on tumor cells vs tumor-infiltrating immune cells**

Because PD-L1 expression on both TC and IC appeared to be independently associated with response to atezolizumab, this scoring approach may help us in understanding more about the discrepancy of the link between PD-L1 expression on TC and response on anti-PD-1/PD-L1 treatment.

In **chapter 2**, we sought to improve insights in the associations of PD-L1 expression and specific patient' or tumor characteristics. Only for PD-L1 expression on TC positive associations were found (*KRAS* mutations and smoking), but none were found for IC. We then explored overlap and differences between expression of PD-L1 on TC and IC. Our interest was raised by the fact that more than half of the cohort had a PD-L1 positive immune infiltrate, which was associated with other immune gene markers, but without upregulation of PD-L1 on TC. In a subsequent analysis, we found that in the subgroup of TC0/IC3 samples an impairment of IFNγ response in the TC might be responsible for the lack of upregulation of PD-L1 in these tumors. From clinical trials we know that this TC0/IC3 subgroup has a higher overall response rate (ORR) compared to the overall TC0 tumors (22% vs. 8%) [15, 16]. These findings may contribute to the understanding why patients with PD-L1 TC negative NSCLC may still (only) require immune checkpoint blockade to gain response and survival benefit.

Protein expression of PD-L1 was assessed by the IHC SP142 assay. In the Blueprint PD-L1 IHC Assay Comparison Project, this specific assay exhibited fewer stained TC overall when compared to the three other assays tested (22C3, 28-8 and SP263) [17]. Therefore, tumors may have been unjustly scored as PD-L1 negative. This may have resulted in an underestimation of the finding of a hampered IFNγ-PD-L1 axis in our TC0 subgroup and might explain why we did not find this impairment in the TC0/IC2 and TC0/IC1 subgroups.

Unfortunately, analyses performed within our cohort cannot be extrapolated towards prediction for response as these resected patients never received immunotherapy. The data of this database enables us to look for associations between different biomarkers, patient and tumor characteristics and prognostic features. So, whether patients with tumors with a hampered IFNγ response would benefit from anti-PD-1/PD-L1 treatment remains unknown. Also, other explanations and hypotheses have been formulated to explain the discrepancy between the level of PD-L1 expression on TC and response to immune checkpoint inhibition: variable PD-L1 antibody assays, different IHC cut-offs, differences in tissue preparation or processing variability, tumor heterogeneity, primary versus metastatic biopsies and oncogenic versus immune-induced PD-L1 expression [18].

**Alternative biomarkers for response to immune checkpoint inhibition**

As mentioned in the recent developments section, clinical decision-making about first-line treatment choices in advanced NSCLC is based on PD-L1 expression on TC as a sole biomarker for immune checkpoint inhibition to date. In recent years, several other biomarkers have been investigated. The tumor mutational burden (TMB) is probably the most prominent one as it has been evaluated in several phase III clinical trials next to PD-L1 expression. Previous studies have reported that PD-L1 and TMB are independent predictors of response to immunotherapy in patients with NSCLC [7, 19, 20]. As smoking is an important cause of DNA damage, TMB in NSCLC is one of the highest among solid cancers [21]. The improved anti-tumor response in the setting of checkpoint inhibitor therapy may be

due to a higher number of tumor mutations increasing the probability of generating a “high quality” immunogenic peptide. The interest of TMB as a predictive biomarker seemed risen especially in the combination of anti-PD-1/PD-L1 with anti-CTLA-4 treatment. In the multi-arm CheckMate-227 study, the combination of nivolumab with ipilimumab significantly improved PFS compared to platinum-based chemotherapy in first-line NSCLC with high TMB (≥10 mutations/megabase (mut/Mb)) and this was irrespective of PD-L1 expression [20]. CheckMate-568 successfully validated the TMB cutoff of ≥10 mut/Mb as a biomarker for improved ORR and PFS for the ipilimumab and nivolumab combination again irrespective of PD-L1 expression in a single arm study design [22]. Another immunotherapy combination of durvalumab with tremelimumab in the MYSTIC trial also showed benefit over first-line chemotherapy in patients with high TMB measured in blood (≥16 mut/Mb), but not for PD-L1 expression of >25% [9]. Unfortunately, in the updated analysis of the Checkmate-227 study, no difference in survival outcomes between patients whose tumors had high or low levels of TMB was observed and the application of frontline approval for the combination of nivolumab and ipilimumab for patients with advanced NSCLC with a TMB of ≥10 mut/Mb was withdrawn [8]. As the updated analysis of the MYSTIC trial showed a negative result for the durvalumab and tremelimumab combination over chemotherapy in the overall study cohort, further clinical development of this treatment option remains unsure. Also, several issues regarding methods of obtaining and reporting TMB in NSCLC need to be mentioned. Some studies report TMB in terms of the absolute number of mutations, while others assess mutations per DNA megabase, i.e. mut/Mb. Additionally, thresholds to establish high TMB vary greatly without a widely used standard currently existing [23]. Besides a relatively long lead time to obtain TMB, tumor purity may also have an effect on TMB measurements [24]. These issues raise questions of utility and reproducibility for TMB as a useful clinical tool for treatment decision making in metastatic setting.

