# BECOMING THE APEX PREDATOR

Improving immunotherapeutic strategies in patients with thoracic malignancies

Daphne Wilhelmina Dumoulin

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# Becoming the Apex Predator

## Improving immunotherapeutic strategies in patients with thoracic malignancies

## Verbetering van immunotherapeutische strategieën bij patiënten met thoracale maligniteiten

**Thesis**

to obtain the degree of Doctor from the
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**Daphne Wilhelmina Dumoulin**
Born in Son en Breugel, The Netherlands

**Erasmus University Rotterdam**

## Doctoral committee

| | |
|---|---|
| **Promotors** | Prof. dr. J.G.J.V. Aerts<br>Prof. dr. A.C. Dingemans |
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*"Een patiënt vertrouwt zijn grootste goed aan je toe: zijn gezondheid.*
*Daar mag je wel wat voor doen."*

Frank Smeenk

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# CHAPTER 1

## GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

# INTRODUCTION

Immunotherapy has proven to be a major breakthrough in the treatment of many cancers and has revolutionized the field of immuno-oncology.[1] Cancer is characterized by genomic instability, among other features resulting in the expression of neoantigens which in turn trigger our immune system to elicit a cellular immune response. However, tumor cells are able to escape from immunosurveillance which can lead to tumor outgrowth. Therefore, counteracting the escape of tumor cells from an immune system attack is necessary for effective tumor cell killing. Immunotherapy can play a role here. The rationale behind immunotherapy is to boost natural defenses in order to eliminate tumor cells.[2]

The first instance of using immunotherapy was discovered in the 18th century when infections were deliberately induced by keeping surgical wounds open to enhance immune responses by expressing antigen-presenting cells (APCs) and cytokines. In 1891, an American surgeon named William Coley developed a vaccine containing a mixture of life and inactivated Streptococcus pyogenes and Serratia marcescens bacteria and injected this vaccine into patients with irresectable soft-tissue sarcomas.[3] Over 1000 patients were treated with this vaccine, of which 51.9% showed complete tumor response and 21.2% had an ongoing remission for at least 20 years after the vaccination. Based on these results, William Coley is known as the father of immunotherapy.[4]

Immunotherapy has undergone significant developments since its inception. Several immunotherapeutic strategies are currently being used or investigated in the treatment of cancer. For betting understanding the mechanisms and pitfalls of these different strategies, knowledge of the normal way our immune system fights cancer is mandatory, which can be best explained by the Cancer-Immunity Cycle described by Chen and Mellman.[5]

**Cancer-Immunity Cycle**

The Cancer-Immunity Cycle consists of several steps.[5] For an effective anti-cancer immune response, every step in this cycle has to be initiated, and the whole cycle has to function for an effective anti-cancer immune response. Each step in this cycle is accompanied by co-stimulatory and co-inhibitory signals, which are responsible for a well-balanced system where immunity is promoted without inducing autoimmunity.

In summary, neoantigens are released and captured by APCs like dendritic cells (DCs). Next, the APCs present the antigens to the T-cells in the lymph node, which in turn become activated and express several activation markers on its surface, like

Programmed Cell Death Protein 1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4). Finally, the activated T-cells traffic to the tumor, where they recognize, attack, and kill the tumor cells, which again leads to the release of tumor-associated antigens (step 1 of this circle).

Tumor cells can evade the attack of the immune system by developing several possible mechanisms in each step, which impedes the circle from advancing to the next step. For example, a tumor can hide by not shedding antigens, or the antigens can act as self-antigen instead of foreign, resulting in the absence of effector T-cell responses. Later in the cycle, tumor cells can escape immune surveillance by preventing T-cells from infiltrating into the tumor, and most importantly, tumor cells can influence the tumor microenvironment in a way that effector cells will be suppressed while negative regulatory pathways will be upregulated. Each mechanism the tumor uses to oppose the next step in the cycle can dampen or arrest the anti-tumor immune response and precludes a self-sustaining cycle of cancer immunity.

**Immune checkpoint inhibitors**

Immunotherapy, predominantly immune checkpoint inhibitors (ICI), is currently widely used as standard of care in the treatment of many types of cancer with sustained clinical responses. The name 'immune checkpoint' is referring to its role as a gatekeeper of immune responses.[6] The most studied ICI are anti-PD-(L)1 and anti-CTLA-4.

PD-1 is a protein that is expressed on T- and B- cells and myeloid cells after activation of antigen-experienced effector T-cells.[6] Those activated T-cells produce IFN-γ, leading to the upregulation of PD-L1 on the surface of the tumor cells or tumor-infiltration immune cells. Binding of PD-1 to its ligand PD-L1 leads to apoptosis of the T cells and therefore an ineffective immune response. To counteract this binding, anti-PD(L)1 antibodies, such as nivolumab, pembrolizumab, atezolimab and durvalumab, have been developed in order to regulate T-cell responses. By using these antibodies, the tumor cells are not able to induce apoptosis of the effector T cells and thus an immune mediated attack of the tumor cells will follow. However, the cancer immunity cycle must have been nearly completed in most patients for anti-PD-(L)1 therapy to be effective. Consequently, the presence of activated effector T-cells in the tumor microenvironment is required for an effective response to anti-PD-(L)1 ICI.[7]

CTLA-4 is a protein that is constitutively expressed by Tregs but can also be upregulated, upon activation, by CD4-T-cells. CTLA-4 binds to its ligands B7.1 (CD80) and B7.2 (CD86) on the APCs with greater affinity than its co-stimulatory receptor CD-28. While CD-28 transmits a stimulatory signal to T-cells, CTLA-4 transmits an inhibitory signal and thus

mediates immunosuppression, mainly by enhancing the immunosuppressive function of Tregs. Thus blocking CTLA-4 by, for instance, ipilimumab causes (re)activation of T-cells leading to a reinvigoration of immune responses.[7]

CTLA-4 regulates T-cell activation early in the immune response and primarily in the lymph nodes, whereas PD-1 suppresses T-cells later in the immune response and primarily at the tumor site.

**This thesis**

As mentioned, several immunotherapeutic strategies are being used in cancer treatment, which can intervene in different steps in the cycle to overcome the diverse immune escape mechanisms. This thesis gives an overview of immunotherapeutic options in small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM) and strategies that could potentially lead to the activation of the anti-tumor immune system to make further necessary improvements possible.

The efficacy of ICI varies between cancer types and individual patients. While patients with non-small cell lung cancer (NSCLC) have relatively high response rates to anti-PD-1 of up to 45% and obtain durable clinical responses with a five-year overall survival of 30%[8], limited benefit is seen in other thoracic malignancies like SCLC and MPM. Both disease entities are extensively described in **Chapter 2** of this thesis. On the other hand, the fact that also in NSCLC the majority of patients do not respond to ICI underlines the variation between patients with the same disease, which remains unexplained.

For instance, SCLC has a high tumor mutational burden (TMB) compared to other tumors, which is supposed to increase responsiveness to ICI, because the more mutations a tumor has, the more neoantigens it has, and thus leading to a higher chance for effective tumor recognition. SCLC is frequently associated with paraneoplastic syndromes, which are in general related to an autoimmune phenomenon, and thus also suggesting suitable for effective ICI therapy. However, monotherapy with ICI failed to show benefit and only a small part of patients with SCLC benefit from the addition of ICI to chemotherapy, corresponding to a modest increase of median OS of approximately 2.0 months and 3 times more patients alive at 3 years (17.6% with chemo-immunotherapy versus 5.8% with chemotherapy).[9–13] Although this benefit is significant and led to approval and registration by FDA and EMA, it does not fulfill the criteria for reimbursement in the Netherlands.[14,15] The reason for this limited responsiveness to ICI is unknown. A comprehensive oversight of immunotherapy in SCLC is given in **Chapter 3**. One of the aspects which have been underestimated so far is the synergy between

chemotherapy and immunotherapy. Different immunostimulatory mechanisms have been ascribed to certain types of chemotherapeutical agents. The absence of the expected effect of ICI in SCLC may be related to the choice of the chemotherapy backbone, which was investigated in **Chapter 4**.

Because SCLC is a rare cancer, performing clinical trials in this population is challenging. Moreover, it seems like the patients in clinical trials do not entirely match the patients seen in daily practice, but data on this are lacking. **Chapter 5** provides insights into the evolving characteristics of the real-world population suffering from SCLC and how this population has changed in the last decades. For instance, the staging system of SCLC has been adapted several times, which could have impacted the outcome. Data of the current SCLC population may give insight in possible new ways to improve the outcome of SCLC patients and can be used in the implementation of new clinical trial designs.

Contradictory to SCLC, MPM has a low TMB. This, in combination with the immuno-suppressive tumor microenvironment in mesothelioma, makes MPM a cold tumor which is characterized by low T cell infiltration into the tumor. Therefore, MPM seems to be less immunogenic and not as suitable for the use of ICI as other tumors.[16]

Studies investigating ICI monotherapy using anti-PD1 showed improved outcomes compared to best supportive care, but a benefit in PFS or OS was lacking compared to chemotherapy.[17,18] Also ICI monotherapy using anti-CTLA-4 failed to improve OS compared to placebo.[19] Because anti-CTLA-4 acts early in the immune response, where it can activate T-cells and inhibit the immunosuppressive function of Tregs, and anti-PD-1 controls T-cell activation at the tumor site, the combination of both ICI seems to be valid. This has been investigated in a phase 3 trial, comparing anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) to chemotherapy, resulting in a significantly improved OS with 3-years OS of 23.2% with ipilimumab and nivolumab compared to 15.4% with chemotherapy. This study led to the approval and registration of this regimen.[20–23] The benefit was higher in non-epithelioid subtypes compared to the epithelioid subtypes, mainly due to the worse effect of chemotherapy in the first group. **Chapter 6** describes the combination of nivolumab and ipilimumab in a real-world setting.

Although the strategy of William Coley using attenuated bacteria to treat malignancies is still used in, for instance, Bacille Calmette-Guérin (BCG) vaccination in bladder cancer, this approach has some limitations.[24] The inability to identify appropriate tumor antigens and the ongoing presence of immunosuppression in the tumor microenvironment makes it challenging to achieve potent cytotoxic T-cell responses in all patients. These limitations may be overcome with adoptive T-cell therapy, like the

injection of genetically modified T-cells. Another option is to activate DCs outside the patient before administering them to present the neo-antigens to the T-cells. DCs are APCs that capture neoantigens that are released by the tumor, in order to present them to the T-cells in the lymph node which will be followed by an adaptive immune response. DCs are among the most potent APCs to activate these T-cells. However, because mesothelioma has low TMB, fewer tumor antigens are expected, and together with the immunosuppressive tumor microenvironment (with the presence of high numbers of M2 macrophages and Tregs and high levels of immune-suppressive cytokines such as VEGF, prostaglandins and TGF-β), DCs will be hampered to exert its function. By the administration of DCs which were cultured and trained to recognize tumor antigens ex-vivo, this problem can be overcome. The DCs will be loaded with tumor cell lysates which may be obtained from autologous or allogeneic tumor cells, with a preference for allogeneic cells to overcome the problem of insufficient amount or unsuitable tumor material. This method of DC vaccination is used in **Chapter 7** of this thesis. In **Chapter 8**, we combined these DCs with anti-PD-1 ICI. Effective DC vaccination aims to induce activation of T-cells, which will be followed by PD-L1 upregulation as a negative feedback. By the addition of anti-PD-1 after DC vaccination, this signal can be neutralized.

Immunotherapy is frequently hampered by a suppressive immune microenvironment, as described above. Several possible drugs are currently being investigated that alter the immune microenvironment in multiple ways. One of these drugs is lurbinectedin. The role of lurbinectedin in SCLC and MPM is discussed in **Chapter 9** of this thesis.

In conclusion, in these rare thoracic malignancies with dismal prognoses, the role of ICI is less established than in some other cancers like NSCLC. Despite some improved outcomes that have been reached using ICI, the benefit of ICI remains limited at the expense of toxicity. In order to promote anti-tumor immunity, a risk of auto-immunity is present, which can result in side effects depending on the affected organ system. One example is inflammation of the kidney. In particular, when ICI is combined with chemotherapy, distinguishing the cause of renal failure, which can either be chemotherapy or immunotherapy, is challenging but extremely relevant. In **Chapter 10**, a tool for clinicians is described to make the correct diagnosis.

## OUTLINE OF THIS THESIS

**Chapter 2** Rare thoracic cancers; a comprehensive overview of diagnosis and management of small cell lung cancer, malignant pleural mesothelioma and thymic epithelial tumors.

**Chapter 3** Immunotherapy in small cell lung cancer: one step a time: a narrative review.

**Chapter 4** A brief report on combination chemotherapy and anti-programmed death (ligand) 1 treatment in small-cell lung cancer: Did we choose the optimal chemotherapy backbone?

**Chapter 5** Trends in epidemiology of small cell lung cancer: a Dutch nationwide population-based study over 1989-2020.

**Chapter 6** Nivolumab and ipilimumab in the real-world setting in patients with mesothelioma.

**Chapter 7** Long-term follow-up of mesothelioma patients treated with dendritic cell therapy in three phase I/II trials.

**Chapter 8** Combination of PD-1/PD-L1 checkpoint inhibition and dendritic cell therapy in mice models and in patients with mesothelioma.

**Chapter 9** Lurbinectedin shows clinical activity and immune-modulatory functions in patients with pre-treated small cell lung cancer and malignant pleural mesothelioma.

**Chapter 10** Renal Toxicity From Pemetrexed and Pembrolizumab in the Era of Combination Therapy in Patients With Metastatic Nonsquamous Cell NSCLC.

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# CHAPTER 2

## RARE THORACIC CANCERS; A COMPREHENSIVE OVERVIEW OF DIAGNOSIS AND MANAGEMENT OF SMALL CELL LUNG CANCER, MALIGNANT PLEURAL MESOTHELIOMA AND THYMIC EPITHELIAL TUMORS

**Daphne W Dumoulin**, Paolo Bironzo, Francesco Passiglia,
Giorgio V Scagliotti, Joachim G J V Aerts; ERN-LUNG Core Network Mesothelioma

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## ABSTRACT

Despite the progress in outcomes seen with immunotherapy in various malignancies including non-small cell lung cancer (NSCLC), the benefits are smaller in small cell lung cancer, malignant pleural mesothelioma and thymic epithelial tumors (TET). New effective treatment options are needed, guided via more in-depth insights into these rare malignancies' pathophysiology. This review comprehensively presents an overview of clinical presentation, diagnostic tools, staging systems, pathophysiology, and treatment options for these rare thoracic malignancies. In addition, opportunities for further improvement of therapies will be discussed.

# INTRODUCTION

Small cell lung cancer (SCLC), malignant pleural mesothelioma (MPM) and thymic epithelial tumors (TET) are rare but aggressive thoracic malignancies. The prognosis of patients is frequently dismal and treatment options are limited. Despite the progress in outcomes seen with immunotherapy in various malignancies, including non-small cell lung cancer (NSCLC), the benefits in these rare thoracic malignancies are smaller for partially unknown reasons. New effective treatment options are needed, guided via more in-depth insights into the pathophysiology of these rare malignancies, as we have learned from the recently performed studies that general principles established in other malignancies do not apply to these rare cancers.

In this review, we provide an overview of the current landscape of these rare thoracic cancers. Based on the completed and ongoing clinical trials, we will suggest therapeutic options for further improvement of therapies in the (near) future.

## SCLC

According to the World Health Organization Classification of Lung Tumors, SCLC is part of the neuroendocrine lung neoplasms, along with large-cell neuroendocrine carcinomas (LCNEC) and carcinoid tumors[1].

### *Epidemiology*

SCLC represents about 15 percent of all lung cancers. With an incidence of 1-5/10.000, SCLC is recognized as an orphan disease[2].

SCLC has a strong correlation with smoking. SCLC used to be more common in men than women, but the male/female ratio became equal due to the increased tobacco consumption in women. Because of a general decrease in the prevalence of cigarette smoking, the incidence of SCLC in the last two decades progressively declined[3]. Of all SCLC diagnoses, only 2% occurs in never-smokers. SCLC in never-smokers may originate from the histological transformation in oncogene driver-mutated lung cancer, for instance in Epidermal Growth Factor Receptor (EGFR) mutated lung cancer who develop resistance to targeted treatment.

Patients with SCLC usually present with small intrapulmonary lesions and bulky mediastinal lymphadenopathies. Distant metastases are most frequently seen in bone, liver, brain and adrenal glands[3]. At diagnosis, approximately 70% of patients already have distant metastases.

*Diagnosis*

The diagnosis of SCLC is based primarily on histological appearance by light microscopy, which demonstrates small tumor cells, poorly defined cell borders, scant cytoplasm and nuclear moulding. The mitotic rate is high with >10 mitoses per $mm^2$, the mean mitoses per $mm^2$ is 60 and the median 80.

The addition of immunohistochemistry can help to distinguish SCLC from other tumors[4]. Because SCLC originates in the lung, these tumors are positive for keratin and epithelial membrane antigen staining, and the majority will also express thyroid transcription factor-1 (TTF1). This helps distinguish small cell tumors that originate in the lung or another organ, like lymphomas which are negative for cytokeratins and express CD45. The most useful neuroendocrine markers include CD56, chromogranin and synaptophysin, which are best used as a panel. CD56 is present in approximately 95% of the patients, whereas up to two-thirds of SCLC will be negative for chromogranin and synaptophysin. In about 10% of the patients with SCLC, all neuroendocrine markers are negative. By using mainly the number of mutations per $mm^2$ and the proliferation marker Ki-67, which is exceptionally high in SCLC (>50%, and usually 80-100%), SCLC can be separated from carcinoid tumors[5].

*Staging*

SCLC used to be divided by the Veterans' Affairs Lung Study Group (VALSG) into limited (tumor confined to the ipsilateral hemithorax and regional nodes able to be included in a single tolerable radiotherapy port) versus extensive stage disease (tumor beyond the boundaries of limited disease)[6]. Although this staging system is still functional and easy to use in clinical practice, it has been replaced by the tumor-node-metastasis (TNM) classification[7]. The TNM classification provides a more detailed staging that better reflects outcome and prognostic information, which is extremely relevant in clinical trials.

At diagnosis, approximately 10% of patients have brain metastases. Most of the patients have symptomatic brain metastases. However, in a substantial part of the asymptomatic patients, MRI detects brain metastases, which are therefore upgraded to stage IV disease[8].

The role of fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) is less clear. PET-CT has been shown to change the staging of the disease in a substantial number of patients, leading to a different treatment strategy[9,10], and thus the recommendation to perform in staging. However, it seems not to influence OS[11].

*Pathophysiology*

SCLC occurs through the inactivation of the tumor suppressor genes TP53 and RB1, which is frequently initiated from exposure to tobacco carcinogens. Besides the loss of p53 and RB1 (in nearly all SCLC tumors), also other alterations have been reported in a subset of patients with SCLC, such as MYC amplification and inactivating mutations in, among others, PTEN, KMT2D, CREBBP and NOTCH genes[12]. So far, no mutationally defined subtypes of SCLC are recognized. However, by using the expression of several specific transcription factors, SCLC can be divided into four biological different subtypes (NAPY classification), which are becoming more and more of interest because of potential new therapeutic options[13]. The most common (70%) is the "classical" SCLC (SCLC-A) which is characterized by ASCL1 expression. It also has high expression of INSM1, L-MYC and delta-like ligand 3 (DLL3), and low NEUROD1 expression. Immunohistochemically, SCLC-A is TTF-1 high and C-MYC low. This subtype can be further divided into SCLC-A and SCLC-A2, based on the expression of HES1[14]. The subtype SCLC-N (11%) is characterized by a high expression of NEUROD1. It has a variable expression of ASCL1, the expression of TTF-1 is low and C-MYC is high. SCLC-P (16%) is characterized by a high expression of POU2F3. The expression of ASCL1 and INSM1 is low. SCLC-Y (3%) is characterized by high YAP1 expression. The expression of ASCL1, NEUROD1 and INSM1 is low. This subtype is further characterized by wildtype/enriched RB1[13]. SCLC-A and SCLC-N show a more neuroendocrine phenotype than SCLC-P and SCLC-Y. Within the neuroendocrine subtypes, another less frequently common subtype is described: NETF, characterized by the expression of ATOH1[15]. Identifying these molecular subtypes could help develop a personalized approach by distinguishing subsets of patients that are most likely to respond to different therapies. For example, it might be that the different subtypes have a different tumor microenvironment (TME). SCLC-Y is enriched for a T-cell inflamed phenotype, making it plausible that this subtype might be most sensitive for therapy with (a combination with) PD-(L)1-inhibitors, while a DLL3- targeted treatment is most logical for SCLC-A[16]. Furthermore, it seems that MYC-high SCLC is especially sensitive to aurora kinase inhibitors[17].

*Treatment*

Chemotherapy has been the backbone in the treatment of all stages SCLC. For several decades, the standard chemotherapy regimen consists of platinum-etoposide. In non-metastatic SCLC, cisplatin is used with the advantage of combining it concurrently with radiotherapy. In metastatic SCLC or patients unsuitable for cisplatin, it can be replaced by carboplatin, without inferiority[18].

cT1-2N0M0, *Very-limited-stage SCLC*

Little evidence is available for the management of patients with very-limited SCLC, defined as cT1-2N0M0 SCLC. It can be considered to treat those patients with local treatment by surgery or radiotherapy, without a preference for one of them[19]. If a resection is performed, adjuvant chemotherapy should follow. Only in cases where an incomplete resection was performed (R1-2 or unforeseen mediastinal lymph nodes), there is a role for adjuvant radiotherapy. Patients not suitable for surgery or with a preference for radiotherapy should be treated with fractionated or stereotactic ablative radiotherapy, combined with chemotherapy before or after[20]. Prophylactic cranial irradiation (PCI) is not recommended in cT1-2N0M0 SCLC because of the relatively low percentages of brain metastases and the risk of neurocognitive toxicities in consideration of the predicted long-term survival[21].

*Stage I-III SCLC*

The recommended treatment for patients with stage I-III SCLC is chemoradiotherapy. Although responses to chemotherapy are exceptionally high in SCLC, relapse will occur soon. By adding radiotherapy to the primary tumor and mediastinal lymph nodes, the 3-year OS can be increased by 5 percent[22]. The most preferred regimen consists of 4 cycles of chemotherapy combined with twice-daily concurrent radiotherapy with 45 Gray[23], starting from the first or second cycle[24]. High-dose once-daily up to 66 Gy radiotherapy seems not superior and toxicity was not significantly different, but this trial was not designed to show equivalence[25]. Therefore, twice-daily radiotherapy remains the standard of care, although for logistic reasons, once-daily radiotherapy could be an alternative option[26]. Higher dose radiotherapy of 60Gy twice daily seems to improve survival in a phase II trial without increasing toxicity[27]. For more frail patients, a sequential chemoradiotherapy approach can be considered. In this scenario, the volume of the pre-chemotherapy primary tumor and the post-chemotherapy nodal volume will be irradiated[28].

PCI in limited-stage SCLC reduced brain metastases and increased OS significantly. However, since magnetic resonance imaging (MRI) of the brain is more frequently used in staging, results have become controversial[29–31]. The currently recommended strategy is to offer PCI to patients with a performance score (PS) of 0-1 who responded to chemoradiotherapy. In patients with stage I-II SCLC, or frailer or older (>70 years) patients, the role of PCI is less clear. For these patients, MRI surveillance could be a worthy alternative and thus shared decision-making is recommended[26]. The recommended dose of PCI is 25 Gy in 10 daily fractions[32]. Because of concerns about late neurocognitive effects, hippocampal avoidant radiotherapy is investigated, however a lower probability of cognitive decline was not found[33].

*Stage IV SCLC*

For many years, the standard chemotherapy used for stage IV SCLC has been 4-6 cycles of platinum combined with etoposide. In terms of efficacy, no differences were found between carboplatin and cisplatin. However, more adverse events were seen with cisplatin, although carboplatin has more hematological toxicity[18]. Continuation of chemotherapy beyond 4-6 cycles is not recommended because of the risk of increased toxicity without improvement of OS.

In recent years, synergistic activity has been reported for the addition of ICI to standard platinum-based chemotherapy, leading to a statistically significant benefit in PFS and/or OS[34–37]. Although median benefits are only modest with an improvement of median OS with around two months, the improvement in the tail of the survival curve suggests that a small proportion of the patients have durable benefit. For instance, atezolizumab combined with chemotherapy improved the 18-months survival rate from 20 to 33% and the 3-years survival rate for durvalumab combined with chemotherapy was improved from 6 to 18%[38]. Dependent on the reimbursement per country, chemotherapy combined with ICI is currently considered the standard first-line therapy for stage IV SCLC.

Several biomarkers were investigated to predict which patients have benefit from ICI[39]. Although in NSCLC PD-L1 expression is used in deciding which therapy is preferred, no correlation was found between PD-L1 expression and efficacy in SCLC. Another biomarker is tumor mutation burden (TMB), which can be measured in the tumor or the blood (bTMB). TMB is defined as the number of somatic mutations found in the DNA of cancer cells per megabase (Mb). Because several trials have shown different correlations of (b)TMB, the role of TMB is still controversial. Currently, no predictive or prognostic biomarker is found.

Almost half of the patients with stage IV SCLC develop brain metastases after the completion of standard chemotherapy. PCI reduces the risk of brain metastases significantly, however, OS seems not to be improved[40]. Active surveillance with MRI might be as effective as PCI[41].

Consolidation thoracic radiotherapy after chemotherapy in the CREST trial did not show an improvement on the primary endpoint 1-year OS and is therefore not recommended. However, in fit patients with residual intrathoracic disease who achieved response after chemotherapy, it could be considered[42].

*Second line*

Despite initial high response rates with chemotherapy, most patients relapse within six months. The second-line treatment depends on the treatment-free interval (TFI) and the response to the first-line therapy. In platinum-sensitive patients with a TFI of at least three months, rechallenge with carboplatin-etoposide can be considered, with a slightly higher PFS than topotecan[43]. Until 2020, topotecan, a topoisomerase one inhibitor, was the only approved treatment in the second line. Despite the modest efficacy and significant toxicity, treatment using topotecan seems to improve OS and quality of life, compared to best supportive care[44]. Oral and intravenous topotecan demonstrated similar efficacy[45].

Following the European Medicines Agency (EMA) granted orphan designation in 2019, in 2020 the US Food and Drug Administration (FDA) granted accelerated approval to lurbinectedin, a selective inhibitor of RNA polymerase II, for patients progressing on or after first-line chemotherapy. This was based on a phase 2 basket trial showing promising results with an ORR of 35%, a median duration of response of 5.3 months, a median OS of 9.3 months, and a manageable safety profile[46]. A phase 3 trial comparing lower dose lurbinectedin combined with doxorubicin versus topotecan or cyclophosphamide/adriamycin/vincristine, showed similar efficacy but a favorable toxicity profile[47].

The PD-1 checkpoint inhibitors nivolumab and pembrolizumab have had FDA approval as monotherapy in third or further line therapy based on phase I/II studies[48,49], however, these approvals were withdrawn when the confirmatory phase III studies failed to reach OS improvement[35,50]. Furthermore, second-line treatment with nivolumab compared to topotecan did not improve OS[50]. Another phase 3 trial comparing topotecan with Rovalpituzumab tesirine (Rova-T), an antibody-drug conjugate containing a DLL3-targeting antibody tethered to a cytotoxic agent, showed decreased OS and increased toxicity with Rova-T[51].

Alternative treatment options without specific approval are cyclophosphamide combined with doxorubicin and vincristine (CAV), paclitaxel, docetaxel, irinotecan and temozolomide[26,52].

*Future perspectives*

The introduction of ICI in several types of cancers did improve patients' survival. Nonetheless, in SCLC this improvement with ICI is only modest. Although SCLC is characterized by a high TMB (which has been shown predictive for ICI efficacy in other cancers[53]) and SCLC is extremely sensitive to chemotherapy (which results in massive

tumor antigen release and potentially reduces the immunosuppressive environment of the tumor), these potentially beneficial characteristics for ICI efficacy do not result in improved outcomes in SCLC. In for instance NSCLC, a synergistic effect between chemotherapy and ICI is seen[54]. In SCLC treated with combination chemotherapy and ICI, response rates do not rise and separation of the PFS and OS curves is seen late after several months, suggesting the absence of a synergistic effect in SCLC.

Many clinical trials using ICI are running to investigate multiple ways to convert SCLC to an immunogenic tumor[39].

While immunogenic cell death (ICD) is crucial for immune modulation by cytotoxic chemotherapies, various chemotherapeutic agents have different capacities to induce ICD. Translocation of the ER protein calreticulin (CALR) to the cell membrane induces activation and maturation of dendritic cells, leading to T-cell activation and proliferation, and is thereby necessary for successful ICD[55,56]. In etoposide-treated mice, CALR translocation was absent, suggesting that the lack of T cell activation during etoposide treatment could be a reason for the reduced efficacy of etoposide in combination with ICI. Therefore, using another chemotherapy backbone in combination with ICI must be considered to reinforce tumor immunogenicity[57].

Since the classification of SCLC in different molecular subtypes, a more personalized approach is trying to be developed. ICI is logically most potent in tumor-associated T-cell immunophenotypes, which are the POU2F3 and YAP1 subtypes, with the highest CD8+ T-cells in the tumors that express none of the molecular biomarkers (NAPY-)[58]. However, an exploratory analysis of the IMpower-133 showed a higher proportion of long-term survivors in one subgroup named SCLC-I, but for both arms (chemotherapy plus placebo group as well as for chemotherapy plus atezolizumab). This SCLC-I subgroup is not well defined but comprises an inflamed subgroup with a high expression of multiple immune genes, including CD8-positive cytotoxic T cells, and lacking the expression of NEUROD1, ASCL1, and POU2F3. The fact that the survival benefit was seen in both arms, suggests that this subgroup might be prognostic[59].

Additionally, several other targets are identified for which separate therapeutic options are being investigated[58]. One of these targets is DLL3, which is highly expressed in SCLC-A. DLL3 is regulated by transcription factor ASCL1, which is an inhibitory ligand of the Notch receptor. Notch signaling is downregulated during neuroendocrine tumor growth, thus the expression of DLL3 promotes the migration of SCLC. DLL3 is expressed in >80% of SCLC and other neuroendocrine tumors, while the expression in normal tissue is limited, making it an interesting target of therapy. Although studies with Rova-T

were disappointing[51,60], other ways to use this target are being investigated, including a chimeric antigen receptor T-cell (CAR T-cell, AMG119[61]) and a bispecific T-cell engager (BiTE, AMG757) of which the phase 1 results are promising (NCT03319940)[62].

Another target is MYC, which is amplified in approximately 20% of SCLC. It is mainly seen in SCLC-N but also in SCLC-A. MYC-high SCLC is more sensitive to targeted therapy with aurora kinase inhibitors[17].

DNA damage plays a major role in SCLC. Therefore, DNA damage repair inhibitors like PARP-1 inhibitors and cell cycle checkpoint kinase inhibitors like WEE1 and cyclin-dependent kinase 7 (CDK7), are of great interest[13]. SLFN11 is used as a biomarker for response to PARP inhibitors and DNA-damaging agents. It is expressed in all molecular subtypes of SCLC, but it was absent in the SCLC tumors which were negative for all the four subtype markers (NAPY-)[58]. Multiple studies investigating DNA damage repair inhibitors are ongoing[63].

In conclusion, new insights into biological subtypes and immunogenicity are recognized as well as the association with some therapeutic response biomarkers. Although the current clinical approach to therapy is still independent of the molecular subtype, a big field of research is ongoing which could have major implications for clinical practice in the (near) future.

To be noted, the best strategy is to prevent SCLC by smoking cessation. Luckily more attention is going to smoking cessation counseling and intervention. Also during treatment, smoking cessation has shown to be beneficial[64].

## MESOTHELIOMA

Malignant mesothelioma is a rare tumor classified by the WHO as directly related to all types of asbestos exposure[65]. It is a fatal neoplasm arising from the mesothelial lining of the lungs, abdomen, heart or testes.

*Epidemiology*

In 2020, 30.000 patients were diagnosed with mesothelioma worldwide. The incidence is higher in males (0.7 per 100.000 persons) than females (0.3 per 100.000 persons) and increases with age. The median age at diagnosis is 76 years. The highest incidence is seen in countries with the greatest previous asbestos use, such as the Netherlands, UK and Australia[66].

Mesothelioma has a strong correlation with asbestos exposure. The greater the asbestos exposure, the greater the risk of developing mesothelioma. People who worked with asbestos products or worked in an environment containing asbestos are at increased risk of developing mesothelioma, but also are at risk individuals who were washing the clothes of someone who worked with asbestos. Although non-occupational exposure to asbestos, including neighborhood, domestic, and household exposure is associated with an increased risk for mesothelioma, a recent meta-analysis indicated that some summary relative risk estimates should be interpreted with caution because of high between-studies heterogeneity[67]. The time to exposure and the onset of mesothelioma is in general >30 years[68]. In some cases, mesothelioma occurs due to a genetic mutation in BRCA-associated protein 1 (BAP1). Patients with mesothelioma usually present with shortness of breath due to pleural effusion, chest pain or weight loss.

The prognosis of mesothelioma is poor. The median survival is only nine months without therapy[69].

Patients with epithelioid histology have a better survival than the other histologies. Also women, younger age and lower stage of cancer are associated with a better prognosis, while higher PS, high platelet counts, high fibrinogen levels, low albumin levels and low glucose levels in the pleural fluid represent adverse prognostic factors. In addition, mesotheliomas arising due to a germline BAP1 mutation have a relatively favorable prognosis[70,71].

*Diagnosis*

Although mesothelioma can be suspected on cytology, tissue biopsy is strongly recommended to establish the diagnosis, preferentially obtaining biopsies from three separate sites[66]. Based on morphology only, mesothelioma cannot be distinguished from metastatic lesions from another primary cancer or from atypia caused by reactive changes. Therefore, additional immunohistochemistry (IHC) investigation is recommended, used in a panel of 3-4 markers. Three histological subtypes are distinguished: epithelioid, sarcomatous and biphasic.

Most mesotheliomas are positive for pan-cytokeratin, independent of subtype, while for instance sarcomas are pan-cytokeratin negative[72]. Epithelioid mesothelioma expresses high levels of calretinin, Wilms' tumour-1 (WT-1) and podoplanin (D2-40). The epithelial markers CEA, Ber-EP4, MOC-31 are usually absent in mesothelioma and claudin-4 is consistently negative, which makes it very useful biomarkers assisting in the differential diagnosis[73]. Sarcomatous mesothelioma does not express high levels of calretinin, but is usually positive for GATA3, which is negative in sarcomatous lung

carcinoma[72]. Conversely, MUC4 is highly expressed in sarcomatous carcinoma of the lung and negative in sarcomatous mesothelioma.

Recently, more investigation was focused on the loss of nuclear BAP1[74]. Loss of BAP1 in IHC corresponds to BAP1 mutation and BAP1 loss is almost 100% specific for malignancy in mesothelial proliferations, however it is not sensitive in distinguishing the different subtypes of mesothelioma[75]. Of all pleural and peritoneal mesotheliomas, 50-70% show BAP1 loss. BAP1 loss is seen in approximately 70% of the epithelioid subtype and 15% of the sarcomatous subtype. In sarcomatous mesothelioma, loss of methyl-thio-adenosine phosphorylase (MTAP) staining is frequently seen, with 96% specificity and 78% sensitivity. MTAP in IHC can act as a surrogate for loss of cyclin-dependent kinase inhibitor 2A *(CDKN2A, which has a prominent role in the* tumor suppressor mechanism*),* which requires fluorescence *in situ* hybridization (FISH)[76].

*Staging*

Mesotheliomas are clinically and pathologically staged using the 8th revision of the UICC TNM staging system[77]. At first, a contrast-enhanced computed tomography (CT) of the thorax and upper abdomen is performed to evaluate the T and N stages. Before performing an extensive staging, it should be considered if the patient is eligible to undergo active treatment[66]. For patients suitable for surgery, additional investigations such as PET-CT, endobronchial ultrasound (EBUS) or mediastinoscopy, are recommended to exclude contralateral lymph nodes and distant metastases[66]. Although survival is similar for all N stages, a survival difference between single compared to multiple metastases was described[78]. Since brain metastases are very rare in mesothelioma, imaging of the brain is only recommended when there is clinical suspicion[79].

*Pathophysiology*

Mesothelioma arises when asbestos fibers enter the pleural or abdominal cavity, causing phagocytosis by macrophages. This can lead to an inflammatory reaction, followed by an increased risk of malignant transformation of mesothelial cells[80]. This risk is higher when the asbestos fibers are longer than 10 µm because of a more difficult clearance by macrophages and thus failed attempts of phagocytosis[81]. Another potential risk factor for the development of mesothelioma, is the fact that asbestos fibers can enter mesothelial cells and directly interfere with mitosis, leading to DNA mutations. Furthermore, mesothelial cells exposed to asbestos fibers release inflammatory cytokines such as tumor growth factor, and vascular endothelial growth factor (VEGF), which generate an ideal environment for tumor growth. These factors are responsible for the increased risk and eventually the development of mesothelioma[80].

*Treatment*

*Surgery*

After adequate staging, surgery may be considered for selected patients where a complete macroscopic resection is to be expected. The overwhelming majority of those surgical cases are stage I mesotheliomas, although upstaging after surgery frequently occurs. The surgery has to be performed in a center of expertise, as part of multimodality treatment. However, the role of surgery is controversial; in the Mesothelioma and Radical Surgery (MARS) study, extrapleural pneumonectomy (EPP) failed to show a benefit to best supportive care (BSC) as an addition to standard chemotherapy treatment[82]. Alternatively, a pleurectomy-decortication (P/D) is a less extensive lung-preserving surgical procedure with a significantly improved perioperative 30-day survival[83]. It must be noted that surgery's positive reported outcomes most likely seem to be based on selection bias[84]. The MARS-2 study comparing P/D to BSC as an addition to standard chemotherapy treatment is currently ongoing[85].

*Systemic treatment*

Due to the widespread pleural metastases, most patients with mesothelioma are not suitable for surgery. For patients eligible for systemic treatment, the doublet combination of cisplatin combined with pemetrexed compared to cisplatin resulted in a survival benefit of 3 months[69]. By adding bevacizumab to pemetrexed plus cisplatin, an additional survival benefit of nearly three months can be achieved, although this treatment is not available in many countries[86]. The role of pemetrexed as maintenance therapy following initial pemetrexed and cisplatin did not result in better PFS[87]. Switch-maintenance gemcitabine significantly improved PFS compared to BSC, but did not improve OS[88].

ICI therapy has been extensively investigated as a treatment option in this setting. In the phase 3 Checkmate 743 trial, nivolumab combined with ipilimumab was compared to standard chemotherapy and resulted in a significantly improved OS[89] with 3-years OS of respectively 23.2% versus 15.4%[90]. This benefit was higher in non-epithelioid subtypes compared to the epithelioid subtype due to the worse efficacy of chemotherapy for non-epithelioid subtypes. In addition, quality of life was better with nivolumab-ipilimumab than with chemotherapy[91].

Since 2020, nivolumab-ipilimumab has been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), without histology or biomarker subtype restriction[92,93].

*Radiotherapy*

The role of radiotherapy in MPM is limited. Prophylactic irradiation was not beneficial in tract metastases rate, chest pain, analgesia requirements, quality of life or OS, and is therefore not recommended. Radiotherapy as part of multimodality treatment, before or after surgery, did not result in a longer relapse-free survival and is therefore not standard of care. Radiotherapy can be considered as palliative treatment in case of mesothelioma induced pain[66].

*Second line*

Based on phase 2 studies, patients can be treated with vinorelbine or gemcitabine in the second or later line, although the impact on survival is questionable and responses are rare[94,95]. In the phase 3 second-line PROMISE-meso study, ICI monotherapy (pembrolizumab) was compared to vinorelbine or gemcitabine which showed an improved response rate, however the study failed to show an additional benefit in PFS or OS[96]. The phase 3 CONFIRM study, compared ICI monotherapy (nivolumab) to BSC, which resulted in improved outcomes with significantly higher OS, PFS and response rates with ICI[97].

*Future perspectives*

Mesothelioma can be classified as a cold tumor characterized by low T cell infiltration into the tumor, which is caused by a low mutational load and an immunosuppressive tumor microenvironment (TME) consisting of increased amounts of myeloid-derived suppressor cells (MDSC), M2 macrophages and regulatory T cells (Tregs). This may result in impaired immune activation.

Several strategies could potentially lead to the activation of the anti-tumor immune system[98]. In NSCLC, the addition of chemotherapy causes cell death leading to the release of tumor neoantigens. In addition, chemotherapy itself can result in a less suppressive tumor microenvironment. After promising results of two phase 2 studies[99,100], the combination chemotherapy with durvalumab as a first-line treatment is currently investigated in the phase 3 DREAM3R study[101]. Furthermore, the phase III BEAT-MESO trial comparing bevacizumab with standard chemotherapy with or without atezolizumab is ongoing[102].

Another strategy to overcome the immune suppression in mesothelioma, considers the potential of dendritic cells (DCs) to activate T-cells. DCs have the capacity to recognize tumor antigens following presentation to the T-cells. The immunosuppressive TME of mesothelioma prevents the maturation and activation of DCs. By the administration of activated and tumor lysate-loaded dendritic cells, this obstacle can be bypassed.

Several phase I/II trials have been performed using DC vaccination therapy. The long-term follow-up of these separate phase I/II trials showed a promising signal, with a 2-year OS of >50% and a 5-year OS of >20%[103]. These studies have led to a currently active randomized phase 3 DENIM trial; the accrual is completed and the results are awaited[104].

Chimeric antigen receptor (CAR) T-cell therapy is an alternative way to overcome the issue with inactivated T cells. Using this strategy, genetically engineered T-cells against a specific tumor-associated antigen like mesothelin, are administrated. This strategy is investigated in several phase I studies, mainly in combination therapies such as ICI[105,106].

Alternative treatment strategies are currently being investigated. ADI-PEG 20 is an enzyme degrading arginine, an amino acid on which mesothelioma cells are dependent. In the ATOMIC phase II/III study, this drug is investigated in addition to first-line chemotherapy compared to placebo[107].

Notably, in the last decades more attention has been given to the prevention of mesothelioma, but currently there is no indication for a systematic early detection program in the exposed population. Asbestos use is currently forbidden or heavily regulated in most Western countries[108].

**Thymic Epithelial Tumors (TETs)**

Thymic epithelial tumors (TETs) are rare neoplasms originating from the thymus.

*Epidemiology*

TETs have an incidence of 1.3 to 3.2 cases per million worldwide[109]. TETs are the most frequent tumors of the anterior mediastinum in adults, with a mean age at diagnosis of 50-60 years.

Approximately 30% of patients diagnosed with thymoma are asymptomatic, however, pain, dyspnea, or cough can occur due to local tumor growth. Rarely, superior vena cava syndrome or phrenic nerve paralysis may occur at presentation, while stridor or dysphagia are late symptoms. Pleural or pericardial effusion suggests metastatic spreading. Up to 35% of patients with thymoma are diagnosed with myasthenia gravis (MG). Among other potential paraneoplastic syndromes associated with thymomas, pure red cells aplasia and hypogammaglobulinemia are the most frequent (2-6%)[110].

Differently from thymomas, thymic carcinomas (TCs) are more aggressive tumors, often presenting with symptoms due to local or distant growth, as well as aspecific

complaints such as weight loss or fever[111]. Only few patients with TC are diagnosed with paraneoplastic syndromes[110].

*Diagnosis*

According to the 5th edition of the World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus and Heart, thymomas represent 75-85% of all TETs and are classified as Type A (11.5%), AB (25%), B1 (17.5%), B2 (26%), B3 (16%), micronodular thymoma with lymphoid stroma (1%), and metaplastic thymoma (0.1%). As most thymomas have non-neoplastic immature T-cells components, TdT immunostaining could be useful for diagnostic purposes, even in metastatic sites. Thymic carcinomas encompass 14-22% of TETs. Although their morphology is that of conventional carcinomas, they often show immunostaining for CD5 and KIT (CD117).

Pretreatment biopsy is not required when TETs are highly suspected based on imaging and upfront surgical resection is feasible. In all other cases, biopsy (respecting pleural spaces to avoid tumor cell seeding) should be performed to inform treatment decision.

*Staging*

For more than 40 years, the Masaoka-Koga classification has been used for TETs staging. Currently, the 8th edition of the Union for International Cancer Control- American Joint Committee on Cancer (UICC/AJCC) TNM classification, developed by the International Association for the Study of Lung Cancer (IASLC)/ International Thymic Malignancy Interest Group (ITMIG), is effective and should be adopted for staging purposes. This classification is based on a retrospective database of more than 8000 cases provided by major global thymic organizations. CT scan is the primary imaging modality that should be used in evaluating TETs. Moreover, MRI has proved to be superior to CT in discriminating TETs from thymic hyperplasia and cysts and so should be used in equivocal cases[112,113]. FDG-PET in thymomas diagnosis and staging can lead to false negative results, as low-grade tumors may lack glucose uptake. At the same time, the differential diagnosis of FDG-PET positive mediastinal masses includes other tumors such as primary mediastinal germ cell tumors and lymphomas as well as non-neoplastic diseases (e.g. infections, thymic hyperplasia, fibrosing mediastinitis). On the other hand, FDG-PET is an essential test to rule out distant metastases in thymic carcinoma patients with localized disease at CT scan.

*Treatment*

*Surgery*

Surgery is the mainstay of treatment of localized TETs. Complete thymectomy is considered the standard approach for patients diagnosed with thymoma and

myasthenia gravis, while thymomectomy can be considered in non-myasthenic patients with stage I disease. Minimally invasive surgery should be considered an option only in early stages and carried out by experienced surgeons. Robot-assisted thoracic thymectomy has proved to be safe and feasible, with two meta-analyses suggesting less operative blood loss and better short-term post-operative outcomes as compared to the video-assisted procedure[114,115]. In locally advanced tumors, en bloc removal of all affected structures should be carried out, including also pleural deposits, whenever feasible. N1 lymphadenectomy is recommended in all TETs, while N2 sampling should be added in stage III/Iva thymomas and in thymic carcinomas and neuroendocrine tumors irrespective of clinical stage[116]. In selected cases, debulking resection of thymomas can be performed to facilitate subsequent radiotherapy (with or without chemotherapy) with radical intent. Such approach should be not pursued in TC. Surgery has also a role in recurrences, as complete resection of recurrent lesions, such as pleural metastases, is a major predictor of favorable outcomes in thymomas[117,118]. It should be underlined that, especially in thymoma management, multidisciplinary discussion is mandatory to optimize patient's outcomes.

*Radiotherapy*

Prospective, multicenter evidence on the role of post-operative radiotherapy (PORT) in TETs is scarce and all available data is based on Masaoka-Koga staging system. As patients with stage I Masaoka-Koga thymoma should not undergo PORT, stage II patients may receive PORT in presence of aggressive histology (type B2/B3) or extensive trans-capsular invasion according to ESMO guidelines. However, PORT is recommended in stage III and IVA thymoma as well as in R1 and R2 resection, despite the stage. For TC, PORT is recommended from stage III to IVA, should be considered for stage II, and is optional for stage I radically resected patients[116]. In unresectable patients with limited disease, radiotherapy can be considered the standard approach as a part of a sequential chemoradiotherapy strategy[116,119].

*Systemic treatments*

Chemotherapy has been historically considered the backbone of advanced, unresectable TETs. However, chemotherapy could be also considered as adjuvant therapy in stage II, III and IVA radically resected TC, and in all TETs after R2 resection. Moreover, some experts suggest to discuss adjuvant chemotherapy also in R1 resected B3 thymomas[120].

In patients with locally advanced TETs, primary/induction cisplatin-based chemotherapy is usually proposed after multidisciplinary discussion. These multi-drugs regimens led to 70-80% response rate (RR) and up to 50% of radical resections[121]. Usually, re-evaluation

to assess resectability is carried out after 2 to 4 cycles. Unfortunately, no prospective comparative trials of neoadjuvant chemotherapy versus chemo-radiotherapy are available, therefore this latter approach is rarely proposed.

Chemotherapy is the standard of care for unresectable or metastatic TETs. No randomized trials have been conducted to compare different regimens. However, the combination of cisplatin, doxorubicin, and cyclophosphamide (PAC) is considered the favored approach for thymomas based on its efficacy and tolerability, while carboplatin plus paclitaxel is usually administered to patients with TCs[122]. Second-line treatment of advanced thymomas could be based on platinum-doublets, the combination of capecitabine plus gemcitabine, or single-agent chemotherapy (including etoposide, pemetrexed, and ifosfamide), with response rates (RR) between 15-40%. Patients not eligible to chemotherapy may be treated with octreotide alone or in combination with prednisone upon *in vivo* demonstration of somatostatin receptors[123]. Second-line chemotherapy for TCs is based on similar regimens as for thymomas, although the expected RR is lower (5-26%).

Among biologic agents, the most promising appear to be the mammalian target of rapamycin (mTOR) inhibitor everolimus which has been evaluated in a phase 2 study of patients with platinum pre-treated TETs, including both thymomas and TCs[124]. The treatment led to a disease control rate (DCR) and mPFS of 93.8% and 16.6 months in 32 patients affected by thymomas, respectively, while DCR and mPFS were 61.1% and 5.6 months in TC patients.

Multi-tyrosine kinase inhibitors (TKI) with antiangiogenic activity such as sunitinib and lenvatinib showed interesting results in TCs. Sunitinib as second-line treatment showed a RR of 26% and mPFS of 7.2 months in 23 patients affected by TC in a phase 2 trial, while lenvatinib achieved a 38% RR and a mPFS of 9.3 months in 42 patients with advanced TC who have progressed to at least one platinum-based regimen in the REMORA phase 2 study[125,126]. As of today, sunitinib represents the second-line treatment of choice in advanced/metastatic TCs.

ICI has been also evaluated in TETs. However, thymomas are characterized by complex interactions with the immune system, as underlined by the frequent co-existence of autoimmune diseases and paraneoplastic immune-mediated syndromes. Trials with ICI showed promising activity in patients affected by thymomas but at the cost of severe toxicities, especially in patients with thymomas[127]. Results from cohort 1 of the phase 2 NIVOTHYM trial in patients with previously treated TETs (n:55, 78% with TC) showed a 6-month PFS rate of 35% and ORR of 12% with nivolumab[128]. Notably, 20% of

patients discontinued treatment due to adverse events, including 3 grade 4 events. A second cohort investigating the combination of nivolumab plus ipilimumab is currently enrolling patients [NCT03134118]. Recently published results from a phase 2 study in the same population (n:32, TC 84%) exploring the combination of the TKI axitinib with the anti-PD-L1 monoclonal antibody avelumab showed an ORR of 34%, with a 12% rate of serious adverse events including grade 3 or 4 polymyositis[129].

*Future perspectives*
Ongoing clinical trials are currently evaluating different systemic approaches in TETs.

Two phase 2 trials are evaluating the activity and safety of the combination of carboplatin plus paclitaxel and the anti-vascular endothelial growth factor receptor-2 (VEGFR-2) monoclonal antibody ramucirumab as first-line treatment of advanced and metastatic TETs [NCT03921671, NCT03694002], while another single-arm trial is evaluating carboplatin plus paclitaxel or nab-paclitaxel with pembrolizumab in the same setting [NCT04554524].

Ongoing trials in second or further treatment lines include those exploring anti PD(L)1 agents alone [NCT03134118, NCT03076554, NCT04321330] or in combination with lenvatinib (PECATI trial, NCT04710628) or sunitinib [NCT03463460].

Another trial is currently investigating the bispecific inhibitor of PD-L1 and CTLA-4 monoclonal antibody KN046 in TCs patients who already received anti-PD(L)1 drugs [NCT04925947]. Finally, bintrafusp alfa, a bifunctional fusion protein targeting transforming growth factor-beta and PD-L1, is also investigated in advanced, pre-treated TETs in a phase 2 trial [NCT04417660].

**Points for clinical practice**
SCLC arises due to the exposure to tobacco carcinogens, which initiate the inactivation of the tumor suppressor genes TP53 and RB1. Due to this correlation with cigarette smoking, patients with SCLC often have smoking related comorbidities like chronic obstructive pulmonary disease (COPD), cardiac disease and hypertension. The diagnosis is based primarily on histological appearance by light microscopy. Immunohistochemistry can play a role in dividing SCLC into four neuroendocrine subtypes. SCLC is staged according to the TNM classification. Chemotherapy still is the backbone therapy in all stages SCLC.

Mesothelioma arises due to the exposure to asbestos fibers, which can lead to an inflammatory reaction and thus an increased risk of malignant transformation of

mesothelial cells. In some cases, mesothelioma occurs due to a germline BAP1 mutation. The diagnosis is preferentially made on histology, additional immunohistochemistry (IHC) can be used to distinguish 3 histological subtypes. Mesothelioma are staged according to the TNM classification. Most patients with mesothelioma are not suitable for surgery due to the widespread pleural metastases, however surgery can be considered for selected patients where a macroscopic complete resection can be expected. Since 2020, nivolumab-ipilimumab has to be considered standard first line therapy, independent on histological subtype.

Thymic epithelial tumors are the most frequent tumors of the anterior mediastinum in adults. The diagnosis is frequently based on imaging; MRI has proved to be superior to CT in discriminating thymoma from thymic hyperplasia and cysts. Up to 35% of patients with thymoma are diagnosed with myasthenia gravis. TNM classification has replaced Masaoka-Koga staging system for thymic epithelial tumors. Surgery is the mainstay of treatment of localized thymic epithelial tumors, followed by post-operative radiotherapy dependent on stage or if not fully resected.

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# CHAPTER 3

## IMMUNOTHERAPY IN SMALL CELL LUNG CANCER: ONE STEP A TIME: A NARRATIVE REVIEW

**Daphne W Dumoulin**, Anne-Marie C Dingemans, Joachim G J V Aerts, Jordi Remon, Dirk K M De Ruysscher, Lizza E L Hendriks

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## ABSTRACT

Chemotherapy with or without radiotherapy has been the standard of care for many years for patients with small cell lung cancer (SCLC). Despite exceptionally high responses (up to 80%) with chemotherapy, the majority of patients relapse rapidly within weeks to months after treatment completion. Therefore, new and better treatment options are necessary. Recently, synergistic activity has been reported for the addition of immune checkpoint inhibitors (ICI) to standard platinum-based chemotherapy in the therapeutic strategy of advanced SCLC. For the first time after several decades, a significant survival improvement was achieved for this population. However, the overwhelming majority of patients do not respond to ICI, or relapse rapidly. There is need for better knowledge about the biology, histopathologic features, and molecular pathways of SCLC. This can probably help to identify the optimal predictive biomarkers, which are warranted to develop an individual therapeutic strategy including the rational use of a combination of immunotherapeutic agents. Here, we provide an overview of the rationale for and clinical results of the completed and ongoing trials using different strategies of immunotherapy in SCLC. In addition, opportunities for further improvement of therapies will be discussed, including the addition of radiotherapy, co-stimulatory antibodies, and other immune modifying agents.

## INTRODUCTION

Lung cancer is a major global health concern, and causes 1.6 million deaths yearly[1]. Small cell lung cancer (SCLC) represents about 10-15% of all lung cancers in Western countries. Smoking is the single most risk factor for developing SCLC[2]. With an incidence of 1-5/10.000, SCLC is recognized as an orphan disease[3]. At diagnosis, approximately 70% of patients already have detectable distant metastases[4]. The prognosis remains poor with a 5-year overall survival (OS) of 25-33% for limited disease SCLC (LD-SCLC) and 3% for extensive disease (ED-SCLC)[5–7]. Unfortunately, little progress has been made over the last decades[8].

For many years, chemotherapy has been the backbone of the treatment for all stages. Despite exceptionally high responses with chemotherapeutical agents like etoposide plus platinum, the majority of patients relapse rapidly[9]. In second line, the only approved treatment is topotecan, with significant toxicity, poor response rates of 24% and a median OS of 6 months[10]. Immune checkpoint inhibitors (ICI) such as programmed cell death 1 (PD-1) inhibitors or the programmed cell death-ligand1 (PD-L1) inhibitors have revolutionized the treatment of stage III and IV non-small cell lung cancer (NSCLC), reporting survival improvement either as monotherapy or in combination with chemotherapy[11–15], but their role in SCLC is less established. This narrative review provides an overview of the rationale for, and clinical results of immunotherapy in SCLC. Furthermore, ongoing challenges and directions for research are discussed.

## METHODS

For this narrative review, a search of the literature on PubMed (last search date Oct 4, 2020), as well as the meeting libraries of the largest oncological conferences (World Conference on Lung Cancer, ASCO, ESMO) was performed (last search date Oct 4, 2020). Only abstracts of full publications in English were considered eligible.

## RATIONALE FOR IMMUNOTHERAPY IN SCLC

Tumors can escape immune surveillance by a number of mechanisms. Inhibitory checkpoints have been recognized to play a key role in this process. By blocking the PD-1 receptor on T cells or PD-L1 receptor on tumor cells, cytotoxic antitumor activity by exhausted CD8+ T cells can be restored[16]. Furthermore, the co-inhibitory receptor cytotoxic T lymphocyte associated antigen 4 (CTLA-4) that is expressed on T cells, can

bind to CD80 and CD86 on antigen presenting cells with a higher affinity than the co-stimulatory receptor CD28, thereby blocking activation and proliferation of T cells[17].

Theoretically, SCLC should be an immunogenic tumor type due to several characteristics. Long-term exposure to carcinogens in cigarettes induces high rates of somatic mutations[18]. This is reflected in the high tumor mutational burden (TMB) of SCLC compared with other tumors[19]. Tumors with a high TMB are generally presenting more neoantigens, which can induce an immune response. In different tumor types, high TMB was indeed associated with response to ICI, although rarely, some cancers with low TMB are amongst the best responders to ICI[20,21]. In addition, SCLC is frequently associated with paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome (LEMS) and anti-Hu[22,23]. Paraneoplastic syndromes occur as a result from an immune response targeting antigens expressed by both SCLC and healthy tissues, in this example the central nervous system. Patients with neurological paraneoplastic syndromes may have a more inflamed tumor micro-environment and better OS compared to those without[24], and it has been suggested that the occurrence of baseline neuronal antibodies may be a potential predictive markers for the efficacy of ICI in SCLC[25,26].

In contrast to NSCLC, PD-L1 expression (associated with anti-PD-(L)1 efficacy) is lower in SCLC. Previous cohorts have reported PD-L1 tumor cell positivity ranging from 10%-19%, with less than 5% of tumors express PD-L1 ≥50%[27], although more recent data report PD-L1 expression in up to 39% of cases[28]. This may be related to the finding that levels of inflammatory cells, such as tumor infiltrating lymphocytes (TILs) are reduced in SCLC compared with NSCLC[29]. These cells are essential for an effective anti-tumor response. In addition, the expression of major histocompatibility antigens (MHC) class I and II are also frequently reduced. As a result, activated TILs are not able to recognize the tumor-associated antigens and cannot establish an anti-tumor response[30]. SCLC also has a high percentage (around 72%) of FOXP3 regulatory T-cells, which are immune suppressive cells that hamper the anti-tumor response[31].

## SCLC IMMUNOTHERAPY TRIAL DATA

### Checkpoint inhibition

*Previously treated SCLC extensive disease*

For second and higher lines of treatment, results of ICI are generally disappointing, although some patients derive long-term benefit. Currently, third line monotherapy treatment with nivolumab or pembrolizumab (both anti-PD-1) is approved by the FDA

(no EMA approval) for patients with metastatic SCLC, independent of PD-L1 expression and based on limited data[32,33].

The approval of nivolumab was based on the pooled data from the non-randomized phase 1/2 CheckMate032 trial (nivolumab or nivolumab combined with ipilimumab for advanced/metastatic solid tumors)[34]. In the randomized SCLC cohort, patients with progression after one or two prior chemotherapy regimens, were treated with nivolumab 3mg/kg every two weeks (Q2W) (N=147) or nivolumab 1mg/kg combined with ipilimumab 3mg/kg Q3W for four cycles followed by nivolumab 3mg/kg Q2W (N=96). Objective response rates (ORR) were 11.6% and 21.9%, respectively (odds ratio 2.12; 95% CI 1.06-4.26; p=0.03). This improved ORR did not result in a better OS: median and 24-months OS were 5.7 months (95% CI 3.8-7.6) and 17.9% (nivolumab monotherapy) and 4.7 months (95% CI 3.1-8.3) and 16.9% (nivolumab/ipilimumab). Among the low percentage of patients responding to nivolumab, 61.5% had a durable response of more than one year, so only a small proportion (about 7%) of patients obtains long-term benefit[35]. Grade 3-4 treatment related adverse events (TRAEs) were much more common with the immunotherapy combination (37.5% vs 12.9%).



Approval of pembrolizumab as a third-line treatment for ED-SCLC was based on the pooled analyses from the phase 1b KEYNOTE-028 (cohort C1, N=24, pembrolizumab 10mg/kg) and the phase 2 KEYNOTE-158 (cohort G, N=107, pembrolizumab 200mg) studies[28,36,37]. In the KEYNOTE-028, a PD-L1 expression ≥1% was mandatory for enrollment. In KEYNOTE-028, the ORR was 33%, the median PFS 1.9 months and the median OS 9.7 months. The median DOR was 19.4 months (3.6 - 20.0+ months). No relationship was shown between efficacy and level of PD-L1 expression[36]. In the KEYNOTE-158, 39% of patients had a PD-L1 expression ≥1%. The ORR was 18.7%, the median PFS 2.0 months and the median OS 8.7 months. PD-L1 correlated with improved ORR (35.7% in PD-L1 positive and 6% in PD-L1 negative tumors) and improved the OS (14.9 compared to 5.9 months). Twelve patients had DOR ≥9 months[28]. In the pooled analyses, 83 patients enrolled had already received ≥ 2 previous lines, 57% were PD-L1 positive. The RR and duration of response were 19.3% and not reached, whereas the median PFS and OS were 2.0 months and 7.7 months, respectively. The grade ≥ 3 immune-related adverse events (ir-AES) were 6%.

Whether there really exists a benefit of anti-PD1 inhibitors as third-line treatment or whether this benefit is just an over-selection of patients enrolled with good prognostic factors remains unknown, as there is no control arm. In the randomized phase III CheckMate 331 trial however, the efficacy of nivolumab was compared to topotecan or amrubicin as second line treatment[38]. The median OS was 7.5 (nivolumab (N=284))

versus 8.4 months (chemotherapy (N=285); hazard ratio (HR) 0.86; 95% confidence interval (CI): 0.72-1.04). Of note, survival curves in the nivolumab arm are under chemotherapy during the first 12 months, suggesting a potential deleterious effect in outcome with ICI in a subgroup of patients with SCLC. However, in an exploratory analyses patients with platinum-refractory SCLC, defined as relapse <90 days after completion of first-line chemotherapy, nivolumab improved the OS compared with chemotherapy (7.0 months versus 5.7 months, HR 0.71 (95% CI 0.54–0.94)). AEs were much more common with chemotherapy than with nivolumab: all grade AEs and grade 3-4 TRAEs were seen in 55% and 4% of nivolumab treated versus 90% and 93% in chemotherapy treated patients.

Atezolizumab (anti-PD-L1) was examined in a phase I trial in 17 patients, from which ≥65% third line[39]. Results were poor, with only 1 responder according to RECIST 1.1 (6%). Based on immune related response criteria, 4 patients (24%) responded, and this response was durable; 4 patients ≥6 months, and 2 of these ≥12 months. Median PFS was 1.5 months (95% CI 1.2-2.7) and median OS 5.9 months (95% CI 4.3-20.1). The most common AE was fatigue in 24% of the patients. Grade 3-5 toxicity was seen in 3 patients, including 1 death due to hepatic failure. These results were not confirmed in a subsequent phase II trial. The phase II, non-comparative IFCT1603 trial, randomizing ED-SCLC patients either to atezolizumab (N=49) or to topotecan (N=24) in second-line, showed disappointing results. After 6 weeks of treatment, 1 patient responded to atezolizumab (2.3%) and disease control rate (DCR) was 20.9% (95% CI 8.8-33.1). Of the chemotherapy treated patients, 2 responded, and DCR was 65%. Median PFS was significantly shorter with atezolizumab (1.4 months, 95% CI 1.2-1.5) compared to topotecan (4.3 months, 95% CI 1.5-5.9) (HR 2.26, p=0.04). OS was 9.4 months for atezolizumab compared to 11.4 months for chemotherapy (HR 0.84, p=0.60). Grade 3 toxicity was seen in 20 patients and consisted only of fatigue.

Another anti-PD-L1 antibody, durvalumab, was investigated as monotherapy in a small phase I/II expansion cohort[40]. Twenty-one patients with ED-SCLC were treated with durvalumab 10mg/kg. Partial response (PR) was seen in only 2 patients (9.5%), however these responses were durable (14.6 and 29.5+ months). Median PFS was 1.5 months (95% CI 0.9–1.8), median OS was 4.8 months (95% CI 1.3–10.4) and 12-month OS rate was 27.6% (95% CI 10.2–48.4). AEs were reported in 7 patients (33%), all grade 1-2.

Durvalumab was also investigated in combination with the anti-CTLA4 antibody tremelimumab for patients with refractory ED-SCLC in the phase II BALTIC study[41]. Preliminary results showed 2 out of 21 PR (9.5%), and a DCR at 12 weeks of 38% (8 patients). Grade ≥3 AEs were reported in 10 patients (48%).

**First line SCLC extensive disease**

Based on promising results of two phase II trials[11,25], the phased administration of ipilimumab in addition to chemotherapy was evaluated in a randomized double-blind phase III trial, enrolling 1132 patients with chemotherapy naïve ED-SCLC, who were randomized to chemotherapy (platinum-etoposide), combined with ipilimumab 10mg/kg or placebo[42]. Chemotherapy was administered for 4 cycles Q3W, ipilimumab or placebo was added from cycle 3 to 6 Q3W, followed by maintenance ipilimumab or placebo Q12W. The trial was negative for its OS primary endpoint (11.0 months versus 10.9 months ; HR 0.94; 95% CI, 0.81-1.09; p=0.377), and no PFS benefit was reported with ipilimumab compared with placebo (HR 0.85; 95% CI, 0.75-0.97, p=0.161), with similar toxicity profile (Grade ³3 TRAE's in 48% versus 45%, respectively). Diarrhea and colitis were the only grade ³3 TRAE's that were more frequently seen in patients treated with ipilimumab; 11% versus 1%.

Despite these negative results, based on the outcome with anti-PD-L1 in SCLC as monotherapy and the synergism observed with anti-PD(L)-1 and chemotherapy in patients with NSCLC, this strategy was explored in patients with SCLC. The randomized phase III IMpower 133 reported in 403 patients with ED-SCLC that the addition of atezolizumab to standard 4-cycles of etoposide-carboplatin chemotherapy followed by atezolizumab / placebo maintenance significantly improved the OS over placebo (12.3 months versus and 10.3 months, HR 0.70; 95% CI 0.54-0.91; p=0.007)[43]. The trial also did achieve the PFS co-primary endpoint (5.2 versus 4.3 months, HR 0.77, 95% CI 0.62-0.96; p=0.02).

In a recently published survival update after a median follow-up of 22.9 months, the OS benefit remained for atezolizumab although with an increasing HR (12.3 versus 10.3 months, HR 0.76; 95% CI 0.60-0.95; descriptive p=0.0154)[44]. Of note, survival rate at 18-months was 13% higher for atezolizumab compared with placebo (33.5% versus 20.4%). Patient characteristics associated with long-term survival (living ≥18 months since randomization) were good performance status, LDH ≤ upper limit of normal and sum of longest diameters of the tumor measurements < the median in the total group. Characteristics specifically predictive for atezolizumab benefit could not be identified[45]. Importantly, crossover to atezolizumab was not allowed in the trial and only 8% of patients received ICI at the time of progression in the control arm. Importantly, the addition of atezolizumab did not result in significantly increased toxicity. Moreover, health-related quality of life (HRQoL) as well as physical function, measured by EORTC QLQ-C30 and QLQ-LC13, improved during therapy with a trend of greater improvement with atezolizumab compared to placebo[46]. Based on these results, atezolizumab in

combination with chemotherapy is approved as a first line treatment for ED-SCLC by FDA and EMA[47,48].

Two other first line phase III trials with the combination of ICI and chemotherapy showed comparable results. The 3-arm CASPIAN trial included 805 patients with treatment-naïve ED-SCLC. Randomization was to platinum-etoposide or platinum-etoposide with durvalumab with or without tremelimumab (anti-CTLA-4)[49,50], stratified by chemotherapy regimen (carboplatin versus cisplatin). In the ICI arms, patients received durvalumab with or without tremelimumab as maintenance treatment in case of no progression to induction treatment. Prophylactic cranial irradiation (PCI) was only allowed in the chemotherapy arm, and crossover was not allowed. Baseline brain metastases were present in 10-14% of the patients (10% for the chemotherapy and chemotherapy plus durvalumab arms, 14% for the chemotherapy-durvalumab-tremelimumab arm), the majority (85-89%) of the patients with brain metastases had not received brain radiation treatment before study entry. Eight percent of patients in the control group were treated with PCI. In an updated analysis, durvalumab achieved the OS primary endpoint, reporting a median OS of 12.9 months compared with 10.5 months in the control group (HR 0.75; 95% CI 0.62-0.91; p=0.0032)[50]. After 18 months, there was an absolute OS benefit of 9% for durvalumab (32% vs 25% alive). The OS benefit with the addition of durvalumab was similar for the subgroup of patients with brain metastases, compared with the group without brain metastases[51]. Interestingly, despite no PCI in the durvalumab arm, the percentage of patients that developed brain metastases was similar to the chemotherapy control arm. The addition of durvalumab did not significantly increase the percentage of ≥ grade 3 toxicity. IrAE's were reported in 20% of patients treated with durvalumab, 5% had grade ³3. Functioning and HRQoL favoured the combination of durvalumab with chemotherapy compared to chemotherapy alone[52]. Durvalumab in combination with chemotherapy is recently approved as a first line treatment for ED-SCLC by the FDA[53]. However, the second experimental arm testing tremelimumab in addition to durvalumab and chemotherapy did not meet its primary endpoint of demonstrating a statistically significant improvement in OS in compared with chemotherapy alone[54]. The median OS was 10.4 months for tremelimumab and durvalumab in combination with chemotherapy compared with 10.5 months in the control group (HR 0.82; 95% CI 0.68-1.00; p=0.0451).

An exploratory subgroup analysis assessing a possible relationship between clinical characteristics and outcomes of patients who derived long-term benefit (PFS ≥12 months), showed that >3 times more patients treated with durvalumab and chemotherapy derived long-term benefit compared to chemotherapy alone. Clinical

characteristics associated with long-term immunotherapy benefit could not be identified[55].

The third first line chemotherapy-ICI combination trial is the KEYNOTE-604[56], which assessed the survival benefit of adding pembrolizumab versus placebo to standard first-line chemotherapy in 453 patients with ED-SCLC. The addition of pembrolizumab improved the median OS: 10.8 months versus 9.7 months (HR 0.80, 95% CI 0.64-0.98, p=0.0164), however the survival improvement did not meet the prespecified criteria for being considered a positive trial. The co-primary endpoint PFS improved significantly (4.8 months versus 4.3 months , HR 0.75, 95% CI 0.61-0.91; p=0.0023). Toxicities with the addition of pembrolizumab were as expected. HRQoL, measured by EORTC QLQ-C30 and QLQ-LC13, was improved during therapy in both arms, with a trend of greater improvement of cough, chest pain and dyspnea with pembrolizumab compared to placebo.



Of note, patients with an ECOG PS of 2 were excluded from all these phase III trials. The phase IIIb MAURIS trial will hopefully provide an answer whether these patients will also benefit from the addition of ICI to chemotherapy[57]. Furthermore, patients with asymptomatic brain metastases are eligible, and thoracic consolidation radiotherapy and PCI are allowed. SPACE (NCT04221529) is a similar trial, including specifically ECOG PS 2 patients.

An overview of the differences in design of the three first line chemo-ICI RCTs is provided in **Table 1**, outcomes of these trials are presented in **Table 2**.

Two recently presented phase II trials obtained similar results as the phase III trials mentioned above, and are summarized in **Table 2**. In contrast to the other trials, in the REACTION trial randomization occurred after 2 cycles of platinum-doublet chemotherapy, and only patients with a response were randomized to chemotherapy and pembrolizumab or chemotherapy alone. By randomizing only responding patients, the benefit of additional immunotherapy could be maximized[58].

*Maintenance strategies*

Initially, maintenance therapy with pembrolizumab after induction chemotherapy was investigated in a small single arm phase II trial (N=45)[59]. The median PFS was 1.4 months (95% CI 1.3-2.8), with a 1-year PFS of 13%. The median OS was 9.6 months (95% CI 7.0-12.0), with a 1-year OS of 37%. Afterwards, an exploratory analysis reported that PD-L1 expression (≥ 1% on stromal cells, N=20) correlated with outcome. Median PFS was 6.8 months for PD-L1 ≥ 1% compared to 1.3 months, and OS was 12.8 months compared to 7.6 months.

**Table 1.** Study design of IMpower133, CASPIAN and Keynote-604

| | IMpower133 | CASPIAN | Keynote-604 |
|---|---|---|---|
| **Number of patients** | 403 | 805 | 453 |
| **ICI** | atezolizumab | durvalumab +/- tremelimumab (3-arms) | pembrolizumab |
| **Phase** | III | III | III |
| **Design** | Double blind, placebo | Open label, sponsor blind | Double blind, placebo |
| **Primary endpoint** | PFS and OS | OS | PFS and OS |
| **Median age (years)** | 64 | 62 | NA |
| **Stratification** | Sex, ECOG, brain metastases | Type platinum | Type platinum, ECOG, baseline LDH |
| **Imaging** | Q6W till week 48, then Q9W | Q6W till week 12, then Q8W | Q6W till week 48, then Q9W |
| **Chemo backbone** | 4 cycles both arms<br>Carboplatin (AUC5)<br>Etoposide 100 mg/m2 | Up to 6 cycles in control arm<br>Carboplatin (AUC 5-6) or cisplatin 75-80mg/m2<br>Etoposide 80-100 mg/m2 | 4 cycles both arms<br>Carboplatin (AUC 5) or cisplatin 75mg/m2<br>Etoposide 100 mg/m2 |
| **Brain metastases** | Eligible if asymptomatic, treated, off steroids | Eligible if asymptomatic, treated, off steroids | Eligible if asymptomatic, treated, off steroids |
| **Presence of brain metastases** | 17% | 10-14% | NA |
| **PCI** | Both arms permitted | Only in control arm permitted | Both arms permitted |
| **Frequency of PCI** | 10% | 8% | NA |
| **TRT** | Not allowed | Not allowed | Not allowed |
| **Treatment beyond PD** | If clinical benefit | If clinical benefit | Permitted at investigators discretion |
| **Cross-over** | No | No | No |

NA = not available

**Table 2.** Outcomes of IMpower133, CASPIAN and Keynote-604

| Median OS (months) | ICI | placebo | HR | 95% CI | p-value |
|---|---|---|---|---|---|
| **IMpower133** | 12.3 | 10.3 | 0.76 | 0.60-0.95 | 0.0154 |
| **CASPIAN (D)** | 12.9 | 10.5 | 0.75 | 0.62-0.91 | 0.0032 |
| **CASPIAN (D+T)** | 10.4 | 10.5 | 0.82 | 0.68-1.00 | 0.0451 |
| **Keynote-604** | 10.8 | 9.7 | 0.80 | 0.64-0.98 | 0.0164 |

| Median PFS (months) | ICI | placebo | HR | 95% CI | p-value |
|---|---|---|---|---|---|
| **IMpower133** | 5.2 | 4.3 | 0.77 | 0.62-0.96 | 0.02 |
| **CASPIAN (D)** | 5.1 | 5.4 | 0.80 | 0.66-0.96 | unknown |
| **CASPIAN (D+T)** | 4.9 | 5.4 | 0.84 | 0.70-1.01 | unknown |
| **Keynote-604** | 4.8 | 4.3 | 0.75 | 0.61-0.91 | 0.0023 |

| 18-months OS | ICI | placebo |
|---|---|---|
| **IMpower133** | 33,5% | 20.4% |
| **CASPIAN (D)** | 32% | 25% |

D = durvalumab
T = tremelimumab
NA = not available



However, maintenance strategy with ICI has not been confirmed in the three-arm randomized phase III CheckMate451 trial. The study enrolled 810 patients and investigated the efficacy of nivolumab with or without ipilimumab (3mg/kg) as maintenance therapy after first line induction platinum-etoposide, compared to placebo[60]. Patient were stratified by ECOG performance score, prior PCI and sex. Out of patients enrolled, 12-16% had brain metastases and PCI was performed in 22% of patients. The primary endpoint was OS for the combination nivolumab and ipilimumab compared to placebo. Nivolumab/ipilimumab was not superior to placebo (9.2 and 9.6 months, HR 0.92; 95% CI 0.75–1.12; p=0.3693), with a 1-year OS rate of 41% and 40%, respectively. Nivolumab alone did neither result in an OS benefit compared with placebo (10.4 months versus 9.6 months, HR 0.84; 95% CI 0.69–1.02), with 1-year OS 44% and 40% respectively. In an OS subgroup analysis, there was an improved OS for patients treated within 5 weeks from last dose of chemotherapy for nivolumab compared to placebo. Remarkably, this benefit was not seen for nivolumab and ipilimumab. Grade ³3 AE were reported in 52% (nivolumab-ipilimumab), 12% (nivolumab) and 8% (placebo). The most common grade ³3 AE was diarrhea, respectively 5%, 1% and 0%. Based on these results switch maintenance treatment is not a standard treatment in patients with ED-SCLC.

A new strategy in clinical trials investigating maintenance therapy is the IMPULSE study. In this trial, the efficacy and safety of the toll-like 9 receptor-agonist lefitolimod was investigated as a maintenance treatment after induction platinum based chemotherapy[61]. No improvement for PFS or OS was found in the whole population, however two predefined patient subgroups showed promising results favoring lefitolimod: patients with a low frequency of activated CD86+ B-cells (HR 0.53, 95% CI 0.26-1.08) and patients with reported chronic obstructive pulmonary disease (COPD) (HR 0.48, 95% CI 0.20-1.1).

**Radiotherapy**

Based on the survival benefit found in the PACIFIC trial (adjuvant durvalumab in stage III NSCLC patients treated with concurrent chemoradiotherapy)[13], the addition of ICI to chemoradiation in SCLC is of interest and several trials are ongoing.

Radiotherapy combined with ICI holds promise for subgroups in stage IV NSCLC, as was shown for instance in the Pembro-RT study[62]. This potential role of thoracic radiotherapy in a concurrent setting with immunotherapy after upfront chemotherapy, was investigated in a phase I/II trial with nivolumab 1mkg/kg and ipilimumab 3mg/kg[63] in SCLC. 21 patients with ED-SCLC were treated with 4-6 cycles platinum-etoposide, followed by combination treatment with thoracic radiotherapy (10 fractions, total of 30 Gy), and ICI (nivolumab and ipilimumab). The six months PFS was 24% (95% CI 9%-43%), which is similar to historical data. This was the reason to discontinue this trial early. The median estimated PFS was 4.5 months (95% CI 2.7-4.6) and the median estimated OS was 11.7 months (95% CI 4.7-16.0).

A comparable phase I trial was performed with pembrolizumab in combination with thoracic radiotherapy after induction chemotherapy for ED-SCLC. 38 patients were treated with 16 cycles pembrolizumab every 3 weeks combined with 45 Gy thoracic radiotherapy in 15 daily fractions. Median PFS was 6.1 months (95% CI 4.1-8.1) and the median OS 8.4 months (95% CI 6.7-10.1)[64].

In conclusion, in line with the results of maintenance systemic treatments in stage IV disease, thoracic radiotherapy with ICI after induction chemotherapy failed to improve the outcome. The role of radiotherapy concurrent with ICI after induction chemo-immunotherapy is currently being investigated in the phase II-III RAPTOR trial (NCT04402788). Furthermore, several trials are ongoing in LD-SCLC, concurrent with chemoradiotherapy (phase II-III NRG-LU005 (NCT03811002), N=506, phase II trial (NCT03585998), N=51) or after concurrent chemoradiotherapy (phase II STIMULI (NCT02046733), N=174, phase II ACHILES study (NCT03540420), N=212, phase III

ADRIATIC study (NCT03703297), N=600). In addition, several comparable phase I trials are ongoing. Only for the STIMULI trial, results have been reported[65]. 153 patients with LD-SCLC were treated with 4 cycles of chemotherapy with concurrent radiotherapy followed by PCI. Non-progressing patients were randomized between adjuvant immunotherapy (nivolumab-ipilimumab) or observation. The primary endpoint, median PFS, was 10.7 months for nivolumab and ipilimumab compared to 14.5 months for observation (HR 1.02, 95% CI 0.66-1.58, p=0.93). Of note, the median time to discontinuation of treatment was 1.7 months for nivolumab and ipilimumab, of which 55% was due to toxicity.



**Vaccination trials**

Another way to induce an immune response is by using tumor vaccines. These vaccines can elicit an in vivo immune response specifically toward tumor-associated antigens formulated in the vaccine, or by directly administering antigen-stimulated T cells or dendritic cells (DCs). Tumor vaccines have already been shown to be promising and safe in NSCLC, however depending on the type of vaccine[66]. Cellular vaccination was found to be more active then peptide vaccination. Several trials using vaccinations were performed in SCLC or are still ongoing.

A randomized phase III trial in LD-SCLC investigated whether Bec2/bacille Calmette-Guerin (BCG) vaccination prolongs survival in patients with LD-SCLC responding to chemoradiotherapy[67]. Bec2 is an anti-idiotypic antibody that mimics GD3, which is expressed on the surface of tumor cells. 515 patients were randomized between five vaccinations or observation. This study did not meet its primary endpoint (OS). Median OS was 14.3 months for vaccination compared to 16.4 months for the observation group (HR 1.12, 95% CI 0.91-1.37, p=0.28). Median PFS was 5.7 months for vaccination compared to 6.3 months for observation (HR 1.11, 95% CI 0.95-1.36, p=0.30).

Another strategy is personalized peptide vaccination (PPV), in which vaccine antigens were selected based on pre-existing host immunity[68]. PPV was tested in 10 patients who failed to respond to chemotherapy with or without radiotherapy. Patients were vaccinated weekly for six consecutive weeks and then bi-weekly thereafter. In four patients, PPV was discontinued during the weekly vaccination due to rapid disease progression. The other six patients experienced a peptide-specific immunological boosting. Four patients had a survival of 25, 9.5, 6.5 and 6 months and 2 patients were still alive at data base lock (survival 24.5 and 10 months).

A comparable phase II trial of PPV was performed in 46 pretreated and patients with treatment-naïve ED-SCLC[69]. 70% of patients had IgG responses to the vaccinated

peptides after 1 vaccination cycle, 95% of patients after 2 cycles. Median OS was significantly improved in patients with augmented IgG responses to a greater number of nonvaccinated peptides after the second cycle of vaccination (1237 vs 382 days, p=0.010).

Two phase I trials were performed with vaccinations administered to patients with LD-SCLC as well as ED-SCLC who had a major response to first line chemotherapy. One of these trials tested the immunogenicity of three different doses of a synthetic version of ganglioside fucosyl-GM1-KLH conjugate[70]. Vaccination was found to be safe an induced an IgM-antibody response. The other trial was using vaccination with polysialic acid (polySA)[71]. PolySa is a polymer side chain bound to the neural cell adhesion molecule that is extensively expressed on the surface of SCLC cells. This vaccination also was found to be safe and resulted in higher antibody responses.

Another trial investigated a vaccine consisting of DC transduced with the full-length wild-type p53 gene delivered via an adenoviral vector in 29 patients with ED-SCLC[72]. P53 is mutated in approximately 90% of SCLC. In this trial, 57.1% of the patients had p53-specific T cell responses to the vaccination. However, only 1 patient showed a clinical response. Interestingly, 61.9% of patients responded to chemotherapy that immediately followed vaccination. These responses were associated with induction of immunologic response to vaccination, which suggests that more effective treatment results are possible by optimal use of vaccination combined with chemotherapy.

In conclusion, to date, the only phase III trial evaluating a tumor vaccine in SCLC has failed to improve OS, other trials are small and benefit of vaccines has not been established. It seems difficult to stimulate the response of the patients' immune system using vaccine therapy. However, the use of a combination of therapies might enhance the effect of tumor vaccines.

**Trials using interferon**

Interferon (IFN) is the first discovered cytokine with efficacy on cancer cells[73]. It was first used in natural form, followed by using in recombinant form. IFN is released by host cells in response to viral stimulation and can activate immune cells, such as macrophages and natural killer cells. In addition, IFN upregulates major histocompatibility complex (MHC) antigens leading to an increased antigen presentation. Furthermore, IFN suppresses angiogenesis and suppresses the proliferation of endothelial cells leading to a decreasing tumor growth.

In the early 1980s, recombinant IFN was investigated in combination with chemotherapy in SCLC patients, with the aim to prevent early relapse after chemotherapy, after in vitro studies have shown a durable effect by using IFN in combination with chemotherapy[74]. These studies showed modest or no improvement in survival[75,76].

In 2013, the effect of interferon combined with chemotherapy was evaluated in a randomized phase II trial with 164 patients[77]. Patients were treated with chemotherapy alone, a combination of chemotherapy with IFN-alfa, IFN-gamma or IFN-alfa and IFN-gamma. Median survival was 10 months for chemotherapy alone (95% CI 9.3–10.6), 10.3 months for chemotherapy with IFN-alfa (95% CI 7.13–13.5), 8.3 months for IFN-gamma (95% CI 6.8–9.8 months), and 11 months for IFN-alfa and IFN-gamma (95% CI 9.2–12.8 months), concluding no significant difference among all four groups. However, looking to only patients with LD-SCLC, a significant survival benefit was found for chemotherapy with IFN-alfa, showing a median OS of 34 months compared to 19 months for chemotherapy alone (p=0.039), 13.6 months for INF-gamma (p=0.005) and 17 months for IFN-alfa and IFN-gamma (p=0.038). IFN remains a potential auxiliary therapy in patients with SCLC, and further trials are needed to identify its effect.



**Trials using novel agents**

Another promising strategy is the use of antibodies against tumor-associated antigens. Ganglioside fucosyl-GM1 (FucGM1) is expressed in SCLC but absent in most normal tissues. Preclinically, the addition of anti-PD-1 or anti-CD137 to BMS-986012 (FucGM1 antibody) improved the therapeutic efficacy of BMS-986012 significantly[78]. BMS-986012 showed early promising safety and efficacy in pretreated SCLC patients when combined with nivolumab.

Stimulating the innate immune system is also an attractive option. Phagocytosis of SCLC cells by macrophages is inhibited by CD47 expression on the SCLC. Preclinically, anti-CD47 resulted in SCLC tumor responses[79]. Phase I trials including patients with solid malignancies are ongoing, also with the addition of pembrolizumab.

**Biomarkers**

With the FDA approval of PD-(L)1 ICI in combination with chemotherapy as a first line treatment in ED-SCLC and PD-1 ICI as a third line option in ED-SCLC, we have made an important advance in treating SCLC patients, whose treatment options have remained unchanged for decades. However, critical analysis of these trials shows that only a small part of the patients benefits from ICI. Identifying these patients is a real clinical challenge.

Several biomarkers have been investigated or are currently being investigated. The most evaluated biomarkers are PD-L1 and TMB.

**PD-(L)1**

PD-1 is a receptor expressed on the surface of T-cells regulating T-cell activation and proliferation. Its ligand PD-L1 is (over)expressed on various tumor cells. In contrast to NSCLC, where PD-L1 expression is used in deciding which therapy is preferred, the clinical relevance of PD-L1 expression in SCLC has remained unclear.

*PD-L1 expression on tumor cells*

In the Checkmate032, PD-L1 expression (tumor cells [TC]) was evaluated in 148 patients with the 28-8 pharmDx antibody[80]. PD-L1 expression was found to be positive »10% of cases, negative in »60% and unknown in »30%. No significant association was found between PD-L1 expression and ORR for nivolumab or combination nivolumab with ipilimumab.

*PD-L1 expression on tumor or immune cells*

Of the 403 included patients in the IMpower133 trial, only 137 patients had evaluable tumor material, which reflects the difficulty in obtaining biopsies in SCLC. PD-L1 expression was analyzed on TC as well as on immune cells (IC) with the VENTANA SP263 antibody[44]. 129 patients (94.2%) had PD-L1 TC expression <1% and 68 patients (49.6%) had PD-L1 IC expression <1%. In subgroup analyses, an inverse correlation was found between PD-L1 expression on TC or IC and OS: a negative PD-L1 expression appeared to be predictive for a better OS (median OS 10.2 months for combination chemotherapy with atezolizumab versus 8.3 months for chemotherapy with placebo, HR 0.51, 95% CI 0.30-0.89). This result is inconsistent with previous data and needs further analysis.

*Combined PD-L1 positive score*

Combined PD-L1 positive score (CPS) has not yet been evaluated in first line therapy, only in relapsed SCLC. CPS is the sum of the number of PD-L1-stained cells, such as TC, lymphocytes and macrophages), divided by the total number of viable TC, multiplied by 100[81]. The maximum score is defined as 100.

CPS was evaluated in the KEYNOTE-028 trial and in the KEYNOTE-158. In the KEYNOTE-028, CPS ≥1% was an inclusion criterion for treatment with pembrolizumab. ORR was 33% (8 of 24)[36]. The KEYNOTE-158 was stratifying patients into a CPS≥1% (n=48) subgroup and a CPS<1% (n=50) subgroup. Results in de CPS≥1% subgroup where an ORR of 35.7%, a 1year OS of 53.1% and a median OS duration 14.6 months, compared

to respectively 6%, 30.7% and 7.7 months for the CPS<1% subgroup. Among patients with unknown PD-L1 status, ORR was 27% (4 of 15)[28].

In conclusion, no correlation was found between PD-L1 expression and efficacy in SCLC. However, looking to KEYNOTE-158 using combined PD-L1 score, CPS might to have predictive value.

**Tumor mutational burden**



TMB is usually defined as the number of somatic mutations found in the DNA of cancer cells per megabase (Mb)[82]. Determination of TMB can be done by several DNA sequencing methods, of which Whole Exome Sequencing (WES) is considered the gold standard. Some trials are using targeted next-generation sequencing (NGS) panels to extrapolate TMB, which leads to a variation in thresholds for TMB and reproducibility of TMB. This causes inter-assay variation, which limits the utility of panel-based TMB. Therefore, WES remains the most comprehensive, reproducible and reliable method to determine TMB[83].

TMB was evaluated in the CheckMate032 using WES. The tertiles were defined as <143 mutations (low), 143–247 mutations (intermediate) and ≥248 mutations (high). Of all treated patients, 61% had sufficient paired tumor and whole blood samples for WES. In 86% of these patients WES could be performed successfully: overall 211 (53%) of all treated patients were evaluable for efficacy analyses by TMB[80]. In the Checkmate032, ORR was 5% in the TMB low subgroup (n=42), 7% in the intermediate subgroup (n=44) and 21% in the TMB high subgroup (n=47). Median PFS was 1.3 months, 1.3 months and 1.4 months, median OS was 3.1 months, 3.9 months and 5.4 months and 1-year OS were 22%, 26% and 35% respectively[80]. For the patients treated with combination nivolumab 1mg/kg and ipilimumab 3mg/kg, results were comparable, showing ORR of respectively 22%, 16% and 46%, median PFS of 1.5 months, 1.3 months and 7.8 months, median OS 3.4 months, 3.6 months and 22 months and 1-year OS of 23%, 20% and 62%.

The KEYNOTE-028 was also evaluating TMB in multiple tumor types. However, in this basket trial consisting of 471 patients in total, TMB data were available for 77 patients, and of these only 4 patients had SCLC[84]. For all tumor types, higher TMB was associated with higher ORR (p=0.018) and longer PFS (p=0.051). The KEYNOTE-158, a basket trial consisting of 1032 patients, contained 751 patients with evaluable TMB in multiple tumor types. Of these, 75 patients had SCLC. The RR for patients with higher TMB was 29% (10 out of 34 patients) compared to 10% for patients with low TMB (4 out of 41 patients)[85].

In conclusion, TMB may be useful to predict benefit from immunotherapy in SCLC. However, the sample size is too limited to draw firm conclusions and the difficulties to encounter when using TMB, makes TMB as a challenging and not preferable biomarker in practice.

**Blood-based tumor mutational burden**

WES using tumor tissue has several disadvantages, for example the need of tumor biopsies, the time-consuming analysis and the costs. BTMB is measured by targeted NGS using cell-free DNA (cfDNA)[86]. Using NGS on blood instead might overcome the previously stated problems.

The IMpower133 investigated the correlation between bTMB and OS[43]. 394 cancer-associated genes were assessed by NGS. Two cut-offs of bTMB were used: 10 mut/Mb and 16 mut/Mb. Using a cut-off of <10mut/Mb (n=139), median OS was 11.8 months for combination chemotherapy and atezolizumab compared to 9.2 months for chemotherapy alone (HR 0.70, 95% CI 0.45-1.07). For patients with bTMB of ≥10 mut/Mb (n=212), median OS was respectively 14.6 months compared to 11.2 months (HR 0.68, 95% CI 0.47-0.97).

A similar improvement in OS was found by using another cut-off of 16mut/Mb: for patients with a bTMB <16mut/mB, median OS was 12.5 months for combination chemotherapy and atezolizumab compared to 9.9 months for chemotherapy alone (HR 0.71, CI 0.52-0.98). For patients with a bTMB of ≥16 mut/Mb, median OS was 17.8 months compared to 11.9 months respectively (HR 0.63, 95% CI 0.35-1.15).

Because the different cut-off subgroups are showing similar improvements in OS, bTMB seems not to be a useful predictive biomarker.

## FUTURE DIRECTIONS

Although the outcome of patients with SCLC has improved by adding ICI to standard first line chemotherapy, and durable responses have been observed with ICI as monotherapy in third line, the majority of the patients do not benefit. Better patient selection is needed, as well as new combinations of drugs. Furthermore, the role of PCI should be redefined in the ICI era. Possible future directions are discussed below.

**Future directions for biomarkers**

The immune contexture of SCLC is often an immune-excluded, non-inflamed T-cell environment[87–89]. Furthermore, SCLC cells often express CD47[79,90], which protects the cells from phagocytosis by macrophages and dendritic cells. Moreover, TILs often express co-inhibitory immune checkpoint proteins such as TIM3, LAG3 and FOXP3[91].

Full characterization of the immune environment is challenging due to the difficulty of obtaining enough tissue and subsequently analyzing this tissue in patients that often have a rapidly deteriorating clinical condition. To date, there are no useful predictive biomarkers to select the subgroup of patients that might have long term benefit from immunotherapy. A recent multicenter retrospective analyses suggested a correlation between irAEs and response to ICI[92]. In 183 patients treated with anti-PD-(L)1 (59.6%) with or without a CTLA-4 inhibitor, irAEs were reported in 39.9%. The ORR of patients who experienced at least one irAE was 27.4%, compared to 3.6% for patients without irAEs. Furthermore, the median PFS was 3.8 and 1.3 months respectively, the median OS 13.8 and 2.9 months. These results were adjusted for age, sex, performance status and presence of brain metastases and need to be prospectively validated.

Biopsies upon progression on ICI are also needed in order to optimize treatment for patients with SCLC. Hopefully the REBIMMUNE trial (NCT04300062) will elucidate some of these mechanisms.

It would also be interesting to evaluate the value of serial circulating tumor DNA (ctDNA) measurements as monitoring option for patients with SCLC treated with ICI. Indeed, in small series, a decrease in ctDNA has been associated with platinum-sensitivity in SCLC[93,94]. Although data exists for NSCLC[95], data for monitoring of ICI efficacy in SCLC are very limited. In a small series (N=27 patients with SCLC, 8 receiving ICI), monitoring by using plasma cell-free DNA (cfDNA) has been described[96]. An increase in cfDNA could be identified before the occurrence of radiological progression. If validated, this could provide a non-invasive option for treatment monitoring.

**Future directions for new combinations of drugs**

Due to the immune-excluded environment of SCLC, the combination of other treatments with immunotherapy seems logical to convert SCLC to an immunogenic tumor. Several options are described below. A summary of all ongoing trials can be found in **Table 3**.