As opposed to TMB, exhaled breath could provide a very accessible biomarker for prediction as its retrieval is rapid and noninvasive. The volatile organic compounds (VOCs) in exhaled breath may represent systemic and local metabolic processes, which can be recognized and analyzed through electronic nose (eNose) technology. In a cohort of 92 advanced NSCLC patients this technique was able to discriminate between responders and non-responders on PD-1 checkpoint blockade at baseline and this finding was validated in a separate cohort of 51 NSCLC patients [25]. The distinction by the exhaled breath analysis was more pronounced compared to assessment of PD-L1 expression and furthermore, with the right cut-off exhaled breath was also more effective in predicting non-response to immune checkpoint blockade compared to PD-L1 expression. However, this biomarker will still need further evaluation. Another field of interest for biomarker research was found in the microbiome of cancer patients. Recent research has characterized the microbiome of NSCLC patients in a Chinese population and found that larger microbiome diversity could serve as a potential biomarker in predicting a favorable response to PD-1 blockade [26]. Apart from being a potential biomarker, influencing the gut microbiome, for example by fecal transplantation, might also lead to overcoming host ‘immune-breaking’ characteristics and therefore serve as potential lead for treatment optimization.

Although promising, as of yet none of these biomarkers have proven themselves more accessible and/or reliable compared to PD-L1 expression on tumor cells by IHC and further research is needed to improve (first-line) treatment selection in advanced NSCLC patients.

**Interaction between tumor and immune cells in adenocarcinoma vs squamous cell carcinoma**

Activated $CD8^+$ T cells are the key players in the anti-cancer immune response that is generated or amplified by immune checkpoint inhibitors. However, this immune response and also the tumor immune microenvironment are heterogeneous entities, involving a whole range of immune cells together with a wide spectrum of soluble chemokines and cytokines. Gene expression analysis allows us to perform excessive immunoprofiling of all the different aspects of the tumor immune microenvironment. In **chapter 3**, we investigated immune gene expression by NanoString of the resection cohort mentioned in the previous chapter. We found that in NSCLC adenocarcinomas (AD) the level of overall inflammation as assessed by immune gene expression was significantly higher compared to squamous cell carcinomas (SCC). This seemed to be related to a higher infiltration rate of immune cells within the tumor bed of AD compared to SCC based on a significant difference in tumor cell percentage between both histologies, i.e. tumor cell percentage being higher in SCC. This may suggest a different interaction of immune cells and tumor cells between the different histologies. Interestingly, a cluster of 34 genes did not correlate with the general level of inflammation, the level of PD-L1 expression or $CD8^+$ T cell infiltration. Expression of this 34-gene cluster, identified by unsupervised clustering, did not differ between AD and SCC histology, but high expression of this signature showed a clear OS benefit in SCC, but not in AD. This finding was validated in two independent NSCLC cohorts. We then tried to allocate the nature of this 34-gene signature and found the strongest correlation with Natural Killer (NK) cell related gene expression. Cell surface genes involved in NK cell recognition and killing - *ULBP2* and *HLA-C* – were significantly different between SCC and AD histology in favor of our hypothesis, namely that SCC may be more susceptible to NK cell killing than AD. Unfortunately, there is no established gold standard for assessing NK cell infiltration and/or activation level in tumor samples. Also, these cells are generally scarce within the tumor microenvironment and our IHC NK cell double-staining was not able to differentiate the 34-gene high from the 34-gene low samples.