**Table 3.** Ongoing trials with immunotherapy in SCLC

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| **LIMITED STAGE** | | | | | | |
| Concurrent with chemoradiotherapy | | | | | | |
| NCT03811002 (NRG-LU005) | II/III | Atezolizumab | Randomized | PFS/OS | 506 | National Cancer Institute (NCI), NRG Oncology |
| NCT03585998 | II | Durvalumab | Single-arm | PFS | 51 | Samsung Medical Center |
| NCT03509012 (CLOVER) | I | Durvalumab +/- tremelimumab + chemoradiotherapy | Non-randomized | safety | 360 | AstraZeneca |
| NCT02402920 | I | Pembrolizumab | Non-randomized | Safety | 80 | M.D. Anderson Cancer Center, National Cancer Institute (NCI) |
| After concurrent chemoradiotherapy | | | | | | |
| NCT03703297 (ADRIATIC) | III | Durvalumab +/- tremelimumab | Randomized | PFS/OS | 600 | AstraZeneca |
| NCT02046733 (STIMULI) | II | Nivolumab + ipilimumab | Randomized | OS | 264 | European Thoracic Oncology Platform, Intergroupe Francophone de Cancerologie Thoracique, Ludwig Center for Cancer Research of Lausanne, Frontier Science Foundation, Hellas, Bristol-Myers Squibb |
| NCT03540420 (ACHILES) | II | Atezolizumab | Randomized | 2y-OS | 212 | multiple |
| **EXTENSIVE STAGE OR RECURRENT** | | | | | | |
| First line | | | | | | |
| NCT04028050 (MAURIS) | IIIB | Atezolizumab + chemotherapy | Single-arm | safety | 150 | Hoffmann-La Roche |
| NCT04256421 (SKYSCRAPER-02) | III | Carboplatin, etoposide, atezolizumab +/- tiragolumab (anti-TIGIT) | Randomized | PFS, OS | 400 | Hoffmann-La Roche |
| NCT04012606 | III | Toripalimab + chemotherapy | Randomized | PFS and OS | 420 | Shanghai Junshi Bioscience Co., Ltd. |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT04005716 | III | Tislelizumab + chemotherapy | Randomized | PFS and OS | 364 | BeiGene |
| NCT04063163 | III | HLX10 (anti-PD-1 ) + chemotherapy | Randomized | PFS | 489 | Shanghai Henlius Biotech |
| NCT03382561 | II | Nivolumab + chemotherapy | Randomized | PFS | 150 | National Cancer Institute (NCI) |
| NCT03568097 (PAVE) | II | Avelumab + chemotherapy | Single-arm | PFS | 55 | Hellenic Cooperative Oncology Group, Merck KGaA, Darmstadt, Germany |
| NCT03041311 | II | Carboplatin, etoposide, atezolizumab +/- trilaciclib (CDK4/6 inhibitor) | Randomized | effect on myelosuppression | 105 | G1 Therapeutics, Inc. |
| NCT04221529 (SPACE) | II | Carboplatin, etoposide, atezolizumab in patients with ECOG PS 2 | Single-arm | OS | 70 | AIO-Studien-gGmbH, Hoffmann-La Roche |
| First line maintenance | | | | | | |
| NCT03516084 | III | Niraparib as maintenance therapy following first-line chemotherapy | Randomized | PFS and OS | 591 | Zai Lab (Shanghai) Co., Ltd. |
| NCT03410368 | II | NK cell-based immunotherapy as maintenance therapy | Randomized | PFS | 120 | jiuwei cui |
| NCT03983759 | II | Chemotherapy plus R-CIK followed by sintilimab as maintenance therapy | Single-arm | OS | 40 | Henan Cancer Hospital |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT04334941 | II | Atezolizumab + talazoparib in patients SLFN11 Positive | Randomized | PFS | 94 | National Cancer Institute (NCI) |
| Second line and beyond | | | | | | |
| NCT02963090 | II | Pembrolizumab | Randomized | PFS | 98 | Alliance Foundation Trials, LLC, Merck Sharp & Dohme Corp. |
| NCT03670056 (CA209-9YT) | II | Nivolumab + ipilimumab | Single-arm | Change in Teff/ Treg | 40 | Yale University |
| Ongoing trials with radiotherapy | | | | | | |
| NCT04402788 (RAPTOR, NRG-LU007) | II/III | Atezolizumab +/- radiotherapy after chemotherapy and atezolizumab | Randomized | PFS and OS | 324 | National Cancer Institute (NCI) |
| NCT02701400 | II | Durvalumab + tremelimumab +/- radiotherapy | Randomized | PFS/ORR | 18 | Emory University, AstraZeneca |
| NCT02934503 | II | Chemotherapy + pembrolizumab +/- radiotherapy | Single-arm | Change in PD-L1 expression | 60 | NYU Langone Health |
| NCT03262454 | II | Sequential hypofractionated radiotherapy followed by atezolizumab | Single-arm | ORR | 35 | National Cancer Center, Korea, Roche Korea co.,Ltd. |
| NCT03262454 | II | Hypofractionated radiation therapy followed by atezolizumab | Single-arm | ORR | 35 | National Cancer Center, Korea |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT03043599 | I/II | Ipilimumab + nivolumab + thoracic radiotherapy after chemotherapy | Single-arm | PFS | 21 | H. Lee Moffitt Cancer Center and Research Institute, Bristol-Myers Squibb |
| NCT02402920 | I | Pembrolizumab + radiotherapy | Non-randomized | Safety | 80 | M.D. Anderson Cancer Center, National Cancer Institute (NCI) |
| NCT03923270 | I | Thoracic radiotherapy + durvalumab +/- tremelimumab or olaparib | Non-randomized | safety, PFS | 54 | H. Lee Moffitt Cancer Center and Research Institute, AstraZeneca |
| NCT03532880 | I | Olaparib + low dose thoracic radiotherapy | Single-arm | safety | 24 | Memorial Sloan Kettering Cancer Center |
| NCT04170946 | I | Talazoparib + low dose thoracic radiotherapy | Non-randomized | safety | 24 | University Health Network, Toronto, Pfizer |
| Ongoing trials with other agents | | | | | | |
| NCT04192682 | II | Anlotinib + sintilimab | Single-arm | PFS | 40 | Changzhou Cancer Hospital of Soochow University |
| NCT02554812 (JAVELIN medley) | II | Avelumab + utomilumab (antiCD137) | Randomized | ORR | 600 | Pfizer |
| NCT03728361 | II | Nivolumab + temozolomide | Single-arm | ORR | 53 | Dwight Owen, National Cancer Institute (NCI) |
| NCT02628067 | II | Pembrolizumab (biomarker analysis) | Single-arm | ORR | 1395 | Merck Sharp & Dohme Corp. |
| NCT03958045 | II | Rucaparib + nivolumab | Single-arm | PFS, OS | 36 | Aman Chauhan |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT03672773 | II | Talazoparib + temozolomide | Single-arm | ORR | 28 | Jonsson Comprehensive Cancer Center, Translational Research in Oncology, National Cancer Institute (NCI) |
| NCT04010357 | II | Abemaciclib (CDK4/6 inhibitor) | Single-arm | ORR | 29 | Case Comprehensive Cancer Center |
| NCT04055792 | II | Sintilimab + anlotinib | Randomized | PFS | 52 | Henan Cancer Hospital |
| NCT03253068 | II | Pembrolizumab + amurubicin | Single-arm | ORR | 25 | Wakayama Medical University, Merck Sharp & Dohme Corp. |
| NCT03994744 | II | Sintilimab + metformin | Single-arm | safety and ORR | 68 | Hunan Cancer Hospital |
| NCT03406715 | II | Nivolumab + ipilimumab + dendritic cell-based p53 vaccination | Single-arm | DCR | 41 | H. Lee Moffitt Cancer Center and Research Institute, Bristol-Myers Squibb, MultiVIr, Inc. |
| NCT04079712 | II | XL184 (Cabozantinib), Nivolumab, and Ipilimumab | Single-arm | ORR | 30 | National Cancer Institute (NCI) |
| NCT03126110 | I/II | INCAGN01876 (anti-GITR) + nivolumab +/- ipilimumab in solid malignancies | Non-randomized | safety, ORR | 285 | Incyte Biosciences International Sàrl |
| NCT03241173 | I/II | INCAGN01949 (anti-OX40), + nivolumab +/- ipilimumab in solid malignancies | Non-randomized | safety, ORR | 52 | Incyte Biosciences International Sàrl |
| NCT02734004 (MEDIOLA) | I/II | Olaparib + durvalumab | Single-arm | safety, DCR, ORR | 427 | AstraZeneca |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT03830918 | I/II | Niraparib + temozolomide | Randomized | safety, PFS | 64 | Jonsson Comprehensive Cancer Center, Translational Research in Oncology, National Cancer Institute (NCI) |
| NCT02247349 | I/II | Nivolumab + BMS-986012 (FucGM1 antibody) | Non-randomized | safety | 172 | Bristol-Myers Squibb |
| NCT03227016 | I/II | Veliparib + topotecan | Single-arm | safety | 30 | Central European Society for Anticancer Drug Research |
| NCT02446704 | I/II | Olaparib + temozolomide | Single-arm | safety and ORR | 106 | Massachusetts General Hospital, AstraZeneca |
| NCT03575793 | I/II | Nivolumab, ipilimumab + plinabulin | Randomized | safety and PFS | 55 | Jyoti Malhotra |
| NCT03554473 | I/II | M7824 + topotecan or temozolomide | Non-randomized | ORR | 67 | National Cancer Institute (NCI) |
| NCT04209595 | I/II | PLX038 (PEGylated SN38) + rucaparib | Non-randomized | safety and ORR | 62 | National Cancer Institute (NCI) |
| NCT03126110 | I/II | INCAGN01876 + nivolumab +/- ipilimumab | Non-randomized | safety and ORR | 285 | Incyte Biosciences International Sàrl |
| NCT02688673 | I/II | Vaccination with dendritic cells plus cytokine-induced killer cells | Single-arm | ORR | 30 | Affiliated Hospital to Academy of Military Medical Sciences |
| NCT03708328 | I | PD-1/TIM-3 Bispecific Antibody (RO7121661) | Non-randomized | safety, ORR, DCR, PFS | 300 | Hoffmann-La Roche |
| NCT04253145 | I | Lurbinectedin + atezolizumab | Single-arm | safety | 25 | Fundacion Oncosur |

**Table 3.** (Continued)

| Trial name / NCT | Phase | Treatment | Allocation | Primary endpoint | Enrollment | Sponsor |
|---|---|---|---|---|---|---|
| NCT03763149 | I | CD47 monoclonal antibody Injection (IBI188) | Single-arm | safety | 42 | Innovent Biologics (Suzhou) Co. Ltd. |
| NCT03013218 | I | Pembrolizumab + ALX148 (anti-CD47) | Non-randomized | safety | 184 | ALX Oncology Inc. |
| NCT03319940 | I | AMG 757 (Bispecific T-cell engager targeting delta-like protein 3) +/- pembrolizumab | Randomized | safety | 162 | Amgen |
| NCT03392064 | I | AMG 119 (Chimeric antigen receptor T-cell therapy targeting delta-like protein 3) | Non-randomized | safety | 6 | Amgen |
| NCT04173325 | I | Nivolumab + irinotecan | Single-arm | safety and ORR | 10 | Augusta University |
| NCT03228667 | I | RO7121661 (PD-1/TIM-3 bispecific antibody) | Non-randomized | safety, ORR, DCR, PFS | 280 | Hoffmann-La Roche |

(Thoracic) radiotherapy is a logical option to add to ICI, or other immune modifying drugs as radiation acts synergistically with immunotherapy[97]. Unfortunately, as described above, TRT (45 Gy) combined with ICI, did not improve outcomes[59,64]. Moreover, median OS was disappointing with 8.4 months, and similar to the CREST-trial[98]. Furthermore, adjuvant nivolumab-ipilimumab failed to improve OS for patients with LD-SCLC treated with chemoradiation[65]. Results from other trials are awaited.

SCLC is very vulnerable to DNA damage; therefore, DNA damage repair inhibitors (like PARP inhibitors) and cell cycle checkpoint kinase inhibitors (inhibition of for example CHK1, WEE1, aurora kinase A (AURKA), cyclin-dependent kinase 7 (CDK7) are of interest[99]. However, results in unselected SCLC patients using monotherapy or combination therapies with chemotherapy were generally disappointing[100]. Combinations with immunotherapy are currently tested preclinically or in early phase trials.



PARP inhibition results in STING pathway activation, interferon signaling and enhancement of Tcel CD4 /CD8 infiltration, at least in vivo[101]. Unfortunately, a phase II trial evaluating the combination of olaparib and durvalumab in relapsed SCLC (N=20) was negative[102]. It could be that more potent PARP inhibitors such as talazoparib or niraparib, or PARP inhibition combined with CHK1 inhibition together with an ICI can obtain better outcomes[101].

Preclinically and in breast cancer patients, cyclin-dependent kinase 4/6 (CDK4/6) inhibition with abemaciclib or palbociclib resulted in enhanced anti-tumor immunity, by increasing the functional capacity of tumor cells to present antigens and by reducing the proliferation of Tregs[103]. CDK4/6 inhibitors are also investigated in SCLC.

Selective CDK7 inhibition (with YKL-5-124) is also promising, as besides inducing DNA replication stress and genomic instability, it also induces immune response signaling[103]. The combination with anti-PD-1 is being tested preclinically.

Lurbinectedin, targeting the enzyme RNA polymerase II, and inducer of DNA double-strand breaks, showed promising activity in phase I and II trials, either as monotherapy or combined with doxorubicin[104–106]. Interestingly, in preclinical models, lurbinectedin acted synergistically with ICI, and reduces tumor associated macrophages[107,108]. The combination of lurbinectedin with atezolizumab is being investigated in SCLC in a phase I trial.

Instead of checkpoint blockade, co-stimulation of T-cell responses with monoclonal antibody agonists is also being explored. Several trials are ongoing with or without combination with ICI and/or chemotherapy.

**Future directions for selection of patients**

As is described above, multiple combination therapies are possible, and it is challenging to select the most promising treatments, and to select the right SCLC patient for each treatment.

In a recently published paper, Rudin *et al* subdivided SCLC into different neuroendocrine subtypes which can be distinguished based on the expression of four key transcriptional regulators: ASCL1 (=ASH1), NEUROD1, POU2F3 and YAP1[99]. Four subtypes were described: The first two are markers of the SCLC neuroendocrine subtypes, the latter two of the non-neuroendocrine ones. The most common (70%) is the "classical" SCLC (SCLC-A) which is characterized by ASCL1 expression. Regarding genomic profile, this subtype has both TP53 and RB1 loss. On a transcriptional level, SCLC-A is characterized by high ASCL1, INSM1, L-MYC and DLL3 expression, and low NEUROD1 expression. Immunohistochemically, SCLC-A is TTF-1 high and C-MYC low. The other neuroendocrine variant (11% of cases) is characterized by NEUROD1 expression and is called SCLC-N. Besides NEUROD1 expression, it differs from SCLC-A in ASCL1 expression (variable) and TTF-1 and C-MYC expression (low and high, respectively). The most common non-neuroendocrine (16% of SCLC cases, SCLC-P) variant is characterized by POU2F3 expression. It further differs from the SCLC-A variant regarding ASCL1 and INSM1 expression (low). The last variant (SCLC-Y) is rare (3%) and is characterized by YAP1 expression. It is RB1 wildtype/enriched, ASCL1, NEUROD1 and ISM1 low[99].

It might be that different subtypes have different immune environments. For example, in a small series of 61 cases of SCLC and pulmonary carcinoids, those with SCLC-Y were enriched for a T-cell inflamed phenotype[109]. These subtypes might be used in the future to select (immune) therapies most suitable for combination with PD-(L)1-inhibitors.

For example, DLL3 expression is high only in SCLC-A. Although theoretically interesting, DLL3 inhibition alone with Rova-T failed to meet the prespecified endpoints in the phase II TRINITY trial including pretreated DLL3 expressing SCLC[110]. It could be interesting to combine Rova-T with ICI in DLL3 high patients. In a phase I/II study (N=42) Rova-T was combined with nivolumab plus ipilimumab (N=12) or nivolumab alone (N=30). The nivo-ipi group was prematurely closed due to toxicity, and also Rova-T plus nivolumab was toxic (53% ≥ grade 3 toxicity of which 10% grade 5). Furthermore, ORR was 22% in the Rova-T nivolumab group with a disappointing median DOR of 3.8 months[111]. Based

on the negative MERU and TAHOE studies, the Rova-T program is now discontinued. Bispecific T-cell engagers and chimeric antigen receptor (CAR) T-cell constructs are currently being investigated. The use of these drugs seems most interesting in DLL3 high patients (SCLC-A subtype), but in both phase I trials, no selection is performed based on DLL3.

As another example, the combination of PARP with anti-PD-L1 inhibition preclinically seems especially promising in SCLC-A[101].



An SVV oncolytic virus has selective tropism for SCLC-N[112]. Furthermore, it seems that MYC-high SCLC is especially sensitive to aurora kinase inhibitors such as alisertib[113]. SCLC-P seems most vulnerable to IGF1R inhibition[114]. However, trial data need to be awaited.

**Future directions for PCI**

The role of PCI has been questioned with the results of a Japanese phase III trial, in which SCLC patients were randomized between PCI and follow-up with brain MRI[115]. A currently ongoing phase III trial is evaluating whether MRI combined with PCI is not inferior tot MRI surveillance alone (NCT04155034), for patients (LD-SCLC as well as ED-SCLC) that have competed their first line treatment (ICI to the discretion of the physician). In NSCLC, it has been suggested that PD-(L)1 inhibition can prevent brain metastases development[13,116]. For SCLC, no data exist to the best of our knowledge, and whether there is a role of PCI in a chemo-ICI regimen should be further evaluated. Furthermore, it is not well known whether PCI can be given safely concurrent with PD-(L)1 inhibition, as only 22 out of the 198 patients in the IMpower133 trial randomized to atezolizumab, were treated with PCI concurrently with atezolizumab and detailed neurotoxicity data have not been reported. However, no grade 3-5 neurological adverse events were reported for the total atezolizumab group.

Numerous retrospective case series in several tumor types suggest that combination of ICI with cranial radiotherapy is safe, however prospective studies are needed to further confirm these findings[117].

## CONCLUSION

SCLC is a disease with a poor prognosis. Even though the incidence of SCLC is decreasing, there is a need for more effective treatment opportunities. With the recent EMA approval of atezolizumab and FDA approval of also durvalumab in combination

with chemotherapy as a first line treatment in ED-SCLC and additional FDA approval for nivolumab/pembrolizumab as a second/third line option in ED-SCLC, we have made an important step in treating patients with SCLC. However, only a small part of the patients benefits from ICI. Numerous studies are currently being performed aiming to improve ICI benefit in SCLC, for example with the addition of radiotherapy, co-stimulatory antibodies, and other immune modifying agents. Prospective trials should include biomarker research and consider the neuroendocrine subtyping of SCLC in order to select patients most likely to benefit.

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# CHAPTER 4

## A BRIEF REPORT ON COMBINATION CHEMOTHERAPY AND ANTI-PROGRAMMED DEATH (LIGAND) 1 TREATMENT IN SMALL-CELL LUNG CANCER: DID WE CHOOSE THE OPTIMAL CHEMOTHERAPY BACKBONE?

Joanne M Mankor*, Fenneke Zwierenga*, **Daphne W Dumoulin**, Jacques J C Neefjes, Joachim G J V Aerts

* These authors contributed equally

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## ABSTRACT

Extensive-stage small-cell lung cancer (ES-SCLC) is an aggressive cancer that remains very hard to treat. The life expectancy of a patient diagnosed with this disease has not changed over the past three decades. Recently, three large clinical studies showed a survival benefit by adding an anti-PD-(L)1 antibody to the current chemotherapy regimen. Although significant and important, the benefit seems less than what has been achieved in NSCLC patients treated with chemo-immunotherapy. A number of hypotheses have been explored in order to explain this discrepancy. Here, we hypothesize that the current chemotherapy backbone in ES-SCLC does not contain the optimal drugs to trigger immunogenic cell death and therefore does not induce a synergy between chemotherapy and immune checkpoint inhibitor (ICI) therapy. Thereby, we advocate that doxorubicin treatment instead of etoposide should be reconsidered as standard of care (SoC) first-line treatment of SCLC.

SCLC is an aggressive type of cancer. At diagnosis, approximately two-third of the patients are diagnosed with extensive stage (ES) disease. ES-SCLC treatment options remain limited, resulting in a poor prognosis that did not improve in the past three decades. For many years, the standard of care treatment regimen most used for patients with ES-SCLC consists of 4-6 cycles of platinum based-chemotherapy (cisplatin/carboplatin) and etoposide. ES-SCLC responds well to chemotherapy, but recurrence of disease develops rapidly [1].

The treatment landscape of thoracic malignancies in general changed dramatically in the past decades, due to the discovery of immune checkpoint inhibitor (ICI) therapy, i.e. programmed death 1 (PD-1), programmed death ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) monoclonal antibodies [2]. Nonetheless, it should be noted that within the thoracic malignancies mainly non-small-cell lung cancer (NSCLC) patients benefit from this discovery[3]. However, two important factors hinted towards a beneficial role for ICI in ES-SCLC. First, the response to chemotherapy, which is seen in the majority of ES-SCLC patients, induces a reduction in tumor burden and thus potentially in the immunosuppressive environment created by the tumor, which is beneficial for ICI response [4]. Second, the high tumor mutational burden (TMB) described in ES-SCLC, potentially resulting in a large number of neoantigens, has been shown to be a promising predictive biomarker of ICI efficacy in several types of cancer. Although the predictive value of TMB appeared to be limited in prospective clinical studies, high TMB in ES-SCLC did raise hope for similar ICI clinical responses in SCLC and NSCLC tumors [5].

Various clinical trials investigated checkpoint blockade in ES-SCLC. Results of single agent checkpoint inhibitor trials in the second-line or later setting, have been disappointing as benefit was limited, in contrast to the results seen in NSCLC. Anti-PD-1 antibody treatment, with or without the addition of anti-CTLA, has been explored in the single arm trials of Checkmate 032[6] KEYNOTE-028[7] and KEYNOTE-158[8]. The pooled analysis of KEYNOTE-028 and KEYNOTE-158 reported a response rate of 19,3%, (95% confidence interval (CI), 11.4–29.4), a median progression-free survival (PFS) of 2.0 months (95% CI, 1.9–3.4) and a median overall survival (OS) of 7.7 months (95% CI, 5.2-10.1)[9]. Checkmate 331[10] and IFCT-1603[11] compared SoC chemotherapy to nivolumab and atezolizumab respectively, but both failed to improve OS in SCLC patients requiring second-line treatment. Furthermore, single agent anti-PD1 treatment in third-line setting has been investigated in single arm trials and FDA approval was granted based on ORRs of only 10 to 20%. In NSCLC in contrast, higher number of responders and more durable responses to single-agent ICI are seen[12–14].

The high sensitivity to chemotherapy that characterizes most SCLCs, results in massive tumor antigen release from dying tumor cells, which theoretically renders these tumors as sensitive to ICI as NSCLC tumors. A number of phase III clinical trials investigated this strategy. The first phase III clinical trial that investigated ICI-therapy in combination with first-line therapy, studied the role of an anti-CTLA-4 antibody in combination with platinum etoposide. No difference was established in PFS nor OS[15]. Furthermore, the IMpower133 phase III randomized trial evaluated the efficacy and safety of atezolizumab (anti-PD-L1) with carboplatin–etoposide. The study showed a significant improvement in PFS (hazard ratio for disease progression or death, 0.77; 95% CI, 0.62-0.96; P = 0.02) and OS (hazard ratio for death 0.70; 95% CI 0.54 to 0.91, p =0.007), but response rates did not differ between the two arms [16]. The phase III, randomized CASPIAN trial assessed first-line durvalumab (anti-PD-L1) and etoposide with either cisplatin or carboplatin versus platinum-etoposide alone. This resulted in significantly longer OS (HR for disease progression or death: 0.73; 95% CI, 0.59-0.91; P=0.0047. Significance of PFS could not be tested due to the study design, but median PFS was 5.1 in the combination treatment arm versus 5.4 months in the platinum-etoposide alone arm, resulting in a HR of 0.78 (95% CI 0.65-0.94)[17]. The KEYNOTE-604 phase III randomized trial evaluated the addition of pembrolizumab (anti-PD-1) to etoposide-platinum (either cisplatin or carboplatin) versus placebo/etoposide-platinum. A prior interim analysis demonstrated a significant improvement in PFS (HR for disease progression 0.75; 95% CI, 0.61-0.91). Although OS did improve as well, the OS results did not meet the criteria for statistical significance per the pre-specified statistical plan (HR for death: 0.80; 95% CI, 0.64-0.98)[18]. In conclusion, the clinical trials summarized here demonstrated significant differences in favor of the chemotherapy ICI combination treatment arm, but only a minority of ES-SCLC patients seems to benefit from ICI in combination with chemotherapy. No biomarker is yet to be found to identify this minority, partly due to the confounding nature of the chemosensitive SCLC tumors. In NSCLC the benefit from chemotherapy ICI combination treatment compared to chemotherapy only, is much more pronounced. KEYNOTE-189 investigated SoC chemotherapy plus pembrolizumab versus SoC chemotherapy plus placebo and found a hazard ratio for progression or death of 0.52 (95% CI, 0.43 to 0.64; P<0.001) and 12-month overall survival rate of 61.7% vs. 52.2% (hazard ratio for death, 0.59; 95% CI, 0.38 to 0.92) in the pembrolizumab vs. placebo groups [19]. Currently, there is also in NSCLC no biomarker available to predict improved outcome on combination treatment.

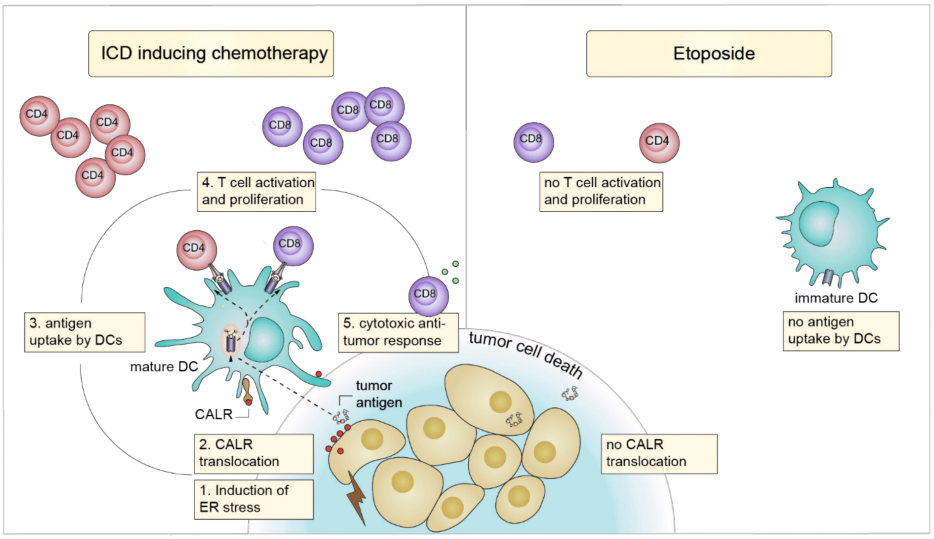
Clearly, there is a discrepancy between NSCLC and SCLC clinical responses to chemotherapy ICI combination treatment. SCLC tends to be intrinsically more resistant to ICI than NSCLC and different mechanisms of resistance are likely to be in place. In NSCLC, benefits in terms of response rates, PFS and OS are potentially due to synergistic

effects of the two treatment modalities. It stands out that in SCLC combination trials, a clear separation of the PFS and OS curves can only be seen after 4 to 7 months. Late separation of the curves in addition to the lack of improvement in response rates do support the absence of a synergistic effect between the two treatment modalities. In this brief report, we explore the optimal chemotherapy backbone for ICI combination treatment in SCLC, aiming for true synergy.

Immunogenic cell death (ICD) is a key mechanism in the process of immune modulation by cytotoxic chemotherapies. ICD results in regulated activation of an immune response, in the absence of "pathogen-associated molecule patterns" (PAMPs). In contrast, "damage-associated molecular patterns" (DAMPs), molecules that are expressed or released upon cellular stress responses or cell death, can exert powerful immunogenic signals by binding to pattern recognition receptors (PPRs) on immune cells. These signals activate a cascade, resulting in the activation of cytotoxic CD8 T cells that can eliminate tumor cells. So far, 4 key DAMPs have been recognized to play an important role in chemotherapy induced ICD [20]. First, the release of the nuclear protein non-histone chromatin-binding protein high-mobility group box 1 (HMGB1) into the extracellular space can activate Toll-like receptor 4 (TLR4) that is expressed on dendritic cells (DCs). Second, type I interferon (IFN) signaling by dying cancer cells can upregulate chemotactic factors on surrounding cells that help attract T cells to the tumor site. Third, if apoptotic cell death is preceded by autophagy, ATP can be released and attract myeloid cells. And last, the ER protein calreticulin (CALR) translocates to the cell membrane in response to the induction of endoplasmatic reticulum (ER) stress, and provides an important "eat-me" signal to antigen-presenting cells (APCs), by interacting with CD91 on the engulfing cell[21]. CALR appears to play a vital role in the context of immunogenic cell death, induced by chemotherapeutic agents.

Obeid et al. performed a number of in vitro and in vivo experiments and concluded that CALR exposure was necessary for successful ICD[22]. First, they found that anthracyclins are highly efficient ICD inducers and that the immunogenicity of anthracyclins could be abrogated by the blockade or knockdown of CALR, which in turn suppressed phagocytosis of dying tumor cells by DCs. On the other hand, CALR translocation was lacking in etoposide treated mice and administration of recombinant CALR could restore ICD and enhance antitumor effects in this treatment regimen. Bezu et al. supported these findings in their review and concluded that, even though etoposide does regulate ATP secretion and HMGB1 release, CALR translocation was inevitable for successful ICD induction[20]. In conclusion, the lack of CALR translocation may be a key feature that is missing in patients treated with platinum-etoposide and ICI (**Figure 1**).

**Figure 1.** Differences in the immunological response to dying tumor cells that is initiated by immunogenic cell death (ICD)-inducing chemotherapies and etoposide.




*The left panel of this figure depicts how ICD inducing chemotherapies can promote a cytotoxic anti-tumor immune response by CD8 T cells. First (1), chemotherapy can induce endoplasmatic reticulum (ER) stress in tumor cells. In response to ER stress, the ER protein calreticulin (CALR) translocates to the tumor cell surface. CALR functions as a damage associated molecular pattern (DAMP) and this damage signal stimulates DCs to take up tumor antigens (3). In response, DCs will mature and prime CD4 and CD8 T cells by displaying the appropriate peptide-MHC ligand, which promotes T cell activation and proliferation (4). Priming of naive CD8 T cells generates cytotoxic effector CD8 T cells that are capable of direct tumor cell killing (5). In contrast, the right panel of this figure depicts the lack of T cell activation during etoposide treatment. Since etoposide is not a potent ICD inducer, CALR is absent on the tumor cell surface, thereby diminishing activation and maturation of DCs. This DCs are less likely to take up tumor antigens and to activate CD4 and CD8 T cells. As a result, T cell activation and proliferation does not occur and a cytotoxic anti-tumor response is not initiated. Abbreviations: CALR: calreticulin, ER stress: endoplasmatic reticulum stress, DC: dendritic cell, ICD: immunogenic cell death.*

Until 2000, anthracyclin-based chemotherapy in the form of doxorubicin was used in Europe as the standard of care regimen for first line treatment in SCLC[23]. Doxorubicin and etoposide share the same molecular targets and induce DNA double-strand breaks in an almost identical manner. Clinical benefit of platinum-etoposide and doxorubicin regimens was shown to be similar in several trials. A meta-analysis of cisplatin containing regimens versus regimens without platinum however, put doxorubicin to the second place of SCLC treatment[24]. Now, in the light of ICD induction, doxorubicin treatment for SCLC should be brought back under consideration.

Since the current regimen consists of etoposide combined with cisplatin or carboplatin, it is inevitable in this context to also consider the immunogenic capacities of platinum

agents. It is important to notice that chemically related chemotherapeutic agents can have entirely different capacities to trigger ICD. Preclinical studies showed that cisplatin is intrinsically incapable of inducing ICD, also due to the lack of ER-stress dependent CALR translocation[25]. Carboplatin induces cell death in a similar manner to cisplatin[26]. Oxaliplatin on the contrary, is known to be a powerful ICD inducer. However, oxaliplatin has not been proved to be effective in SCLC and thus the platinum chemotherapeutics appear not to be the right agents to combine with ICI in the context of SCLC.

It should be mentioned, that etoposide combination treatment was also one of the potential agents used in the PACIFIC trial. This trial investigated the benefit of adding durvalumab as maintenance treatment in stage III NSCLC[27]. Approximately one quarter of patients received etoposide, and up to this date, no data are present on differences in efficacy of the chemotherapy arms. But one should realize that these patients were irradiated concurrently and radiotherapy is known to be a potent ICD inducer. This may have reduced the need for DAMP release induced by chemotherapy.



In conclusion, although exciting new treatment options are developed by combining chemotherapy and ICI, the combination should be designed with care. By adding chemotherapy to ICI, we should aim to reinforce tumor immunogenicity and alleviate immunosuppression. Therefore, we argue that a systematic investigation of ICD inducing capacities of currently available chemotherapies for SCLC is urgently needed. This knowledge should be the basis for further clinical investigations.

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# CHAPTER 5

## TRENDS IN EPIDEMIOLOGY OF SMALL CELL LUNG CANCER: A DUTCH NATIONWIDE POPULATION-BASED STUDY OVER 1989-2020

**Daphne W Dumoulin**, Mieke Aarts, Dirk K M De Ruysscher, Joachim G J V Aerts, Anne-Marie C Dingemans

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## ABSTRACT

### Introduction

This study describes the evolving characteristics of patients with small cell lung cancer (SCLC) from 1989 to 2020 in the Netherlands to analyze how the population of patients with SCLC has changed in the last decades, hypothesizing that this might explain the little progress made in SCLC.

### Methods

Patients with SCLC diagnosed from 1989 to 2020 were selected from the Dutch cancer registry. Incidence, patient and disease characteristics, treatments, and OS were analyzed. Joinpoint analyses were used to test annual percentage changes for statistical significance.

### Results

A total of 52,527 patients were diagnosed with SCLC. The absolute numbers of patients with SCLC remained equal over the years, however the incidence rates decreased from 15.01 to 8.93 per 100,000 person-years. The proportion of women increased from 22% to 50%, and those aged ³75 years increased from 20% to 25%. The latter coincided with a higher proportion receiving only best supportive care over the years (18% to 24%). The use of surgery in stage I increased from 2% to 37%. The proportion of patients diagnosed with stage IV increased from 46% to 70% due to better staging. The OS improved for all stages, with a 2-year OS rate for stage IV doubling from 3% to 6%.

### Conclusion

The incidence of SCLC has significantly decreased over the last 30 years, with an increasing proportion of elderly and women. The male-female ratio became similar and the OS improved. As a consequence of more elderly and probably more vulnerable patients, more patients received only best supportive care.

## INTRODUCTION

Small cell lung cancer (SCLC) accounts for 10-15 % of all lung cancer diagnoses, corresponding to an incidence of 1-5 patients per 10,000 persons.[1] Historically, SCLC used to be divided into limited disease (LD) (primary tumor and regional lymph nodes in a single radiation field; potentially curable) and extensive disease (ED) (others).[2] Since 2010, the TNM staging is recommended, in which LD mainly corresponds to TNM stages I-III and ED to stage IV.[3]

Since 1985, the combination of platinum plus etoposide has been the backbone for the treatment of patients with SCLC.[4] Although the majority of patients benefit from a fast response, most relapse rapidly after completion of chemotherapy and develop acquired resistance. At diagnosis, at least 70% of patients with SCLC have distant metastases, and 10-15% already have brain metastases, leading to an OS rate at 5 years of 15% for LD-SCLC and 3% for ED-SCLC.[5-7]



The concurrent addition of atezolizumab or durvalumab to standard chemotherapy followed by ICI maintenance as first-line therapy significantly improved the OS of patients with stage IV SCLC without increasing toxicity, which has led to a Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved first-line treatment option for patients with stage IV SCLC.[8,9]

With the decreasing incidence of SCLC and these new treatment options, further in-depth analysis of the current population of patients who suffer from SCLC is warranted. This may give insight in possible new ways to improve the outcome of SCLC patients.

As to the best of our knowledge, since the report of Govindan et al., describing the epidemiology of SCLC in the US from 1973 to 2002[10], no similar studies have been published. We therefore investigated the characteristics of patients diagnosed with SCLC between 1989 and 2020 in the Netherlands.

## MATERIALS AND METHODS

**Study population/Database**

Patients diagnosed with a first primary SCLC between 1989 and 2020 were selected from the population-based Netherlands Cancer Registry (NCR), which is maintained by the Netherlands Comprehensive Cancer Organisation. Trained registration personnel actively collects information on patient, disease, and the first-line treatment given

from the hospitals' medical records.[11] WHO performance status (PS) is registered from 2015. Patients diagnosed at autopsy or who resided or received treatment abroad were excluded. TNM editions 4 (to 1992), edition 4 2nd revised edition (1993-1998), edition 5 (1999-2002), edition 6 (2003-2009), edition 7 (2010-2016) and edition 8 (from 2017) were used. Pleural effusion was not registered separately but was defined as metastatic disease since the introduction of the TNM 7th edition. Mortality is updated annually through linkage with the Dutch Personal Records Database. Follow-up data were complete until February 1, 2022.

**Analyses**

Characteristics and treatments were analyzed according to 5-year periods, except for the last period including six years. Chi-Square tests were used to test significance of the distribution of the variables over time. Wilcoxon signed rank test was used to test significance of median ages. Trends in survival were calculated by median survival and its 95% confidence interval (CI) for each period and by 5-year (stages I-III) and 2-year OS (stage IV) rates. Analyses were stratified by gender and stage of disease, where stage I and II were combined in case of small numbers of patients. Pathological stage was used if available, otherwise clinical stage was imputed. For some patients in older years, neither pTNM nor cTNM was registered. In case no additional information could be obtained about the extent of disease, these were included in stage unknown (N=170). Multivariable proportional hazards regression analysis was used to discriminate independent risk factors for death. A model was built including gender, age, year of diagnosis, stage and best supportive care.

Incidence rates were calculated by adjusting for the revised European standard population and reported as European standardized rates per 100,000 person-years (RESR). JoinPoint regression analyses were performed to test for incidence trends and compute the annual percentage changes (APC).

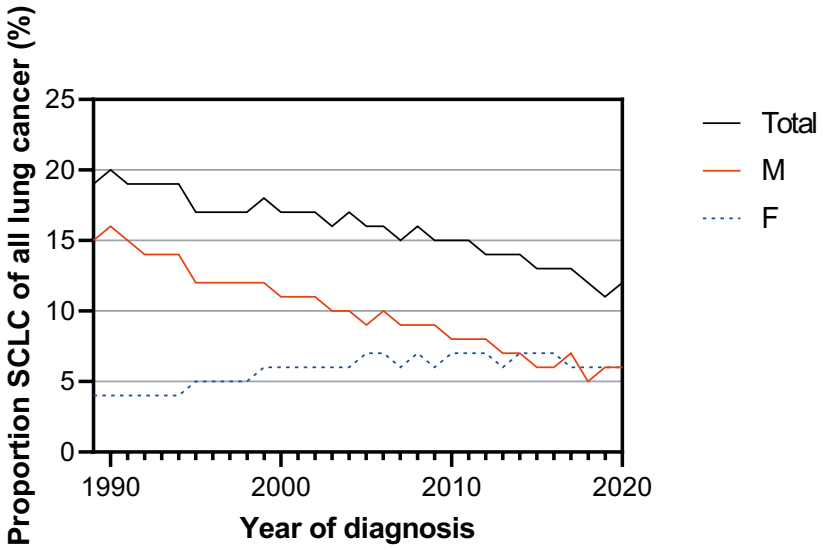
P-values <0.05 were considered statistically significant, and all tests were two-sided. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, USA) and JoinPoint version 4.9.1.0.

## RESULTS

### Incidence

The Dutch population increased from 14.8 million inhabitants in 1989 to 17.4 million in 2020. Between 1989 and 2020, 340,514 patients were diagnosed with first primary lung cancer, of which 52,527 (15%) patients with SCLC. Of all lung cancers, the proportion SCLC decreased from 19% in 1989 to 12% in 2020 (p-value <0.0001) (**Figure 1**). The absolute number of patients with SCLC remained similar over the years: ~1600. The incidence rates decreased from 15.01 in 1989 to 8.93 per 100,000 person-years (RESR) in 2020.

**Figure 1.** The proportion of SCLC among all lung cancers in the Netherlands, 1989-2020, according to sex



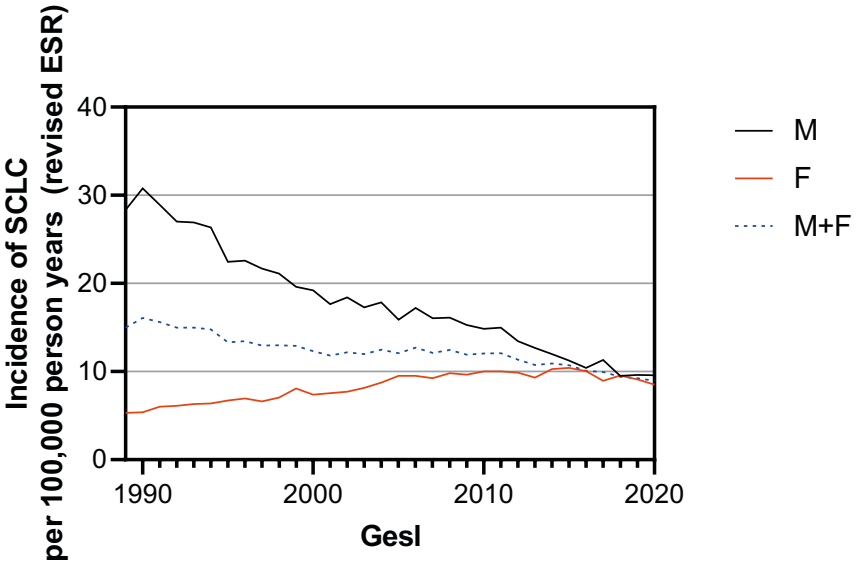

M = male
F = female

### Sex

Among all patients diagnosed with SCLC, the initial male-female ratio in 1989-1994 was 3.6 to 1 (**Table 1**). Subsequently, the ratio gradually equalizes to 1 to 1 from 2015-2020 (**Figure 1** and **3**). During the study period, the incidence of SCLC for men decreased from 28.35 (1989) to 9.55 (2020) per 100,000 person-years (RESR), with an APC of 4.3% to 2001, 2.0% until 2009, and 4.8% from 2009 (**Figure 2**). Among females, the incidence of SCLC increased from 5.31 (1989) to 10.02 (2010) per 100,000 person-years,

corresponding to an APC of 3.1%, after which it slightly decreased to 8.50 per 100,000 person-years in 2020, APC -1.3% (**Figure 2**).

**Figure 2.** Trends in incidence of SCLC by gender in the Netherlands, 1989-2020




| | Annual percent change |
|---|---|
| *Males* | |
| 1989-2001 | -4.28%* |
| 2001-2009 | -1.98%* |
| 2009-2020 | -4.75%* |
| | |
| *Females* | |
| 1989-2010 | 3.05%* |
| 2010-2020 | -1.33%* |
| | |
| *Males+Females* | |
| 1989-2011 | -1.22%* |

## Age

From 1989 to 2020, the proportion of patients aged ³70 years increased from 38% to 46%, most prominently in the 75+ group (20% to 25%) (**Table 1**, **Figure 3**). Females were younger than males, with a median age of 68 compared to 70 years in 2015-2020 ($p<0.0001$).

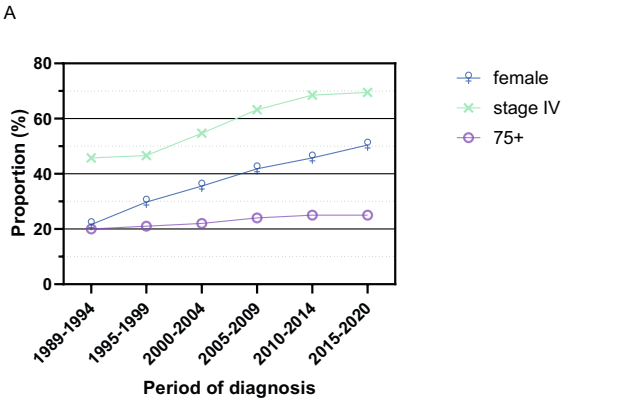
**Table 1.** Characteristics of patients with small-cell lung cancer in the Netherlands, according to period of diagnosis, 1989-2020

| | | 1989-1994 | 1995-1999 | 2000-2004 | 2005-2009 | 2010-2014 | 2015-2020 | Total |
|---|---|---|---|---|---|---|---|---|
| Numbers | | 9965 | 7695 | 7683 | 8430 | 8740 | 10014 | 52527 |
| Gender (%) | M | 78 | 70 | 64 | 58 | 54 | 50 | 62 |
| | F | 22 | 30 | 36 | 42 | 46 | 50 | 38 |
| | M:F | 3.6 | 2.4 | 1.8 | 1.4 | 1.2 | 1.0 | 1.7 |
| Age (y) | Median | 66 | 67 | 67 | 67 | 68 | 69 | 67 |
| Age (%) | 0-39 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 40-69 | 61 | 60 | 58 | 58 | 57 | 54 | 58 |
| | 70-74 | 18 | 19 | 19 | 18 | 18 | 21 | 19 |
| | 75+ | 20 | 21 | 22 | 24 | 25 | 25 | 23 |
| Stage (%) | I | 9 | 7 | 5 | 3 | 2 | 2 | 5 |
| | II | 2 | 2 | 2 | 2 | 2 | 3 | 2 |
| | III | 35 | 39 | 36 | 31 | 27 | 25 | 32 |
| | IV | 46 | 47 | 55 | 63 | 68 | 70 | 58 |
| | Unknown | 8 | 5 | 3 | 1 | 0 | 0 | 3 |
| WHO PS* (%) | 0 | | | | | | 21 | |
| | 1 | | | | | | 29 | |
| | 2 | | | | | | 11 | |
| | 3,4 | | | | | | 6 | |
| | Unknown | | | | | | 32 | |
| Metastases (%) | Bone | 1 | 14 | 15 | 16 | 28 | 33 | 26 |
| | Liver | 1 | 23 | 26 | 30 | 46 | 47 | 38 |
| | Brain | 6 | 7 | 9 | 15 | 16 | 18 | 13 |

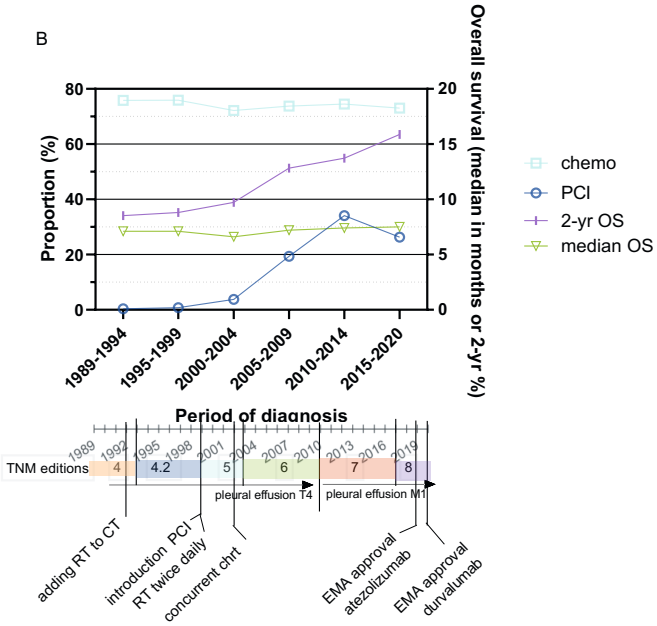
* available from 2015

**Figure 3.** Characteristics of patients with small cell lung cancer in the Netherlands, 1989-2020, according to period of diagnosis

**A. characteristics of the patients**
**B. characteristics of treatment and outcome**
**PCI=prophylactic cranial irradiation**
**OS=overall survival**




| | 1989-1994 | 1995-1999 | 2000-2004 | 2005-2009 | 2010-2014 | 2015-2020 |
|---|---|---|---|---|---|---|
| Female (% | 22 | 30 | 36 | 42 | 46 | 50 |
| Stage IV (% | 46 | 47 | 55 | 63 | 68 | 70 |
| 75+ (%) | 20 | 21 | 22 | 24 | 25 | 25 |




| | 1989-1994 | 1995-1999 | 2000-2004 | 2005-2009 | 2010-2014 | 2015-2020 |
|---|---|---|---|---|---|---|
| chemotherapy (%) | 76 | 76 | 72 | 74 | 74 | 73 |
| prophylactic cranial irradiation (% | 0 | 1 | 4 | 19 | 34 | 26 |
| 2-year overall survival (%) | 9 | 9 | 10 | 13 | 14 | 16 |
| median overall survival (months) | 7.1 | 71 | 6.6 | 7.2 | 7.4 | 7.5 |

**Staging**

Among all stages, the proportion of stage IV increased from 46% (1989-1994) to 70% (2015-2020) (**Figure 3**). Of the patients with stage IV disease in 1989-1994, metastases were mainly reported in bone (14%), liver (23%), and brain (6%). These percentages increased to respectively 38%, 47%, and 18% in 2015-2020 (**Table 1**).

**WHO performance status**

From 2015 (as WHO PS was registered), 50% of the patients had PS 0 or 1 (**Table 1**).

**Treatment**

Patients with SCLC have been less frequently treated for their cancer over the years: 18% (1989-1994) compared to 24% (2015-2020) (**Table 1**) received best supportive care (BSC). The proportion was highest among stage IV, of which 24-29% only received BSC. Most important reasons were preference of patients/family (39%) and PS or comorbidity of the patient (23%). Multivariable logistic regression analyses show more BSC in older patients and in 2000-2009 (corrected for stage, sex and age). The proportion of patients receiving chemotherapy fluctuated between 77% and 52% in stage I, between 69% and 80% in stage II, around 80% in stage III and around 70% in stage IV. The type of platinum-based chemotherapy was only recorded in our database from 2018; the majority of patients received carboplatin, ranging from 64% in 2018 to 77% in 2020. The use of surgery in stage I increased from 2% in the first period to 37% in the last period, and from 5% to 18% in stage II.

Since its introduction, the use of PCI increased to 34% in 2010-2014 and was most frequently used in stage II: max 63% (2010-2014). In the period from 2015, the use of PCI has decreased to 26% for all stages. The use of PCI was irrespective of age. However, overall, the use of PCI was lower among patients aged 70 +.

**Survival**

The median OS of patients with SCLC was 7.2 months (**Figure 3B** and **Supplemental(S)1**). The median OS in stage I SCLC increased from 11.1 months in 1989-1994 to 32.2 months in 2015-2020, from 12.6 to 21.5 in stage II, from 9.3 to 15.8 in stage III, and from 4.2 to 5.6 in stage IV (**Figure S1**). Median OS is 1 to 3.7 months higher among women. Year of diagnosis, stage and receiving BSC contributed only little to the difference in survival between males and females (multivariable-adjusted HR 0.83 (0.82-0.85)).

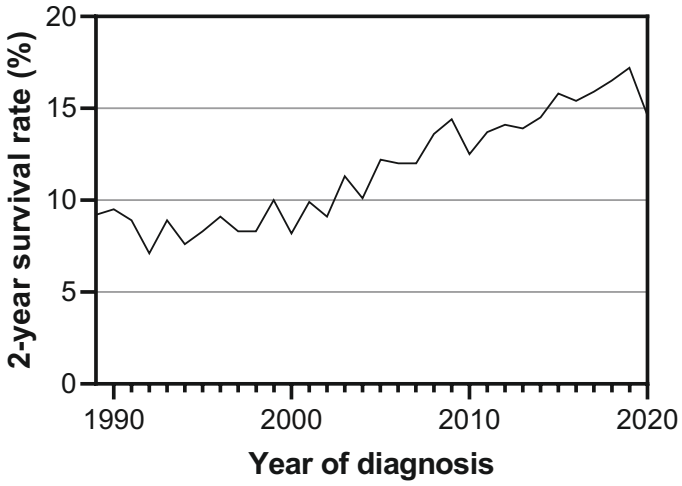
In multivariable Cox regression analyses adjusted for gender, age, and stage, the overall hazard of death decreased over time from 1.0 in 1989-1994 to 0.64 (0.62-0.66) in 2015-2020. Stratifying these analyses according to BSC-status led to a HR of 0.96

(0.90-1.02) in 2015-2020 in the BSC group and a HR of 0.57 (0.55-0.59) in the non-BSC group, compared to the period 1989-1994. Thus, the net improvement of the OS in the total group of SCLC is due to improvement in the group who received tumor-directed treatment, and irrespective of the increased proportion of patients receiving BSC.

The 2-year OS rate increased from 9% (1989) to 16% (2020), with an APC of 3.0% from 1992 (**Figure 3** and **4**). More specifically, a slight improvement was seen in stage IV; from 3% in 1989 to 6% in 2020 (**Figure 5**). The 2-year OS rate among women with stage IV SCLC is higher compared to men (**Figure S2**). However, the 2-year OS among men with stage IV SCLC increased by 5.0% annually, while the APC in women is 3.5% (**Figure S2**).

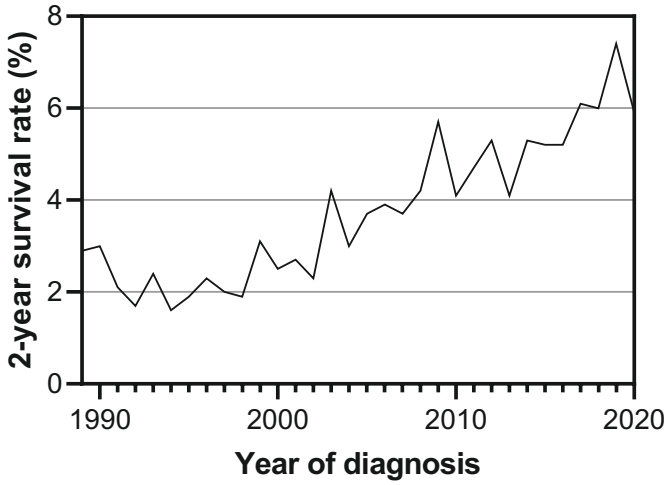
The 5-year OS increased from 8% in 1989 to 40% in 2017 in stage I, from 6% to 29% in stage II and from 4% to 18% in stage III, respectively (**Figure S3**). Joinpoint analysis confirmed these increases. (**Figure S3**). The accompanying survival rates for men and women including joinpoint analysis, are depicted in **Figure S4**.