Many endeavors of gene expression-based exploration of the immune tumor microenvironment have been performed using RNA sequencing platforms. RNA sequencing allows for a broader number of genes to be assessed per sample compared to the NanoString technique used in our cohort, but this goes at the expense of sensitivity. Almost all genes within our 34-gene signature could not be adequately measured by RNA sequencing technique due to low expression levels of the genes in the signature. To further investigate the biological rationale of such low-level signatures newer techniques might come of aid. Single cell sequencing using NanoString or microarray-based techniques may be able to further dissect the different aspects of the tumor immune microenvironment with a higher sensitivity together with assessing the level of activation of these different immune cells. This information could bring new insights in the role of immune cells that are present within the tumor infiltrate in low quantities, like NK cells. Several clinical phase I/II trials in various solid tumors manipulating the anti-cancer immune response through activation of NK cells are currently ongoing. Trials within this field are investigating the safety and efficacy of i) infusing NK cells as monotherapy or in combination with immune checkpoint inhibitors, chemotherapy or targeted drugs; ii) new molecules that target NK cells and T-cell activation signals to specific receptors on cancer cells, like antibody-dependent cell-mediated cytotoxicity (ADCC);

iii) chimeric antigen receptor T (CAR-T) cells or CAR-NK cells to redirect and activate NK cells into the tumor bed; iv) hematopoietic stem cell transplantation; v) cytokines and immunostimulatory drugs to boost the anti-tumor activity of NK cells [27].

**PART II. Modulating the tumor immune microenvironment**

As mentioned earlier, long-lasting responses in patients with advanced NSCLC on PD-1 blockade have been established with a reported estimated 5-year OS ranging between 15 - 27% [11, 12]. Although these are numbers previously unheard of in advanced NSCLC, there is still an urgent need for further improvements, especially for those patients not responding to immune checkpoint blockade. Radiation therapy (RT) could be a potent modulator of the tumor microenvironment and could augment the antitumor immune response when combined with immune checkpoint inhibition. In **chapter 4**, we provided a review about the off-target effects of RT, the so-called abscopal effect. We describe how RT may counteract the mechanisms of failure of immunotherapy and an oversight of pre-clinical and clinical data supporting augmentation of abscopal events by RT when combined with immune checkpoint inhibition is presented.

Based on these biological principles and at that time mainly pre-clinical results, we set up the PEMBRO-RT trial. In this multicenter study, patients with advanced NSCLC that had received at least one prior line of chemotherapy but were immunotherapy-naïve, were randomized between pembrolizumab treatment (control arm) vs pembrolizumab treatment within one week after three doses of 8 Gy on a single tumor lesion (experimental arm). Stratification was based on smoking status: <10 pack years vs ≥10 pack years. The primary end point was ORR at 12 weeks from randomization according to Response Evaluation Criteria in Solid Tumors (RECIST). The results of the PEMBRO-RT trial are presented in **chapter 5** [28]. The intention-to-treat (ITT) population consisted of 76 patients. Although the ORR at 12 weeks doubled in the experimental arm compared to the control arm (36% vs 18%), this difference was not statistically significant (p=0.07). The PD-L1 negative subgroup experienced a significant PFS and OS benefit in the experimental arm compared to the control arm, but no differences were seen in the overall ITT population regarding these outcomes. No increase in treatment-related toxicity was observed in the experimental arm. So, although an augmenting effect of RT on the response to PD-1 blockade in patients with metastatic NSCLC was observed, the study did not meet its primary end point of prespecified criteria for meaningful clinical benefit.

At the time of publication of the PEMBRO-RT trial, the MD Anderson Cancer Center (MDACC) was analyzing results from a similar randomized trial of pembrolizumab alone vs pembrolizumab in combination with RT [29]. In this study, patients with advanced NSCLC that were treatment-naïve or who had received prior chemotherapy both were allowed to participate. Patients were randomized between pembrolizumab treatment (control arm) vs pembrolizumab treatment with concurrently applied RT with the first dose of immunotherapy (experimental arm). Stratification was based on amenability of a lung or liver lesion to one of two RT regimens: 50 Gy in 4 fractions (50Gy/4, stereotactic body radiotherapy (SBRT) vs 45 Gy in 15 fractions (45Gy/15, traditional RT). The primary end point was disease response according to immune related response criteria (irRC). Preliminary results were

presented at ASCO 2019 and although response rates and PFS were similar between the RT and the control arm in the overall cohort, the 50Gy/4 SBRT subgroup showed a non-significant improvement in response rate in the non-irradiated lesions with a significant improvement in PFS compared to the traditional fractionated RT. Based on these results, it was hypothesized that a possible augmentation of an antitumor immune response on immune checkpoint inhibition may only exist when a SBRT regimen, but not traditional fractionation, is applied.