**Figure 4.** 2-Year overall survival of patients with SCLC in the Netherlands, according to year of diagnosis.




| | Annual percent change |
|---|---|
| 1989-1992 | -7.84% |
| 1992-2020 | 2.97%* |

**Figure 5.** 2-Year overall survival of patients with stage IV SCLC in the Netherlands, according to year of diagnosis.




| | Annual percent change |
|---|---|
| 1989-1992 | -17.48% |
| 1992-2020 | 5.01%* |



## DISCUSSION

In this study, we analyzed the evolution of the characteristics of patients diagnosed with SCLC, in the period from 1989 to 2000. SCLC is mainly diagnosed in older people. It is known that the Dutch population has become older over the years. While 13% of the Dutch population was 65+ years in 2000, the proportion of 65+ has risen to 20% in 2022.[12] Our analysis shows that the median age of the patients diagnosed with SCLC increased from 66 to 69 years, and the proportion of 75+ increased from 20% to 25%. However, the overall incidence of SCLC in the Netherlands has clearly decreased over the last decades. The decrease in the overall incidence of SCLC is the net result of a significantly decreasing trend in men, while the incidence in women has slightly increased, which can be explained by the correlation between SCLC and cigarette smoking. While around 60% of the Dutch population was smoking in the sixties, this was 26% in 2014 and 21% in 2021.[13,14] Remarkably, in a Spanish cohort registration of patients with advanced SCLC, the percentage active smokers between 2016-2020 was still 60%, accompanied by almost 80% of the patients with male gender, showing the existence of differences in smoking behaviour and, therefore, patient characteristics between countries.[15]

The risk of SCLC is related to the duration and intensity of smoking and can be increased up to 38 times higher risk.[16] While the consumption of cigarettes among men has decreased over the years, this has conversely risen for women. Furthermore, at the same tobacco exposure, women have a higher risk for developing SCLC than men.[17] Smoking cessation decreases the risk of developing SCLC from an odds ratio of 14.5 in current smokers, to 10.9 for those who quit smoking <4 years and 2.2 after >25 years of smoking abstinence.[18]

Besides a decrease in incidence, this analysis also shows a remarkable shift in stage distribution, with an increase from 46% of patients diagnosed with stage IV SCLC in 1989-1994 to 70% in 2015-2020. Several reasons can explain this increase. First, the staging systems are adjusted regularly. According to the VALG staging system, patients with malignant pleural effusion were defined as having limited-stage SCLC.[2] Since the introduction of the TNM 7$^{th}$ edition in 2009, pleural effusion has been defined as metastatic disease. Because pleural effusion was not registered separately, we cannot distinguish between stage IV due to pleural effusion or other metastases.

Another reason for the shift towards more advanced stage SCLC is the major improvement in the available diagnostic methods.

First, the sensitivity and specificity of CT scans and the use of contrast protocols have improved over the last decades.[19,20] Second, Positron Emission Tomography (PET) scanner was increasingly used to stage lung cancer patients since its introduction in clinical practice in 1996, leading to a clear stage migration towards higher stages.[21]

Third, brain imaging with dedicated CT protocols or with MRI scans is increasingly used.[22]

Our analysis also shows changes over time regarding treatment, particularly in stages I-III. As was described by Evers et al., surgery in stage I has increased over time, as well as the use of chemoradiotherapy, reflecting the guidelines which changed clinical practices after the introduction of new treatments (**Figure 3B**).[23] Additionally, the role of PCI has changed over time. In contrast to the initial increase in PCI use since its introduction[24], a decline has been observed in 2015-2020 in all stages of SCLC. In stage IV SCLC, this might be explained by the in 2017 published Japanese phase 3 trial, showing no OS benefit of PCI together with MRI surveillance compared to periodic MRI surveillance during follow-up.[25] This approach is more controversial for stages I-III due to the lack of prospective randomized studies. The use of PCI was lower among

patients aged 70+, however, the reduction in the proportion of patients receiving PCI was independent of age.

In our analysis, the OS over the years has increased. This can be explained, as mentioned, by the shift in stage distribution due to a more accurate staging system and, possibly more important, the improvement in diagnostic methods. This stage migration towards higher stages leads to improved prognoses for all stages. The OS improvement is more pronounced in stages I-III than in stage IV disease. This might be caused by the increase in surgical procedures. The introduction of twice-daily radiotherapy in 1999 could also play a role in this OS improvement. However, the implementation in daily practice was limited, with only half of the patients with stage II-III SCLC treated with concurrent chemoradiotherapy receiving an accelerated scheme. Compliance with chemotherapy also improves OS, however, our database did not register how many patients received the total planned number of chemotherapy cycles or if dose reduction had to be performed. Furthermore, as mentioned, PCI also influences OS in stages I-III. Noteworthy, the improvement of OS was irrespective of the increased proportion of patients receiving only BSC. The risk of death in the group of patients that received tumor-directed treatment decreased over time, while this remained stable for the patients only receiving BSC. The OS benefit may, therefore, be subscribed to the improvement in the group of patients receiving tumor-directed treatment.



Several limitations must be noted for our study. First, our registry does not include diagnostic imaging procedures. Although it seems logical that the implementation of FDG-PET-CT and brain MRI has led to a shift towards stage IV SCLC, it is impossible to determine the extent to which this plays a role.

A second limitation that influences the stage migration, is the adaption of the TNM staging classification, by which pleural effusion has been defined as stage IV SCLC since 2009. Our database did not register pleural effusion separately, making it impossible to distinguish between stage IV due to pleural effusion or other metastases.

A third limitation is that only the given treatment is registered in our database. Insights into treatment adjustments are lacking. In addition, in our database only the first given treatment is registered. Therefore, information about longitudinal sequences of treatments is lacking. Furthermore, our database does not contain data about the scheme of radiotherapy, and whether this was performed concurrently or sequentially is only available in recent years.

Another limitation is that only a limited number of patients were treated with ICI. Although atezolizumab (September 2019) and durvalumab (September 2020) received EMA approval[8,9], first line chemotherapy-ICI is not reimbursed in the Netherlands. Therefore, immunotherapy was only applied in clinical studies, which accounts for less than 3% of the patients per year.

The aim of this study was to analyze the changes in the population suffering from SCLC, with the hypothesis that our current population had become older and more fragile. We indeed observed that patients diagnosed with SCLC are older and more often have metastatic disease, leading to less anti-cancer treatment. Comparing the current population suffering from stage IV SCLC with the population that had been treated in clinical trials, we found some remarkable differences in particularly age, presence of brain metastases and PS. Median age in our real world treated stage IV SCLC population was 67 years (60% 65+), against 62-64 years (45% 65+) in clinical trials. Brain metastases were present in 20% (real world), compared to 8-14% in trials. Also, excluding PS 2 in clinical trials leads to differences to real-world treated patients as these account for about 18% of the population. These patient characteristics of the patients in our real-world cohort seems to be comparable to that of, for instance, a Spanish cohort registration, showing 20% of the patients having brain metastases and 24% PS≥2.[15] However, of the patients with stage IV SCLC, almost 30% of the patients in daily practice were not treated. Therefore, patients included in clinical trials (36-41% PS=0, 59-64% PS=1) represent only a subgroup of patients with SCLC in clinical daily practice. While a clinical need remains to improve the prognosis of these more fit patients, there is an accompanying clinical need for the older or less fit patients.

## CONCLUSIONS

The incidence of SCLC has significantly decreased, with and increasing proportion of elderly and women. The male-female ratio became equal. Interestingly, more patients did not receive active anti-cancer treatment, probably because of an increasing proportion of elderly and thereby more vulnerable patients, however, a significant increase in OS was observed.

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# SUPPLEMENTAL DATA

**Table S1.** Treatment characteristics (%) of patients with small-cell lung cancer in the Netherlands, according to period of diagnosis, 1989-2020

| | 1989-1994 | 1995-1999 | 2000-2004 | 2005-2009 | 2010-2014 | 2015-2020 | Total |
|---|---|---|---|---|---|---|---|
| **Treatment +** | | | | | | | |
| All stages combined | | | | | | | |
| BSC # | 18 | 20 | 25 | 23 | 23 | 24 | 22 |
| Surgery | 1 | 2 | 2 | 2 | 2 | 3 | 2 |
| PCI $ | 0 | 1 | 4 | 19 | 34 | 26 | 15 |
| Radiotherapy | 3 | 16 | 22 | 24 | 26 | 29 | 20 |
| Chemotherapy | 76 | 76 | 72 | 74 | 74 | 73 | 74 |
| Chemoradiation | 2 | 14 | 20 | 23 | 25 | 27 | 18 |
| Stage I | | | | | | | |
| BSC | 15 | 16 | 18 | 12 | 8 | 11 | 15 |
| Surgery | 2 | 7 | 11 | 24 | 32 | 37 | 12 |
| PCI | 1 | 1 | 10 | 28 | 42 | 15 | 10 |
| Radiotherapy ⌘ | 3 | 25 | 37 | 47 | 55 | 47 | 26 |
| Chemotherapy | 73 | 77 | 75 | 73 | 64 | 52 | 72 |
| Chemoradiation | 3 | 22 | 36 | 41 | 37 | 23 | 20 |
| Stage II | | | | | | | |
| BSC | 8 | 9 | 21 | 13 | 8 | 11 | 11 |
| Surgery | 5 | 27 | 16 | 21 | 18 | 18 | 17 |
| PCI | 2 | 3 | 8 | 36 | 63 | 37 | 27 |
| Radiotherapy | 5 | 27 | 37 | 58 | 69 | 67 | 46 |
| Chemotherapy | 77 | 74 | 69 | 76 | 84 | 80 | 77 |
| Chemoradiation | 4 | 23 | 34 | 53 | 65 | 62 | 42 |
| Stage III | | | | | | | |
| BSC | 10 | 13 | 18 | 15 | 13 | 14 | 14 |
| Surgery | 0 | 1 | 1 | 1 | 1 | 2 | 1 |
| PCI | 1 | 2 | 8 | 35 | 55 | 45 | 22 |
| Radiotherapy | 5 | 28 | 44 | 56 | 66 | 70 | 42 |
| Chemotherapy | 84 | 83 | 79 | 80 | 85 | 83 | 82 |
| Chemoradiation | 4 | 25 | 41 | 54 | 64 | 68 | 40 |
| Stage IV | | | | | | | |
| BSC | 24 | 25 | 29 | 28 | 28 | 29 | 27 |
| Surgery | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| PCI | 0 | 0 | 1 | 11 | 25 | 19 | 11 |
| Radiotherapy | 1 | 4 | 6 | 6 | 8 | 12 | 7 |
| Chemotherapy | 72 | 73 | 69 | 71 | 71 | 70 | 71 |
| Chemoradiation | 1 | 3 | 5 | 6 | 8 | 11 | 6 |

+ combinations possible
# BSC = best supportive care, includes radiotherapy on metastases
$ PCI = prophylactic cranial irradiation
⌘ radiotherapy concerns radiotherapy on primary tumour

**Figure S1.** Median overall survival rate of patients with SCLC in the Netherlands, according to year of diagnosis.

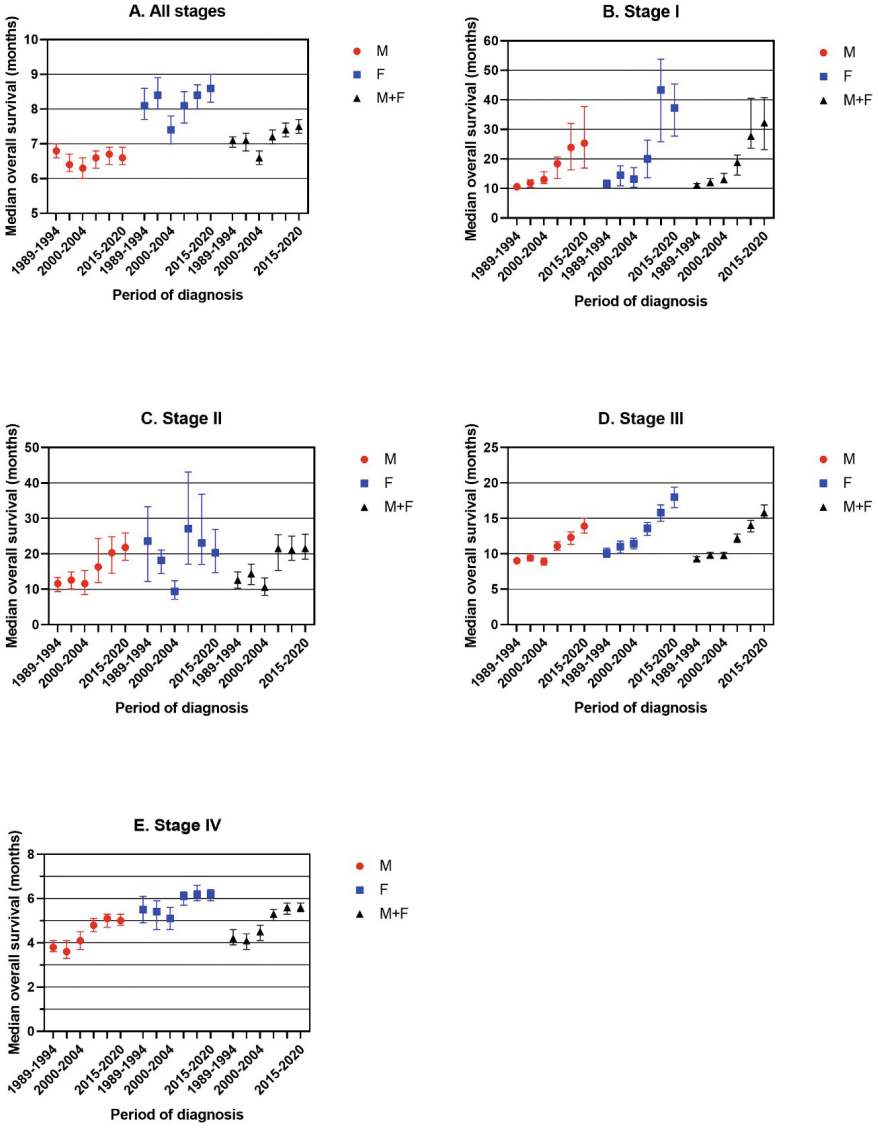
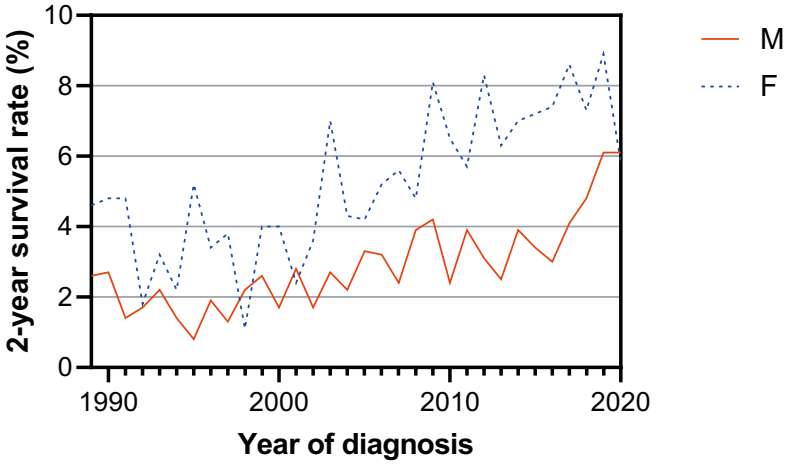
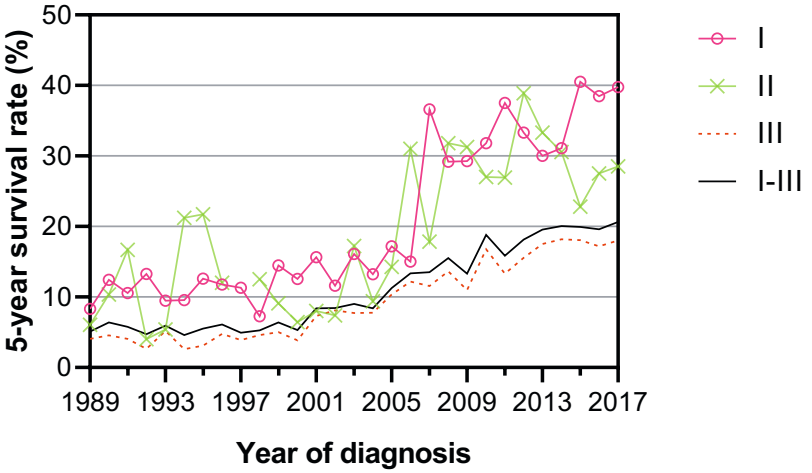


**Figure S2.** 2-Year overall survival of stage IV SCLC in the Netherlands, by sex, according to year of diagnosis.



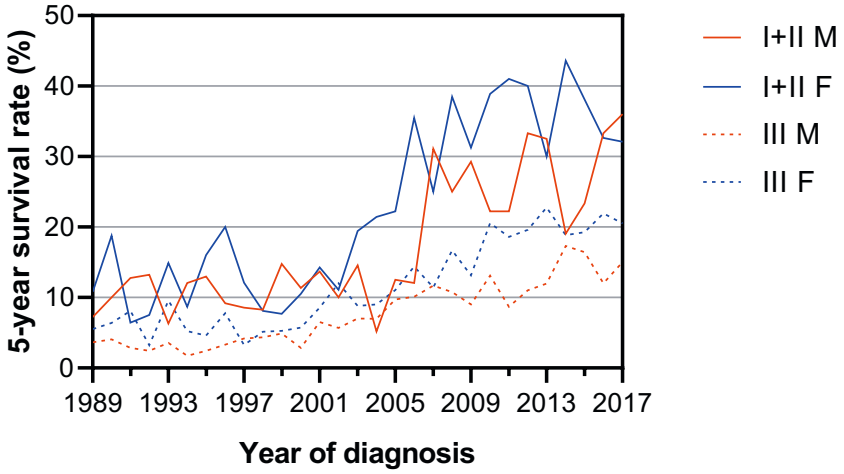

| | Males: Annual percent change |
|---|---|
| 1989-2020 | 3.46%* |
| | |
| | Females: Annual percent change |
| 1989-2020 | NA |

**Figure S3.** 5-Year overall survival of stage I-III SCLC in the Netherlands, according to year of diagnosis.




| | Annual percent change |
|---|---|
| *Stage I* | |
| 1989-1998 | 0.39% |
| 1998-2017 | 7.92%* |
| | |
| *Stage II* | NA |
| | |
| *Stage III* | |
| 1989-1994 | -4.06% |
| 1994-2017 | 8.66%* |
| | |
| *Stage I-III* | |
| 1989-1998 | -0.61% |
| 1998-2008 | 11.16%* |
| 2008-2017 | 3.87%* |
| | |
| | |

**Figure S4.** 5-Year overall survival of stage I-II and III SCLC in the Netherlands, by sex, according to year of diagnosis.




| | Annual percent change |
|---|---|
| *Stage I-II Males* | |
| 1989-2017 | 5.08%* |
| *Stage I-II Females* | |
| 1989-2000 | -0.78% |
| 2000-2008 | 17.13%* |
| 2008-2017 | -0.80% |
| *Stage III Males* | |
| 1989-1992 | -14.30% |
| 1992-2017 | 8.26%* |
| *Stage III Females* | |
| 1989-2017 | 6.25% |

*Stages I and II are combined due to small numbers of patients (and similar trends).*

# CHAPTER 6

## NIVOLUMAB AND IPILIMUMAB IN THE REAL-WORLD SETTING IN PATIENTS WITH MESOTHELIOMA

**D.W. Dumoulin***, L.H. Douma*, M.M. Hofman, V. van der Noort, R. Cornelissen, C.J. de Gooijer, J.A. Burgers, J.G.J.V. Aerts

* These authors contributed equally

*Submitted*

## ABSTRACT

### Objectives

Nivolumab (anti-PD-1) plus ipilimumab (anti-CTLA-4) is a new first-line treatment combination for patients with pleural mesothelioma. Nivolumab-ipilimumab improved the survival, however, 30.3% of the patients suffered from grade 3-4 treatment related adverse events (TRAE's) and TRAE's led to discontinuation in 23.0% of all patients. Here, we present the first real-world data of nivolumab plus ipilimumab in patients with malignant mesothelioma treated in two mesothelioma expert centers.

### Methods

Clinical data of patients with mesothelioma treated with nivolumab and ipilimumab were prospectively collected. Clinical parameters were obtained every visit, CT scans were evaluated every 12 weeks and adverse events were assessed continuously during the treatment. Data on grade 2-5 TRAE's and activity (overall response rate (ORR), duration of response (DOR), disease control rate (DCR), median progression-free survival (mPFS) and median overall survival (mOS) were reported.

### Results

Between January 2021 and August 2022, 184 patients were treated with nivolumab plus ipilimumab. The median follow-up was 12.1 months (95%CI 11.1 – 13.1). Grade 3-4 TRAEs were seen in 27.7% of the patients and 25.0% discontinued immunotherapy treatment early because of TRAE's. ORR was 21.7% (95% CI 15.7-27.7), median DOR was 5.7 months (IQR 3.2-8.7) and DCR at 12 weeks 56.0% (95% CI 48.8-63.2). The mPFS was 5.5 months (95%CI 4.1-6.9), mOS was 14.1 months (95% CI 11.1-18.2).

### Conclusions

Nivolumab plus ipilimumab had an equal efficacy in a real-world comparable population but also a high risk of TRAE's, leading to discontinuation of treatment in 25% of the patients.


**Keywords**: checkpoint inhibition, nivolumab, ipilimumab, malignant pleural mesothelioma/ MPM, immunotherapy, immune monitoring

# INTRODUCTION

Mesothelioma is a rare and aggressive malignancy with a poor prognosis. Without treatment, the median survival time ranges between six and nine months.[1] As the diagnosis usually is made at an advanced stage most patients are not eligible for surgery and designated for palliative systemic treatment.[2] Treatment in first-line with a combination of platinum and pemetrexed resulted in a median survival benefit of three months.[3] The addition of bevacizumab resulted in an additional survival benefit of nearly three months.[4] Recently, a randomized phase 3 trial showed clinically meaningful activity with a significant improvement of overall survival (OS) using the combination of nivolumab (anti-programmed cell death protein-1 (PD-1)) and ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) compared to chemotherapy (18.1 months (95% CI 16.8-21.4) and 14.1 months (95% CI 12.4-16.2), respectively, HR=0.74, p=0,0020).[5,6] In this study, 30% of the patients treated with nivolumab plus ipilimumab suffered from grade 3 and 4 adverse events (26% and 4%, respectively), and 23.0% discontinued at least one of the treatment components due to treatment-related adverse events (TRAE's). This has led to a Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved first-line treatment option for patients with unresectable mesothelioma.[7,8]



The patients enrolled in clinical trials are often subjected to stringent selection criteria that may not necessarily reflect the real-world population. Because side effects might be more prominent in a more fragile patient population and survival benefit less pronounced, a description of real-world data on nivolumab plus ipilimumab combination in patients with mesothelioma is urgently needed. This article describes safety and activity of this treatment combination in patients who were treated in an expanded access program (EAP) in the Netherlands from January 2021 to August 2022.

# METHODS

### Study design and procedures

Data were collected from patients with mesothelioma, who were treated with nivolumab intravenously at a dose of 360 mg or 4.5mg/kg every 3 weeks and ipilimumab at a dose of 1mg/kg every 6 weeks as part of a named patient program (NPP). Data was collected prospectively in the Erasmus Medical Center (Rotterdam, the Netherlands) and the Antoni van Leeuwenhoek Hospital (Amsterdam, the Netherlands), who serve as referral centers for patients with mesothelioma in the Netherlands. These two

hospitals accounted for 97% of all patients treated with nivolumab plus ipilimumab in the Netherlands in the given time period.

A detailed description of eligibility criteria and procedures of the clinical study is provided in the **Data Supplements**. We cross-checked the number of patients in our study with the data from the Expanded Access Program by BMS. The data cut-off was January 15th, 2023 for all analyses, except for overall survival, for which the data cut-off was July 1st, 2023. All patients who received at least 1 cycle of nivolumab-ipilimumab were included in the toxicity and response analysis. Clinical parameters were obtained every visit. CT scans were evaluated using modified RECIST version 1.1 every 12 weeks.[9] Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0[10] and assessed continuously during the treatment and for patients who discontinued until 30 days after the last treatment. All procedures were conducted in accordance with the Declaration of Helsinki. According to national guidelines, no ethical committee approval was needed for the collection of the clinical data.

The primary objective was to investigate safety in terms of TRAE's. We report data on grade 2-5 TRAE's which were requiring steroid treatment, and/or were reason for discontinuing immunotherapy. Secondary objective was to describe the real-world activity of nivolumab plus ipilimumab. A detailed description of the outcome measurements is provided in the **Data Supplements**.

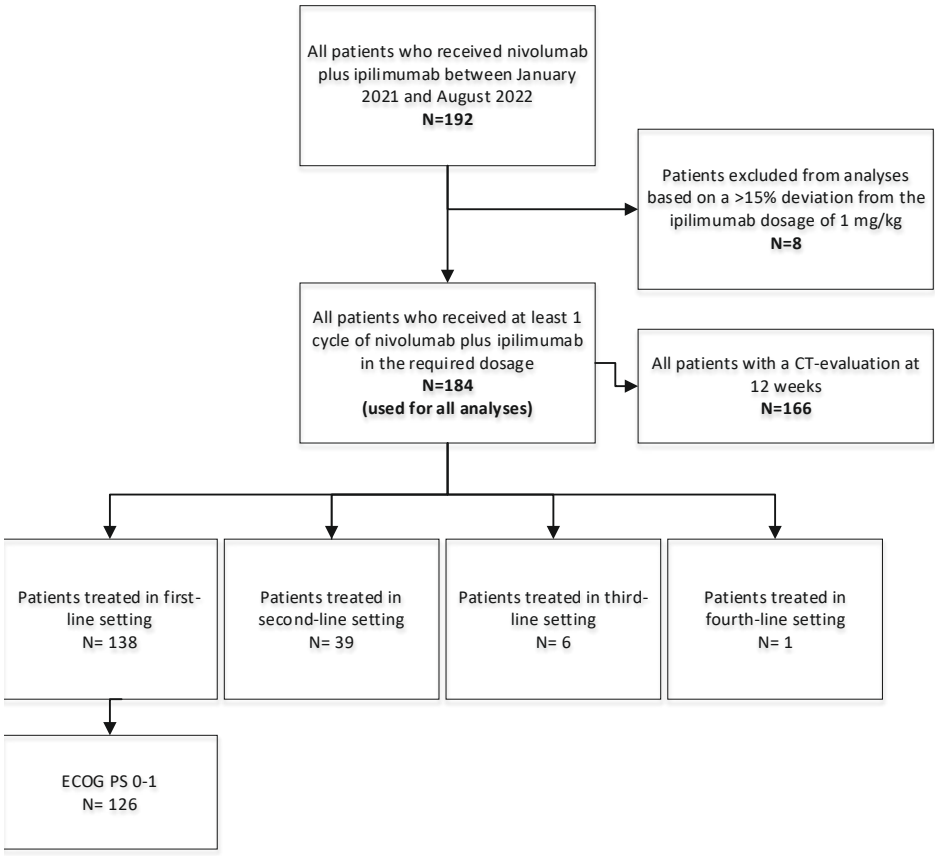
The statistical analysis is described in the **Data Supplements**.

## RESULTS

### Patient characteristics

Between January 1st, 2021, and August 1st, 2022, 192 patients started treatment with nivolumab plus ipilimumab. Eight patients were excluded from analyses due to >15% deviation in the administered dosage of 1 mg/kg ipilimumab (**Figure 1**).

**Figure 1.** Consort diagram




184 Patients were included in our analyses of which 86.4% were men. The median age at start of treatment was 71 years (IQR 66-76), with the highest percentage in the subcategory 65-75 years (48.7%). 53 patients (29%) had an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0 and 113 patients (61%) had an ECOG PS of 1 at the start of treatment. 136 patients (74%) of the patients had not received any previous line of treatment. (**Table 1**).

**Table 1.** Baseline characteristics

| | **All patients (n=184)** |
|---|---|
| **Age, median in years (IQR)** | 71 (66-76) |
| < 65 | 39 (21.2%) |
| ≥ 65 to < 75 | 91 (49.5%) |
| ≥ 75 | 54 (29.3%) |
| **Sex, n** | |
| Male | 159 (86.4%) |
| Female | 25 (13.6%) |
| **ECOG performance status, n** | |
| 0 | 53 (28.8%) |
| 1 | 113 (61.4%) |
| 2 | 8 (4.3%) |
| 3 | 2 (1.1%) |
| Missing | 8 (4.3%) |
| **Histology, n** | |
| Epithelioid | 103 (56.0%) |
| Non-epithelioid | 76 (41.3%) |
| Sarcomatoid | 48 (26.1%) |
| Mixed | 28 (15.2%) |
| Epithelioid peritoneal | 3 (1.6%) |
| Mixed peritoneal | 1 (0.5%) |
| Missing | 1 (0.5%) |
| **Line of treatment, n** | |
| No pre-treatment | 138 (75.0%) |
| 2nd line | 39 (21.2%) |
| 3rd line | 6 (3.3%) |
| 4rd line | 1 (0.5%) |

## Clinical outcomes in the real-world setting

The median follow-up time of all patients was 12.1 months (n=184; 95% CI 11.1 – 13.1 months) with a minimum of 5.5 months follow-up. For OS an extra survival sweep was done with a median follow-up time of 17.1 months (95% CI 16.4-18.5). The patients received a median number of 6 cycles of nivolumab (range: 1-29) and 3 cycles of ipilimumab (range: 1-14). Ninety patients (49%) received ≥4 cycles.

TRAE's of grade 2-4 that required additional treatment were observed in 86 patients (46.7%), including 51 patients (27.7%) with grade 3 or 4 TRAE's (**Table 2**). The most common grade 3 or 4 TRAE's were hepatitis (7.1%) and colitis (6.5%). No grade 5

TRAE's were found. Infusion related reactions occurred in 45 of 184 patients (24.5%). Multiple grade 2-4 toxicities occurred in the 86 patients. Of those, 75 patients received corticosteroids. Within this group, some patients received additional immunosuppressant agents; infliximab was administered in five patients, cellcept, tocilizumab, methotrexate and azathioprine were administrated in one patient. Other TRAE-treatment included thyroid suppletion or antidiabetica. Twenty-five percent of the patients discontinued nivolumab plus ipilimumab treatment earlier due to TRAE's (**Table S1**). This was 30% of the patients who had discontinued treatment at time of data cut-off. Only one patient (0.5%) discontinued ipilimumab earlier. The median time to develop toxicity in all patients with TRAE's was 9 weeks. The timing of any TRAE after initiation of treatment is shown in **Figure S1**.

**Table 2.** Number of patients experiencing most common TRAE's requiring immunosuppressant treatment

| | All patients (n=184) | | |
|---|---|---|---|
| | **Grade 2 N (%)** | **Grade 3 N (%)** | **Grade 4 N (%)** |
| Any toxicity | 35 (19) | 43 (23.4) | 8 (4.3) |
| Colitis | 7 (3.8) | 12 (6.5) | 0 (0) |
| Pneumonitis | 5 (2.7) | 8 (4.3) | 1 (0.5) |
| Hepatitis | 4 (2.2) | 11(6.0) | 2 (1.1) |
| Endocrinopathy | 16 (8.7) | 3 (1.6) | 1 (0.5) |
| Dermatitis | 4 (2.2) | 4 (2.2) | 0 (0) |
| Nephritis | 2 (1.1) | 1 (0.5) | 1 (0.5) |
| Myocarditis | 1 (0.5) | 0 | 2 (1.1) |
| Musculoskeletal toxicity | 22 (12) | 4 (2.2) | 0 |
| Myasthenia | 0 | 1 (0.5) | 0 |
| Other toxicities | 4 (2.1) | 6 (3.3) | 1 (0.5) |

The objective response rate (ORR) was 21.7% (40 out of 184 patients; 95% CI 16.0-28.4), and 40% had stable disease (SD) as the best result (**Table 3**), resulting in a disease control rate (DCR) at 12 weeks of 56.0% (95% CI 48.8-63.2). mPFS was 5.4 months (95% CI 4.5-6.4) and mOS was 14.1 months (95% CI 11.6-16.6) (**Figure 2**). The 6-months PFS rate was 46% (95% CI: 38.8-53.6) and the 6-months OS rate was 76% (95% CI: 69.9-82.1). The duration of response was 5.7 months (IQR 3.2-8.7).

**Table 3.** Objective response rate by mRECIST per histological subtype of all patients who were evaluable for response.

| Histology | | Frequency (N) | Percentage (%) | Cumulative Percent |
|---|---|---|---|---|
| All histologies | Complete response | 1 | 0.5 | 0.5 |
| | Partial response | 39 | 21.2 | 21.7 |
| | Stable disease | 63 | 34.2 | 56.0 |
| | Progressive disease* | 81 | 44.0 | 100 |
| | Total | 184 | 100 | |
| Epithelioid | Complete response | 0 | 0 | 0 |
| | Partial response | 20 | 20.6 | 51.5 |
| | Stable disease | 30 | 30.9 | 30.9 |
| | Progressive disease | 47 | 48.5 | 100 |
| | Total | 97 | 100 | |
| Non-epithelioid | Complete response | 1 | 1.6 | 100 |
| | Partial response | 18 | 28.1 | 75.0 |
| | Stable disease | 30 | 46.9 | 46.9 |
| | Progressive disease | 15 | 23.4 | 98.4 |
| | Total | 64 | 100 | |
| Epithelioid peritoneal | Stable disease | 3 | 100 | 100 |
| Mixed peritoneal | Partial response | 1 | 100 | 100 |
| Subtype unknown | Stable disease | 1 | 100 | 100 |

* 18 patients had clinical deterioration

**Figure 2.** Progression-Free Survival (PFS) and Overall Survival (OS) in months

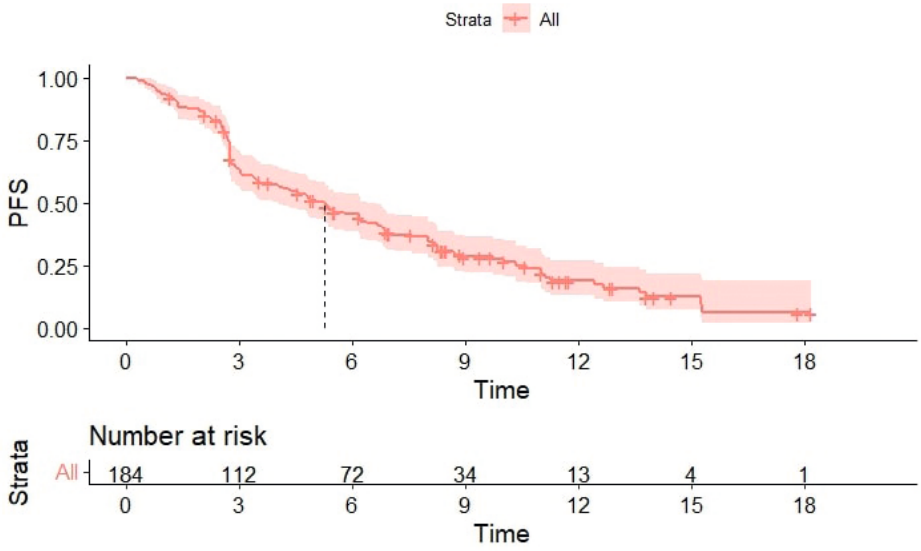



**Figure 2A.** PFS in months, dotted line shows median PFS (5.4 months (95% CI 4.5-6.4))



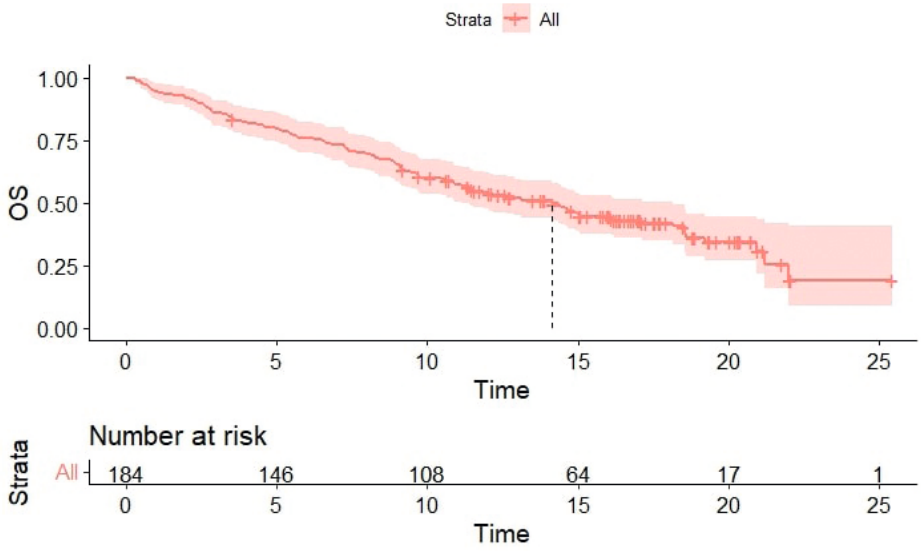



**Figure 2B.** OS in months, dotted line shows median OS (14.1 months (95% CI 11.1-18.2))

We also performed subgroup analyses on PFS (**Figure S2-S4**) and OS (**Figure S5-S7**). PFS and OS seem to be correlated with ECOG PS; a worse ECOG PS results in impaired PFS and OS. We did not find a correlation between PFS and OS with age or histologic subtype.

**Additional analyses**

As a consequence of the nature of real-world setting, the included patient population exhibited a meaningful heterogeneity. Subsequently, we performed several subgroup analyses.

*Adverse events in patients treated with a different dosage*

Eight patients had received a deviating dosage of treatment and therefore excluded from the analyses (**Figure 1**). Two of them experienced a TRAE. One patient had hyperthyroidism, which occurred 9 weeks after initial treatment that required medication, while the other patient had grade 3 musculoskeletal toxicity, which occurred 19 weeks after the initial treatment and required corticosteroids. Both patients had received 100 mg ipilimumab with an average weight of 70 kg.

*Corticosteroids*

Seventy-five patients needed corticosteroids due to adverse events, of whom 26 patients had stable disease and 25 patients had partial response. Twenty-four patients had progressive disease.

*Performance status <2 and first-line treatment (inclusion criteria of the CheckMate-743)*

In our cohort, 5.4% of the patients had an ECOG PS of ≥ 2 and 25% of the patients were treated in second or further lines of treatment. In a subgroup analysis excluding these patients (**Figure 1**; total included patients n=126), we found a median PFS of 6.2 months (95% CI 4.8 – 7.6), ORR of 26.3% and DCR 62.7% (53.6-71.1). Median OS was 14.9 months (11.6 – 18.3) (**Figure S8**).

*TRAE's and discontinuation of treatment related to age and ECOG PS*

Since we were interested in safety, we performed several analyses to evaluate the frequency of TRAE's and the numbers of patients who discontinued treatment due to toxicity between age categories and ECOG PS (**Table S2**-**S5**). Relatively most toxicities occurred in the elder patient population. In addition, among the patients aged > 75 years, 29.6% discontinued treatment due to TRAEs, compared to 24.2% in patients aged 65-74 years and 20.5% in patients aged <65 years. Toxicity did not seem to be correlated to ECOG PS.

## DISCUSSION

To the best of our knowledge this is the first full paper reported real-world cohort study conducted to date to evaluate the safety and activity of nivolumab plus ipilimumab in patients with mesothelioma. In the Netherlands, two hospitals have been designated as center of expertise for patients with mesothelioma: Erasmus Medical Center (Rotterdam) and Antoni van Leeuwenhoek Hospital (Amsterdam). All patients who were treated in one of these hospitals in the given time period are reported, which accounted for 97% of all patients treated with nivolumab plus ipilimumab in the Netherlands. This 97% is based on the data from the number of applications to BMS for acces to the drugs and is based on the fact that other hospitals in the Netherlands were in a preliminary stage of implementing the combination treatment nivolumab plus ipilimumab. As both centers already had experience with this combination treatment and were both amongst the highest including centers in the CheckMate-743 study both centers are well experienced in recognizing and treating TRAE's.

Regarding toxicity, the frequency of TRAE's in our real world population was comparable to the CheckMate-743 study[5] (28% versus 30%), and no new safety signals were reported (**Table 2**). We found a similar percentage of discontinuation of treatment due to TRAE's among the patients in our real-world cohort compared to the CheckMate-743 study (25% versus 23%).

In our real-world population, we found a numerically lower PFS of 5.4 months compared to 6.8 months in the CheckMate-743 trial (**Table 4**) and OS (14.1 months versus 18.1 months). This can be explained by the fact that the characteristics of the patients treated in our real-world cohort did not match well with those of the patients in the CheckMate-743 trial. The most relevant difference is the proportion of patients in our cohort with a higher ECOG PS (**Table 4**) as this factor is known to be negatively associated with outcome and shown in **Figure S5**. We also observed a lower duration of response and objective response rate.

**Table 4.** Comparison between the patients treated with nivolumab and ipilimumab in the CheckMate-743 trial and our real-world setting.

| | CheckMate-743 | This study | This study exclusively 1st line + ECOG 0-1 |
|---|---|---|---|
| Patient number | 303 | 184 | 126 |
| Median age, years (IQR) | 69 (65-75) | 71 (66-76) | 72 (66-77) |
| Proportion men, n (%) | 234 (77%) | 159 (86%) | 87% |
| 1st Treatment line, n (%) | 303 (100%) | 138 (75%) | 100% |
| Epithelial histology, n (%) | 229 (76%) | 103 (56%) | 55% |
| ECOG PS 0, n (%) | 114 (38%) | 53 (29%) | 34% |
| Median duration of treatment, months (IQR) | 5.6 (2.0-11.4) | 3.0 (1.0 – 6.0) | 4.1 (2.1 – 6.2) |
| Median number nivolumab cycles, n (IQR) | 12.0 (5.0-23.5) | 6 (range 1-29) | 7 (4-12) |
| Median number ipilimumab cycles, n (IQR) | 4.0 (2.0-7.0) | 3 (1-14) | 4 (2-6) |
| DCR, % (95%CI) | 77% (71.4-81.2) | 56.0% (48.8-63.2) | 62.7% (53.6-71.1) |
| ORR, % (95% CI) | 40% (34.1-45.4) | 21.7% (16.0-28.4) | 26.3% (18.7-34.8) |
| Median duration of response, months | 11 (95%CI 8.1-11.5) | 5.7 (IQR 3.2-8.7) | 5.7 (IQR 3.3 -8.5) |
| Median PFS, months (95%CI) | 6.8 (5.6-7.4) | 5.4 (4.5-6.4) | 6.2 (4.8-7.6) |
| Median OS, months (95%CI) | 18.1 (16.8-21.4) | 14.1 (11.1-18.2) | 15.01 (12.1-18.0) |
| Median follow-up time, months (IQR) | 29.7 (26.7-32.9) | 9.8 (5.9-13.2) | - |
| Patients with TRAE's grade 3-4, n (%) | 91 (30%) | 51 (28%) | 34 (27) |
| TRAE's as reason of discontinuation nivo/ipi, n (%) | 69 (23%) | 46 (25%) | 31 (25) |
| Early discontinuation ipilimumab, continuing monotherapy nivolumab | 9% | 0.5% | - |

Abbreviations: DCR, disease control rate; ORR, overall response rate, PFS, progression-free survival; OS, overall survival; IQR, interquartile range

It is noteworthy to mention that the small differences we found in the median age of our population (71 years compared to 69 years observed in the clinical trial, the bigger proportion of men (86% compared to 77%) and the lower proportion of epithelioid histology (56% compared to 76%), seem to have only a small influence on the outcomes as in subgroup analyses no differences were observed between the groups (**Figure S3, S7**).

A large difference with the CheckMate-743 was seen in baseline histologic subtype, where we included more patients with sarcomatoid subtype (**Table 4**). This is likely caused by

a referral bias, as the benefit from nivolumab plus ipilimumab in the CheckMate-743 trial was more prominent in the non-epithelial subgroup. As a consequence that part of the epithelial subgroup will not be referred to one of the referral centers and be treated with chemotherapy. Chemotherapy could be administered at the local hospital, whilst nivolumab plus ipilimumab was only available at EMC and NKI. Hence, we believe the difference in histological subtype reflects the real-world situation at the time. At present doublet immunotherapy is available in more centers as registered treatment for all histological subtypes.

We included all different lines of treatment in all analyses. In an extensive prognostic model, developed by de Gooijer et al, the value of line of treatment seemed limited.[16] Thus, possibly this is not the most important baseline characteristic to take into account in the analyses. The fact that we even found patients treated in a different line or histology signifies the thoroughness of our search to include all mesothelioma patients treated with nivolumab plus ipilimumab.

Our study is subject to several limitations. Due to its clinical character, some information, such as low-grade adverse events, was not reported, because this might have been without therapeutic consequences. Moreover, our study included three patients with peritoneal mesothelioma, which was not an exclusion criterion in the named patient program. Whether the outcomes of ICI in peritoneal mesothelioma are comparable to that of pleural mesothelioma is unclear. The patient population in a real-world cohort differs from a trial cohort is commonly seen.[11,12]



Due to a limited number of patients, adequate statistical testing to confirm correlations was not possible. To address this limitation, a larger database is required to increase the statistical power and ensure that any observed trends or associations are robust. Nevertheless, possible trends in descriptive statistics are informative as well. For example, the observation that a higher incidence of TRAE's may be present, despite being treated in highly experienced centers, warrants clinicians to be cautious when prescribing nivolumab and ipilimumab and to closely monitor patients for potential adverse events. Also, we advise centralization of this treatment to ensure that patients are treated by a dedicated and experienced team.

Furthermore, this study involved a heterogeneous population including patients with a fixed dosing scheme based on a pharmacological rationale.[13] In addition, nationwide new immunotherapy dose schemas are under development or are being tested.[14,15] To be sure that the results of the patients in our study were not dose dependent and to allow proper comparison with the CheckMate-743 trial, we calculated the dose deviation

for each patient and excluded those who deviated more than 15% from the standard dosage of ipilimumab.

Our real-world data of patients with mesothelioma treated with nivolumab plus ipilimumab confirmed activity at the expense of a substantial number of TRAE's. The median PFS of patients treated in our real-life program is comparable with the study population, but only when the same selection criteria are applied, omitting patients with poor prognostic characteristics. We recommend to prescribe nivolumab-ipilimumab with caution.

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# DATA SUPPLEMENTS

**Eligibility criteria, study procedures and outcomes**

Potentially eligible patients were presented to a central medical board that confirmed eligibility and recommended the patients to the Named Patient Program team. From June 2022 onwards, patients were not treated as part of the program, but as regular standard of care. Eligible patients were adults (≥18 years old) with pathologically confirmed unresectable mesothelioma (cytology was also approved). No recent tumor biopsy was required. All histological subtypes were allowed. Peritoneal mesothelioma was not an exclusion criterion. Eligible patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤3 adequate hematological, renal, metabolic, and hepatic function and evaluable disease on CT scan. Patients with a history of chronic inflammatory or autoimmune disease or an interstitial lung disease that was symptomatic or may interfere with the detection or management of suspected drug-related pulmonary toxicity were excluded, as well as patients requiring steroids >10mg daily prednisone (or equivalent).



During the program, patients received nivolumab 360 mg fixed dose or 4.5mg/kg every 3 weeks intravenously (Q3W) and ipilimumab 1 mg/kg every 6 weeks intravenously (Q6W) until disease progression or unacceptable toxicity for a maximum of 2 years. From May 2022 onwards, a new dosing scheme for ipilimumab was introduced in the Netherlands Cancer Institute according to weight class (40-70 kg, 71-120 kg, and 121-150 kg receive 50, 100, or 150 mg, respectively). The equivalent range in mg/kg is 0.71-1.25 mg/kg, 1-1.4 mg/kg, and 1-1.5 mg/kg. From November 2022 onwards, also a new scheme for ipilimumab according to weight class (44-59kg, 59-65kg, 65-88kg, 88-118kg, 118-147kg receive 50, 60, 75, 100 or 125mg, respectively) was introduced in Erasmus MC. Patients who deviated more than 15% from the 1mg/kg dosage were excluded from our primary analysis.[13]

Clinical data of the patients was collected from the digital patient register. The following variables were collected and used for analysis: diagnosis, date of the first diagnosis, age, sex, histological subtype (epithelioid, non-epithelioid subdivided in sarcomatoid and mixed), ECOG PS, line of treatment, the start date of nivolumab + ipilimumab, best response to treatment, date of progression after the start of treatment, date of death, toxicities requiring steroids and toxicities requiring interruption or discontinuation of the treatment.

Radiological tumor assessment was performed at baseline and every 2 cycles after the start of treatment using computed tomography (CT) using modified RECIST v.1.1.[9]. A confirmatory CT scan of progressive disease was not mandatory.

The data cutoff for this study was set at January 15, 2023, as this ensured that the results would be sufficiently matured, allowing the majority of patients to have undergone at least one response CT-scan.

Progression-free survival (PFS) was defined as the time interval from the first nivolumab plus ipilimumab administration until the earliest date of clinical or radiological progression or death from any cause or were censored at the date of last evaluable tumor assessment. Overall survival (OS) was defined from the date of the first immunotherapy administration until patient death from any cause. A patient who was still alive was censored or were censored at the time of data cut-off (July 1st). The objective response rate (ORR) was defined as the proportion of patients who had as best response partial (PR) or complete response (CR) to therapy, whereas the disease control rate (DCR) was defined as the percentage of patients who achieved a CR, PR, or stable disease (SD) around 12 weeks of treatment. Duration of response was defined as time between the date of first documented response (CR or PR) to the date of the first documented tumor progression per modified RECIST v 1.1. Only patients who were evaluable with a CT-scan were included for objective response rate, disease control rate, duration of response, and progression free survival. All patients were included for the safety analysis. Adverse events (AEs) were graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events (CTCAE) v.5.0.

**Statistical analyses**

Response rates with 95% confidence intervals (CI) were reported using exact methods. Progression-free survival and survival data was analyzed using Kaplan-Meier estimates. Patients who discontinued or for whom no observation was available were censored. Subgroup analyses of categorical variables were performed with Pearson's Chi-Square tests. All calculations were performed using IBM SPSS statistics version 27.0 (Armonk, NY: IBM Corp). Rstudio version 2023.06.0 was used for the Kaplan-Meier curves.

**Figure S1.** Timing of TRAE's (any grade) after the start of the treatment with nivolumab plus ipilimumab.

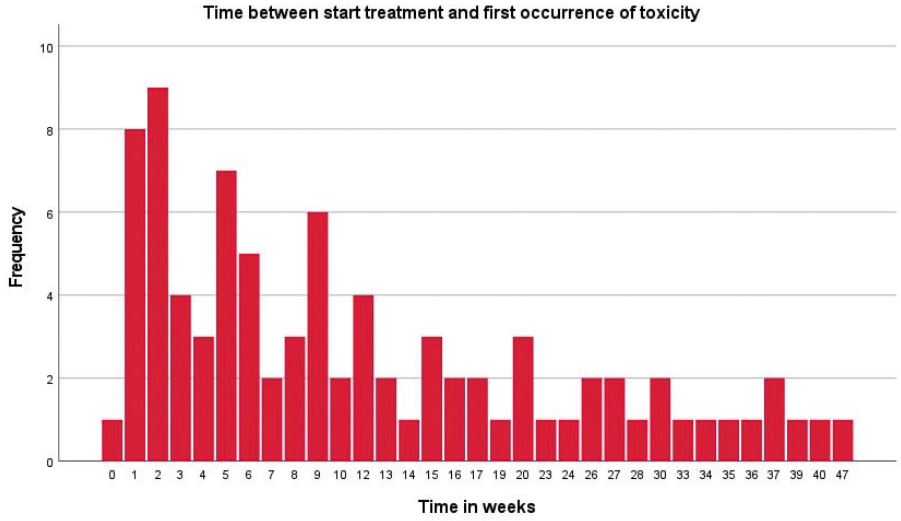




**Figure S2.** Progression-Free Survival by ECOG PS 0-3

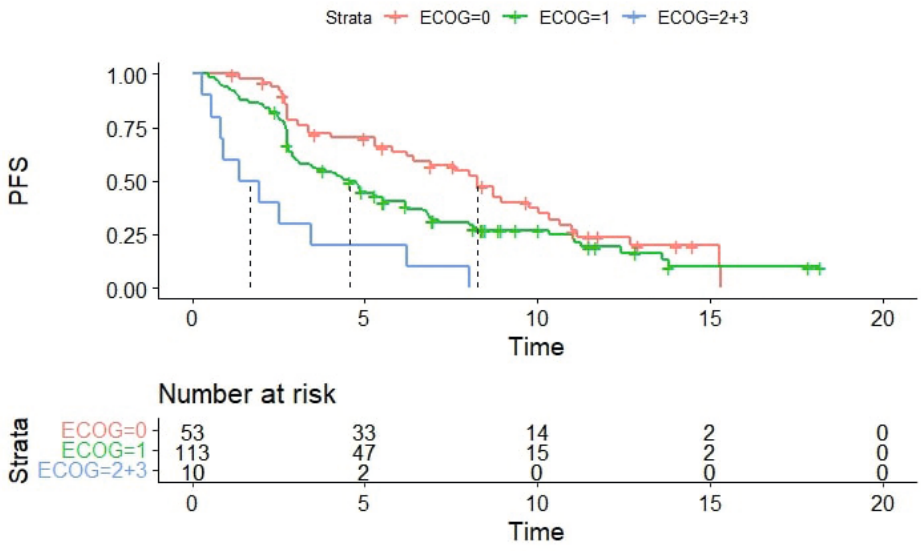

**Figure S3.** Progression-Free Survival by age categories

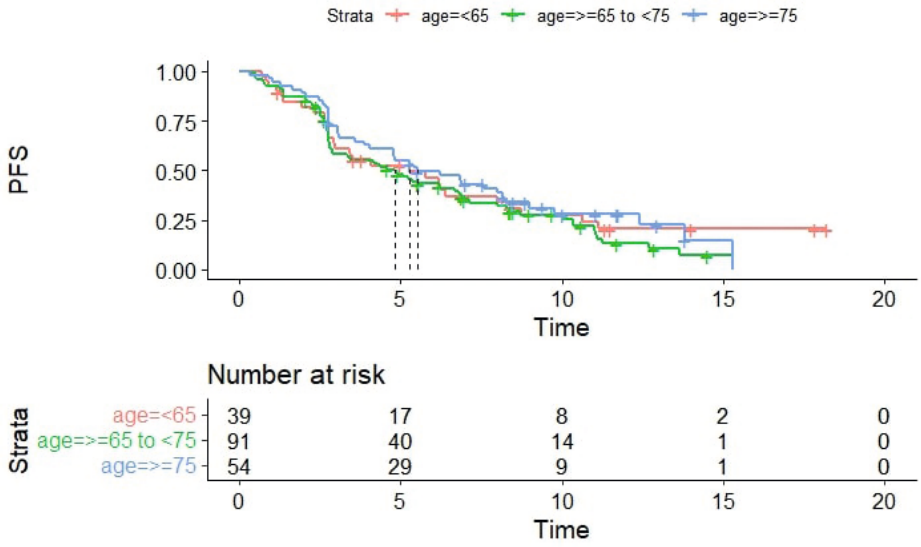


**Figure S4.** Progression-Free Survival by histological subtype

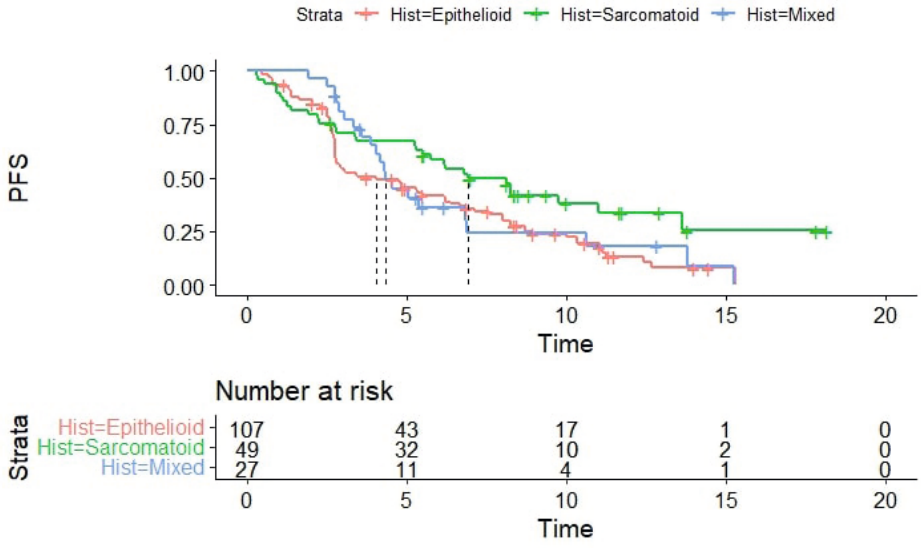

**Figure S5.** Overall Survival by ECOG PS 0-3

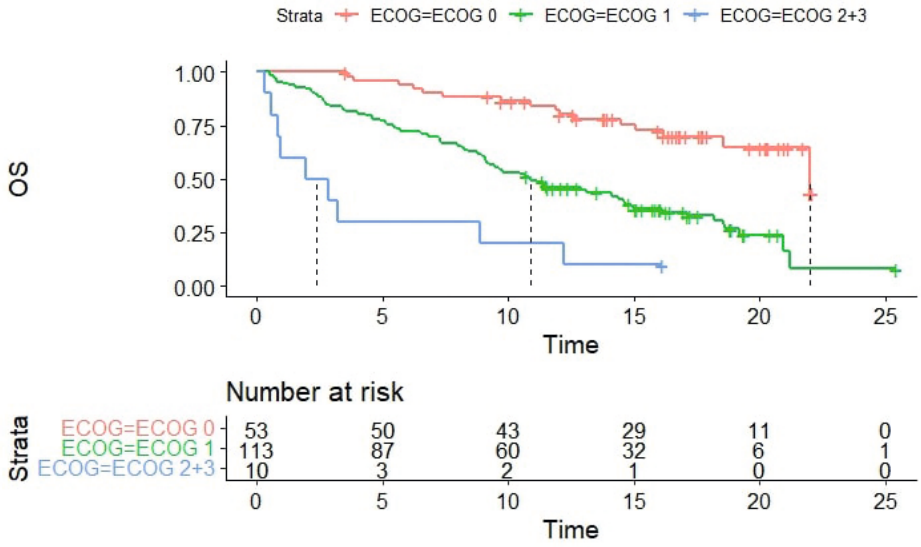




**Figure S6.** Overall survival by age categories

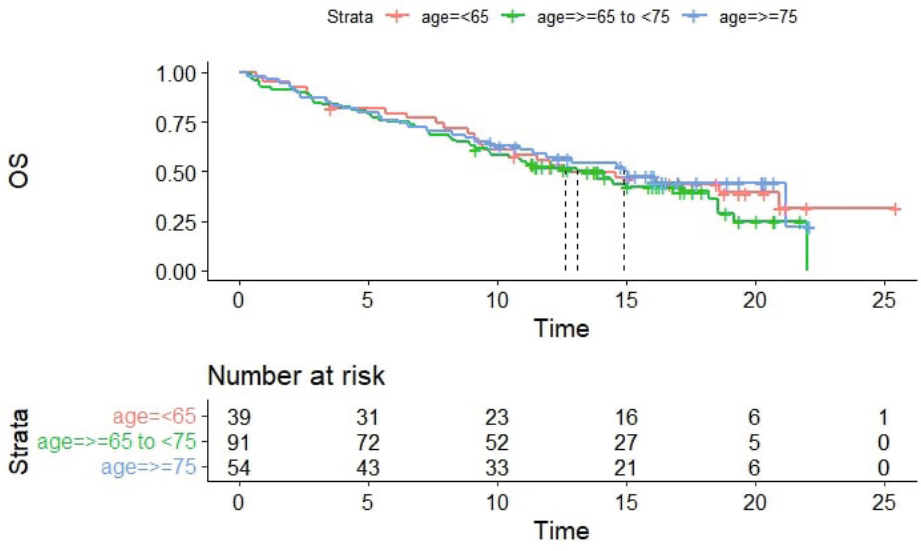

**Figure S7.** Overall Survival by histological subtype. mOS epithelioid, sarcomatoid and mixed were 14.4 months (95% CI 10.8 – NR), 14.6 (95% CI 11.4 –NR), and 11.9 (95% CI 8.8 – NR), respectively.

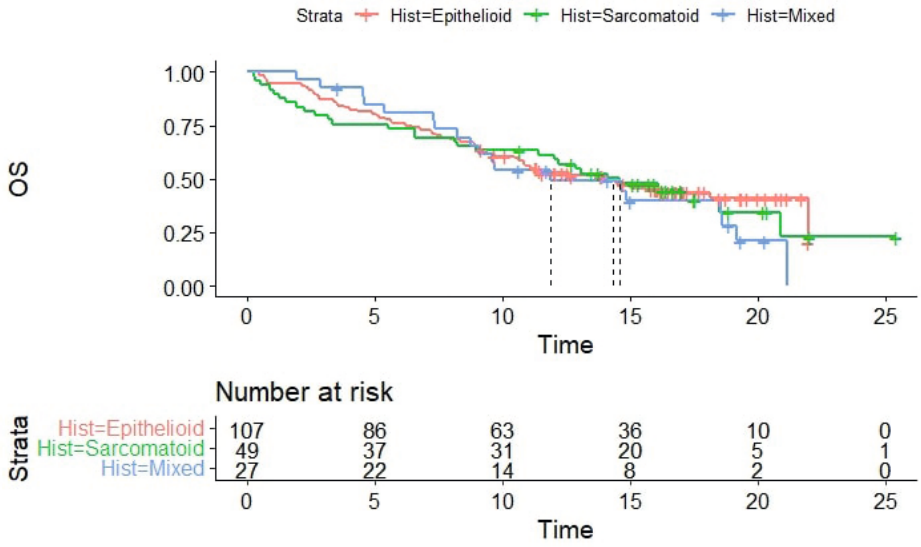


**Figure S8.** Overall Survival in patients with performance status <2 and first-line treatment, dotted line reflects median OS (14.9 months (95% CI 11.6-18.3)).

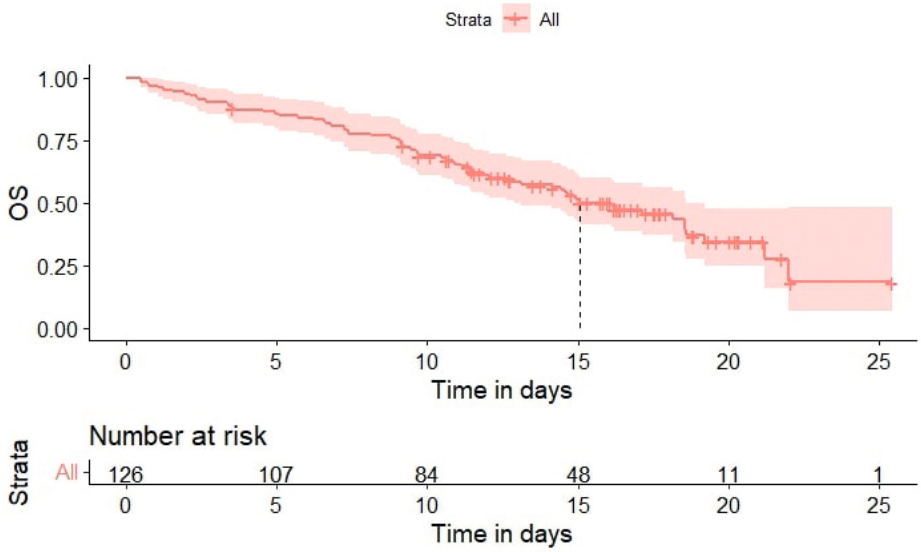

**Table S1.** Reason for discontinuation treatment

| | Frequency (N) | Percentage (%) | Percentage of patients who discontinued treatment (%) |
|---|---|---|---|
| On treatment at data cut-off | 29 | 15.8 | NA |
| Progression | 92 | 50.0 | 59.4 |
| Toxicity | 46 | 25.0 | 29.7 |
| Passed away prior to discontinuation | 8 | 4.3 | 5.2 |
| Passed away by euthanasia | 2 | 1.1 | 1.3 |
| Withdrawal at patient's request | 3 | 1.6 | 1.9 |
| Referral to other center | 4 | 2.1 | 2.6 |
| Total | 184 | 100% | 100% |

**Table S2.** Toxicity divided by age categories

| | | Age | | | |
|---|---|---|---|---|---|
| | | <65 n (%) | >=65 to <75 n (%) | >= 75 (n%) | Total |
| Toxicity | Absent | 23 (59.0) | 50 (54.9) | 25 (46.3) | 98 (53.3) |
| | Present | 16 (41.0) | 41 (45.1) | 29 (53.7) | 86 (46.7) |
| Total | | 39 (100) | 91 (100) | 54 (100) | 184 (100) |

**Table S3.** Toxicity divided by ECOG Performance score

| | | ECOG PS | | | |
|---|---|---|---|---|---|
| | | 0 (n%) | 1 (n%) | 2 +3 (n%) | Total |
| Toxicity | Absent | 28 (52.8) | 59 (52.2) | 7 (70) | 94 (53.4) |
| | Present | 25 (47.2) | 54 (47.8) | 3 (30) | 82 (46.6) |
| Total | | 53 (100) | 113 (100) | 10 (100) | 176 (100) |

**Table S4.** Reason of discontinuation divided by age

| | <65 (n%) | >=65 to <75 | >=75 | Total |
|---|---|---|---|---|
| On treatment at data cut-off | 5 (12.8) | 17 (18.7) | 7 (13.0) | 29 (15.8) |
| Progression | 22 (56.4) | 46 (50.5) | 24 (44.4) | 92 (50.0) |
| Toxicity | 8 (20.5) | 22 (24.2) | 16 (29.6) | 46 (25.0) |
| Passed away prior to discontinuation | 1 (2.6) | 4 (4.4) | 3 (5.6) | 8 (4.3) |
| Passed away by euthanasia | 0 (0) | 1 (1.1) | 1 (1.9) | 2 (1.1) |
| Withdrawal at patient's request | 1 (2.6) | 1 (1.1) | 1 (1.9) | 3 (1.6) |
| Referral to other center | 2 (5.2) | 0 (0) | 2 (3.7) | 4 (2.1) |
| Total | 39 (100) | 91 (100) | 54 (100) | 100 |

**Table S5.** Reason of discontinuation divided by ECOG PS. 8 patients missing value for ECOG PS.

| | WHO 0 N (%) | WHO 1 N (%) | WHO 2+3 N (%) | Total N (%) |
|---|---|---|---|---|
| On treatment at data cut-off | 12 (22.6) | 15 (13.3) | 0 (0) | 27 (15.3) |
| Progression | 25 (47.2) | 56 (49.6) | 8 (75) | 89 (50.6) |
| Toxicity | 14 (26.4) | 29 (25.7) | 1(12.5) | 44 (25.0) |
| Passed away prior to discontinuation | 0 (0) | 8 (7.1) | 1 (12.5) | 9 (5.1) |
| Withdrawal at patient's request | 0 (0) | 3 (2.7) | 0 (0) | 3 (1.7) |
| Referral to other center | 2 (3.8) | 2 (1.8) | 0 (0) | 4 (2.2) |
| Total | 53 | 113 | 10 | 176 |

# CHAPTER 7

## LONG-TERM FOLLOW-UP OF MESOTHELIOMA PATIENTS TREATED WITH DENDRITIC CELL THERAPY IN THREE PHASE I/II TRIALS

**Daphne W Dumoulin**, Robin Cornelissen, Koen Bezemer, Sara J Baart, Joachim G J V Aerts

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## ABSTRACT

### Background

Malignant pleural mesothelioma (MPM) is a fatal neoplasm with, if untreated, poor survival of approximately nine months from diagnosis. Until recently, phase II-III immunotherapy trials did not show any significant benefit. The lack of immunotherapy efficacy can be explained by the fact that mesothelioma is a tumor with an "immune-desert" phenotype, meaning a non-inflamed tumor characterized by low T-cell infiltration. By administration of DCs, which were *ex-vivo* cultured, exposed to (tumor associated) antigens, and subsequently activated, this "immune-desert" phenotype might be turned into an "inflamed" phenotype. Three phase I studies have been performed and published using activated DCs, which support this concept. We here report on the long-term survival of the patients treated with DCs in these three phase 1 studies.

### Methods

Survival data of the phase 1 trials using DC therapy in MPM patients was obtained and subsequently analysed. In the first two trials, DCs loaded were loaded with autologous tumor lysate, while in the third trial DC were loaded with allogeneic mesothelioma tumor cell line lysate.

### Results

In the three studies combined, 29 patients with MPM were treated with DC vaccination between 2006 and 2015. At data cut-off, the median OS was 27 months (95% CI: 21 – 47 months). OS at 2 years was 55.2% (95% CI: 39.7%-76.6%), OS at 5 years was 20.7% (95% CI: 10.1%-42.2%).

### Conclusions

The long-term survival of DC therapy in MPM in these three trials is promising, which led to the randomized phase II/III DENIM study. This DENIM study is currently enrolling, and the results have to be awaited for definite conclusions.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a fatal neoplasm of the pleural lining with poor survival of approximately nine months from diagnosis without treatment. Currently, treatment options are limited. Treatment with the combination of cisplatin with an anti-folate (pemetrexed or raltitrexed) resulted in a survival benefit of nearly three months[1,2]. Checkpoint inhibition therapy using pembrolizumab in second line (PROMISE-MESO), improved response rate (RR) but did not improve progression-free survival (PFS) or overall survival (OS) compared to chemotherapy[3]. Recently, the phase 3 randomized CheckMate-743 trial showed an improvement in OS of four months in previously untreated malignant pleural mesothelioma with nivolumab (anti-programmed death-1 (anti-PD-1)) and ipilimumab (cytotoxic T-lymphocyte associated protein 4 (anti-CTLA-4)) compared to chemotherapy, leading to FDA approval[4]. The efficacy in the non-epithelioid subgroup was impressive, with an improvement in median OS of nearly ten months. For the epithelial subgroup, this improvement was, although significant, more modest; over 2 months and long-term survival is unknown[5]. It should be noted, however, that both of these are prespecified subgroup analyses. This first positive trial regarding immunotherapy in MPM is in contrast with non-small cell lung cancer (NSCLC), where mono- immunotherapy targeting PD-(L)1 has become standard of care based on many positive trials from 2015, showing long term overall survival[6–8]. However, in mesothelioma flattening of the curve is so far absent[5].



The lack of immunotherapy efficacy can be explained by the fact that mesothelioma is a tumor with an "immune-desert" phenotype[9], meaning a non-inflamed tumor characterized by low T-cell infiltration. T-cells need to be activated by antigen-presenting cells; dendritic cells (DCs) are among the most potent antigen-presenting immune cells to activate these T-cells[10]. In mesothelioma, a low tumor mutational burden results in low numbers of tumor associated antigens (TAA), leading to a challenging tumor recognition by DCs. Furthermore, the immunosuppressive tumor microenvironment, characterized by high numbers of immunosuppressive cells such as M2 macrophages and regulatory T-cells and high levels of immune-suppressive cytokines such as VEGF, hinder the maturation of DCs, and cause the absence of activated dendritic cells.[10]. Given that mature DCs are mandatory for an effective immune response, focusing on this step in the immune cycle could lead to a probably more viable treatment option for patients with mesothelioma[11]. DC's can be maturated in several ways, either in vivo or ex vivo. A disadvantage of in vivo generation of DCs is that they may become inactivated by the immunosuppressive environment of the tumor. By generating DCs ex vivo, this can be prevented. DCs can be derived from monocytes, or they can be isolated in low levels from peripheral blood. For cancer immunotherapy, the ideal target for activating DCs

would be a TAA that is exclusively expressed on all tumor cells without being present in normal tissues to prevent autoimmunity. Targeting multiple TAAs, as in tumor cell lysates, may overcome several disadvantages that can arise when using a single TAA[12]. For example, a single TAA may not be expressed on all tumor cells. Furthermore, when a single TAA is downregulated by the tumor, this will result in avoidance of immune detection. Polyvalent tumor cell lysates may be obtained either from autologous or allogeneic tumor cells.

In mesothelioma, three phase I/II studies have been performed using activated DCs, which were cultured, activated, and exposed to antigens ex-vivo in order to overcome the problem of absent tumor recognition and absent maturation of DCs[13–15]. We hypothesize that treatment with activated dendritic cells could preclude this T-cell exhaustion and therefore increase long term survival. Therefore, we collected the long-term survival of the patients treated with DCs in these three phase I/II studies.

## MATERIAL AND METHODS

For this study, the survival data of three phase 1 data was combined. In short, the studies were performed as follows:

In the first clinical trial (Study 1), safety and immunological response by administering tumor lysate-pulsed dendritic cells were analyzed in patients with MPM[15]. Ten patients were treated with four cycles of standard chemotherapy followed by three vaccinations of mature DCs loaded with autologous tumor lysate and keyhole limpet hemocyanin (KLH) as a surrogate marker in 2-week intervals. Each vaccination consisted of 50 million DCs. The vaccinations were given 1/3 intradermally and 2/3 intravenously. In addition, peripheral blood mononuclear cells were drawn during the treatment in order to analyze immunological responses.

In a follow-up trial, the decrease in the number of regulatory T cells and immunological responses in peripheral blood during treatment with autologous dendritic cell vaccination combined with low-dose cyclophosphamide were analyzed (Study 2)[14]. Cyclophosphamide was added to reduce the number of Tregs. Ten patients were treated with four to six cycles of platinum and pemetrexed, followed by DC-vaccination combined with low-dose cyclophosphamide intermittently. In five of these patients, an additional pleurectomy/decortication was performed before DC-vaccination. As in the first trial, the vaccinations of mature DCs were loaded with autologous tumor lysate and keyhole limpet hemocyanin (KLH) as a surrogate marker and were given 1/3

intradermally and 2/3 intravenously. The vaccinations were given with a 2-week interval 3 times, followed by revaccination after 6 and 12 months. Each vaccination consisted of 50 million DCs. Cyclophosphamide was administered daily starting 1 week prior to vaccination until the day of vaccination, in a dose of 100mg a day.

Using autologous tumor cell lysate, the number of patients eligible for vaccinations was limited due to insufficient amount and unsuitable tumor material, which hampers a similar but larger multicenter clinical trial. To overcome this challenge, a follow-up trial using an allogeneic mesothelioma tumor cell line lysate was performed (Study 3). The aim of this trial was to investigate the efficacy of allogeneic lysate-pulsed DC vaccination in mice and safety in humans[13]. Nine patients were treated with DC vaccinations consisting of autologous monocyte-derived DCs pulsed with tumor lysate originating from five different mesothelioma cell lines. DC vaccinations were given with a 2-week interval 3 times, followed by revaccination after 3 and 6 months. The vaccinations were administered 1/3 intradermally and 2/3 intravenously. The setup of this trial was a 3+3 dose escalation safety analysis. Therefore, 3 patients received 10, 3 patients 25, and 3 patients 50 million DCs per vaccination.

**Statistical analysis**

In the current analysis, we collected the survival data of these three phase 1 trials. The median OS, 2-year OS, and 5-year OS, including 95% confidence intervals were calculated based on the Kaplan-Meier curve. The analyses were repeated stratified per study. R version 3.6.2 (R Core Team, 2019) was used for the statistical analysis.



## RESULTS

In the three studies combined, 29 patients with MPM were treated with DC vaccination between 2006 and 2015. Patient characteristics are summarised in **Table 1**. At data cut-off, the median OS was 27 months (95% confidence interval (CI): 21 – 47 months). OS at 2 years was 55.2% (95% CI: 39.7%-76.6%), OS at 5 years was 20.7% (95% CI: 10.1%-42.2%).

Four patients are still alive, at respectively 71, 77, 114, and 128 months. The first two patients were treated with DC vaccinations containing allogeneic tumor lysate; the latter two patients were treated with autologous dendritic cell vaccination combined with low-dose cyclophosphamide; one patient has had a pleurectomy/decortication before the DC vaccination.

The survival of all 29 patients is shown in **Figure 1**.

The survival analysis for the 3 separate trails are shown in **Table 2**. The first trial showed a median OS of 15 months, a 2-year OS of 20% (95% CI 5.8% - 69.1%) and a 5-year OS of 10.0% (95% CI 1.6% - 64.2%). Study 2 showed a median OS of 26 months, a 2-year OS of 60% (95% CI 36.2 – 99.5%) and a 5-year OS of 30.0% (95% CI 11.6% - 77.3%). Study 3 showed a median OS of 31 months, a 2-year OS of 88.9% (95% CI 70.6% - 100%) and a 5-year OS of 22.0% (95% CI 6.6% - 75.4%), **Table 2**.

**Figure 1.** Overall Survival of the combined phase I/II trials

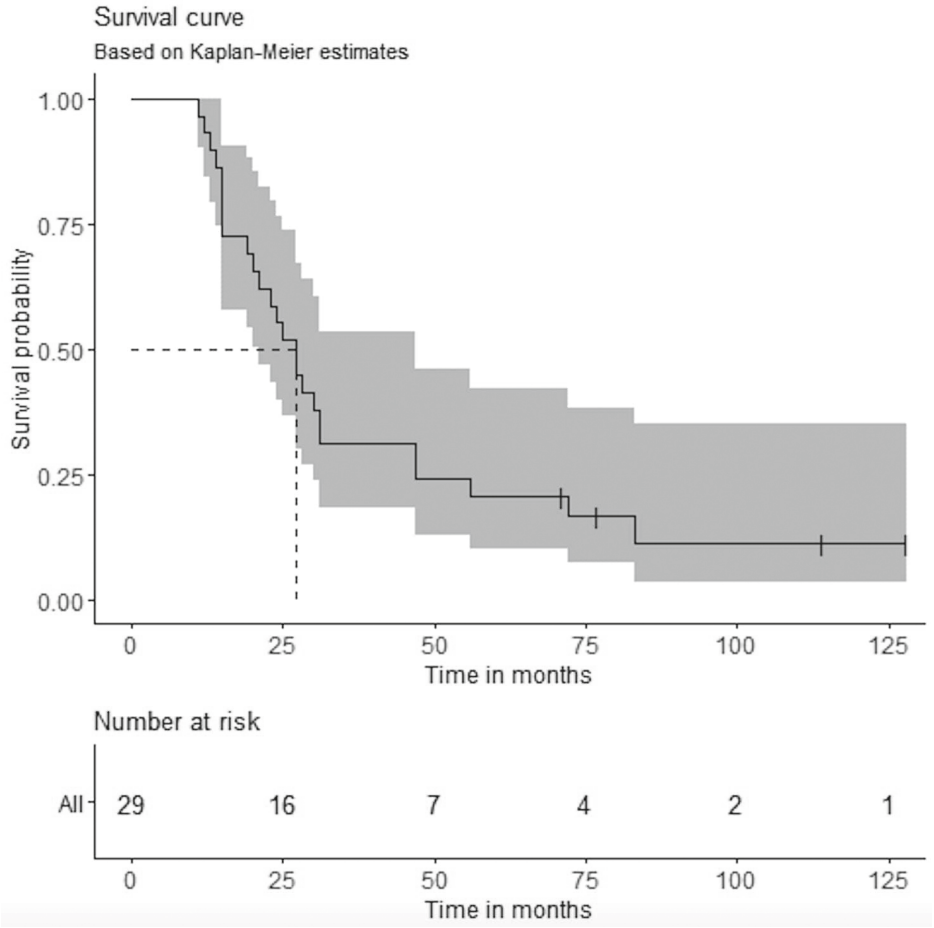

**Table 1.** Patient characteristics

| | Gender | Age | Histology | Extended P/D | Chemotherapy | Response on DC | Survival (months) | Alive |
|---|---|---|---|---|---|---|---|---|
| study 1 (N=10) | male | 68 | epithelial | no | yes | PR | 23 | no |
| | male | 63 | epithelial | no | yes | PD | 72 | no |
| | male | 55 | epithelial | no | yes | PR | 19 | no |
| | male | 66 | epithelial | no | yes | PR | 30 | no |
| | male | 71 | epithelial | no | yes | SD | 15 | no |
| | male | 64 | epithelial | no | yes | PD | 13 | no |
| | male | 75 | epithelial | no | yes | PD | 11 | no |
| | male | 77 | epithelial | no | yes | PD | 15 | no |
| | male | 70 | epithelial | no | yes | PD | 15 | no |
| | male | 58 | epithelial | no | yes | PD | 15 | no |
| study 2 (N=10) | male | 62 | epithelial | no | yes | SD | 24 | no |
| | male | 71 | epithelial | no | yes | SD | 25 | no |
| | male | 78 | epithelial | no | yes | SD | 14 | no |
| | male | 55 | epithelial | no | yes | CR | 114 | yes |
| | male | 75 | epithelial | no | yes | SD | 27 | no |
| | male | 63 | epithelial | yes | yes | SD | 20 | no |
| | male | 58 | biphasic | yes | yes | PD | 12 | no |
| | female | 35 | epithelial | yes | yes | PR | 128 | yes |
| | female | 55 | biphasic | yes | yes | SD | 56 | no |
| | male | 48 | epithelial | yes | yes | SD | 83 | no |
| study 2 (N=10) | male | 79 | epithelial | no | no | SD | 47 | no |
| | male | 69 | epithelial | no | no | SD | 31 | no |
| | male | 44 | epithelial | no | yes | SD | 77 | yes |
| | female | 59 | epithelial | no | yes | PR | 47 | no |
| | male | 73 | epithelial | no | no | PR | 71 | yes |
| | male | 67 | epithelial | no | yes | SD | 28 | no |
| | male | 68 | epithelial | no | no | SD | 21 | no |
| | male | 71 | epithelial | no | yes | SD | 31 | no |
| | male | 60 | epithelial | no | yes | SD | 27 | no |

**Table 2.** Overall Survival analysis based on the Kaplan Meier curves

| | Median OS (95% CI) | OS – 2 years (95% CI) | OS – 5 years (95% CI) |
|---|---|---|---|
| Overall | 27 months (21 - 47) | 55.2% (39.7% - 76.6%) | 20.7% (10.1% - 42.2%) |
| Study 1 | 15 months (15 - Inf) | 20.0% (5.8% - 69.1%) | 10.0% (1.6% - 64.2%) |
| Study 2 | 26 months (20 - Inf) | 60.0% (36.2 – 99.5%) | 30.0% (11.6% - 77.3%) |
| Study 3 | 31 months (28 - Inf) | 88.9% (70.6% - 100%) | 22.2% (6.6% - 75.4%) |

## DISCUSSION

The long-term follow-up of MPM patients treated with DC vaccination in the three separate phase I/II trials show a promising signal, with a 2-year OS of over 50% and a 5-year OS of over 20%. In addition, 2 patients are alive to date after 10 years of treatment. In our opinion, these findings show the potency of DC vaccination therapy in the long-term activation of the immune system. Translational research performed in these studies did reveal that DC vaccination was able to induce a tumor directed anti T-cel response, the essential step for effective immunotherapy[16]. This opens the potential for combination immunotherapy with DC therapy as backbone.

The three separate phase I/II trials had more or less similar study designs; In study 2, 5 patients underwent additional debulking surgery. With regard to immunotherapy, checkpoint inhibition therapy has shown to be more useful in patients with a low to modest tumor burden in melanoma and NSCLC patients[17–19]. A less prominent immunosuppressive tumor microenvironment can explain the improved effectiveness of immunotherapy in these studies in patients with a smaller tumor volume. Whether debulking surgery can cause an effect similar to an “earlier stage” cancer has to be determined. Also, whether a reduced tumor load is beneficial for DC vaccination therapy is a field of further research. As already mentioned, cyclophosphamide was added to this treatment regimen in order to reduce the number of Tregs. Subsequently, this effect was measured in peripheral blood analysis, and this strategy could be used in further research[20,21].

The use of autologous tumor material to load the DCs was labor-intensive and cumbersome as in the majority of screened patients, not enough viable cells could be obtained to generate a lysate for DC loading. In addition, the need for viable tumor

material resulted in only part of patients being eligible for participation in the first two studies. Therefore, an allogeneic tumor lysate was produced, which meant that an "off-the-shelf" product is now readily available, which is also used in the current phase II/III trial. The long-term survival of the patients in the third study is, in fact, not inferior to the patients treated in the previous trials.

There are several limitations to this study. First, the primary outcomes of these phase I/II trials were safety and feasibility. OS was an exploratory outcome. Second, the three trials had a different setup; therefore, combining the outcomes should be approached with some caution. Third, there is no control group. The survival in mesothelioma patients is known to be variable, and long-term survivors do exist. However, when looking at survival data from historical data, the outcomes of patients treated with DC-therapy are promising. Given that the patients treated in our three trials had to be non-progressive on the platinum-pemetrexed treatment, a comparison with the recently published NVALT-19 study is the most logical one. In the NVALT-19 study, patients were treated with switch-maintenance gemcitabine or best supportive care (BSC) if there was no progression on platinum-pemetrexed treatment[22]. In this study, OS at 2 years is approximately 25% in the gemcitabine group and 20% in the BSC group, comparing to 55.2% OS for DC vaccination. Fourth, the treatment which was given after patients progressed after DC therapy varied because over the years. Most patients were treated with second-line therapy in trials of named-patient programs, with checkpoint inhibition therapy being increasingly given over the years. In fact, eight of nine patients in the last trial were treated with immune checkpoint inhibition (ICI) therapy at some point in time. In theory, adding ICI therapy after DC therapy seems very logical. The T-cells are activated by the administration of the DC vaccines. In turn, these activated T-cells can be blocked by a PD-L1 expression of the tumor. ICI therapy would, therefore, be an ideal partner compound. If the seemingly improved survival is a result of this remains to be elucidated.

DC vaccination therapy in MPM patients is currently being investigated in a large randomized phase II-III trial (NCT03610360)[23]. In the DENdritic cell Immunotherapy for Mesothelioma (DENIM) study, patients are randomized to allogenic tumor lysate loaded DC vaccination therapy and BSC versus BSC alone after completion of 4-6 cycles of platinum-pemetrexed chemotherapy. The primary endpoint is OS. 230 patients will be enrolled at 6 sites in 5 countries. Enrolment is currently ongoing, and the trial is expected to complete enrolment in 2021.

## CONCLUSION

The long-term survival of DC therapy in mesothelioma in three phase I/II trials is promising. Results of the randomized phase II/III DENIM study, which is currently enrolling, have to be awaited for definite conclusions. Additional biomarker studies, as well as treatment combinations with, for example ICI, could further improve the outcomes of this treatment strategy.

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# CHAPTER 8

## COMBINATION OF PD-1/PD-L1 CHECKPOINT INHIBITION AND DENDRITIC CELL THERAPY IN MICE MODELS AND IN PATIENTS WITH MESOTHELIOMA

Mandy van Gulijk*, Bob Belderbos*, **Daphne Dumoulin**, Robin Cornelissen, Koen Bezemer, Larissa Klaase, Floris Dammeijer, Joachim Aerts

* These authors contributed equally

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## ABSTRACT

Immunotherapy with anti-PD1/PD-L1 is effective in only a subgroup of patients with malignant pleural mesothelioma (MPM). We investigated the efficacy of a combination of anti-PD1/PD-L1 and dendritic cell (DC) therapy to optimally induce effective anti-tumor immunity in MPM in both humans and mice. Data of nine MPM patients treated with DC therapy and sequential anti-PD1 treatment were collected and analyzed for progression-free survival (PFS) and overall survival (OS). Survival and T-cell responses were monitored in AC29 mesothelioma-bearing mice treated concurrently with the combination therapy; additionally, the role of the tumor-draining lymph node (TDLN) was investigated. The combination therapy resulted in a median OS and PFS of 17.7 and 8.0 months, respectively. Grade 3-4 treatment-related adverse events had not been reported. Survival of the mesothelioma-bearing mice treated with the combination therapy was longer than that of untreated mice, and coincided with improved T-cell activation in peripheral blood and less T-cell exhaustion in end stage tumors. Comparable results were obtained when solely the TDLN was targeted. We concluded that this combination therapy is safe and shows promising OS and PFS. The murine data support that PD-L1 treatment may reinvigorate the T-cell responses induced by DC therapy, which may primarily be the result of TDLN targeting.

## INTRODUCTION

The median survival after diagnosis for patients with malignant pleural mesothelioma (MPM) remains between 13 and 18 months[1,2]. Therefore, novel therapeutic strategies that effectively induce anti-tumor responses are warranted. PD-1 checkpoint inhibition has shown remarkable responses in multiple cancer types. Anti-PD-1 therapy induces responses in 9-29% of MPM patients and as second line treatment it has been associated with a median progression-free survival (PFS) of 2.5 months and median overall survival (OS) of 10.7 months[1,3-5]. In combination with ipilimumab (anti-CTLA-4), response rates were even higher and more durable. Still, the majority of patients failed to respond which could be due to lack of T-cell infiltration before treatment[2,6].

Dendritic cell (DC) therapy has been shown to be safe, feasible and able to induce radiological responses in MPM coinciding with enhanced intratumoral T-cell infiltration[7-9]. As DC therapy-induced infiltrating T cells may in turn become exhausted through PD-1/PD-L1 signaling, we investigated the efficacy of adjuvant anti-PD-1 immunotherapy in DC-treated MPM patients. Additionally, as PD-L1 is expressed on DCs, the effects of concurrently combining DC- and anti-PD-L1 therapy were analyzed in a MPM murine model.

## MATERIAL AND METHODS

*Patient data collection.* Data were collected of nine patients with histologically proven MPM treated in second or third line with CI therapy after progression on treatment with autologous monocyte-derived DCs (moDCs) loaded with allogeneic (n=8) or autologous (n=1) tumor lysate (NCT02395679, NCT01241682). Five patients had received first line chemotherapy prior to DC therapy.

*Patient treatment.* Intravenous anti-PD-1 treatment, consisting of nivolumab (3 mg/kg every 2 weeks) or pembrolizumab (2 mg/kg every 3 weeks) was administered, irrespectively of PD-L1 expression. One patient received nivolumab and ipilimumab at dosages described in the INITIATE trial[10].

*Patient response evaluation.* Radiological tumor evaluation was done 6 weeks after start of treatment and every 4 to 12 weeks thereafter; the interval depended on the previous CT evaluation. The tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) for mesothelioma (final data check November 19th, 2021)[11]. OS was defined as the time from start of CI therapy until death. PFS was determined from the time of start of CI therapy until radiological progression or

death of any cause. The overall response rate was defined as the percentage of patients with a partial response (PR) or complete response. Disease control rate was defined as the percentage of patients without progressive disease as best overall response (BOR).

*In vivo experiment in murine AC29 tumor model*. Female 8-12-week-old CBA/J mice and C57BL/6 mice were purchased from Envigo and housed under specific pathogen-free conditions in individually ventilated cages at the animal care facility of the Erasmus University Medical Center (Erasmus MC), Rotterdam.

For tumor inoculation, mice were intraperitoneally (i.p.) injected with $10^6$ AC29 mesothelioma tumor cells (RRID:CVCL_4407) in 300 µl PBS, as described previously[12]. Mice with established i.p. tumors were killed at indicated time points for immune cell profiling or when profoundly ill according to the body condition score for therapy efficacy experiments. Mice were randomly assigned to experimental groups.

For bone marrow derived dendritic cells (BMDC)-transfer, AC29 tumor lysate was produced and DCs were cultured as previously described[13]. Briefly, tumor lysate was produced by disrupting frozen tumor cells by four cycles of freeze-thaw cycles with liquid nitrogen followed by sonication. BMDCs were generated using recombinant murine GM-CSF (provided by B. Lambrecht VIB)., Ghent) in DC-culture medium followed by loading with tumor lysate and activation with CpG (Invitrogen) on day 9 and injection at day 10. Where applicable, DCs were labeled at day 10 with carboxyfluorescein succinimidyl ester (CFSE) according to the manufacturer's instructions (Thermo Fisher). Dependent on treatment arm, mice were treated with either 200 µg isotype (clone 2A3, BioXCell) or 200 µg anti-PD-L1 antibody (clone MIH5, provided by L. Boon, Bioceros B.V., Utrecht, the Netherlands) in 300 µl PBS in the peritoneal cavity. In case of intrapleural injection, 200 µL PBS was injected in the pleural cavity of mice that were under short-term anesthesia. All experiments were performed with mycoplasma-free cells.

*Preparation of single cell suspensions from mouse tissues*. Single-cell suspensions were generated from isolated inguinal lymph node (non-tumor draining lymph node (non-TDLN), mediastinal lymph node (TDLN), blood and tumor tissue of mice from each group as previously reported[13]. In brief, 30 µl blood was collected in EDTA tubes (Microvette CB300, Sarstedt) and erythrocytes were lysed using osmotic lysis buffer (8.3% NH4Cl, 1% KHCO3, and 0.04% Na2EDTA in Milli-Q). Tumors were collected and dissociated using a validated tumor dissociation system (Miltenyi Biotec) according to protocol.

*Statistical analysis.* Median OS and PFS were estimated using a Kaplan Meier curve in combination with a log-rank (Mantel-cox) test. Survival data were plotted as Kaplan-

Meier survival curves, using the log-rank test to determine statistical significance. A P-value of 0.05 or below was considered to indicate statistical significance. All reported p-values were two tailed. Statistical analyses were performed using R 3.6.0 (R Foundation for Statistical Computing) or Graphpad Prism 8.0.

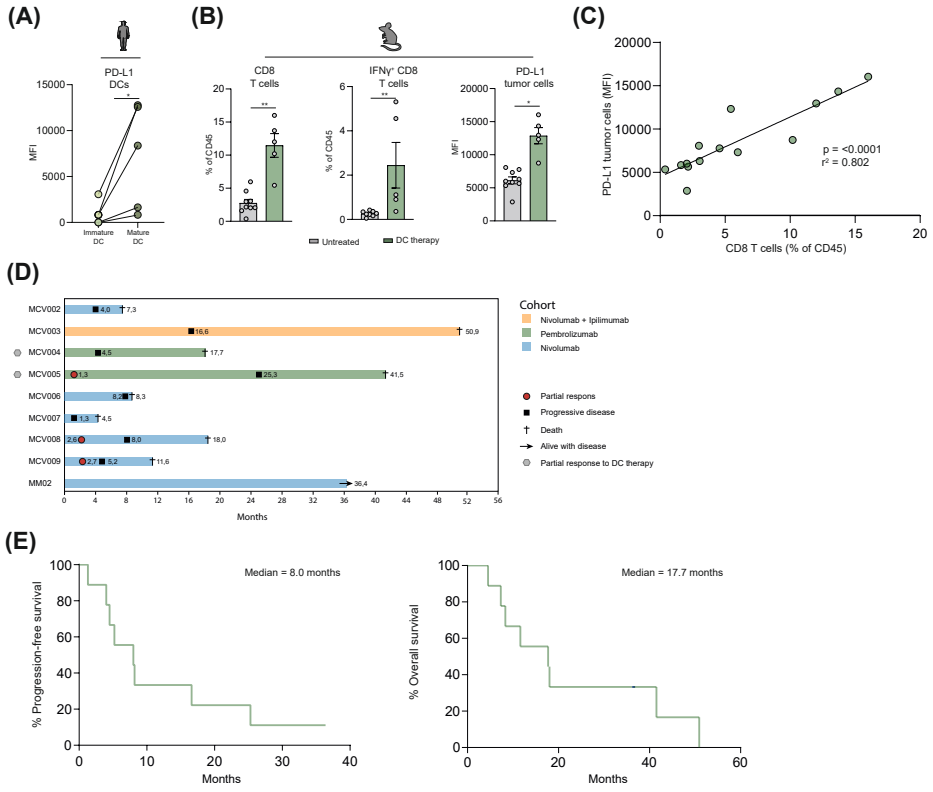
## RESULTS

We identified strong PD-L1 upregulation on *in vitro* matured patient-derived moDCs used for vaccination (**Figure 1A**). Vaccination of mice with DCs induced $CD8^+$ T-cell (CTL) infiltration, which coincided with increased PD-L1 expression by tumor cells, likely due to increased IFN-γ production by CTLs (**Figure 1B-C**). Due to the upregulation of PD-1 on CTLs and PD-L1 on both tumor cells and exogenous DCs, we investigated whether checkpoint blockade could re-induce T-cell mediated immunity and responses in patients. We assessed nine MPM patients receiving pembrolizumab (n=2) or nivolumab (n=7; one patient combined with ipilimumab) upon progression after DC therapy (**Figure 1C**). The median PFS following start checkpoint blockade was 8.0 months and the median OS was 17.7 months (**Figure 1E**). Three patients exhibited partial responses, five stable disease, and one progressive disease; thus, the objective response rate was 33% (**Figure 1D**). At 6 months, five patients (55.6%) showed disease control. Application of the Common Terminology Criteria for Adverse Events (CTCAE 5.0) did not reveal any grade 3/4 adverse events.

Similar to the PD-L1 upregulation on patient-derived moDCs, we identified increased PD-L1 expression on both transferred and endogenous DCs in the TDLNs of tumor-bearing mice (**Figure 2A**). Therefore, we wondered whether concurrent treatment with DC therapy and checkpoint blockade could enhance anti-tumor immunity. To investigate this, we concurrently treated mesothelioma-bearing mice with DC therapy and anti-PD-L1, enabling us to assess PD-1 expression on T cells following treatment. This combination treatment resulted in longer survival compared to untreated mice (**Figure 2B-C**). This was accompanied by synergistic and rapid CD69 upregulation (early activation marker) on T cells in peripheral blood, followed by increased proliferation (assessed by Ki-67), which was most prominent for CD4-Th cells (**Figure 2D**, **S1A**). Moreover, the expression of the exhaustion-program driver TOX on tumor-infiltrating CTLs was most profoundly decreased following combination treatment, indicating a less-exhausted T-cell phenotype. This phenotype was confirmed by a decreased percentage of cells positive for PD-1, TIM3 and CD39, and lower TOX expression within this triple-positive cell subset (**Figure 2E**, **S2B**).

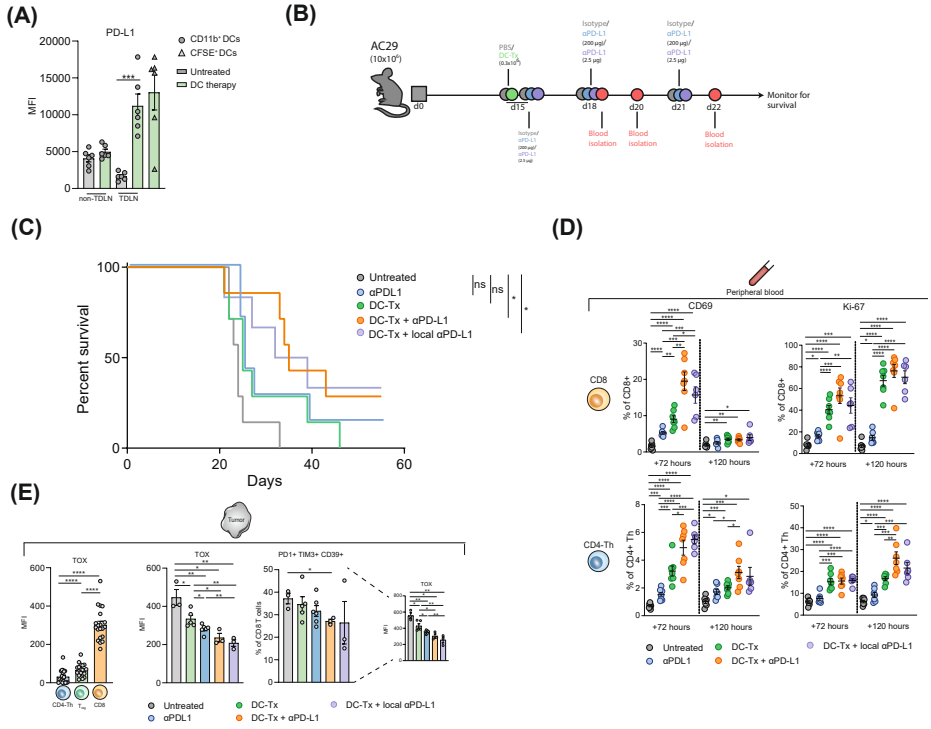
We have previously shown a critical role for PD-L1-expressing DCs in suppressing anti-tumor T cells in TDLNs of mesothelioma-bearing mice[12]. This potentially suggest that TDLNs may be important in mediating the efficacy of PD-L1 blockade combined with DC therapy. To investigate whether the efficacy in mice resulted from PD-L1 blockade in TDLNs or in tumors, we targeted PD-L1 specifically and solely in TDLNs using an established method in which anti-PDL1 is administered at a low dose in the pleural cavity[12]. Blocking PD-L1 solely in TDLNs mimicked systemic anti-PD-L1 treatment for survival (**Figure 2C**) and for alterations in immune phenotype (**Figure 2D-E**). This implies that the efficacy of concurrent combination treatment may primarily depend on blocking the PD-1/PD-L1 axis in the TDLN, thereby resulting in improved T-cell priming by DCs. These findings could indicate the importance of optimizing T-cell priming in TDLNs for maximum anti-tumor T-cell capacity and provide a preclinical rationale for concurrent treatment in MPM patients.