In **chapter 6**, we present the results of the pooled analysis of these two randomized trials. By exploring the possible abscopal effect in a larger cohort of advanced NSCLC patients, we found not only a significant improvement of abscopal response rate (ARR) in the experimental arm compared to the control arm, but also a significant PFS and OS benefit was observed in the patients treated with pembrolizumab and RT. Because RT regimen was not applied randomly, but rather based on trial variability and/or physicians' discretion, statistical comparison between RT schemas was not feasible. However, the 45Gy/15 subgroup showed an ARR similar to the control group, both around 20%. However, the other two RT regimens produced an ARR over two times as high. Exploration of the absolute lymphocyte count (ALC) showed a more pronounced ALC decline in the 45Gy/15 subgroup, which provides a hypothesis of a detrimental effect on immune response by traditional fractionation that requires further investigation.

In the PEMBRO-RT study, expression of PD-L1 was assessed after termination of the trial. At the time of writing of the study protocol in 2014/2015, the role of PD-L1 expression on clinical decision making was still under debate and not yet accessible in the clinical setting. The distribution of PD-L1 expression between the control arm and the experimental arm was skewed, leading to a higher number of patients with high PD-L1 expression, i.e. ≥50%, in the experimental arm at the expense of PD-L1 negative tumors, i.e. 0%. Fortunately, in our pooled analysis this imbalance in PD-L1 distribution between arms was corrected. Subsequently, no association between PD-L1 expression or benefit of pembrolizumab combined with RT could be established. Although comparison between RT regimens was limited by confounding, the baseline characteristics between the control and experimental arm of the overall intention-to-treat (ITT) population were well balanced, making these interpretations statistically sound.

Although these data suggest that RT is able to augment systemic immunotherapy responses and improve outcomes for patients with advanced NSCLC, these results are not yet convincing enough to change clinical decision making. Not only will we need more data on the optimal timing of application of RT and start of immunotherapy, selection of number and location of RT lesions and ideal RT regimen, but also what tumor and/or patient characteristics are more prone to benefit from this combination. One of the special requirements of this study was the collection of tumor biopsies before and after 6 weeks of therapy of non-irradiated tumor lesion. Ongoing translational research of blood and tumor samples collected during the trial will hopefully bring insights in associations between tumor and/or patient characteristics and abscopal benefit. Whole exome sequencing (WES) of all baseline tumor samples is currently obtained for determination of TMB, identification of possible neoantigens involved in abscopal responses, enrichment in mutation pathways, clonal composition and other gene alterations. T cell receptor (TCR) sequencing will be performed of matched baseline and on-treatment samples collected from non-irradiated tumor lesions to evaluate broadening of the TCR repertoire during treatment to allow

identification of a true antitumor immune response compared to overall induction of inflammation by either treatment regimens, i.e. RT or pembrolizumab, and compare the amplitude between responders in the control arm to responders in the experimental arm. Also, may sufficient material remain, RNA sequencing will be performed of these matched samples to explore pathway activation and the evolution of the composition of the immune infiltrate during treatment. This can be compared to the changes in T cell subsets and up- or downregulation of several immune checkpoints within the tumor immune microenvironment as assessed by multiplex immunohistochemistry (mIHC) using the Vectra technology. Patterns of circulating tumor DNA will be analyzed to explore differences between arms and between responders and non-responders and relate these to the sequencing data. Peripheral blood mononuclear cells (PBMCs) have been collected during treatment and will be used to perform functional neoantigen screens by pulse autologous T cells based on WES and TMB. A proteomics profile in plasma able to predict response on anti-PD-1 treatment in advanced melanoma patients has been established [30]. This was later also shown in advanced NSCLC [31, 32]. This proteomics signature will be determined at baseline and after application of RT, but before first pembrolizumab dosage, to explore whether RT would be able to transform a previous 'resistant' tumor into a 'sensitive' one. Lastly, baseline and on-treatment imaging will be assessed through a radiomics analysis pipeline possible enhancing our understanding of response to treatment, tumor heterogeneity and tumor immune microenvironment additionally to the already mentioned analyses of more invasively obtained materials. Hopefully these results may guide us how to proceed clinical implementation of the abscopal phenomenon.