**Figure 1.** Rationale and clinical responses to treatment with checkpoint blockade upon progression to DC therapy.




MFI of PD-L1 on immature and mature patient-derived DCs cultured *in vitro* for therapy (A). $CD8^+$ T-cell infiltration, IFNγ production and PD-L1 expression on tumor cells was assessed in AC29 bearing female CBA/J mice untreated (n=9) or treated with DC therapy (n=5) on day 15 (B). Correlation of PD-L1 expression on tumor cells and $CD8^+$ T-cell levels and a Pearson correlation coefficient was calculated ($r^2$) (n=14) (C). Swimmer plot of patients treated with checkpoint blockade upon progression to DC therapy. Overall survival of patients since date of first vaccination is represented by the filled bars. Start and end of RECIST responses are depicted by the red circles and black squares, respectively. First evaluation of response was after 6 weeks for all patients. (D) Kaplan-Meier curves showing progression-free survival (left) and overall survival (right) for all patients. (E) Means and SEMs are shown and Mann-Whitney U tests were performed indicating statistical significance. *$p < 0.05$, **$p < 0.01$.

**Figure 2.** Concurrent treatment with anti-PD-L1 and DC therapy results in improved survival and anti-tumor immunity.




MFI of PD-L1 on injected CFSE labeled DCs and endogenous DCs in non-TDLN and TDLN of AC29 mesothelioma bearing CBA/J mice (n=6/group; total n=12) 24 hours after DC therapy (A). Mice bearing AC29 tumors (n=7/group; total n=35) were treated with DC therapy or PBS at day 15. At day 15, 18 and 21, mice were also treated with either isotype, low-dose anti-PD-L1 (2.5 μg) or systemic dose (200 μg) (B). Mice were monitored for survival which is depicted in a Kaplan-Meier curve (C). From the experiment in B, blood was isolated at day 18 and 20 and expression of early-activation marker CD69 and proliferation marker Ki-67 were determined for $CD8^+$ T cells and CD4-Th cells (D). Expression of TOX on CD4-Th cells, $T_{reg}$ cells and $CD8^+$ T cells and the percentage of triple-positive ($PD\text{-}1^+$ $TIM3^+$ $CD39^+$) and their TOX expression level was determined in end-stage tumor material from experiment in B (E). Means and SEMs are shown and paired- and unpaired t tests were performed indicating statistical significance. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. MFI = median fluorescence intensity, non-TDLN = non tumor-draining lymph node, TDLN = tumor-draining lymph node, CD4-Th = CD4 T helper, SEM = standard error of the mean.

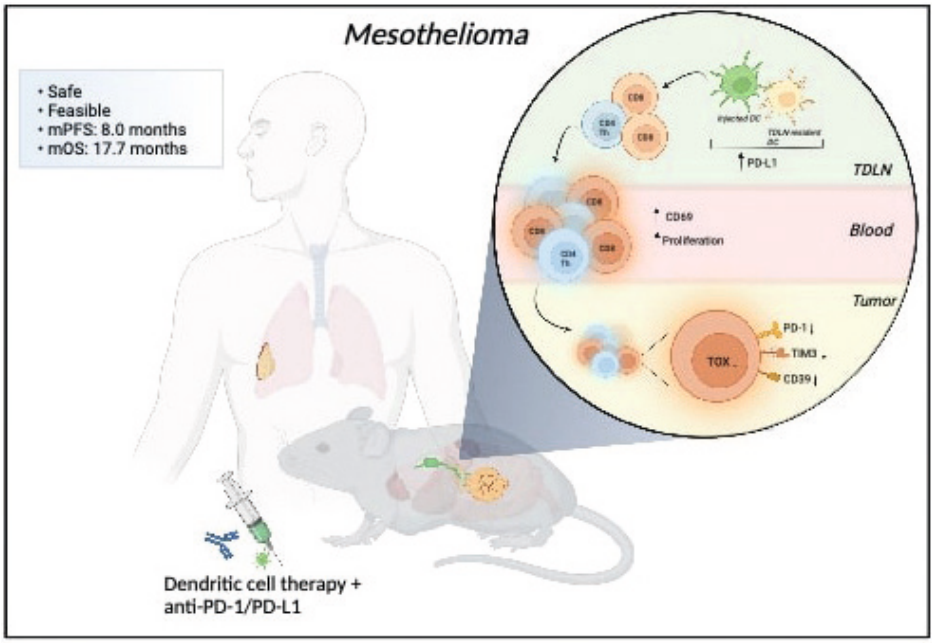
## DISCUSSION

In this study, we have shown that anti-PD-1 following DC therapy is safe and feasible in MPM patients. The response rate (33%), PFS (8.0 months) and OS (17.7 months) are promising when compared to anti-PD-1 monotherapy. Still, the potential bias in patient selection calls for caution in the interpretation of these findings. To support the potential synergy, combining DC therapy with concurrent blockade of the PD-1/PD-L1 axis reinvigorated T cells and prolonged survival in the mesothelioma-bearing mice. This synergistic effect of concurrent treatment may be the result of the blockade of high PD-L1 expression on DCs *in vivo* and on moDCs given as DC therapy. The data suggest that this effect could be primarily derived from the TDLN, as TDLN-specific blockade of PD-L1 resulted in comparable immune-stimulating effects as did systemic anti-PD-L1 treatment. By releasing progenitor-exhausted tumor-specific T cells, PD-L1 blockade on DCs in the TDLN has been shown to induce effective tumor immunity[12,14]. As we observed a less-exhausted tumor-infiltrating CTL phenotype in combination therapy-treated mice, these results could indicate that concurrent treatment may eventually result in more efficient T-cell priming in the TDLN by DCs. Since we treated MPM patients sequentially with PD-1 blockade, our data indicate that clinical responses might even be further improved by concurrent treatment.

Limitations of our study include the lack of pre-and post-treatment biopsies in MPM patients treated with DC therapy. This precluded investigation of the PD-L1 upregulation that we observed in mice. Furthermore, due to rapid tumor growth, we could not include a murine treatment arm of DC- and anti-PD-L1 therapy administered sequentially. Lastly, while MPM patients were treated with PD-1 blocking agents, mesothelioma-bearing mice were treated with antibodies blocking its ligand, PD-L1. Although both antibodies block the same axis, it has recently been demonstrated that anti-PD-1 and anti-PD-L1 may have different immune modulating effects due to *cis* interactions with CD80 on antigen-presenting cells which could potentially influence efficacy of the combination treatment[15]. Whether anti-PD-L1 leads to suboptimal anti-tumor immunity, compared to anti-PD-1, needs to be further investigated in our models.

In conclusion, our data from both patients and mice indicate that the combination of DC therapy and anti-PD-1/PD-L1 could be a promising treatment for MPM, as it was found feasible and safe, and did show clinical efficacy.

**Graphical abstract**



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# CHAPTER 9

## LURBINECTEDIN SHOWS CLINICAL ACTIVITY AND IMMUNE-MODULATORY FUNCTIONS IN PATIENTS WITH PRE-TREATED SMALL CELL LUNG CANCER AND MALIGNANT PLEURAL MESOTHELIOMA

**Daphne W Dumoulin***, Luca Cantini*, Robin Cornelissen, Madelief Vink, Larissa Klaase, Kick Slooff, Nura Tebayna, Joanne M Mankor, Sara J Baart, Rudi Hendriks, Anne-Marie C Dingemans, Marcella Willemsen, Joachim G J V Aerts

* These authors contributed equally

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## ABSTRACT

### Purpose

Lurbinectedin is a promising new drug being investigated in pre-treated patients with small cell lung cancer (SCLC) or malignant pleural mesothelioma (MPM). Its clinical activity in the real-world setting has not been investigated yet.

### Patients and Methods

Clinical data of patients with SCLC and MPM who were treated with lurbinectedin were prospectively collected. Comprehensive immune cell profiling by flow cytometry was performed on screening and on treatment peripheral blood samples.

### Results

A total of 95 patients (43 SCLC and 52 MPM) were treated, mostly as ≥3-line of therapy. In the SCLC cohort, median progression free survival (mPFS) was 1.5 months (95% CI: 1.4–3.0), and median overall survival (mOS) was 7.0 months (95% CI: 4.7–not reached). Objective radiological response and disease control rate (DCR) after 12 weeks were 16% and 28%, respectively. In the MPM cohort, mPFS was 2.8 months (95% CI: 1.4–4.2), and mOS was 7.2 months (95% CI: 5.9–not reached). DCR after 12 weeks was 29%, whereas no partial responses were registered. No new safety signals were observed. Lurbinectedin treatment was significantly associated with depletion of circulating classical monocytes, which correlated with a better PFS in SCLC patients. Lurbinectedin increased proliferation of $CD4^+$ and $CD8^+$ T cells (SCLC), and NK and NKT cells (SCLC and MPM) and altered co-stimulatory and co-inhibitory receptor expression on circulating lymphocytes.

### Conclusion

Lurbinectedin has a manageable safety profile and shows clinical activity in pre-treated patients with SCLC and MPM. Its immune-modulatory functions make lurbinectedin a potential platform for immunotherapy combinations.

## INTRODUCTION

Small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM) are both aggressive thoracic malignancies with a dismal prognosis. Despite the addition of immune checkpoint inhibitors (ICI) to the treatment armamentarium[1–3], overall survival (OS) remains poor, and there is a lack of treatment options after first-line treatment failure.[4,5] Thus, identification of new effective treatment strategies for both diseases represent an utmost clinical challenge.

Lurbinectedin (Zepzelca®) is a promising new agent that is currently being investigated in patients with SCLC or MM after failure of at least first-line systemic therapy.[6–8] Lurbinectedin recognizes specific sequences within the promoters of actively transcribed genes, blocks the binding of oncogenic transcription factors to their target sequences and promotes the irreversible proteasomal degradation of RNA polymerase II.[9,10] As a consequence of its mechanism of action, lurbinectedin induces double-strand breaks in the DNA, triggers an extended delay in the transition through the S phase of the cell cycle with an arrest in the G2/M phase, and finally leads to tumor cell death by apoptosis.[11] Apart from its direct cytotoxic effect on the tumor cells, lurbinectedin presents a marked effect on the tumor microenvironment by inhibiting transcription and secretion of tumor-growth promoting cytokines by tumor associated macrophages (TAMs).[12] TAMs are responsible for an immune-suppressive tumor microenvironment and their reduction may lead to a more effective anti-tumor immune response.[13] Based on a phase 2 basket trial with 105 patients with stage IV SCLC pre-treated with one chemotherapy regimen (immunotherapy was allowed, combined with chemotherapy or alone), in 2019, the EMA granted orphan designation. Subsequently, in 2020 the FDA granted accelerated approval to lurbinectedin for patients with metastatic SCLC with disease progression on or after platinum-based chemotherapy.[6]



In another phase 2 trial, 42 patients with progressive MPM were treated with lurbinectedin in 2nd line. Although this trial met its primary endpoint, progression free survival (PFS) at 12 weeks, this did not lead to registration for this indication.[8,14]

As far as we know, no real-world data on the efficacy of lurbinectedin has been published. Lurbinectedin has previously been reported to deplete monocytes (specifically Ly6c$^{high}$CD11b$^{+}$CD115$^{+}$ monocytes) in mice,[12] but whether this occurs in patients with SCLC and MPM remains largely unknown. Here, we present real-world data of two large cohorts of patients with SCLC or MPM treated with lurbinectedin in a Dutch tertiary referral university medical cancer centre on a named patient program.

We also report on the immune-modulatory effect of lurbinectedin, as determined by the circulating immune profile of these patients.

## METHODS

### Study design and procedures

Data from patients with SCLC or MPM treated with lurbinectedin intravenously at a dose of 3·2 mg/m$^2$ every 3 weeks, as part of a named patient program in Erasmus Medical Center (Rotterdam, the Netherlands), were prospectively collected. A detailed description of eligibility criteria and procedures of the clinical study is provided in the **Data Supplement**. The database lock for the current analysis was March 19$^{th}$, 2021. All patients with a follow-up shorter of 3 months before data cut-off were excluded except when progression was established before data cut-off or death. Of all included patients, blood samples were collected for immune monitoring analysis. All study procedures were conducted in accordance with the Declaration of Helsinki. Blood samples were obtained after patient's informed consent. According to national guidelines, no ethical committee approval was needed for the prospective collection of the clinical data.

The primary objective was to describe the real-world efficacy of lurbinectedin in patients with SCLC and MPM. Secondary and exploratory objectives were to investigate safety and immune-modulatory properties of lurbinectedin. A detailed description of the outcome measurements is provided in the **Data Supplement**.

The statistical analysis are described in the **Data Supplement**.

## RESULTS

### Patient characteristics

From November 29$^{th}$, 2019 to December 22$^{th}$, 2020 a total of 95 patients (43 SCLC and 52 MPM) started treatment with lurbinectedin. Patients had a median age of 67 years (range: 40-82) and 75 patients (90%) had a good Eastern Cooperative Oncology Group (ECOG) performance status score of 0/1 at the start of treatment. All patients with SCLC and 81% of patients with MPM had received at least two previous lines of treatment (**Table 1**).

**Table 1.** Patient and disease baseline characteristics.

| Characteristic | SCLC (n=43) | MPM (n=52) |
|---|---|---|
| **Median age, years (range)** | 62 (40-77) | 71 (52-82) |
| **Gender, male, No. (%)** | 19 (44) | 46 (87) |
| **Median time from diagnosis to start of lurbinectedin, months (IQR)** | 15.2 (9·9-22·0) | 18·7 (12·8-27·1) |
| **Smoking status, No. (%)** | | |
| Former/current | 31 (72) | 29 (55) |
| Never | 2 (5) | 13 (26) |
| Unknown | 10 (23) | 10 (19) |
| **ECOG PS at start of lurbinectedin, No. (%)** | | |
| 0 | 5 (12) | 10 (19) |
| 1 | 34 (79) | 26 (50) |
| ³2 | 3 (6) | 5 (10) |
| Unknown | 1 (3) | 11 (21) |
| **Histological subtype, No. (%)** | | |
| Epithelioid | NA | 41 (79) |
| Mixed/Sarcomatoid | NA | 9 (17) |
| Peritoneal mesothelioma (epithelioid) | NA | 2 (4) |
| **Previous line(s) of treatment, No. (%)** | | |
| 1 | 0 (0) | 10 (19) |
| 2 | 21 (48) | 25 (48) |
| ³3 | 22 (52) | 17 (33) |
| **Median previous line(s) of therapy (range)** | 2 (2-6) | 2 (1-8) |
| **Prior chemotherapy, No. (%)** | 43 (100) | 52 (100) |
| **Prior immunotherapy, No. (%)** | 8 (19) | 43 (83) |
| **Time since last cycle of systemic treatment, months (range)** | 1·9 (0·8-10·8) | 1·6 (0·5-21·2) |
| <90 days | 31 (72) | 36 (69) |
| ≥90 days | 10 (23) | 16 (31) |
| Unknown | 2 (5) | 0 (0) |
| **Type of last systemic treatment, No. (%)** | | |
| Chemotherapy | 43 (100) | 17 (33) |
| Immunotherapy | 0 (0) | 35 (67) |
| **Best response to last line of systemic treatment, No. (%)** | | |
| PD | 24 (54) | 19 (37) |
| SD | 8 (19) | 21 (40) |
| PR/CR | 10 (22) | 12 (23) |
| Unknown | 2 (5) | 0 (0) |
| **Median albumin, g/l (range)** | 39 (28-46) | 35 (22-45) |
| **Median LDH, U/L (range)** | 277 (150-1537) | 184 (125-370) |

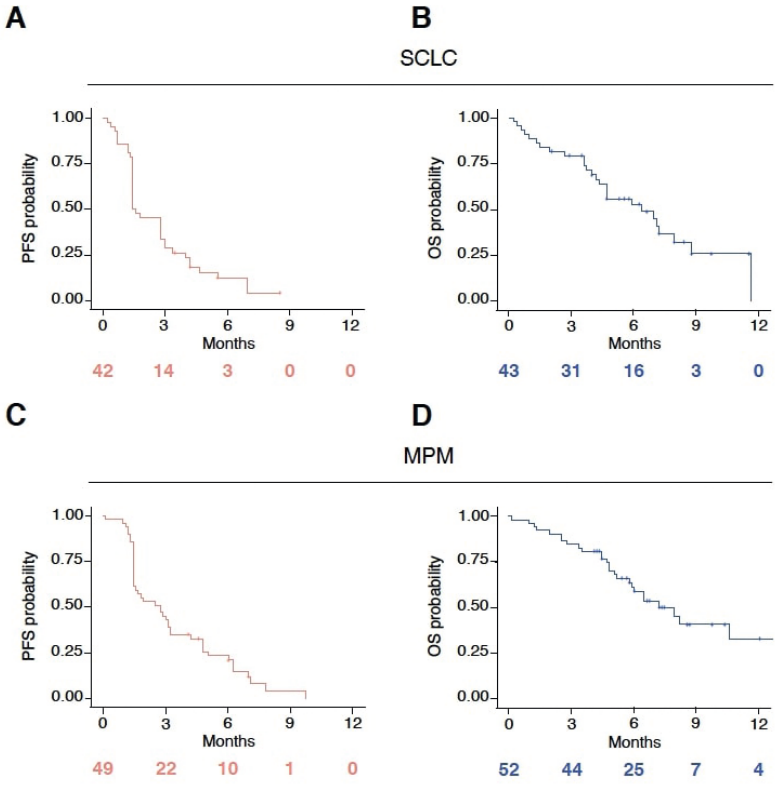
Abbreviations: SCLC, small cell lung cancer; MPM, malignant pleural mesothelioma; IQR, Interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance score; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; LDH, lactate dehydrogenase.

**Clinical outcomes and safety of lurbinectedin in the real-world setting**

Patients with SCLC received a median number of lurbinectedin cycles of 2 (range: 1-12), whereas those with MPM received a median of 3 cycles (range: 1-13) with 12 (28%) and 8 (15%) patients receiving ≥6 cycles respectively.

In the SCLC cohort, with a median follow-up time of 7.2 months, 39/43 patients had progression of disease and 23/43 died. Median PFS (mPFS) was 1.5 months (95% CI: 1.4–3.0) (**Figure 1A**), and median OS (mOS) was 7.0 months (95% CI: 4.7–not reached) (**Figure 1B**). The 6-month PFS rate was 12% (95% CI: 5–28%) and the 6-month OS rate was 57% (95% CI: 43–75%). Regarding the overall lurbinectedin activity, 7/43 patients had a tumor response (16.3% ORR) and five (11.6%) had SD as the best result after 12 weeks of treatment, resulting in a DCR of 27.9%.

**Figure 1.** Kaplan Meier analyses in patients with small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM).




**A: Progression-free** survival of SCLC patients (entire cohort). **B**: Overall survival of SCLC patients (entire cohort). **C**: Progression-free survival of MPM patients (entire cohort). **D**: Overall survival of MPM patients (entire cohort).

Univariable Cox proportional hazard regression analysis in patients with SCLC revealed no major clinical parameters able to predict the outcome, outside known prognostic factors (**Data Supplement Table 2**).

In the MPM cohort, the median follow-up time was 7.3 months. Forty-four out of 52 patients had progression of disease and 28/52 died. Median PFS was 2.8 months (95% CI: 1.4–4.2) (**Figure 1C**), and mOS was 7.2 months (95% CI: 5.9–not reached) (**Figure 1D**). The 6-month PFS rate was 20% (95% CI: 11–36%) and the 6-month OS rate was 58% (95% CI: 46–74%). No tumor responses were registered, and 15/52 patients obtained SD after 12 weeks of treatment for a DCR of 28.8%.

Univariable Cox proportional hazard regression analysis in patients with MPM revealed no major clinical parameters able to predict the outcome, outside known prognostic factors (**Data Supplement Table 3**).

The treatment safety profile was consistent with previous studies, and no new safety signals were reported (**Table 2**). Lurbinectedin-related adverse events (AEs) of any grade were observed in 83/95 pts (87.4%) and grade 3/4 AEs in 25/95 patients (26.3%). The most common grade 3/4 AEs were neutropenia (11% SCLC, 16% MM) and fatigue (2% SCLC, 6% MM). Febrile neutropenia was documented in two MPM patients (4%). There was no association between chemotherapy free interval (CFI) and neutropenia onset in the whole cohort ($P$ = 0.30, Wilcoxon signed-rank test).

Dose reductions were performed in 27% of patients and were mainly due to hematologic toxicity and fatigue. Two patients stopped the treatment due to AEs; one due to persisting thrombocytopenia, the other one due to persisting neutropenia. Treatment delays occurred at least once in 6 patients with SCLC (14%) and 17 patients with MM (33%) (**Data Supplement Table 4**).

**Table 2.** Treatment-related adverse events (SCLC n=45; MPM, n=52).

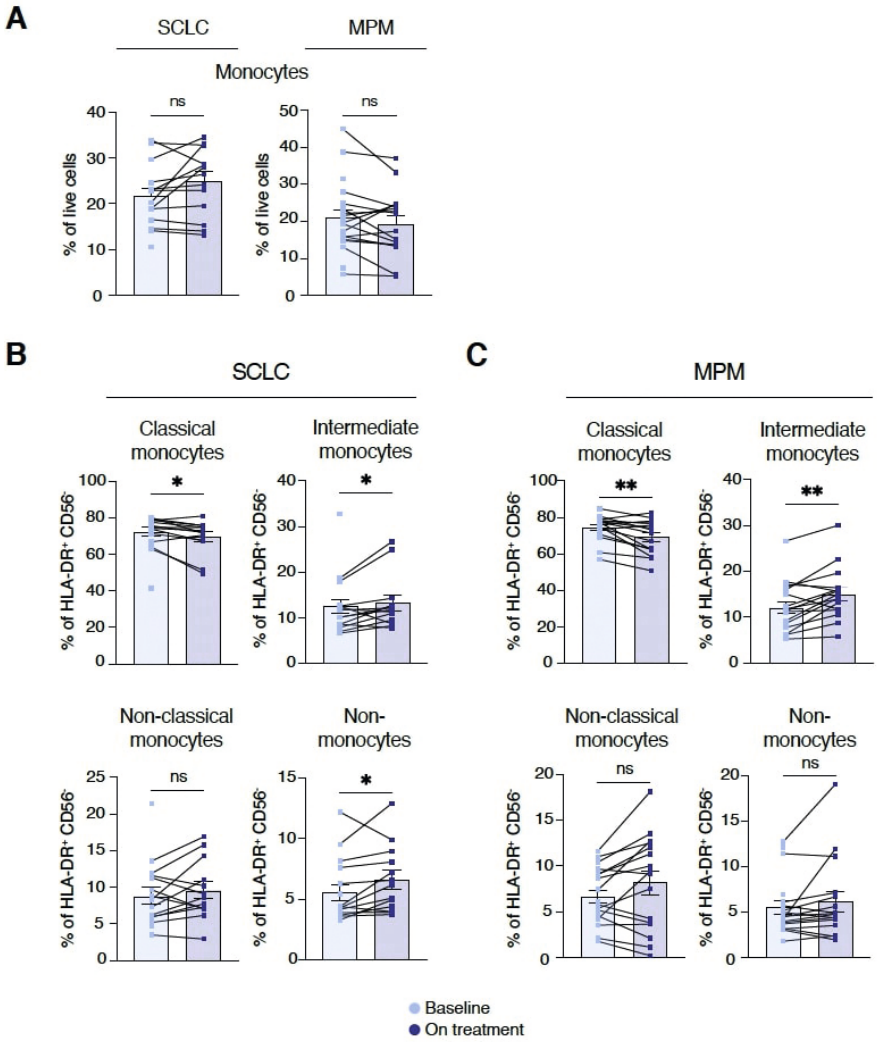
| | Grade 3 | Grade 4 |
|---|---|---|
| Any | 20 (21) | 5 (5) |
| Anemia | 2 (2) | 0 |
| Neutropenia | 8 (8) | 5 (5) |
| Thrombocytopenia | 1 (1) | 1 (1) |
| Creatinine increased | 0 | 0 |
| Alanine aminotransferase increased | 0 | 2 (2) |
| Aspartate aminotransferase increased | 2 (2) | 0 |
| γ-glutamyl transferase increased | 2 (2) | 0 |
| Alkaline phosphatase increased | 0 | 0 |
| Fatigue | 4 (4) | 0 |
| Nausea | 0 | 0 |
| Dysgeusia | 0 | 0 |
| Vomiting | 0 | 0 |
| Diarrhea | 1 (1) | 0 |
| Constipation | 0 | 0 |
| Febrile neutropenia | 2 (2) | 0 |
| Hiccups | 1 (1) | 0 |
| Dyspnea | 2 (2) | 0 |
| Mucositis | 1 (1) | 0 |
| Rash | 0 | 0 |

**Immunological phenotyping**

Major baseline characteristics and clinical outcome of the patients of whom peripheral blood samples were collected (SCLC n=20 and MPM n=19) did not differ from the whole group of patients. (**Data Supplement Table 5, Data Supplement Figure 2**).

Although the relative proportion of the total monocyte population did not change significantly during therapy (**Figure 2A**), lurbinectedin significantly reduced the proportions of $HLADR^{+}CD56^{-}CD14^{+}CD16^{-}$ classical monocytes within the total monocyte population, both in SCLC and in MPM patients (**Figure 2B** and **2C;** see for gating: **Supplement Figure 2**). This decrease of classical monocyte frequencies was paralleled by a significant relative increase of intermediate monocytes in both SCLC (**Figure 2B**) and MPM (**Figure 2C**). Interestingly, we found that SCLC patients with lower frequencies of classical monocytes before treatment with lurbinectedin, had a longer PFS (**Data Supplement Figure 3**).

**Figure 2.** Lurbinectedin treatment is associated with depletion of the classical monocyte subset.



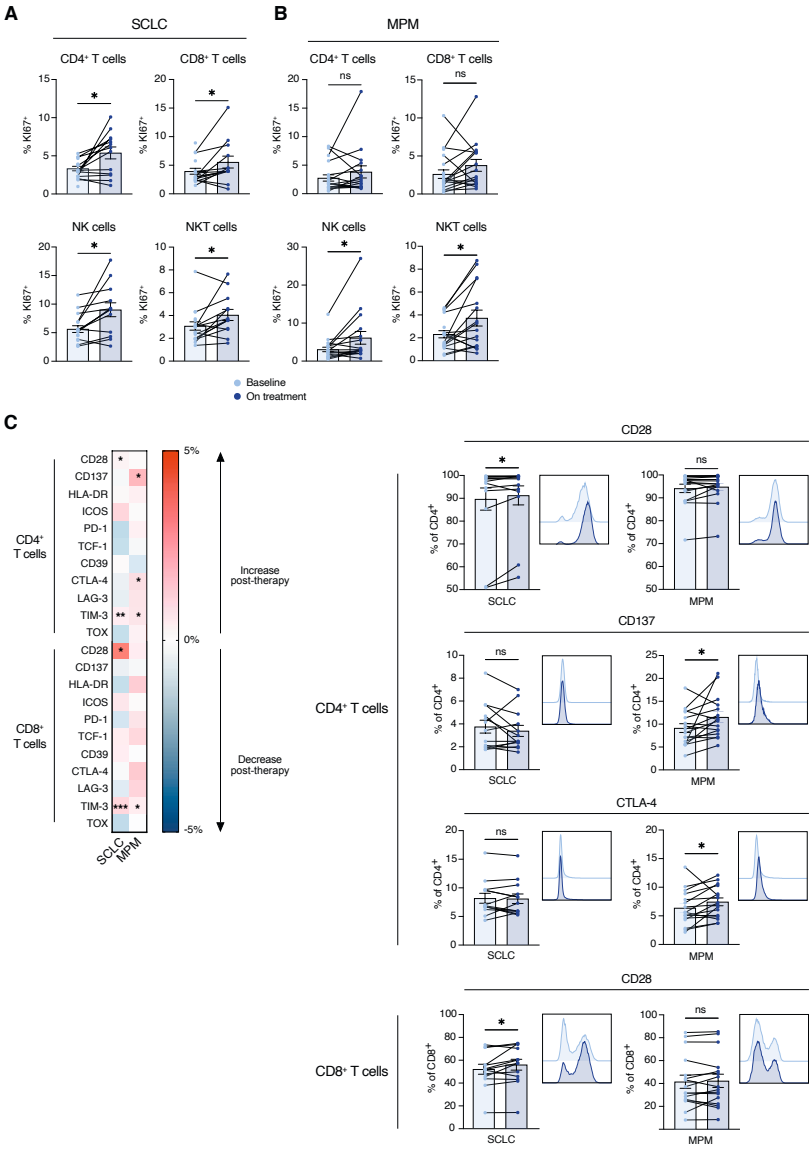

**A**: Percentage of monocytes (CD14$^{+}$ CD16$^{+/-}$ and CD14$^{-}$ CD16$^{+}$) at screening an on-treatment time points in small cell lung cancer (SCLC) patients (left) and malignant pleural mesothelioma (MPM) patients. **B**: Percentage of HLA-DR$^{+}$CD56$^{-}$ cell subsets, at screening and on-treatment time points in small cell lung cancer (SCLC) patients. **C**: Percentage of HLA-DR$^{+}$ CD56$^{-}$ cell subsets, at screening and on-treatment time points in malignant pleural mesothelioma (MPM) patients. Wilcoxon matched-pairs signed rank tests or Student's *t*-tests were performed to calculate statistical significance. Paired samples are shown connected by black lines. Bars depict mean values with standard error of the mean. A total of 29 patients had data available at both time points and were included in the analysis (n = 13 SCLC; n = 16 MPM). ns = not significant, * = p<0.05, ** = p<0.01

We subsequently analyzed whether treatment with lurbinectedin also affected lymphocytes. The treatment did not result in changes in the proportions of $CD4^+$ and $CD8^+$ T cells, NK cells and NKT cells within the lymphocyte compartment in both SCLC and MPM patients (data not shown). Next, proliferation was assessed by Ki67 expression, a cell cycle marker expressed by dividing or recently divided cells. Lurbinectedin increased the frequencies of $Ki67^+$ proliferating cells within the $CD4^+$ and $CD8^+$ T cell populations specifically in SCLC patients (**Figure 3A**), and of NK and NKT cells in both SCLC and MPM **(Figure 3B**). This increase in proliferation was independent of clinical response (**Data Supplement Figure 4A-B**). We also examined whether differences in the proliferation of $CD8^+$ T cells prior to treatment could help identify patients with longer PFS under lurbinectedin. Log rank test revealed that SCLC patients with a higher proportion of $CD8^+$ proliferating T cells (cut-off based on the median proportion) at screening, had a significantly longer PFS upon lurbinectedin (mPFS: 4.7 vs. 2.1 months, $p = 0.04$) (**Data Supplement Figure 4C**)

We also investigated different T cell subsets. (**Data supplement Figure 5A** and **5B**) Even though proliferating $CD4^+$ and $CD8^+$ T cells and $T_{EM}$ cells were increasing upon treatment in SCLC, no correlation was noted between the decrease of classical monocytes and the increase of proliferating $CD8^+$ total, $CD8^+$ $T_{EM}$, $CD4^+$ total or $CD4^+$ $T_{EM}$ cells in SCLC (**Data Supplement Figure 6**).

In addition to T cell proliferation, we assessed the expression of a variety of co-stimulatory and –inhibitory receptors on circulating T cells (**Figure 3C**). The frequency of both $CD4^+$ and $CD8^+$ T cells that expressed the co-receptor CD28 slightly, but significantly, increased upon treatment in patients with SCLC, indicating that lurbinectedin induced T cell activation. Contrary to CTLA-4 which was significantly increased upon treatment in $CD4^+$ T cells in patients with MPM only, the inhibitory receptor TIM-3 changed with similar dynamics both on $CD4^+$ and $CD8^+$ T cells and both in SCLC and MPM (**Figure 3C**). These findings suggest that lurbinectedin induced a two-side alteration of the circulating T cell phenotype, with upregulation of co-stimulatory receptors being counterbalanced by contemporary upregulation of co-inhibitory markers. These findings should help the implementation of rational combination therapies.

**Figure 3.** Lurbinectedin modulates proliferation and alters phenotype of circulating lymphocyte subsets




**A**: Percentage of Ki67⁺ CD4⁺ T cells, CD8⁺ T cells, NK and NKT cells, at screening and on-treatment time points in small cell lung cancer (SCLC) patients. **B**: Percentage of Ki67⁺ CD4⁺ T cells, CD8⁺ T cells, NK and NKT cells, at screening and on-treatment time points in malignant pleural mesothelioma (MPM) patients. **C**: Heatmap, graphs and (representative) histograms showing mean percentage of change and paired analyses of co-stimulatory and co-inhibitory receptor expression during lurbinectedin in SCLC and MPM patients. Wilcoxon matched-pairs signed rank tests or Student's *t-tests* were performed to calculate statistical significance. Paired samples are shown connected by black lines. Bars depict mean values with standard error of the mean. A total of 29 patients had data available at both time points and were included in the analysis (n = 13 SCLC; n = 16 MPM). ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001.

## DISCUSSION

To the best of our knowledge, this is the first prospective real-world dataset from patients with SCLC and MM treated with lurbinectedin mostly as third or further-line treatment.

When comparing our real-world data to the clinical trials in SCLC and MM, our results are inferior (**Table 3**).[6,8,14] This result is expected considering that our unselected and heterogeneous patient cohort represented a more frail and more heavily pre-treated population.

**Table 3.** Main efficacy outcomes in SCLC and MPM patients treated with lurbinectedin monotherapy in the context of phase 2 trials and in the Erasmus MC real-world experience.

| | Trigo et al. (SCLC) | Dumoulin et al. (SCLC) | Metaxas et al./Mark et al. (MPM) | Dumoulin et al. (MPM) |
|---|---|---|---|---|
| Patient number | 105 | 43 | 42 | 52 |
| Treatment line | 2-3 | 3-4 | 2-3 | 2-3 |
| Median follow-up | 17·1 months | 7·2 months | 32.8 | 7·3 months |
| Median pts CFI | 3·5 months | 1·9 months | unknown | 1·6 months |
| DCR 12 weeks | 68% | 28% | 52% | 29% |
| ORR 12 weeks | 35% | 16% | 4% | 0% |
| Median PFS | 3·5 months | 1·5 months | 4·1 months | 2·8 months |
| Median OS | 9·3 months | 7·0 months | 11·5 months | 7·2 months |

Abbreviations: CFI, chemotherapy-free interval; DCR, disease control rate; ORR, overall response rate, PFS, progression-free survival; OS, overall survival.

Comparing the results of lurbinectedin in our real-world SCLC cohort with those obtained with topotecan, which is the standard of care according to the guidelines after failure of first-line chemotherapy[15], we found a promising ORR of 16% in our cohort compared to 5% (for chemotherapy-refractory disease) and to 17% (for chemotherapy-sensitive disease) with topotecan. Of note, this relatively high response rate in our patients was seen despite of the fact that the patients were heavily pre-treated and largely being pre-treated with topotecan as second-line treatment.

Recently, in the randomized phase 3 ATLANTIS study, the combination of lurbinectedin (at a 2 mg/m2 dosage) with doxorubicin as second-line treatment for SCLC did not improve OS when compared to topotecan or cyclophosphamide/doxorubicin/vincristine (CAV)[16]. However, the safety profile of lurbinectedin was better and a model developed by investigators (based on exposure-response analysis) predicted that usage of single-

agent lurbinectedin at 3.2 mg/m$^2$ (its approved dose) would have yielded significantly higher response rates and significantly longer survival. In this context, our real-world clinical data offer further support for the efficacy of lurbinectedin in thoracic neoplasms.

Combinations of lurbinectedin with other cytotoxic agents or ICI are being explored based on the hypothesized immunological effects of lurbinectedin (NCT04358237, NCT04610658, NCT04253145, NCT02611024). We further explored this immune modulating effect in patients. Our study, by using comprehensive immune monitoring, demonstrated that lurbinectedin induces a relative reduction of circulating classical monocytes. These effects on the myeloid compartment have not been previously reported in patients, and further deepen previous pre-clinical observations showing that lurbinectedin induces a dose- and time-dependent death in cultured monocytes and monocytic myeloid derived suppressor cells (Mo-MDSC).[17] Our study showed that despite lurbinectedin-mediated depletion of classical monocytes, only patients with SCLC with lower frequencies of classical monocytes prior to start of treatment seem to benefit, while patients with MPM seemed not to be affected, to signify that different (immunological) mechanisms might also play a role in response to lurbinectedin.

Looking at modulation of the lymphoid subset, in this study lurbinectedin was found to increase proliferation of CD4$^+$ and CD8$^+$ T cells specifically in patients with SCLC, and of NK- and NKT- cells in both SCLC and MPM. This proliferation was irrespective of clinical response, which can be ascribed to a number of mechanisms, but open the field of research by combining lurbinectedin with other immune modulating agents. This is supported by the effect found on the circulating T- cell phenotype, with both activation (CD28 on CD4$^+$ T cells in SCLC) and inhibitory markers (CTLA-4 on CD4$^+$ T cells in MPM, and TIM-3 on CD4$^+$ and CD8$^+$ T cells in both SCLC and MPM) being upregulated upon treatment. The increased expression of these markers on lymphocytes following lurbinectedin suggests that the combination of lurbinectedin with immunotherapy might be efficacious[18]. In our study, alteration of T cell phenotype involved different markers and was dependent on tumor type, suggesting that development of future combinational therapy should come along with in-depth immune-monitoring investigations.

Noteworthy, neither T cell proliferation nor the activation phenotype related to monocytes frequencies. These findings are in line with previous observations from our group showing that depletion of TAM is not sufficient *per se* to enhance CD8$^+$ T cell proliferation and effector phenotype, and combination with other type of immunotherapies such as dendritic cell vaccination is needed to improve T cell memory responses and consequentially survival.[13]

Apart from this, the observed increase of T cell proliferation ($T_{EM}$ cells specifically) may be an indirect result of the cytotoxic effect from lurbinectedin on tumor cells (probably involving an increased release of tumor-derived antigens) rather than a direct drug-mediated modulation of immune cells.

Despite its prospective design and the use of an extensive cohort of SCLC and MPM for the immune monitoring analysis, this study has some limitations. Because this study is not a randomized controlled trial, there is no control group. The absence of a control group precludes formal conclusions to be made on the immune-modulatory functions of lurbinectedin that should be considered exploratory and need confirmation in the context of larger randomized trial. However, most of the immune-related changes were observed early on treatment (6 weeks), making tumor response/progression less likely responsible for the observed modifications.

Furthermore, the widespread effects of lurbinectedin on a variety of immune cells *in vivo*, the absence of available tissue sample and the lack of functional *in vitro* data, precludes us to provide clear mechanistic insights about how lurbinectedin may modulate the anti-tumor immune response.

Nonetheless, our real-world data confirmed activity of lurbinectedin in a cohort of heavily pre-treated SCLC and MPM patients. Lurbinectedin monotherapy appears to be an alternative therapeutic option of interest for these patients with a dismal prognosis of which the efficacy might be positively influenced by the combination with other agents, based on the results of our exploratory study. In fact, our study suggests that lurbinectedin might have immune-modulatory functions by promoting proliferation and phenotype shifting of anti-tumor immune cell populations, making lurbinectedin an interesting chemotherapy backbone on which to build better immunotherapy combination options for patients with SCLC and MPM.

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# SUPPLEMENTAL METHODS

**Eligibility criteria, study procedures and outcomes**

Eligible patients were adults (≥18 years old) with either pathologically proven and unresectable small cell lung cancer (SCLC), progressing after at least one platinum-etoposide chemotherapy, or patients with histologically confirmed malignant pleural mesothelioma (MPM) and progression during or after at least one course of platinum-pemetrexed chemotherapy. All eligible patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2, adequate hematological, renal, metabolic, and hepatic function, no active uncontrolled infection or symptomatic, steroid-requiring, or progressive central nervous system involvement. Unfit patients or those who refused systemic treatment were not included in the trial and were candidate to best supportive care.

Lurbinectedin was given intravenously at a dose of 3·2 mg/m$^2$ every 3 weeks until progression or unacceptable toxicity. Dose reductions were performed in steps of 0·6 mg/m$^2$, with a minimal dose of 2·0 mg/m$^2$. Antiemetic prophylaxis using corticosteroids and, if needed, 5-HT3 antagonists were administered before every cycle of lurbinectedin.

Clinical data of the patients was collected from the digital patient register. The following variables were collected and used for analysis: diagnosis (for MM also histologic subtype: non-epithelioid vs. epithelioid), date of the first diagnosis, age, gender, ECOG performance score at the start of treatment, line of treatment, response to previous anti-cancer therapy, the start date of lurbinectedin, chemotherapy-free interval (CFI) since the last cycle of chemotherapy or interval since the last cycle of systemic treatment until the start of lurbinectedin (≥90 days vs. <90 days), best response to lurbinectedin, date of progression after the start of lurbinectedin, date of death, toxicities requiring dose delay or reduction, and onset of neutropenia.

Radiological tumor assessment was performed at baseline and every 2 cycles after the start of treatment using computed tomography (CT) using Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 for patients with SCLC and modified RECIST v.1.1 for patients with MPM. Blood was drawn at baseline and on treatment time points in EDTA tubes and processed. In those patients who gave informed consent, peripheral blood mononuclear cells (PBMCs) were purified from whole blood by density-gradient centrifugation (Ficoll Plaque™, GE Healthcare, Chicago, IL, USA) and cryopreserved before analysis.

Progression-free survival (PFS) was defined as the time interval from the first lurbinectedin administration until the earliest date of clinical or radiological progression or death from any, whereas overall survival (OS) was accounted from the date of the first lurbinectedin administration until patient death from any cause (censored at the last tumor assessment date for patients who were alive at the time of data cut-off). The objective response rate (ORR) was defined as the proportion of patients who had a partial (PR) or complete response (CR) to therapy at 12 weeks of treatment, whereas the disease control rate (DCR) was defined as the percentage of patients who achieved a CR, PR, or stable disease (SD) at 12 weeks of treatment. Adverse events (AEs) were graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events (CTCAE) v.5.0.

Fluorochrome-conjugated antibodies are listed in **Data Supplement Table 1**. Cells were first stained for membrane markers. Secondly, cells were stained with Fixable Viability Dye, followed by fixation and permeabilization using the FoxP3 Transcription Factor Staining Buffer Set (both eBioscience, ThermoFisher, Waltham, MA, USA). Subsequently, intracellular proteins were stained and FACS acquisition was performed on a FACSymphony A5 using BD FACSDiva software (both BD Biosciences, Franklin Lakes, NJ, USA). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA). The gating strategy can be found in **Data Supplement Figure 1.**

**Statistical analysis**

All statistical analyses were executed using Graphpad Prism software (Graphpad Software Inc., San Diego, CA, USA) and R software version 3.6.1. The demographic and baseline characteristics of patients are depicted by the descriptive statistics. Categorical variables were presented as absolute and relative frequencies and numerical variables as median (range or interquartile range [IQR]). Median PFS and OS and their fixed-time estimations were estimated according to the Kaplan-Meier method (with corresponding 95% confidence intervals ([CI]) and were compared using a log-rank test. Associations between covariates and time-to-event outcomes (i.e. PFS and OS) were analyzed with univariate Cox proportional hazards models, while associations between clinical covariates and objective response rate (ORR) and disease control rate (DCR) were analyzed with univariate logistic regression analyses. Safety outcomes were described as counts and percentages.

For longitudinal analysis of blood samples (baseline vs on treatment), Wilcoxon signed rank tests (non-parametric, paired data) and Student's *t* test (parametric, paired data) were used. Only when the paired sample was available, the samples were included in the analyses. P-values less than 0.05 were considered statistically significant.

# SUPPLEMENTAL RESULTS

### Immunological phenotyping

When we investigated different T cell subsets (see for gating: **Data Supplement Figure 1**), lurbinectedin was found to significantly increase the proliferation of CD4+ central memory (TCM) and effector memory (TEM) T cells and of CD8+ TEM cells among SCLC (**Data Supplement Figure 5A**). In MPM, lurbinectedin increased more specifically the proliferation of CD4+ TEM cells, while CD8+ T cell subsets were not significantly affected (**Data Supplement Figure 5B**).

**Supplemental Table 1.** Antibodies used for flow cytometry staining.

| Antibody | Fluorochrome | Manufacturer | CAT number |
|---|---|---|---|
| CD45RA | PE-TxR | Life technologies | MHCD45RA17 |
| CD3 | APC-Cy7 | Invitrogen | 47-0038-42 |
| CD4 | BV785 | BD | 563877 |
| CD8 | AF700 | Biolegend | 344724 |
| CCR7 | BV412 | Biolegend | 353208 |
| CD56 | BV605 | BD | 562780 |
| CD28 | Pe-Cy7 | Biolegend | 302926 |
| CD137/4-1BB | PerCP-Cy5.5 | Biolegend | 309814 |
| PD-1 | APC | Biolegend | 329908 |
| HLA-DR | BV711 | BD | 563696 |
| ICOS | BV650 | BD | 563832 |
| Human TruStain | | Biolegend | 422302 |
| Aqua L/D | BV510 | eBioscience | 65-0866-14 |
| Ki-67 | FITC | Invitrogen | 11-5699-42 |
| TCF1 | PE | Biolegend | 655208 |
| LAG-3 | Pe-Cy7 | Biolegend | 369310 |
| TIM-3 | BV650 | Biolegend | 345028 |
| CD39 | BV711 | BD | 563680 |
| TOX | PE | Miltenyi | 130-120-716 |
| CTLA-4 | PerCP-Cy5.5 | Invitrogen | 46-1529-42 |
| CD16 | Fitc | BD | 555406 |
| PD-L1 | PE-CF594 | BD | 563742 |
| CD56 | Pe-Cy7 | BD | 557747 |
| CD15 | APC | Biolegend | 301908 |
| CD3 | AF700 | eBioscience | 56-0038-82 |
| CD19 | AF700 | Invitrogen | 56-0199-42 |
| CD20 | AF700 | BD | 560631 |
| CD86 | bio | BD | 555656 |
| CD137L | BV421 | BD | 744392 |
| CD11c | BV605 | Biolegend | 301636 |
| CD123 | BV650 | BD | 563405 |
| CD14 | BV785 | BD | 563699 |

**Supplemental Table 1.** (Continued)

| Antibody | Fluorochrome | Manufacturer | CAT number |
|---|---|---|---|
| strep | APC-Cy7 | Invitrogen | 47-4317-82 |
| IRF4 | PE | Invitrogen | 12-9858-82 |
| IRF8 | PerCp-Cy5.5 | Invitrogen | 46-9852-82 |
| Granzyme B | FITC | Biolegend | 372205 |
| FoxP3 | PE | Invitrogen | 12-4777-42 |
| IL-10 | Pe-Cy7 | Biolegend | 501420 |
| TNFa | PerCP-Cy5.5 | Invitrogen | 45-7345-42 |
| IL-2 | BV650 | BD | 563467 |
| IFN-y | BV711 | BD | 564039 |

**Supplemental Table 2.** Univariable analysis of PFS, OS and DCR (at 12 weeks) for clinically important factors in patients with small cell lung cancer.

| Parameter | PFS | | | OS | | | DCR | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* | HR | 95% CI | *P* | OR | 95% CI | *P* |
| **ECOG PS (≥1 vs 0)** | 0·67 | 0·25-1·77 | 0·42 | 1·43 | 0·33-6·20 | 0·63 | 1·11 | 0·71-1·71 | 0·64 |
| **Age (>65 vs ≤65)** | 1·48 | 0·74-2·95 | 0·26 | 0·49 | 0·16-1·47 | 0·20 | 0·89 | 0·66-1·20 | 0·48 |
| **Gender (male vs female)** | 0·90 | 0·46-1·71 | 0·74 | 0·78 | 0·33-1·91 | 0·60 | 1·05 | 0·79-1·40 | 0·70 |
| **Line of treatment (≥4 vs 3)** | 0·53 | 0·22-1·28 | 0·16 | 0·51 | 0·26-1·01 | 0·06 | 1·20 | 0·92-1·59 | 0·18 |
| **CFI (≥90 vs <90 days)** | 0·46 | 0·19-1·13 | 0·09 | 0·29 | 0·07-1·08 | 0·06 | 1·30 | 0·94-1·80 | 0·11 |
| **Time interval from diagnosis to lurbinectedin (>median vs ≤median)** | 0·36 | 0·18-0·73 | <0·01 | 0·21 | 0·08-0·56 | <0·01 | 1·24 | 0·94-1·63 | 0·12 |
| **LDH (>ULN vs ≤ULN)** | 1·45 | 0·74-2·84 | 0·27 | 1·00 | 0·40-2·51 | 0·98 | 0·90 | 0·67-1·20 | 0·48 |
| **Albumin (>median vs ≤median)** | 0·92 | 0·46-1·84 | 0·82 | 0·66 | 0·26-1·64 | 0·37 | 1·10 | 0·81-1·50 | 0·51 |

Abbreviations: PFS, progression-free survival; OS, overall survival; DCR, disease control rate; ECOG PS, Eastern Cooperative Oncology Group performance score; CFI, chemotherapy-free interval; LDH, lactate dehydrogenase.

**Supplemental Table 3.** Univariable analysis of PFS, OS and DCR (at 12 weeks) for clinically important factors in patients with malignant pleural mesothelioma.

| Parameter | PFS | | | OS | | | DCR (at 12 weeks) | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* | HR | 95% CI | *P* | OR | 95% CI | *P* |
| **ECOG PS (≥1 vs 0)** | 1·26 | 0·56-2·83 | 0·57 | 2·22 | 0·73-6·76 | 0·16 | 1·07 | 0·75-1·53 | 0·70 |
| **Age (>65 vs ≤65)** | 0·35 | 0·17-0·72 | <0·01 | 1·37 | 0·51-3·64 | 0·52 | 1·37 | 1·01-1·84 | 0·04 |
| **Gender (male vs female)** | 0·27 | 0·11-0·67 | <0·01 | 0·75 | 0·22-2·52 | 0·64 | 1·44 | 0·97-2·13 | 0·08 |
| **Histologic subtype (non-epithelioid vs epithelioid)** | 1·56 | 0·73-3·32 | 0·24 | 5·10 | 2·0-12·98 | <0·01 | 0·88 | 0·62-1·25 | 0·50 |
| **Line of treatment (≥3 vs 2)** | 0·80 | 0·37-1·77 | 0·59 | 2·68 | 0·63-11·4 | 0·18 | 1·16 | 0·83-1·61 | 0·37 |
| **Time since last systemic treatment (≥90 vs. <90 days)** | 0·84 | 0·41-1·72 | 0·64 | 0·64 | 0·26-1·6 | 0·35 | 1·01 | 0·74-1·39 | 0·90 |
| **Time interval from diagnosis to lurbinectedin (>median vs ≤median)** | 0·56 | 0·31-1·04 | 0·07 | 0·70 | 0·32-1·50 | 0·36 | 1·29 | 0·99-1·68 | 0·06 |
| **Albumin (>median vs ≤median)** | 0·74 | 0·39-1·40 | 0·36 | 0·62 | 0·27-1·42 | 0·26 | 1·14 | 0·86-1·52 | 0·34 |

Abbreviations: PFS, progression-free survival; OS, overall survival; DCR, disease control rate; ECOG PS, Eastern Cooperative Oncology Group performance score

**Supplemental Table 4.** Treatment dose reductions, delays, and discontinuation on lurbinectedin.

| | SCLC (n=43) | MPM (n=52) |
|---|---|---|
| **Treatment dose reductions, No. (%)** | 8 (19) | 18 (35) |
| **Hematological toxicity** | 3 (7) | 6 (12) |
| **Fatigue/QoL deterioration** | 4 (7) | 10 (19) |
| **Treatment delays, No. (%)** | 6 (14) | 17 (33) |
| **Treatment discontinuation[a], No. (%)** | 2 (5) | 8 (15) |
| **Hematological toxicity** | 0 (0) | 2 (4) |
| **Fatigue/QoL deterioration** | 2 (5) | 6 (12) |

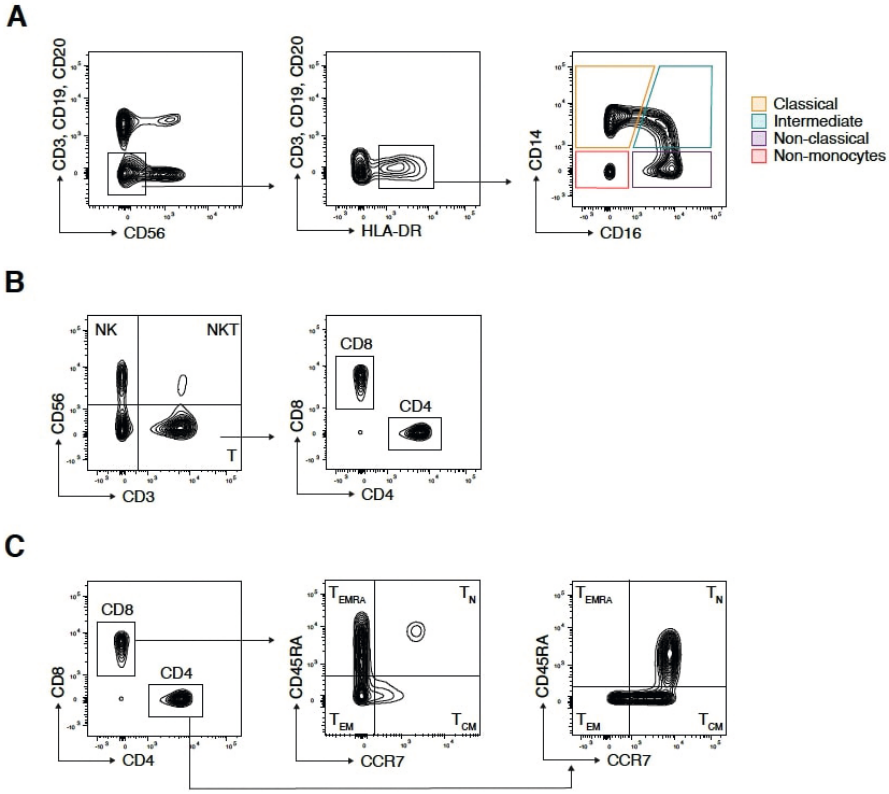
[a]Treatment discontinuation caused by disease progression is not taken into account for this estimate.
Abbreviations: SCLC, small cell lung cancer; MPM, malignant pleural mesothelioma; QoL, quality of life.

**Supplemental Table 5.** Patient and disease baseline characteristics of patients included in the immune monitoring study.

| Characteristic | SCLC (n=20) | MPM (n=19) |
|---|---|---|
| **Median age, years (range)** | 65 (56-77) | 73 (58-79) |
| **Gender, male, No. (%)** | 9 (45) | 17 (89) |
| **Median time from diagnosis to start of lurbinectedin, months (IQR)** | 14.0 (11.0-22.8) | 13.0 (10.4-26.3) |
| **Smoking status, No. (%)** | | |
| Former/current | 12 (60) | 11 (58) |
| Never | 1 (5) | 3 (16) |
| Unknown | 7 (35) | 5 (26) |
| **ECOG PS at start of lurbinectedin, No. (%)** | | |
| 0 | 2 (10) | 3 (16) |
| 1 | 17 (85) | 12 (63) |
| ³2 | 0 (0) | 1 (5) |
| Unknown | 1 (5) | 3 (16) |
| **Histological subtype, No. (%)** | | |
| Epithelioid | NA | 17 (90) |
| Mixed/Sarcomatoid | NA | 1 (5) |
| Peritoneal mesothelioma (epithelioid) | NA | 1 (5) |
| **Previous line(s) of treatment, No. (%)** | | |
| 1 | 0 (0) | 6 (32) |
| 2 | 12 (60) | 9 (47) |
| ³3 | 8 (40) | 4 (21) |
| **Prior immunotherapy, No. (%)** | 2 (10) | 43 (83) |
| **Time since last cycle of systemic treatment, months (range)** | 1.9 (0.8-7.4) | 1·6 (0·8-21·2) |
| <90 days | 18 (90) | 13 (68) |
| ≥90 days | 2 (10) | 6 (32) |
| Unknown | 0 (0) | 0 (0) |
| **Response to lurbinectedin, No. (%)** | | |
| PD | 13 (65) | 12 (63) |
| SD | 3 (15) | 7 (37) |
| PR | 3 (15) | 0 (0) |
| Unknown | 1 (5) | 0 (0) |

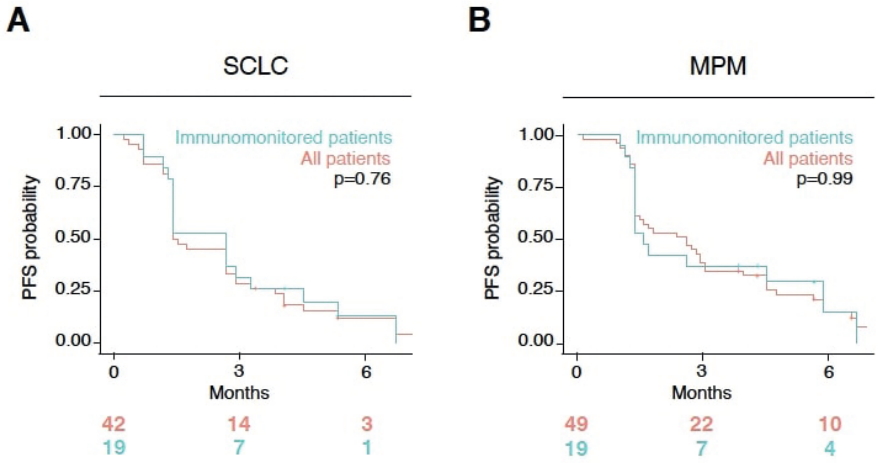
Abbreviations: SCLC, small cell lung cancer; MPM, malignant pleural mesothelioma; IQR, Interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance score; PD, progressive disease; SD, stable disease; PR, partial response

**Supplemental Figure 1.** Gating strategies.



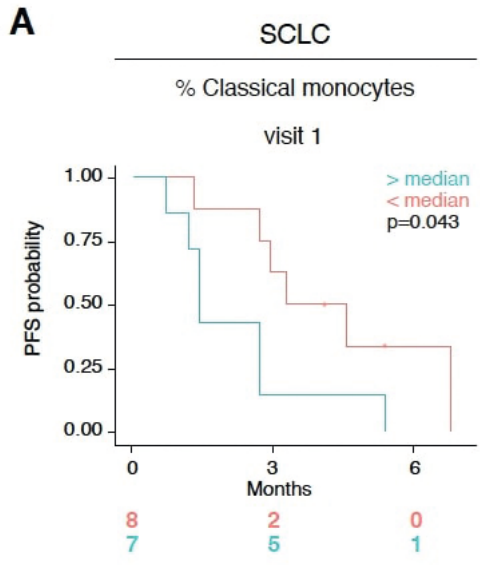

**A:** Gating strategy for the circulating myeloid subsets. **B:** Gating strategy for NK cells, NKT cells and T cells. **C:** Gating strategy for $CD4^+$ T cell and $CD8^+$ T cell subsets.

**Supplemental Figure 2.** No differences in progression-free survival (PFS) between immunomonitored patients and the complete cohort.

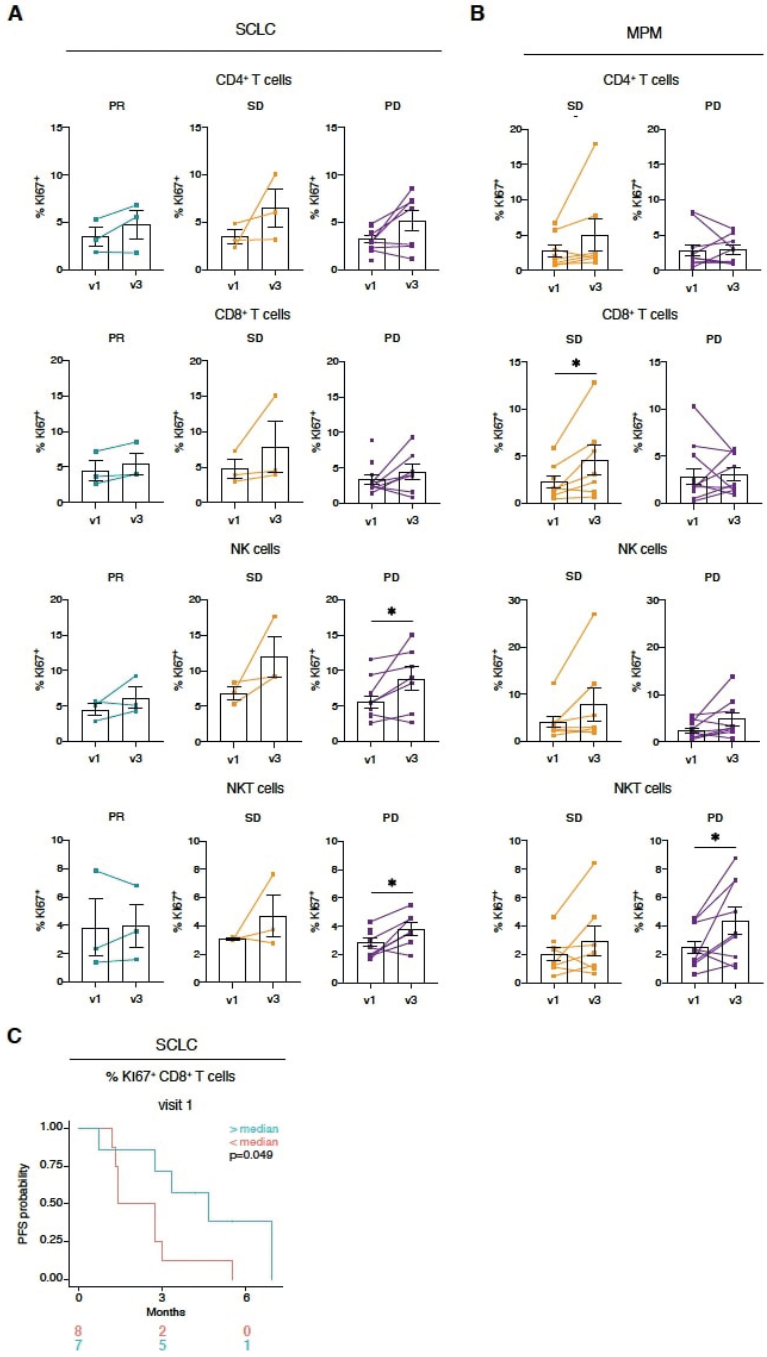



**A:** PFS of small cell lung cancer patients included in the immune monitoring study (blue) vs all (red). **B:** Progression-free survival (PFS) of malignant pleural mesothelioma patients included in the immune monitoring study (blue) vs all (red). Significance was determined using the log rank test.

**Supplemental Figure 3.** Kaplan–Meier analysis showing differences in progression-free survival between SCLC patients exhibiting a lower (red) or higher (blue) proportion of classical monocytes prior to treatment.

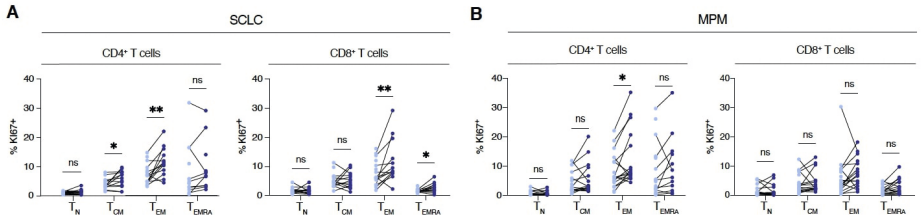


**Supplemental Figure 4.** Lurbinectedin effect on proliferation of circulating lymphocytes is independent of clinical response.



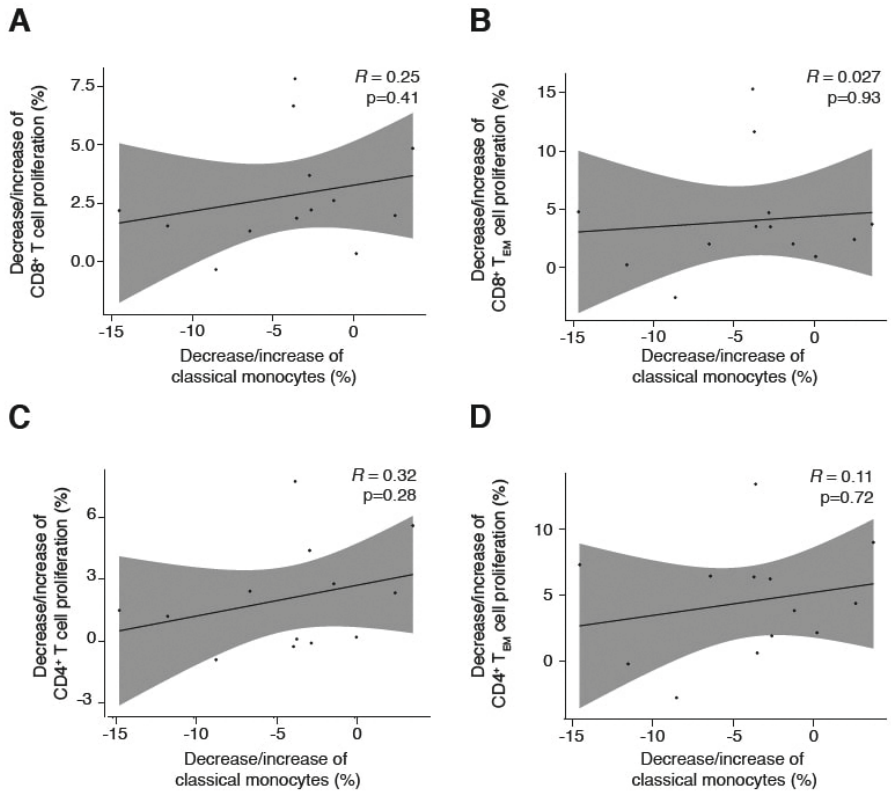
**Supplemental Figure 4. A**: Comparison between small cell lung cancer (SCLC) patients with partial response (PR), stable disease (SD) and progressive disease (PD) for the percentage of Ki67$^+$ CD4$^+$ T cells, CD8$^+$ T cells, NK- and NKT cells, at screening and on-treatment time points. **B**: Comparison between malignant pleural mesothelioma (MPM) patients with partial response (PR), stable disease (SD) and progressive disease (PD) for the percentage of Ki67$^+$ CD4$^+$ T cells, CD8$^+$ T cells, NK- and NKT cells, at screening and on-treatment time points. Wilcoxon matched-pairs signed rank tests or Student's *t*-tests were performed to calculate statistical significance. Paired samples are shown connected by black lines. Bars depict mean values with standard error of the mean. A total of 29 patients had data available at both time points and were included in the analysis (n = 13 SCLC; n = 16 MPM). Only significant differences are indicated. * = p<0.05. **C**: Kaplan–Meier analysis showing differences in progression-free survival between patients exhibiting a higher (blue) or lower (red) proportion of Ki67$^+$ CD8$^+$ T cells prior to treatment. 15 lurbinectedin-treated SCLC patients were included in the analysis, and log-rank test was applied.

**Supplemental Figure 5.** Titel?




**A**: Percentage of Ki67$^+$ CD4$^+$ T cell subsets and CD8$^+$ T cell subsets, at screening and on-treatment time points in SCLC patients. **B**: Percentage of Ki67$^+$ CD4$^+$ T cell subsets and CD8$^+$ T cell subsets, at screening and on-treatment time points in MPM patients.

**Supplemental Figure 6.** T cell proliferation does not relate to monocytes frequencies in SCLC patients.




**A**: Correlation between the decrease of classical monocytes and the increase of proliferating $CD8^+$ T cells. **B**: Correlation between the decrease of classical monocytes and the increase of proliferating $CD8^+$ effector memory T cells. **C**: Correlation between the decrease of classical monocytes and the increase of proliferating $CD4^+$ T cells. **D**: Correlation between the decrease of classical monocytes and the increase of proliferating $CD4^+$ effector memory T cells.

# CHAPTER 10

## RENAL TOXICITY FROM PEMETREXED AND PEMBROLIZUMAB IN THE ERA OF COMBINATION THERAPY IN PATIENTS WITH METASTATIC NONSQUAMOUS CELL NSCLC

**Daphne W Dumoulin***, Sabine Visser*, Robin Cornelissen, Teun van Gelder, Johan Vansteenkiste, Jan von der Thusen, Joachim G J V Aerts

* These authors contributed equally

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## ABSTRACT

The combination of chemotherapy and immune checkpoint inhibition (ICI) therapy is the current standard of care for the majority of patients who are fit to undergo treatment for metastatic NSCLC. With this combination, renal toxicity was slightly higher than with chemotherapy alone in initial clinical trials, but in recent real-world data kidney function loss is reported to be quite more frequent. Both chemotherapy and ICI therapy can induce renal impairment, although the mechanism of renal damage is different. Renal injury due to chemotherapy is often ascribed to acute tubular injury and necrosis (ATN), while the main mechanism of injury due to ICI therapy is acute tubulointerstitial nephritis (ATIN). In case of concomitant use of chemotherapy and ICI therapy, it is a challenge to distinguish the cause of the renal failure. Discriminating between these etiologies is of utmost importance for assessing which drug can be safely continued and which drug must be halted. The aim of this review is to describe the underlying mechanisms of the renal side effects caused by chemotherapy and ICI therapy, leading to a suggested diagnostic and treatment algorithm based on clinical, laboratory, radiographical and pathological parameters. This algorithm may be a supportive tool for clinicians to diagnose the underlying cause of the acute kidney injury in patients treated with combination chemo- and immunotherapy.

## INTRODUCTION

For many years, first-line treatment for advanced non-small-cell lung cancer (NSCLC) was platinum-based combination chemotherapy. Based on the Keynote-024 study, in patients with stage IV NSCLC without EGFR mutation or ALK translocation and programmed-death-ligand-1 (PD-L1) expression of ≥50%, pembrolizumab became the standard first-line therapy because of a significantly longer progression-free and overall survival compared to chemotherapy.[1] Recently, the phase 3 Keynote-189 trial showed that in previously untreated patients with advanced non-squamous NSCLC without EGFR mutation or ALK translocation, the progression-free and overall survival were significantly longer with addition of pembrolizumab to platinum-pemetrexed chemotherapy than with chemotherapy alone, irrespective of PD-L1 expression of the tumor.[2] This combination therapy is now considered a standard of care for the majority of patients, who are fit to undergo treatment for advanced non-squamous NSCLC.

One of the major concerns about combination treatment with different anti-tumor drugs is toxicity, as this may have major impact on quality of life and may lead to withdrawal of effective treatment in patients. Although the overall reported frequency is still low, renal toxicity seems more frequent in the setting of the chemotherapeutic agent pemetrexed in combination with the immune checkpoint inhibitor (ICI) pembrolizumab. According to Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0), in the Keynote-24 trial, comparing pembrolizumab to standard chemotherapy in the first-line setting, nephritis grade 3-5 was seen in 0.6% of patients having immunotherapy.[1] In addition, an increased creatinine was reported in 1.9% of these patients. In the Keynote-189 study, acute kidney injury (AKI), defined according to CTCAE v4.0, was observed in 5.2% of the patients in the pembrolizumab-combination group compared to only 0.5% in the placebo-combination group. A total of 12.2% of patients treated with pembrolizumab and carboplatin-pemetrexed showed an all grade increased blood creatinine, of which 0.7% grade 3-4. Renal adverse events in the pembrolizumab-combination group led to treatment discontinuation in 2% of the patients. The majority of patients in this trial received chemotherapy with carboplatin as the platinum compound, and only about 25% received the more nephrotoxic cisplatin. While initial clinical trials reported a low incidence of immunotherapy-related nephrotoxicity, emerging data suggest a higher incidence rate between 13.9-29%, especially when chemo- and immunotherapy are combined.[3]

Discrepancies between results of clinical trials and real-world data are also present with regard to pemetrexed-induced nephrotoxicity. In the pivotal PARAMOUNT trial, only <10% of patients treated with pemetrexed maintenance therapy experienced renal

impairment, and <5% had to discontinue treatment due to nephrotoxicity.[4] Several retrospective studies already described a higher incidence (17-21%) of renal impairment with pemetrexed.[5,6] In a recent prospective cohort study by our group, frequencies of approximately 30% acute kidney disease (AKD) and up to 20% treatment discontinuation were reported during pemetrexed maintenance treatment.[7]

As platinum, pemetrexed and pembrolizumab are now often combined, it is a challenge to distinguish between chemotherapy- and pembrolizumab-induced renal adverse events. However, discriminating between these etiologies is of utmost importance as misdiagnosis of the causative agent may provoke wrong interventions, which potentially lead to further deterioration of renal toxicity or/and interruption or even cessation of an effective treatment. The aim of this review is to describe the mechanisms of the renal side effects caused by the frequently used combination of platinum, pemetrexed and pembrolizumab, leading to a suggested diagnostic and treatment algorithm. Other oncological therapeutic agents will not be covered in this manuscript.

**Definition of renal toxicity**

Estimations of the frequency of kidney injuries in clinical studies depend on how kidney injury has been defined. In the field of oncology, (renal) adverse events are reported according to the descriptive terminologies of CTCAE, providing a grading (severity) scale for each adverse event (**Table 1**).[8] In CTCAE version 4.0, an important adjustment has been made that takes into account the absolute increase of creatinine and its relative increase from baseline. Notably, in the newest version 5.0 lower grades (1/2) AKI are not defined anymore and severe AKI (grade>3) only depends on need of hospitalization or dialysis and not on measured kidney function. The Acute Kidney Injury Working Group of KDIGO (Kidney Disease: Improving Global Outcomes) proposed the most commonly used definitions of kidney disease nowadays and they divided renal injury into three categories based on the duration of renal function deterioration: AKI, AKD and chronic kidney disease (CKD) (**Table 1**).[9] All individuals, including elderly, with a glomerular filtration rate (GFR) < 60 mL/min are considered to have CKD.[9] Although some decline of GFR is expected with age, most healthy older individuals do not necessarily have a decreased GFR.[9] Moreover, also in older people decreased GFR is associated with increased risk of mortality and kidney failure.[10] In an earlier study by our group, renal adverse events were graded according to CTCAE 4.03 as well as to CTCAE 3.0, to allow for comparison with data from the registration trial of pemetrexed maintenance treatment.[7] From the patients who developed AKD during maintenance pemetrexed therapy according to KDIGO definitions, 77% had all grades renal adverse events according to CTCAE 4.03 and only 54% using CTCAE 3.0. Hence, using CTCAE 3.0 we found only 16% of patients experienced renal adverse events in contrast to

30% using the KDIGO definitions. This study illustrates probable underestimation of renal toxicity by using the CTCAE 3.0 and 4.03 compared to AKD (KDIGO). By taking into account absolute increases of creatinine and its relative increase from baseline, the results of the updated version CTCAE 4.03 corresponded better with the AKD results.

**Mechanisms of renal toxicity**

Anti-tumor drugs can cause renal toxicity by different mechanisms. Renal injury due to chemotherapy is often ascribed to acute tubular injury and necrosis (ATN) while the main mechanism of injury due to immunotherapy is acute tubulointerstitial nephritis (ATIN).[11,12] AKI is associated with immediate and long-term unfavorable outcomes and the development of CKD[13] so it is of utmost importance to rapidly identify the cause and start the appropriate management. Uncovering the underlying mechanisms can be key in the management of AKI during combination treatment of chemotherapy and immunotherapy. In the case of ATIN, timely administration of steroids can salvage kidney tissue by reducing the amount of tubulointerstitial fibrosis that may ultimately develop.[14]

Below, we discuss several separate chemotherapeutic agents used in the treatment of NSCLC in the Keynote-189 trial, followed by ICI.

**Table 1.** Definitions and classifications of kidney injury according to CTCAE and KDIGO

| **CTCAE** | | | | | | |
|---|---|---|---|---|---|---|
| | **Grade 1** | | **Grade 2** | **Grade 3** | **Grade 4** | |
| version 3.0 | | | | | | |
| *creatinine* | >ULN-1.5 x ULN | | >1.5-3.0x ULN | >3.0-6.0 x ULN | >6.0 x ULN | |
| *GFR* | <75-50% LLN | | <50-25% LLN | <25% LLN, chronic dialysis not indicated | Chronic dialysis or renal transplant indicated | |
| version 4.03 | | | | | | |
| *AKI* | Creatinine level increase of >0.3mg/dL (26.5 µmol/L); creatinine 1.5-2.0 x above baseline | | Creatinine 2-3 x above baseline | Creatinine >3 x baseline or >4.0 mg/dL (354 µmol/L); hospitalization indicated | Life-threatening consequences; dialysis indicated | |
| version 5.0 | | | | | | |
| *AKI** | - | | - | Hospitalization indicated | Life-threatening consequences; dialysis indicated | |
| **KDIGO** | | | | | | |
| *AKI* | Increase in serum creatinine by 50% within 7 days *OR*<br>Increase in serum creatinine by 0.3mg/dL (26.5 mmol/L) within 2 days *OR*<br>Oliguria† | | | | | |
| | Stage 1 | | Stage 2 | Stage 3 | | |
| | Creatinine: 1.5-1.9 x baseline OR ³ 0.3mg/dL (26.5 mmol/L) | | Creatinine: 2.0-3.0 x baseline | Creatinine: > 3.0 x baseline OR ³ 4.0 mg/dL (354 mmol/L) | | |
| *AKD* | AKI *OR*<br>GFR < 60mL/min per 1.73m² for < 3 months *OR*<br>Decrease in eGFR by > 35% *OR*<br>Increase in serum creatinine > 50% for < 3 months | | | | | |
| *CKD* | GFR < 60 mL/min per 1.73m² for > 3 months | | | | | |
| | G1 (normal) | G2‡ | G3A | G3B | G4 | G5 (renal failure) |
| | GFR ³ 90 | GFR 60-89 | GFR 45-59 | GFR 30-44 | GFR 15-29 | GFR < 15 |

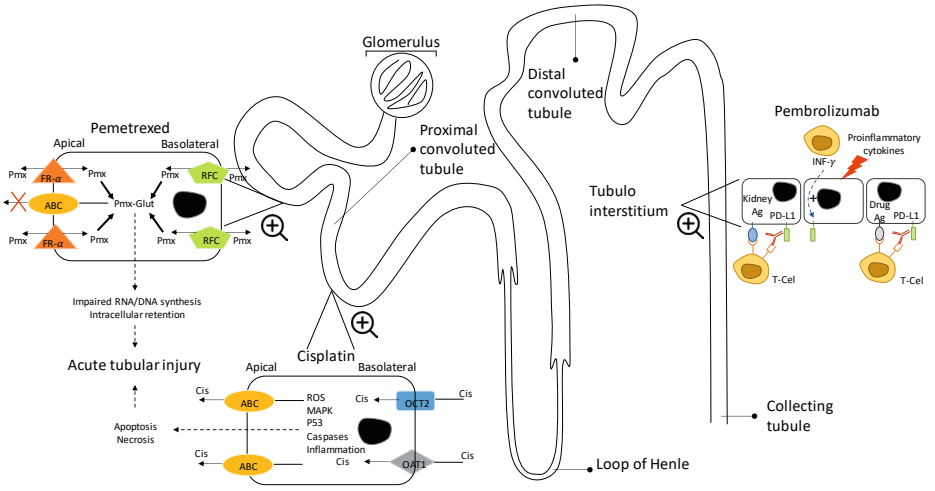
* A disorder characterized by the acute loss of renal function (within 2 weeks). † Oliguria is also used in staging of AKI, but not further discussed here. ‡ GFR 60-89 mL/min is considered to be mildly decreased, but the threshold of GFR < 60mL/min (G3a-G5) is chosen for CKD. Abbreviations: (e)GFR, glomerular filtration rate; AKI, acute kidney injury; AKD, acute kidney disease; CKD, chronic kidney disease

**Cisplatin**

Cisplatin is a platinum compound that is widely used as a cornerstone of chemotherapeutic therapy for many carcinomas, sarcomas and lymphomas. One of its major adverse events is nephrotoxicity, which is often (partially) reversible but may be permanent.[15] Cisplatin is principally excreted by the kidneys and thus its concentrations in the renal cortex are high compared to plasma and other organs.

A key role in the development of cisplatin-mediated nephrotoxicity might be ascribed to basolateral drug transporters, as the expression of proximal tubule organic cation transporter-2 (OCT2) has been shown to influence intracellular accumulation.[16] After cisplatin enters the tubular cell, multiple intracellular injury pathways including inflammation, oxidative stress, apoptotic pathways, cytoplasmic organelle dysfunction and DNA damage can contribute to kidney injury.[17] The renal tubular cell injury ultimately leads to clinical AKI by ATN and apoptosis (**Figure 1**). Another commonly observed manifestation of nephrotoxicity is hypomagnesemia by decreased renal tubular reabsorption, which occurs in 40-100% of patients.[18] Less common manifestations of nephrotoxicity are thrombotic microangiopathy (TMA), Fanconi like syndrome, distal tubular acidosis and renal concentrating defect.[17] Despite renoprotective strategies using hydration and diuresis, magnesium supplementation and mannitol, still approximately one-third of patients treated with cisplatin develop renal impairment after the initial dose. Cisplatin-induced nephrotoxicity is dose-dependent and also increases with recurrent drug administration.[19] In patients with thoracic malignancies (majority NSCLC), cisplatin induced AKI was observed in 21% of the patients.[15] In a recent study by our group, the frequency of acute kidney disease accumulated from 20% during cycle 1 to 50% during cycle 4 in patients treated with combined cisplatin-pemetrexed treatment.[7]

**Figure 1.** Mechanisms underlying chemotherapy- and immune checkpoint-induced kidney injury.




After entrance of tubular cells, polyglutamation leads to entrapment of pemetrexed in the cell as these polyglutamates are no substrate for ABC transporters. Impaired RNA and DNA synthesis leads to acute tubular injury. Cisplatin induces multiple intracellular injury pathways including inflammation, oxidative stress, apoptotic pathways and DNA damage mediating renal tubular cell injury. Immune activation by checkpoint inhibitors leads to the development of autoimmunity, reactivation of memory T-cells previously primed by exogenous drug exposure and increase in proinflammatory cytokines/ chemokines in kidney tissue. FR-a, folate receptor alpha; ABC, ATP binding cassette transporter; Pmx, pemetrexed; Pmx-glut; pemetrexed polyglutamates; RFC, reduced folate carrier; OCT2, organic cation transporter 2; OAT 1, organic anion transporter 1; Cis, cisplatin; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; PD-L1, programmed cell death- ligand 1; INF-g, interferon gamma

## Carboplatin

Carboplatin has a less nephrotoxic profile than cisplatin, in spite of the fact that the elimination of carboplatin is primarily renal via glomerular filtration. Most likely, its lower nephrotoxic potential can be explained by a lack of cell transport by OCT-2, thereby reducing proximal tubular intracellular accumulation. In addition, the chloride at cis-position in cisplatin is replaced by carboxylate which is described to further reduce toxicity.[11] Another explanation for the lower incidence of renal toxicity of carboplatin is the fact that dosing is based on the renal clearance of the patient. Thus, in case of declining kidney function the dose of carboplatin will be adapted, which is not the case with cisplatin treated patients. Nevertheless, renal adverse events are observed during carboplatin-based chemotherapy with direct tubular injury most commonly as the primary mechanism, followed by magnesium wasting. A meta-analyses based on (individual patient) data from phase II and III trials showed a significantly higher incidence of grade 3-4 nephrotoxicity in patients treated with various combinations of chemotherapy combined with cisplatin compared with carboplatin (1.5% vs 0.5%,

p = 0.018).[20] In a real-life setting approximately 20% of the patients having carboplatin-pemetrexed treatment developed acute kidney disease.[7]

**Pemetrexed**

Pemetrexed is an antifolate agent that inhibits multiple enzymes involved in the synthesis of purine and thymidine nucleotides. After cell entrance, pemetrexed undergoes rapid intracellular polyglutamation resulting in polyglutamates that are more potent inhibitors of the enzymatic processes involved in de novo DNA synthesis. Pemetrexed does not undergo significant metabolization and the unchanged parent compound is primarily eliminated via the kidneys, with 70-90% of the administered drug excreted unchanged into urine within 24 hours.[21] Although pemetrexed is often combined with cisplatin or carboplatin, also pemetrexed monotherapy can cause renal failure. While the pathogenic mechanism of renal injury of pemetrexed is not fully understood, histopathology in several case reports described distinct patterns of tubular toxicity.[11] Reduced folate carrier (RFC) is the main entrance transporter of pemetrexed and is expressed on basolateral membranes of kidney tubules, while the folate receptor-alfa (FR-α) provides drug uptake at the apical site.[11] Pemetrexed polyglutamation results in prolonged retention of polyglutamates intracellularly, which in turn may lead to an increase of impaired RNA and DNA synthesis and ultimately tubular injury (**Figure 1**). Cumulative systemic dose of pemetrexed might play a role in the development of nephrotoxicity.[22] Permanent impairment of the kidney function after discontinuation of pemetrexed maintenance therapy has been reported.[23]

**Immune checkpoint inhibitors (ICI)**

ICI are monoclonal antibodies targeted at a specific receptor, either PD-1 or PD-L1, to counteract the blockade of cytotoxic T cells by PD-L1 upregulating tumor cells. Using this mechanism, the inhibition of T cells is released and the immune system can effectively kill the cancer cells. However, PD-L1 is also constitutively expressed on renal cells, and is upregulated by IFN-gamma.[24] By administrating an anti-PD1 or anti-PD-L1 antibody, the PD-1 receptor will be blocked causing proliferation of T-cells and cytotoxic injury of the kidney. It has been speculated that PD-L1 inhibitors potentially lead to less autoimmune toxicity due to diminished blockade of the negative inhibitory signal, caused by the persistent interaction between PD-1 and and its other ligand PD-L2. A systematic review showed similar incidence of adverse events in patients treated with PD-1 and PD-L1 inhibitors.[25] Although renal toxicity was not described separately, there was a trend towards higher incidence of the overall rate of immune related adverse events (irAE) with PD-1 inhibitors, but the number of grade ³3 irAE was comparable.

Thus, kidney injury might be due to loss of peripheral tolerance of self-reactive T-cells against endogenous kidney antigens leading to an auto-immune variant of interstitial nephritis.[26] Alternatively, ICI may induce reactivation of drug-specific T-cells primed by nephrotoxic drugs (e.g. NSAIDs).[12] As associations between drug-specific T-cells and ATIN have been described, it is plausible that ICI may reactivate these latent drug-specific T-cells.[27] Another hypothesis-driven explanation is that the increase of proinflammatory cytokines/chemokines may mediate inflammatory injury in kidney tissue.[28] In contrast to the pharmacokinetics of mentioned chemotherapeutic agents, ICI are not eliminated by the kidneys but cleared primarily by proteolytic degradation in plasma and peripheral tissues.[29]

Renal parenchymal damage due to ICI can be subdivided into two types: ATIN and more rarely glomerular diseases.[3] In addition one case report described thrombotic microangiopathy (TMA) as a result of checkpoint inhibition.[30] However, TMA is also associated with malignancies in general, which makes it uncertain if TMA can be caused by checkpoint inhibition.[31] TMA is characterized by hemolytic anemia due to red blood cell fragmentation, thrombocytopenia due to platelet consumption, and end-organ damage due to microvascular thrombi.[32] Drug-induced TMA has also been reported after treatment with a number of chemotherapeutic agents, including gemcitabine and the already mentioned cisplatin.[33] The exact incidence of drug-induced TMA is difficult to estimate, as cases are underreported, and as clinical presentation is sometimes confused with other causes. The mechanism by which the chemotherapeutic agent induces TMA can either be non-dose dependent (immune-related) or, more frequently, dose-related (toxic).[34] In a patient with severe acute renal failure after treatment with nivolumab/ipilimumab combination therapy a combination of acute interstitial nephritis and TMA-like lesions were found in the renal biopsy.[35]

ATIN induced by ICI is caused by migration of T-cells into the kidneys, resulting in severe inflammatory cell infiltrates with or without granuloma. This mechanism can occur as early as days after treatment initiation, but a considerable delay in development of AIN is often observed with a median time of three months and even reporting of events as late as 12 months.[12,36] Immune-mediated kidney involvement is relatively rare compared to other organs such as the skin, gastrointestinal tract, endocrine glands, and liver, but when ICI cause nephrotoxicity, it can be severe and treatment must be initiated quickly. Timely administration of steroids can salvage kidney tissue by reducing the amount of tubulointerstitial fibrosis that may ultimately develop.[14]

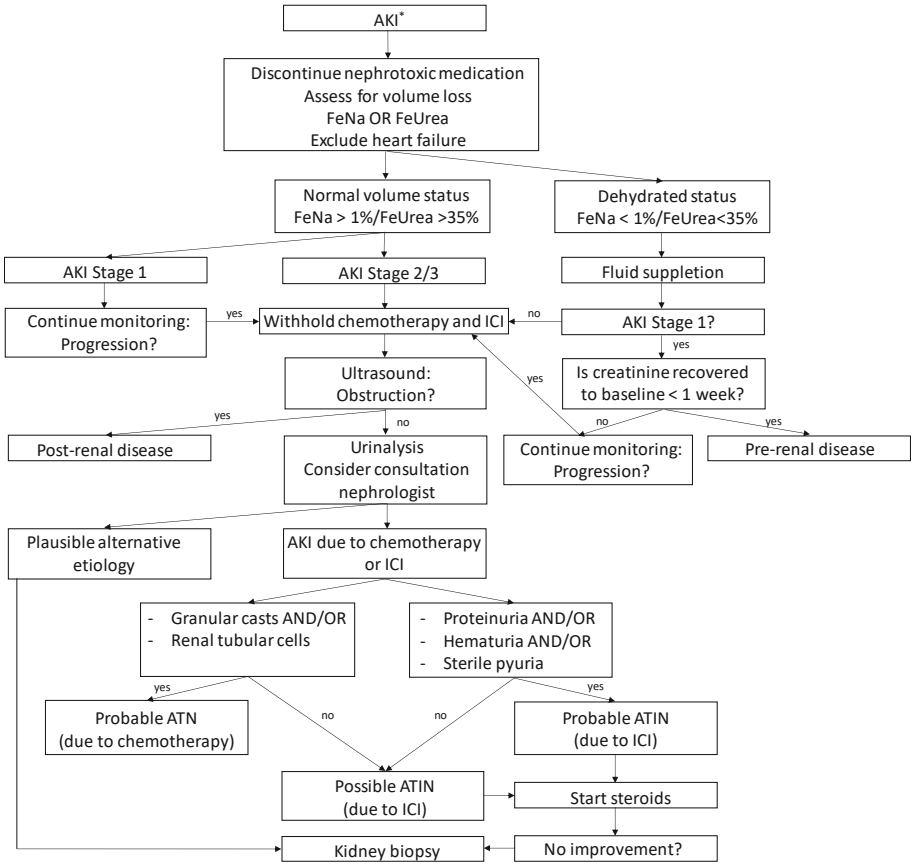
**Evaluation and management of acute kidney injury**

As described above, renal impairment during both treatment with chemotherapy and ICI is common, but their pathophysiologic mechanisms are different. The presence of CKD (eGFR < 60 mL/min) prior to treatment is a known risk factor for AKI. Baseline renal function should be measured before the start of platinum-pemetrexed treatment and immunotherapy, as – even mildly (eGFR 60-90 mL/min)- decreased renal function can predispose to chemotherapy-induced nephrotoxicity.[7,37] In addition to a baseline values of creatinine and eGFR, monitoring these parameters during treatment prior to each next administration is needed. Some important pitfalls with regard to measuring renal function must be addressed. First, estimated GFR is only reliable when plasma creatinine is in steady-state, which is not the case in AKI. Therefore KDIGO states that only an absolute or relative change of creatinine within 48-hours and 7 days respectively (or loss of urine output) can be used for diagnosis of AKI (**Table 1**). The AKD definition takes into account changes in both creatinine and eGFR. In clinical practice, using AKD definition is more convenient as it allows for comparsion between these values with a time interval up until three months. Second, estimations of GFR are dependent on creatinine values. In patients with high age, muscle wasting and poor nutritional status, the use of eGFR may lead to an overestimation of actual renal function.

Before starting chemotherapy/ICI, withdrawal of potential nephrotoxic comedication should be considered. The use of high-dose NSAIDs are (relatively) contra-indicated in the days before and after pemetrexed administration, and contra-indicated in patients with impaired renal function at baseline (FDA label pemetrexed). Besides NSAIDs, it should be considered to interrupt the use of diuretics and angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB), as different studies showed an association between nephrotoxicity and the use of these agents during platinum chemotherapy.[38,39] Of patients treated with ICI, 60% were taking drugs known to potentially cause ATIN.[40] Discontinuation of these drugs should be considered.

A diagnostic algorithm for AKI during treatment of chemotherapy in combination with immunotherapy has been developed based on clinical, laboratory, radiographical and pathological parameters (**Figure 2**).

**Figure 2.** Diagnostic and treatment algorithm for renal injury during combination chemotherapy/ immunotherapy




*AKI is defined and staged according to the KDIGO guideline[9] but decreases in eGFR and a longer time interval (< 3 months) for renal injury to develop based on the AKD definition should be taken into account. Abbreviations: AKI, acute kidney injury; FeNa, fractional excretion of sodium; FeUrea, fractional excretion of urea; ICI, immune checkpoint inhibitor; ATN, acute tubular necrosis; ATIN, acute tubulointerstitial nephritis.

### *Clinical evaluation*

When AKI is observed during treatment, it is important to critically evaluate again whether all potential nephrotoxic medication has been withdrawn if possible. Another mechanism which may contribute to renal failure in patients treated with systemic therapy for lung cancer, is intravenous contrast administration during imaging procedures. These agents cause contrast-induced acute kidney injury by direct and indirect nephrotoxic effects.[41] Patients treated with chemotherapy and immunotherapy are frequently exposed to contrast agents, since they undergo follow-up CT scans

regularly to evaluate response to treatment. The KDIGO working group defined contrast-induced AKI (definition **Table 1**) as AKI after exposure to a contrast medium. Pre-existent CKD is the strongest independent risk factor for contrast-induced acute kidney injury.[41] For this reason the use of intravenous contrast must be carefully considered in each patient but especially in patients with pre-existent kidney disease. Although increments of plasma creatinine levels meeting the AKI criteria are not uncommon the incidence of severe AKI due to contrast-enhanced CT is low with a rate of 0.3% post-procedure dialysis.[42]

Therefore, in the context of the frequently detected decreasing renal function in patients undergoing systemic treatment for lung cancer, the risk of intravenous contrast should be carefully weigthed against the benefit and not as a routine procedure when a CT scan is ordered.

Symptoms may be observed with ATIN, like generalized malaise, fatigue, weakness, fever and anorexia. Obviously, it will be impossible to distinguish the cause of these non-specific symptoms in the presence of malignant disease. Interestingly, 60% of the patients in a recent case series reporting on clinical features of immuno-therapy induced AKI, at least one extra-renal immune-related adverse events was documented prior or concurrently to AKI onset.[37] Additionally, the time of onset of AKI seems to be delayed with a median of 91 days (IQR 60-183 days) and patients could still develop ATIN two months after treatment discontinuation.[12] Thus, concomitant extra-renal irAE at the time of AKI may raise the suspicion of immunotherapy-related renal toxicity. Timing of AKI is unlikely to be helpful in distinguishing between immunotherapy- or chemotherapy-related renal toxicity during combination treatment, except for patients who have a very rapid onset of renal impairment after initiation of treatment, which is suggestive of chemotherapy-related toxicity.

*Blood testing*

No blood tests are helpful in pointing the differential diagnosis of AKI toward ATIN. Serum eosinophils may be moderately or highly elevated (up to 50-75% of the total white blood cell count).[43] However, in a case-series on renal failure only one of 12 (8.3%) patients treated with ICI had eosinophilia.[12] Eosinophilia is also associated with NSCLC and the use of immunotherapy and therefore is not a specific marker.[44]

Blood tests in combination with urine chemistries may be helpful to distinguish prerenal renal injury from ATN. Fractional excretion of sodium (FeNa) and urea (FeUrea) can be calculated and are measures of tubular resorption of sodium and urea, respectively. A FeNa<1% in volume-depleted patients is suggestive of prerenal acute kidney injury,

however its value is unreliable during the use of diuretics.[45] In that case, fractional excretion of urea (FeUrea) is more accurate, with the FeUrea usually <35% in prerenal disease.[46] Patients with ATIN may have FeNa values <1% and >1% and therefore FeNa is useless for diagnosing ATIN.[45] FeUrea has not been properly examined in this population.

As mentioned above, it is important to take into account the pretreatment kidney function, as a decreased creatinine clearance at baseline may be predictive of sensitivity to kidney dysfunction during treatment. In our previous study we also established that a decline in renal function during treatment is predictive for developing renal failure.[7] Additionally, the trend of renal function during treatment should be noted. Although values may still be within a normal range, a decreasing renal function during induction treatment may predict the occurrence of AKI during maintenance treatment.[7]

*Urinalysis*

Urinalysis is a simple test but is the most important noninvasive test in the workup of AKI in general (**Table 2**). In ATIN sterile pyuria is present in most cases, as well as microscopic hematuria without casts suggesting non-glomerular disease. Proteinuria is mild, generally demonstrating protein concentrations <2g/d. White blood cell casts may be observed, but sensitivity is low.[47] ATN is characterized by the presence of (deeply-pigmented) granular and/or renal tubular epithelial cell casts with or without free renal tubular epithelial cells.[48]

**Table 2.** Urinalysis in acute tubulointerstitial nephritis (ATIN) and acute tubular necrosis (ATN)

| | ATIN | ATN |
|---|---|---|
| WBC | +* | 0 |
| WBC casts | + | 0 |
| RBC | + | 0 |
| Protein | + | +/- |
| Renal tubular cell casts | +- | + |
| Granular casts | 0 | + |

* eosinophiluria may be present. Abbreviations: WBC, white blood cells; RBC, red blood cells

PD-1 related ATIN seems to present similar to other causes of ATIN, with evidence of pyuria and sub-nephrotic range proteinuria in 60% and 50% of the patients, respectively.[12] Red blood cells were also detected in approximately 60% of the patients. Urinary cytokine interleukin-9 and tumor necrosis factor-a effectively distinguished ATIN from other renal lesions in patients treated with ICI, but these biomarkers still need validation.[49]

*Imaging*

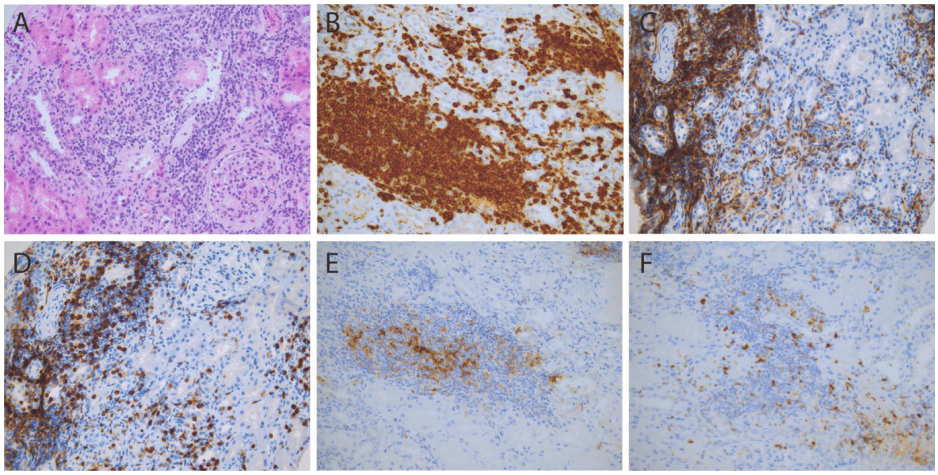
If prerenal disease is excluded or severe AKI is present, an ultrasound should be performed to rule out postrenal disease by urinary tract obstruction. A CT may be performed when hydronephrosis or urinary tract obstruction cannot be reliable excluded by ultrasound. Kidney imaging with $^{67}$gallium scintigraphy has been proposed in the evaluation of ATIN, as positive enhancement is seen if administered $^{67}$gallium binds to lactoferrine, which is released by leukocytes within the kidney interstitium. However, sensitivity (58-100%) shows a large variety and specificity (50-60%) is low.[50] The role of imaging during the workup of AKI during chemotherapy/immunotherapy combination is limited to excluding postrenal disease. However, when imaging procedures are requested, the use of intravenous contrast must be carefully considered to prevent further decrease of kidney function.