**Future perspectives on the investigation of abscopal responses**

Due to the success of PD-1/PD-L1 inhibition in advanced NSCLC, immunotherapy also found its way in the treatment of earlier and therefore curable stages of disease. In 2018, the PACIFIC-trial showed improvement of PFS and OS from one-year adjuvant durvalumab over placebo for patients that had not developed progression after treatment with concurrent chemoradiation (CCRT) for locally advanced irresectable NSCLC [33]. This adjuvant treatment is now the first application of immunotherapy as SoC in earlier stage NSCLC. Also, the concurrent administration of nivolumab with CCRT proved feasible and safe based on results from a formal interim safety analysis in the NICOLAS-trial [34]. Two courses of neo-adjuvant nivolumab in resectable NSCLC was also deemed safe and showed major pathological responses (MPR) grossly irrespective of pre-operative radiologic assessments [35]. Preliminary results of ongoing neo-adjuvant phase II trials with immunotherapy previous to resection also showed a beneficial safety profile, discordant radiological and pathological responses and a presumably higher MPR rate and memory TILs induction of the combination of ipilimumab with nivolumab over nivolumab alone [36, 37].

Many trials are currently ongoing exploring the safety and efficacy of adjuvant treatment with PD-1/PD-L1 blockade after resection, SBRT and CCRT. Clinical outcomes of trials investigating concurrent application of immune checkpoint inhibition with CCRT are awaited as well. The Induction trial, currently running at the Netherlands Cancer Institute (NKI) is the only study to date evaluating the safety of neo-adjuvant treatment of immunotherapy -dual checkpoint inhibition with durvalumab and tremelimumab- in locally advanced CCRT setting (NCT04287894). Also, further exploration of neo-adjuvant treatment

in resectable disease will provide us with improved insights in the antitumor effects of immunotherapy, but may provide improvements in patient outcomes as well. Combining immunotherapy -two doses of pembrolizumab- with SBRT and comparing this regimen to either treatment alone in neo-adjuvant resectable setting, as is performed in the NKI-based trial NCT03446911, will allow to investigate loco-regional pathological investigation as well as systemic immune responses.

In the current treatment landscape of advanced NSCLC, investigation of underlying mechanisms and tumor-immune interactions for abscopal responses by the addition of RT to immunotherapy has become challenging. PD-1/PD-L1 blockade is now combined with platinum-doublet chemotherapy and only in a specific already immunogenic subgroup of tumors with PD-L1 expression of ≥50% monotherapy with immune checkpoints remains an option. It is difficult, maybe even impossible to date, to establish whether response is attributable to chemotherapy alone, immunotherapy alone or the combination specifically. Advancements of the role of immunotherapy into earlier stages of disease might provide opportunities to further explore the combination of RT with checkpoint inhibition. A challenging factor in stage I-III disease is that all (known) tumor lesions receive ablative local therapy with either resection or RT, thereby disabling exploration of a possible off-target invigorated antitumor immune response. However, in resectable stage with more than one tumor location, i.e. a primary tumor with N1 or uni-level N2 disease, a neo-adjuvant design with a combination of immunotherapy and RT could be proposed. By only radiating the primary tumor, evaluating an off-target effect in lymph node metastases would remain possible and a subsequent resection would be yield material for elaborate translational research. Still, in advanced NSCLC, an interesting approach of investigating the RT-immunotherapy combination could be performed in second-line setting as a means to overcome primary or secondary resistance to immunotherapy. Re-invigoration of responses to immunotherapy through the addition of RT after development of secondary resistance have been described [38, 39]. In this setting, a multi-arm optimal dose/fractionation-finding study could be performed; also, a comparison of monotherapy or combinations of immune modulating systemic treatment with RT could be tested this way.

Hopefully, the translational endeavors from the PEMBRO-RT trial may bring useful insights and biomarkers to better identify the abscopal effect in a clinical setting. These may assist us in optimization further research protocols investigating the possible advantage of adding RT to systemic treatment like immunotherapy in particular in NSCLC.

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