*Renal biopsy*

The gold standard for distinction between chemo- or immunotherapy induced renal toxicity is a renal biopsy. Renal toxicity caused by chemotherapy shows ATN, while renal toxicity as a consequence of immunotherapy shows ATIN (**Figure 3**). ATIN is characterized by marked mononuclear cell infiltration and a variable number of lymphoid follicles and tubulitis. There is a strong infiltration of mainly CD3+ T cells, many of which are CD4+ T helper cells with a mild infiltrate of CD8+ cytotoxic T cells and CD20+ B lymphocytes.[12] CD68+ and CD163+ macrophages are also seen, together with CD1c+ dendritic cells. More uncommon mechanisms of immunotherapy-induced renal disease have previously been published as case reports, and these include TMA, minimal change disease, immune complex glomerulonephritis, as well as drug-induced lupus nephritis.[51–53] While TMA can be diagnosed histomorphologically, minimal change disease can only be diagnosed with confidence using electron microscopy, and the latter two require confirmation by demonstration of a characteristic immunofluorescence staining pattern.

The timing of when to consider a kidney biopsy is discutable and often depends on the subjective judgement of the clinician. Empirical treatment with steroids after ruling out pre- and postrenal causes of renal injury is recommended for most patients. A renal biopsy is indicated directly for patients who are likely to have an alternative etiology of renal injury, such as glomerulonephritis not ICI-related, and for patients who do not recover with high doses of steroids.

**Figure 3.** Kidney biopsy with tubulointerstitial nephritis




A. Hematoxylin-eosin stain, demonstrating extensive immune cell infiltration in the kidney parenchyma, affecting and displacing tubules but not encroaching on glomeruli (bottom right). B. Immunohistochemical stain for CD3, demonstrating aggregates of T lymphocytes, and tubulitis. C. CD4 stain, positive in histiocytes and helper T cells in interstitial stroma, but not present in tubules. D. CD8, positive in cytotoxic T cells in stroma, and present in intratubular lymphocytes. E. PD-L1, limited to lymphoid aggregates, likely positive in dendritic / antigen presenting cells. F. PD-1 stain, positive in lymphocytes, within and outside of aggregates / follicles.

*Management*

It is recommended to continue ICI and monitor closely in grade 1 AKI, while grade 2-4 AKI should prompt to discontinue treatment, explore the exact cause of AKI, and start with steroids.[54] In patients with grade 4 AKI, immunotherapy should not be restarted. A recent review of observational studies showed that the majority of patients received corticosteroids (80%) and immunotherapy was discontinued (90%) if ATIN was considered during treatment with ICI, but the approach with regard to dose and length of corticosteroid treatment was highly variable.[40] Only one third of these patients had a complete recovery of their kidney function and 10% of the patients needed renal replacement therapy. There is a need for better immuno-pathophysiological knowledge and biomarkers, to develop more personalized therapeutic drug regimens for severe and refractory immune-related adverse events.[55]

In case of severe kidney injury most likely to be caused by chemotherapy, dose reductions or discontinuation should be considered although extensive data supporting such recommendations are lacking.[56,57] According to Kintzel et al., in patients treated with cisplatin a dose reduction of 25% is suggested for creatinine clearance (CrCl) 46-60 mL/min and a 50% dose reduction for CrCl 30-45 mL/min[56], while Aronoff

et al. even recommend cisplatin administration in patients with more severe renal impairment.[57] Substituting cisplatin by carboplatin is a pragmatic approach in most patients with advanced NSCLC. For carboplatin renal function-based dose adjustments are recommended by using the Calvert formula, capping the maximum carboplatin dose based on target AUC. In patients treated with pemetrexed, dose adjustment is not necessary in patients with a CrCl ³ 45mL/min and it is not recommended to use the drug in patients with a CrCl <45ml/min, although data are scarce in these patients.[58] Pemetrexed dosing is BSA-based, however increasing evidence suggests renal function is a main predictor of pemetrexed clearance and thus exposure.[59] Therefore renal-based dosing may result in a more stable exposure and less toxicity. Currently, a phase II study is assessing the feasibility of renal function-based dosing of pemetrexed in patients with an impaired renal function CrCl<45ml/min (IMPROVE-I, ClinicalTrials.gov Identifier: NCT03656549).

## DISCUSSION

Combination chemo- and immunotherapy with PD-1/PD-L1 inhibition improves survival in patients with NSCLC. The hypothesis is that chemotherapy increases the responsiveness to ICI causing some synergistic effects with outcomes superior to the administration of both therapies in a sequential way. This also holds true for the maintenance phase, in which it is recommended to continue treatment with pemetrexed in combination with pembrolizumab.

The gain in survival benefit due to combination of chemotherapy and immunotherapy, probably increases the willingness of patients to undergo treatment. This will lead to a larger treatment population in clinical practice, including frail patients who are more prone to side effects of treatment. Given the higher age and the cardiovascular comorbidities often seen in lung cancer patients, renal side effects are more frequently seen in a general population then reported in clinical trials.[7]



Some important challenges are encountered in clinical practice when dealing with renal injury during chemotherapy/ICI treatment. We do not only need to be aware of underestimation of kidney injury in clinical trials, but also large variations in incidence may be found due to use of different definitions. Especially the latest CTCAE (v5.0) may falsely report low numbers, as only kidney disease leading to hospitalization will be scored. Additionally, rather than using single eGFR and creatinine measurements alone, we emphasize to look at the trend during total treatment period. Not only the absolute value of kidney function but also its decrease during treatment may predict

further complications during maintenance treatment. For this reason, defining (sub) acute renal injury according to the AKD definition seems most appropriate.

Proper diagnosis of causes of the side effects in these patients is of upmost importance, to preclude worsening of side effects and decreases in quality of life. The algorithm described in this paper may be a help for clinicians to diagnose acute kidney injury in patients treated with combination chemotherapy and ICI.

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# CHAPTER 11

## GENERAL DISCUSSION

## GENERAL DISCUSSION

Immunotherapy, mainly immune checkpoint inhibitors (ICI), is currently widely used as the standard of care in the treatment of many types of cancer. It is the first therapy in metastatic non-small cell lung cancer (NSCLC) without targetable molecular aberrations that has shown durable responses, which is a major difference with chemotherapy. The first approved ICI ever was the anti-CTLA-4 antibody ipilimumab, which was approved by the FDA in 2011 for the first- or second-line treatment in patients with melanoma.[1] In a pooled analysis with 1861 patients from 10 prospective and two retrospective trials with ipilimumab for melanoma, including 1257 previously treated and 604 treatment-naïve patients, 3-year overall survival (OS) was seen in 20% of the patients (compared to historically 10%), which was maintained for over 10 years.[2] Nevertheless, ICIs efficacy varies between cancer types. Monotherapy with anti-CTLA-4 proved not beneficial in NSCLC, and a phase II study investigating the combination of ipilimumab with chemotherapy also did not improve median OS.[3] However, another form of ICI therapy targeting the PD-1/PD-L1 checkpoint with anti-PD-1 antibodies pembrolizumab and nivolumab showed improved survival in second-lined line treatment in NSCLC.[4–6] In addition, treatment with pembrolizumab as monotherapy in the first-line setting in patients with NSCLC and a PD-L1 expression of at least 50% doubled the 5Y-OS to 32% compared to chemotherapy.[7] Also, in melanoma, the anti-PD-1 antibodies had superior efficacy compared to ipilimumab, with a 5-year OS of 43-44%.[8]

The above-mentioned results of these studies with major improvements in long-term survival show that it is debatable whether the endpoints previously used in clinical trials (mainly focusing on the median or hazard ratio) are valid to conclude if a treatment is superior. Using the example of ipilimumab in melanoma, the 3-year OS doubled, which was the reason for approval. However, the median OS was 11.4 months (95% CI, 10.7 to 12.1 months), compared to 8-10 months for other approved therapies for stage IV melanoma. This indicates that for those treatments that could be beneficial for only part of the patients, the focus should be more on the long-term survival rates, as opposed to medians. Notably, not every patient will respond to chemotherapy so also this treatment is beneficial for only part of the patients. However, with the thought that immunotherapy can cure, in contrast to chemotherapy, long-term survival outcomes become more relevant.

In contrast to NSCLC, the majority of patients suffering from mesothelioma or small-cell lung cancer (SCLC) do not respond to immunotherapy or relapse rapidly with the present forms of ICI.[9,10] In Chapter 2, an overview of SCLC and mesothelioma is given, showing that in both entities, only a small proportion of patients have durable benefit from ICI.

In addition, monotherapy ICI in SCLC and mesothelioma failed to show improvement in OS over chemotherapy.[9,10] Furthermore, in contrast to NSCLC, a synergistic effect of the currently used chemotherapy combined with ICI in SCLC seems to be absent despite the exceptional sensitivity of SCLC to chemotherapy. It could be that the currently used chemotherapy agent in SCLC is not the most optimal for triggering the immune system, which is described in Chapter 4. Also in mesothelioma the synergistic effect is less evident. Apparently, SCLC and mesothelioma are tumors that are, at least, less sensitive to ICI than other tumors for reasons incompletely understood. To achieve long-term survival benefit also in these entities, more in-depth insights into the mechanisms for primary ICI resistance are necessary.

**Tumor characteristics contributing to ICI resistance**

For an effective anti-cancer immune response, as widely discussed in this thesis, every step in the Cancer-Immunity Cycle must function.

*Mesothelioma*

As described in Chapter 2, several steps are hindered in the immune- cycle in mesothelioma, contributing to an ineffective immune response. Cancer genes in mesothelioma have relatively low numbers of genetic aberrations, leading to a low tumor mutational burden (TMB).[11] In addition, mesothelioma is surrounded and invaded by immunosuppressive cells; the infiltration of asbestos fibers leads to the release of inflammatory cytokines and vascular endothelial growth factor (VEGF), which attracts immune stimulatory macrophages resulting in further stimulation of cytokine release. Due to this chronic inflammatory response, immunosuppressing cells such as myeloid-derived suppressor cells (MDSC), M2 macrophages, and regulatory T cells (Tregs) are attracted.[12] Thus, the microenvironment of mesothelioma is enriched in myeloid cells; the infiltration of CD8+ lymphocytes is relatively limited, accompanied by an impaired function due to the presence of Tregs and suppressive immune checkpoints.[13] Furthermore, while DCs can activate T-cells, the immunosuppressive tumor microenvironment of mesothelioma prevents the maturation and activation of DCs, thus hindering T-cell activation. Hence, in mesothelioma, the low mutational load and the immunosuppressive tumor microenvironment may result in escape from immune surveillance.



*SCLC*

In contrast to cancer genes in mesothelioma harboring a low TMB, SCLC is, due to the association with smoking resulting in high rates of somatic mutations, characterized by a high TMB. As described in Chapter 2, despite the high TMB, which induces the presence and release of neoantigens and is thought to have a predictive role in response to

ICI, the benefit of ICI in SCLC is limited compared to, for instance, NSCLC.[14] The exact reasons for the lack of efficacy of ICI in SCLC are not fully understood, but several factors could play a role. SCLC cells express CD47, which protects the cells from phagocytosis by macrophages and dendritic cells, enabling tumor cells to escape immune surveillance.[15] Also, as we described in Chapter 3, PD-L1 expression is lower in SCLC (15%) than in NSCLC (60%), suggesting a less immunogenic phenotype, although the clinical relevance of PD-L1 expression in SCLC has remained unclear.[16,17] Moreover, CD8+ tumor-infiltrating lymphocytes (TILs) are less often present in SCLC (around 13%) than in NSCLC, while SCLC harbors higher numbers of immune suppressive cells like Forkhead box P3 (FOXP3) positive Tregs and MDSCs.[18,19] Furthermore, in up to 90% of SCLC, the tumor suppressor gene RB1 is inactivated, which may mediate myeloid-derived suppressor cell accumulation.[20] Thus, in SCLC, the immune-excluded environment may contribute to evading the immune response. Notably, more elderly patients are diagnosed with SCLC over time as described in Chapter 5. Although only a small number of older patients were enrolled in ICI-studies, it seems like immunosenescence does not affect efficacy of ICI and does therefore not play a major role in immune escape.[21]

**Mechanisms to potentially improve immunotherapeutic strategies in mesothelioma and SCLC**

In order to improve survival outcomes in mesothelioma and SCLC, we investigated several ways to counteract the escape from immune surveillance.

To overcome the inability for potent T-cell activation due to immature and inactivated DCs in mesothelioma, the administration of activated and tumor-lysate-loaded dendritic cells (DCs) ex-vivo was investigated in Chapter 7. Based on the promising signal we found with a 2-year OS of >50% and a 5-year OS of >20%, this strategy has led to the phase 3 DENIM trial. Although vaccinations with activated DCs led to a T-cell response, they did not improve the OS, which was the primary endpoint.[22] This could potentially be explained by the fact that despite the effective T-cell activation induced by the DC vaccinations, this, in turn, can be followed by the upregulation of PD-L1 on TILs as negative feedback. Therefore, a combination of DC vaccination with anti-PD(L)1 was investigated in Chapter 8, which supported the hypothesis that this strategy could reinvigorate the T-cell response. Additionally, ex-vivo activation of large numbers of DCs possibly results in over-upregulation of PD-L1 and, therefore, a diminished effect of DC vaccination immunotherapy. By administrating ICI concurrently with DC vaccination, this problem can be counteracted. In addition, PD-L1 was not only upregulated in activated DCs ex-vivo, but similar upregulation was found in tumor-draining lymph nodes (TDLNs) of tumor-bearing mice. TDLNs seem to have a crucial role in a durable anti-tumor immune response, as immune activation primarily takes place there.[23] In the

TDLNs, our group showed that PD-1/PD-L1 interaction takes place between DCs and T-cells prohibiting sustained T-cell activation. Blockade of this PD-1/PD-L1 interaction specifically and only in the TDLN using ICI, enhances anti-tumor T-cell immunity and thus improves tumor control. This supports the idea of concurrent administration of ICI with DC vaccination. However, if this preclinical mouse model can be used as a comparative situation to humans, is unclear. In humans, DCs were injected intradermally, not specifically targeting the TDLNs. In contrast, the mice in this experiment received the injections in the intraperitoneal cavity, which is draining on the mediastinal lymph nodes: the TDLNs. To imitate the situation of mice in humans and investigate if long-term tumor control can be reached by targeting the TDLNs, it would be interesting to target the TDLNs by injecting them directly.

Notable, the fact that the control group in the DENIM trial also received ICI after progression on chemotherapy could have impacted the OS. Therefore, long-term survival outcomes are of interest, which has to be awaited to conclude the additive value of DC vaccination therapy.

Another option that can be used to stimulate T-cell responses is to block the CTLA-4 receptor, which is constitutively expressed on Tregs but can also be upregulated, upon activation, by CD4-T-cells. CTLA-4 mediates immunosuppression; thus, by blocking this antibody, the immunosuppressive function of Tregs could be diminished. Further improvement can be reached by the complementary blockade of PD-(L)1, which controls T-cell activation at the tumor site. Based on this strategy, treatment with anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) was approved and registered after the positive results of the phase 3 Checkmate-743 study.[24] A real-world cohort of patients treated with this combination ICIs was described in Chapter 6, which confirmed efficacy comparable to the results found in the Checkmate-743 study. The treatment is accompanied by a substantial number of immune-related adverse events (irAEs) which leads to treatment continuation in 25% of the patients. Although these numbers of toxicity were expected as they are comparable to the toxicity found in the Checkmate-743 study, this observation underlines the importance of being aware of ICI-induced side effects and managing these effects as soon as possible. In addition, it underlines the need for a probable less toxic regimen of ICI treatment, for instance, by limiting the number of treatment cycles. This idea can be supported by the fact that, based on the results of subgroup analysis from other studies using combination ICIs, the OS of the patients who discontinued the treatment with ICI because of irAEs have improved even more.[25] Apparently, durable benefit from ICI treatment can also be seen with limited numbers of ICI treatment cycles.

Noteworthy to mention is that the role of Tregs in resistance against ICI (anti-PD(L)1) may be underestimated. Tregs are potent suppressors of immune cells, including DCs and CD4+ and CD8+ T-cells.[26] To exert this immunosuppressive function, Tregs have to be TCR-activated in the presence of IL-2, which can be consumed due to the high CD25 expression.

Next to CTLA-4, PD-1 is expressed on Tregs. In patients with gastric cancer, it was shown that an increased PD-1 expression in tumor-infiltrating Tregs compared with circulation Tregs before PD-1 blockade was predictive of resistance to anti-PD-(L)1 therapy and was correlated with hyper progressive disease.[27] We, therefore, investigated, outside of this thesis, if we could identify the role of anti-PD-(L)1 blockade in facilitating the immunosuppressive function of Tregs and, thereby, immune resistance. Our study found increased proliferation of PD-1+ Tregs in the peripheral blood of patients with SCLC, NSCLC, and mesothelioma after treatment with anti-PD-(L)1, mainly in the patients who did not respond.[28] Furthermore, after the depletion of Tregs followed by anti-PD-L1 treatment in mice, a decrease in tumor burden and an increase in TILs were found. These findings suggest that treatment with anti-PD-(L)1 blockade can negatively impact immune surveillance by activating Tregs, which provides a rationale for combining anti-PD-(L)1 with, for instance, anti-CTLA-4.

Another checkpoint that promotes the immunosuppressive function of Tregs by direct FOXP3 binding is the T-cell immunoreceptor with Ig and ITIM domains (TIGIT).[29] While CTLA-4 is expressed in 69% of the Tregs, TIGIT is expressed in 73%.[13] TIGIT can also be expressed on effector T-cells and NK-cells.[30] TIGIT can bind to the ligands Poliovirus receptor (PVR) and PVRL2 (nectin-2), which are expressed by tumor cells and APCs. By blocking TIGIT, the immunosuppressive function of Tregs can be compromised.[31] In a phase 2 study in PD-L1 positive NSCLC, the combination of anti-PD-L1 antibody atezolizumab and anti-TIGIT antibody tiragolumab resulted in an improved PFS and OS compared to anti-PD-L1 alone.[32,33] However, despite TIGIT being expressed on 74% of limited-stage SCLC samples[34], the same combination of anti-PD-L1 and anti-TIGIT in a phase 3 study in SCLC failed to show improvement for reasons unknown.[35]

Next to the immune checkpoints PD-1, CTLA-4, and TIGIT, other checkpoints are known and under investigation for their role in the immune system to exert its protective function.

LAG-3 is mainly expressed on the surface of Tregs and can also be expressed on effector T-cells, natural killer (NK)-cells, B-cells, and DCs. By binding LAG-3 to MHCII molecules expressed on APCs, it exerts a negative regulatory function on the

proliferation and activation of T-cells and enhances the suppressive activity of Tregs.[36,37] In addition, a phase 2-3 trial in melanoma showed improved survival by the addition of anti-LAG-3 to anti-PD-1.[38] The role of LAG-3 in immune suppression has already been proven in early-phase clinical trials in various cancers, and currently, several LAG-3-targeting agents are being investigated.[39] Although LAG-3 is not directly expressed by cancer cells, its expression on cytotoxic T-cells in at least mesothelioma patients is elevated.[40] In addition, LAG-3 is frequently detected in pleural effusion of patients with mesothelioma.[41] Therefore, LAG-3 inhibitors, with or without other checkpoint inhibitors, could be an interesting target in mesothelioma.

Also, the expression of TIM-3 on cytotoxic T-cells is elevated in patients with mesothelioma.[40] Next to T-cells, TIM-3 can also be expressed on macrophages, DCs, and mesothelioma tumor cells.[42] TIM-3 binds to its ligand galectin-9, followed by T-cell apoptosis. Furthermore, most TIM-3+ T-cells co-express PD-1, which can bind galactin-9, leading to apoptosis.[43] Blockade of PD-(L)1 and TIM-3 in allogenic mesothelioma PBMCs, resulted in a high concentration of IFNγ.[44] Galectin-9 is upregulated by IFNγ and IFNβ. IFNβ is produced by intratumoral DCs when they signal dying tumor cells, and IFNγ is released by T-cells when they become activated. Thus, while an anti-tumor immune response occurs, galactin-9 will be upregulated, resulting in T-cell apoptosis and, therefore, immune resistance. In addition, galactin-9 is also expressed by Tregs, which contributes to a suppressive immune response.[43]

However, as we found with TIGIT in SCLC, the fact that these coinhibitory receptors are expressed in mesothelioma does not consequently mean that targeting these receptors will be effective. Further research is needed to investigate the role of blockade of these particular checkpoints on efficacy.

As opposed to the coinhibitory receptors that can dampen the immune response, costimulatory molecules are able to enhance anti-tumor immunity. T-cells can express several molecules from the tumor necrosis factor receptor superfamily (TNFRSF), such as OX40, CD40, and 4-1BB.[31] These TNF receptors can play an important role in Treg exhaustion and effector T-cell activation. If stimulating these receptors leads to efficacy in mesothelioma has to be investigated.



Furthermore, traditional treatment with chemotherapy could be of added value in combination strategies with ICI. First, chemotherapy induces immunogenic cell death, which can lead to the release of tumor neoantigens. Second, chemotherapy can induce a less suppressive tumor microenvironment by several immunostimulatory mechanisms. Combining chemotherapy with ICI is already the standard of care for

several malignancies like NSCLC.[45] Due to the immune-excluded SCLC environment, combining chemotherapy with immunotherapy seems logical to convert SCLC into an immunogenic tumor. In SCLC, chemotherapy combining anti-PD-(L)1 has been investigated in the first-line setting in three randomized phase III (IMpower 133, CASPIAN, and KEYNOTE-604[46–48]) and two randomized phase II trials (REACTION and ECOG-ACRIN5161[49,50]), all showing improved OS compared to chemotherapy alone, although not all significant. Of note, the addition of only anti-CTLA4 to chemotherapy did not prolong OS compared to chemotherapy alone in patients with newly diagnosed stage IV SCLC.[51] In mesothelioma, the combination of chemotherapy with anti-PD-1 is recently presented and shows significant but limited improvement of OS compared to chemotherapy.[52] Two other phase III trials combining chemotherapy with anti-PD-(L)1 are currently ongoing.[53,54]

Investigating chemotherapy with both anti-PD-(L)1 and anti-CTLA4 could be viable, based on the earlier mentioned rationale, and supported by the fact that this strategy is already proven effective in NSCLC.[55,56] This strategy is not investigated in patients with mesothelioma yet. However, in mesothelioma cell lines, the effect of chemotherapy on the expression of several immune stimulatory checkpoints is studied, showing that chemotherapy predominantly induces downregulation of the checkpoints and their ligands.[57] In SCLC, the addition of anti-CTLA4 to anti-PD-L1 and chemotherapy in the CASPIAN trial was not of additive value.[47]

Noteworthy, many chemotherapeutic agents have different characteristics and, therefore, different ways to influence the tumor microenvironment. Although in clinical trials as well as in clinical practice, usually a particular chemotherapeutic agent is used, it could be that another agent has a more pronounced influence on the tumor microenvironment. This role of the backbone chemotherapy for SCLC was described in Chapter 4, with a rationale to switch this backbone if ICI is added to chemotherapy in order to reinforce tumor immunogenicity. Another cytostatic agent that aims to influence the tumor microenvironment in patients with mesothelioma and SCLC is lurbinectedin, discussed in Chapter 9 of this thesis. Lurbinectedin inhibits transcription by RNA polymerase II, resulting in double-strand breaks in the DNA, leading to apoptosis. In addition, our study found a shift in monocytes in favor of intermediate monocytes, which can contribute to better antigen recognition. Despite the disappointing clinical results of lurbinectedin in mesothelioma, we found an increase of inhibitory markers such as CTLA-4 upregulated upon treatment. Furthermore, the expression of the inhibitory receptor TIM-3 was upregulated in SCLC and mesothelioma. This would suggest that a combination of lurbinectedin with ICI could be effective. The combination of lurbinectedin with anti-PD(L)1 is presently under investigation in multiple tumor

types (NCT05572476, NCT05091567, NCT05574504). Whether the combination with other immune checkpoints like CTLA-4 and TIM-3 is beneficial has to be investigated.

Although the combination of chemotherapy with anti-PD(L)1 was beneficial in SCLC and led to approval by the Food and Drug Administration (FDA[58–60]) and European Medicines Agency (EMA[61,62]), the benefit was only modest compared to for instance, NSCLC. Only a limited number of patients suffering from SCLC seem to have a (durable) response to anti-PD(L)1. In the Netherlands, a committee has been appointed that assesses the clinical value of new oncolytic registered medicines or indications through the use of the so-called "PASKWIL-criteria", including the elements 'Palliative', 'Adjuvant', 'Specific toxicity', 'Quality of life', 'Impact of treatment' and 'Level of evidence'.[63] These criteria use mOS and hazard ratio in the in the assessment whether a drug may be prescribed in the Netherlands. Consequently, based on these criteria, the combination chemotherapy with anti-PD(L)1 is rejected in the Netherlands and is, therefore, currently not reimbursed. Nevertheless, although less efficacy was reached in SCLC compared to other tumor types, it remains a matter of debate whether the 10% increase in 2Y-OS with anti-PD(L)1 plus chemotherapy compared to chemotherapy alone in SCLC is of no added value for patients and their families. This long-term OS improvement is currently not involved in the above-mentioned criteria that only using the medians and hazard ratios. For this, it would be very relevant to identify which patients could derive benefit from the addition of ICI.

Recently, a new classification for SCLC was developed based on gene expression profiles, which could help predict which patients could potentially have clinical benefit of immunotherapy.[64] Four major subtypes can be distinguished by differential expression of four key transcriptional regulators: ASCL1 (=SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P), and YAP1(SCLC-Y)]. SCLC-Y is enriched for a T-cell inflamed phenotype with the highest CD8+ T-cells in the tumors, making it plausible that this subtype might derive the most benefit from ICI.[65] However, an exploratory analysis of the KEYNOTE-604 study comparing chemotherapy plus pembrolizumab to chemotherapy plus placebo in stage IV SCLC did not show an OS difference between the transcriptional subgroups and neither an additional benefit of pembrolizumab in one of the subgroups.[66]

## PROPOSAL FOR FUTURE RESEARCH

Immunotherapy has revolutionized the landscape of cancer treatments, but improvement is still needed. From my point of view, a more personalized treatment is necessary. Because of the heterogeneity of the tumor and its environment with the presence or absence of, among others, immune checkpoints, TILs, Tregs, and other immune-suppressive or stimulating cells, there is no one-size-fits-all solution to overcome all the challenges.

As mentioned, in SCLC, it is worth first investigating the role of a more immunomodulating cytotoxic agent as a backbone and analyzing if we could find a subgroup that seems more prone to respond on ICI, like the YAP1. Second, in this selected group of patients, I would suggest investigating the new chemotherapy backbone in combination with anti-PD-(L)1 and anti-TIGIT, because TIGIT is expressed in the majority of SCLC patients. Targeting TIGIT in combination with anti-PD-L1 could also be beneficial in mesothelioma, as our group found upregulation of TIGIT following PD-L1 blockade.[28]

Another interesting treatment for both SCLC and mesothelioma could be anti-TIM-3 combined with lurbinectedin, as we found an increased upregulation of TIM-3 upon treatment. Furthermore, lurbinectedin functions through the depletion of the classical monocyte subsets and a shift in macrophage subtypes, which, therefore, leads to an increase in M1 macrophages and a more immune-activating environment.[67] Also, upregulation of CTLA-4 was seen upon treatment with lurbinectedin in patients with mesothelioma, which suggests that the combination of lurbinectedin combined with anti-CTLA-4 (eventually plus anti-PD-(L)1)) could be beneficial. Because of the probably significant role of the immunosuppressive tumor microenvironment in mesothelioma in immune resistance, particularly Tregs, there seems to be a rationale to reduce the (activity of) Tregs. Based on the rationale mentioned above, targeting LAG-3 in combination with anti-PD-(L)1 and anti-CTLA-4 could be an attractive strategy to suppress the activity of Tregs. In addition, in an exploratory biomarker analysis of the Checkmate-743 study, LAG-3 measured by RNA sequencing as a part of a four-gene inflammatory signature score together with CD8A, STAT1, and CD274 (PD-L1), was associated with longer OS in patients receiving immunotherapy, which supports this suggestion.[68]

Thus, next to PD-(L)1 and CTLA-4, there seems to be a role for a combination treatment with other immune checkpoints. However, the expression of these checkpoints will not per se translate into clinical benefit. Further research is needed to explore.

Of note, investigating classical chemotherapy combined with anti-PD(L)1 and anti-CTLA-4 in mesothelioma could be interesting. As shown in Chapter 6, the combination of nivolumab plus ipilimumab in patients with mesothelioma is accompanied by frequently reported irAEs (30% grade ≥3). In contrast, the combination chemotherapy with anti-PD-L1 plus anti-CTLA-4 in NSCLC in the Poseidon study reports 10% irAEs grade ≥3.[56] Although the patient population differs in this study, in my opinion, it would be interesting to investigate the role of chemotherapy in preventing irAEs. On the other hand, a chemotherapy-free regimen could be preferable because of the vulnerable population of patients, particularly in case of mesothelioma. In our analysis of the evolution of characteristics of patients suffering from SCLC described in Chapter 5, we found an increased proportion of patients aged ³70 years over time and more patients that were treated with only best supportive care, suggesting that also in SCLC less toxic treatment regimens are desired. Whether a chemotherapy-free treatment for SCLC is realistic has to be found out.

The first step to a more personalized and maybe chemotherapy-free treatment is distinguishing which patient will derive benefit from which type of immunotherapy. Therefore, better biomarkers are required. In mesothelioma, the earlier mentioned four-gene signature could maybe play a role. Next, BAP1 loss in mesothelioma might be a predictive biomarker for response to ICI, as BAP1 loss is associated with a more inflamed tumor microenvironment.[69] Furthermore, activated dendritic cells seem more prominent in mesothelioma with BAP1 loss. Combining clinical with translational research is the way to achieve a more personalized approach to immunotherapy treatment.

Nonetheless, in view of toxicity and from an economic perspective, biomarkers are also warranted to prevent possible unnecessary complications and costs. Although hybrid-dosing strategies are already adopted to reduce medical costs while maintaining efficacy[70], in most patients suffering from mesothelioma or SCLC, ICI does not prolong OS. These patients are exposed to toxicity from ICI, for which hospitalization and expensive treatments could be necessary, leading to impaired quality of life and even more financial toxicity.



Overall, future clinical trials on immunotherapy in patients with mesothelioma and SCLC should focus on rational synergic combination treatments. These trials should be based on disease characteristics and combined with extensive exploratory analyses to find biomarkers for response to treatment, while treatment related toxicity, including financial toxicity, should be taken into account.

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# APPENDICES

# ENGLISH SUMMARY

Immunotherapy has proven to be a major breakthrough in the treatment of many cancers and has revolutionized the field of immuno-oncology.[1] In **Chapter 1**, we describe the cancer immune cycle which explains how our immune system fights cancer.[2] We also describe which mechanisms the tumor can use to evade the attack of the immune system, and in which ways we can counteract this escape.

Subsequently, we investigated some strategies that could potentially lead to the activation of the anti-tumor immune system. These are explained in the following chapters:

In **Chapter 2**, small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM) are extensively described regarding diagnosis and treatment and future perspectives. In both entities, only a small proportion of patients have durable benefit from immune checkpoint inhibitors (ICI), which is an immunotherapy treatment that is currently widely used as standard of care in the treatment of many types of cancer with sustained clinical responses. Apparently, SCLC and mesothelioma are tumors that are, at least, less sensitive to ICI than other tumors for reasons incompletely understood. Biomarkers able to select the patients who could derive benefit from ICI are currently lacking.

A comprehensive overview of immunotherapy in SCLC is given in **Chapter 3**. SCLC has a higher tumor mutational burden (TMB) than other tumors, leading to more neoantigens and, therefore, a higher chance for effective tumor recognition. In addition, SCLC is exceptionally sensitive to chemotherapy, which also leads to more neoantigens. However, a synergistic effect of combining chemotherapy with ICI is lacking. This may be caused by the chemotherapeutic agent that is currently used in SCLC, which might be less immunogenic. This is described in **Chapter 4**. The exact reasons for the lack of efficacy of ICI in SCLC are not fully understood. Several factors could play a role, which was discussed in **Chapter 3**, like the relatively low Programmed Cell Death Ligand 1 (PD-L1) expression, the low number of CD8+ tumor-infiltrating lymphocytes (TILs), and the relatively high presence of immune suppressive cells like regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).[3,4] Furthermore, the characteristics of the population suffering from SCLC have changed over time, which were investigated in **Chapter 5**. We found a decreased incidence of SCLC, while the absolute number of patients with SCLC remained similar over the years. We also found that patients were diagnosed with SCLC at an older age and that those patients are usually excluded from clinical trials. However, it seems like immunosenescence (the development of immune dysfunction as a result of aging) does not affect the efficacy of ICI and does,

therefore, not play a major role in immune escape.[5] We also found that more patients were diagnosed with metastatic disease, probably due to a changed staging system over time and better staging tools. The older age, in combination with more often metastatic disease, probably resulted in more vulnerable patients, which can explain why fewer patients received anti-cancer treatment over time. Although these evolving characteristics do not directly influence ICI efficacy, they might give insight into which patients need to be treated and thus can be used to implement into new clinical trial designs.

Contradictory to SCLC, MPM has a low TMB, which, in combination with the immunosuppressive tumor microenvironment in MPM, leads to low T cell infiltration into the tumor. These characteristics make MPM not as suitable for the use of ICI as other tumors.[6] However, although ICI monotherapy using anti-PD1 or anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) failed to show improvement over chemotherapy, the combination of both significantly improved overall survival (OS), which resulted in the approval and registration of this regimen.[7–10] Because the results of clinical trials may overestimated the effects in the real-world, we investigated this new combination treatment in a real-world setting, as described in **Chapter 6**. In our real-world population, we also included patients in further lines of therapy and patients with a higher performance score who were excluded in the pivotal phase 3 trial. We found that a worse performance score resulted in impaired progression free survival (PFS) and OS. Excluding these patients from the analyses resulted in comparable efficacy to the phase 3 trial. We also found a high but comparable frequency of immune-related adverse events (irAEs); 46.7% of the patients experienced grade 2-4 irAEs requiring additional treatment, and 25% discontinued treatment because of irAEs. Elderly patients had the highest risk of irAEs. Performance score was not shown to be correlated to irAEs.

Although the combination ICI therapy in MPM showed improved OS, most patients do not have any or durable response. As mentioned, a limitation for ICI response in MPM seems to be the low TMB in combination with the immunosuppressive tumor microenvironment, leading to the inability to activate T-cells. This can be overcome by dendritic cell (DC) therapy, which was investigated in **Chapter 7**. DCs were cultured and trained to recognize tumor antigens outside the patient. Subsequently, the DCs were administered to the patient, which led to neo-antigen presentation to the T-cells, resulting in T-cell activation. In this way, we found a promising signal with a 2-year OS of >50% and a 5-year OS of >20%, which has led to the phase 3 DENIM trial. Although this DC-vaccination strategy led to a T-cell response, it did not improve OS.[11] This could potentially be explained by PD-L1 upregulation as negative feedback after T-cell activation. By adding anti-PD-1 after DC vaccination, this signal can be neutralized,

which was investigated in **Chapter 8** and supported the hypothesis that administrating ICI with DC vaccination could reinvigorate the T-cell response. In addition, we found that PD-L1 was not only upregulated in activated DCs ex-vivo but similar upregulation was found in tumor-draining lymph nodes (TDLNs) of tumor-bearing mice. TDLNs seem to have a crucial role in a durable anti-tumor immune response, as immune activation primarily takes place there.[12] Blockade of the PD-1/PD-L1 interaction specifically and only in the TDLN using ICI enhances anti-tumor T-cell immunity and thus improves tumor control.

In **Chapter 9**, we investigated the role of lurbinectedin in SCLC and MPM. Lurbinectedin aims to influence the tumor microenvironment, which could be of added value in patients with MPM and SCLC. Although we found disappointing clinical results regarding PFS and OS in particular MPM using lurbinectedin as monotherapy, we found a shift in monocytes in favor of intermediate monocytes, which can contribute to better antigen recognition. Furthermore, we found increased expression of inhibitory markers such as CTLA-4 and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), which provides a rationale for combining lurbinectedin with ICI.

In order to promote anti-tumor immunity by using ICI, a risk of auto-immunity is present, which can result in side effects depending on the affected organ system. This was already shown in **Chapter 6**. Because chemotherapy is frequently combined with ICI, and both treatments can induce renal impairment, we designed a tool for clinicians to distinguishing the cause of renal failure. This was described in **Chapter 10**. Renal injury due to chemotherapy is often ascribed to acute tubular injury and necrosis (ATN), while the main mechanism of injury due to ICI therapy is acute tubulointerstitial nephritis (ATIN). By using clinical, laboratory, urine, radiographical and pathological parameters, the underlying cause of the kidney injury can be discriminated.

Finally, in **Chapter 11**, we discuss the findings of our investigations and how we can use this in future research to contribute to further improvement of immunotherapy treatment.

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# NEDERLANDSE SAMENVATTING

Met de komst van immunotherapie is er een grote doorbraak opgetreden in de behandeling van vele kankertypes en hiermee is een revolutie onstaan op het gebied van immuno-oncologie.[1] In **Hoofdstuk 1** beschrijven we de kankerimmuniteitscyclus die uitlegt hoe ons immuunsysteem kanker bestrijdt.[2] Ook beschrijven we welke mechanismen de tumor kan gebruiken om de aanval van het immuunsysteem te ontwijken, en op welke manieren we deze ontsnapping kunnen tegengaan.

Vervolgens hebben we enkele strategieën onderzocht die mogelijk kunnen leiden tot de activering van het anti-tumor immuunsysteem. Deze worden in de volgende hoofdstukken toegelicht:

In **Hoofdstuk 2** worden diagnosestelling, behandeling en toekomstperspectieven van het kleincellig longkanker (SCLC) en maligne pleuraal mesothelioom (MPM) uitgebreid beschreven. In beide entiteiten heeft slechts een klein deel van de patiënten langdurig baat bij immuun checkpoint remmers (ICI): een immunotherapiebehandeling die momenteel op grote schaal wordt toegepast als standaardbehandeling bij de behandeling van vele soorten kanker met aanhoudende respons als resultaat. Blijkbaar zijn SCLC en mesothelioom tumoren die op zijn minst minder gevoelig zijn voor behandeling middels ICI dan andere tumoren om redenen die we nog niet volledig begrijpen. Biomarkers die in staat zijn de patiënten te selecteren die baat zouden kunnen hebben bij ICI behandeling ontbreken momenteel.

Een uitgebreid overzicht van immunotherapie bij SCLC wordt gegeven in **Hoofdstuk 3**. SCLC heeft een hogere tumormutatielast (TMB) dan andere tumoren, wat leidt tot meer neoantigenen en daardoor een grotere kans op effectieve tumorherkenning. Bovendien is SCLC uitzonderlijk gevoelig voor chemotherapie, wat ook leidt tot meer neoantigenen. Een synergetisch effect van het combineren van chemotherapie met ICI ontbreekt echter. Dit kan worden veroorzaakt door het soort chemotherapie dat momenteel wordt gebruikt bij SCLC, welke mogelijk minder immunogeen is. Dit wordt beschreven in **Hoofdstuk 4**. De exacte redenen voor het gebrek aan werkzaamheid van ICI bij SCLC zijn niet volledig bekend. Verschillende factoren kunnen een rol spelen, zoals besproken in **Hoofdstuk 3**, zoals de relatief lage Programmed Cell Death Ligand 1 (PD-L1) expressie, het lage aantal CD8+ tumor-infiltrerende lymfocyten (TIL's), en de relatief hoge aanwezigheid van onderdrukkende immuuncellen zoals regulerende T-cellen (Tregs) en "myeloid-derived suppressor cellen" (MDSC's).[3,4] Bovendien zijn de kenmerken van de populatie die lijdt aan SCLC in de loop van de tijd veranderd, hetgeen werd onderzocht in **Hoofdstuk 5**. We vonden een verminderde incidentie van

SCLC, terwijl het absolute aantal patiënten met SCLC door de jaren heen gelijk bleef. We ontdekten ook dat SCLC bij patiënten op oudere leeftijd werd gediagnosticeerd en dat deze patiënten meestal worden uitgesloten van klinische onderzoeken. Het lijkt er echter op dat immunosenescentie (de ontwikkeling van immuun dysfunctie als gevolg van veroudering) geen invloed heeft op de werkzaamheid van ICI en daarom geen grote rol speelt bij het ontsnappen aan het immuunsysteem.[5] We ontdekten ook dat bij meer patiënten gemetastaseerde ziekte werd gediagnosticeerd, waarschijnlijk als gevolg van een veranderd stadiëringssysteem in de loop van de tijd en betere stadiëringsmiddelen. De hogere leeftijd, in combinatie met vaker uitgezaaide ziekte, resulteerde waarschijnlijk in kwetsbaardere patiënten, wat kan verklaren dat in de loop van de tijd minder patiënten een antikankerbehandeling kregen. Hoewel deze zich ontwikkelende kenmerken van de populatie SCLC niet direct van invloed zijn op de werkzaamheid van ICI, kunnen ze inzicht geven voor welke patiënten een behandeling gewenst zou zijn en kunnen ze dus worden gebruikt in de implementatie van nieuwe onderzoeksvoorstellen.

In tegenstelling tot SCLC heeft MPM een lage TMB, wat, in combinatie met de immunosuppressieve tumormicro-omgeving in MPM, leidt tot lage T-celinfiltratie in de tumor. Deze kenmerken maken MPM niet zo geschikt voor het gebruik van ICI als andere tumoren.[6] Hoewel ICI-monotherapie met anti-PD1 of anti-cytotoxisch T-lymfocyt-antigeen-4 (CTLA-4) geen verbetering liet zien ten opzichte van chemotherapie, verbeterde de algehele overleving (OS) met combinatie van beiden aanzienlijk, wat resulteerde in de goedkeuring en registratie van dit behandelregime.[7–10] Omdat de resultaten van klinische onderzoeken vaak een overschatting zijn van datgene wat gezien wordt in de dagelijkse praktijk, hebben we deze nieuwe combinatiebehandeling in de praktijk onderzocht, zoals beschreven in **Hoofdstuk 6**. In onze dagelijkse populatie hebben we ook te maken met patiënten die reeds een andere behandeling hadden gehad, of patienten die minder fit waren. Deze populatie was uitgesloten in de fase 3 studie, maar werd in onze analyse wel meegenomen. We ontdekten dat een minder goede conditie resulteerde in een verminderde progressievrije overleving (PFS) en OS. Het uitsluiten van deze minder fitte en/of voorbehandelde patiënten in onze analyses resulteerde in een vergelijkbare werkzaamheid als de fase 3-studie. We vonden ook een hoge maar vergelijkbare frequentie van immuungerelateerde bijwerkingen (irAE's); 46,7% van de patiënten kreeg graad 2-4 irAE's waarvoor aanvullende behandeling nodig was, en 25% stopte vroegtijdig met de behandeling vanwege irAE's. Oudere patiënten hadden het hoogste risico op irAE's. Conditie bleek niet gecorreleerd te zijn met het optreden van irAE's.

Hoewel de combinatie ICI-behandeling bij MPM een verbeterde in OS aantoonde, hebben de meeste patiënten geen of geen duurzame respons. Zoals vermeld, lijken bij MPM de lage TMB in combinatie met de immunosuppressieve micro-omgeving van de tumor een beperking te geven voor ICI-respons, wat leidt tot het onvermogen om T-cellen te activeren. Dit kan worden ondervangen door het toepassen van dendritische celtherapie (DC), wat werd onderzocht in **Hoofdstuk 7**. DC's werden buiten het lichaam gekweekt en getraind om tumorantigenen te herkennen. Vervolgens werden de DC's aan de patiënt toegediend, wat leidde tot presentatie van neo-antigeen aan de T-cellen, resulterend in activatie van T-cellen. Op deze manier vonden we een veelbelovend signaal met een 2-jaars OS van >50% en een 5-jaars OS van >20%, wat heeft geleid tot de fase 3 DENIM trial. Hoewel deze DC-vaccinatiestrategie leidde tot een T-celrespons, verbeterde het de OS niet.[11] Dit zou mogelijk kunnen worden verklaard door PD-L1-upregulatie als negatieve feedback na T-cel activatie. Door na DC-vaccinatie anti-PD-1 therapie toe te voegen, kan dit signaal worden geneutraliseerd. Dit werd onderzocht in **Hoofdstuk 8** en ondersteunde de hypothese dat het toedienen van ICI met DC-vaccinatie de T-celrespons nieuw leven zou kunnen inblazen. Bovendien ontdekten we dat PD-L1 niet alleen buiten het lichaam werd opgereguleerd in geactiveerde DC's, maar dat vergelijkbare opregulatie werd gevonden in lymfeklieren nabij de tumor (TDLN's) van tumordragende muizen. TDLN's lijken een cruciale rol te spelen in een duurzame anti-tumor immuunrespons, aangezien de immuunactivatie voornamelijk daar plaatsvindt.[12] Blokkade van de PD-1/PD-L1-interactie specifiek in de TDLN met behulp van ICI verbetert de antitumor T-celimmuniteit en verbetert zo de tumorcontrole.

In **Hoofdstuk 9** hebben we de rol van lurbinectedin in SCLC en MPM onderzocht. Lurbinectedin heeft tot doel de micro-omgeving van de tumor te beïnvloeden, wat van toegevoegde waarde kan zijn bij patiënten met MPM en SCLC. Hoewel we teleurstellende klinische resultaten vonden in OS en PFS met lurbinectedin als monotherapie bij voornamelijk MPM, vonden we een verschuiving in monocyten ten gunste van intermediaire monocyten, wat kan bijdragen aan een betere antigeenherkenning. Verder vonden we een verhoogde expressie van remmende markers zoals CTLA-4 en T-celimmunoglobuline en mucinedomein-bevattend eiwit 3 (TIM-3), wat een grondgedachte vormt voor het combineren van lurbinectedin ICI.

Terwijl middels ICI wordt gepoogd de anti-tumor immuniteit te stimuleren, bestaat hierbij het risico op auto-immuniteit, wat kan leiden tot bijwerkingen, afhankelijk van het aangetaste orgaansysteem. Dit werd al aangetoond in **Hoofdstuk 6**. Omdat chemotherapie vaak wordt gecombineerd met ICI, en beide behandelingen nierinsufficiëntie kunnen veroorzaken, hebben we een stroomdiagram ontworpen voor clinici waarmee de oorzaak van nierfalen kan worden onderscheiden. Dit werd

beschreven in **Hoofdstuk 10**. Nierbeschadiging als gevolg van chemotherapie wordt vaak toegeschreven aan acute tubulus necrose (ATN), terwijl het belangrijkste mechanisme van nierschade als gevolg van ICI-therapie acute tubulo-interstitiële nefritis (ATIN) is. Door klinische, laboratorium-, urine-, radiografische en pathologische parameters te gebruiken, kan de onderliggende oorzaak van de nierbeschadiging worden onderscheiden.

Ten slotte bespreken we in **Hoofdstuk 11** de bevindingen van onze onderzoeken en analyses en hoe dit in toekomstig onderzoek kan worden gebruikt om bij te dragen aan verdere verbetering van immunotherapiebehandeling.

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# PHD PORTFOLIO

**Summary of PhD Training and Teaching**

**Name PhD student:** D.W. Dumoulin
**Erasmus MC Department:** Pulmonary Medicine
**PhD Period:** 2020-2023
**Promotor:** prof. dr. J.G.J.V. Aerts
**Promotor:** prof. dr. A.C. Dingemans

**General Courses**

2017 Erasmus MC: Basis Course for Clinical Investigators
2020 Erasmus MC: Good Clinical Practice
2022 Erasmus MC: Good Clinical Practice
2022 Erasmus MC: Basis Course for Clinical Investigators
2022 Erasmus MC: The advanced immunology Course

**(International) Scientific Presentations**

2015 WCLC Denver: Large variation between hospitals in the percentage of patients with stage IIIB / IV lung cancer who receive chemotherapy and strong correlation of being treated with chemotherapy and survival (poster presentation)
Lung cancer award.

2019 ELCC Geneve: Renal toxicity from platinum/pemetrexed and pembrolizumab in the era of combination therapy (poster presentation)

2019 ELCC Geneve: Paclitaxel/ carboplatin/ bevacizumab in non-small cell lung cancer patients induces peripheral effector CD8 T cell proliferation that could be prone for treatment with checkpoint inhibitors (poster presentation)

2019 WCLC Barcelona: Real-world data of nivolumab in chemotherapy pre-treated Mesothelioma patients (mini-oral)

2021 ASCO Chicago: Lurbinectedin in pre-treated patients with SCLC and mesothelioma (mini-oral)

2021 ASCO Chicago: Long-term follow up of patients with mesothelioma treated with dendritic cell therapy in three phase I-II trials (poster presentation)

2023 IMIG Lille: Nivolumab and ipilimumab in the real-world setting in patients with mesothelioma (poster presentation)

**Teaching**

2018 Immunotherapy and toxicity. Albert Schweitzer hospital Dordrecht
2018 Update on immunotherapy. Maasstad hospital Rotterdam
2018 Regional seminar on thoracic oncology: Combination therapies in NSCLC
2018 Comprehensive cancer network, oncologic policy
2019 NVALT oncology course for Dutch pulmonologists in training
2019 National web-symposium on immuno-oncology
2019 Sharing Practice Utrecht: SCLC and mesothelioma
2019 Academic center of excellence thoracic oncology collaboration
2020 Amsterdam Lung Cancer Course
2020 NVPA Advanced course PD-L1
2020 Teaching medical doctors/residents
2021 Medtalks update immunotherapy
2021 Medtalks Lungcancer update
2021 Telereview ASCO
2021 Telereview ESMO/WCLC
2021 Teaching medical doctors/residents
2022 Virtual tumor board
2022 Telereview ESMO/WCLC
2022 Erasmus MC MolMed Immunotherapy
2022 Medtalks update immunotherapy
2022 IASLC Global Lung Cancer Academy
2022 Teaching medical doctors/residents
2023 Bronkhorst Colloquium Brugge
2023 Lung cancer Preceptorship
2023 Webinar dual immunotherapy in NSCLC
2023 Telereview ESMO/WCLC

# LIST OF PUBLICATIONS


1. Dumoulin DW, Aerts JG, Cornelissen R. Is immunotherapy a viable option in treating mesothelioma? Future Oncol Lond Engl. augustus 2017;13(20):1747-50.

2. Dumoulin D, Goeje P de, Poncin M, Bezemer K, Groen HJM, Smit EF, e.a. Paclitaxel/ carboplatin/ bevacizumab in non-small cell lung cancer patients induces peripheral effector CD8 T cell proliferation that could be prone for treatment with checkpoint inhibitors. Ann Oncol. 1 april 2019;30:ii7.

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# ABOUT THE AUTHOR

Daphne Dumoulin was born February 25th, 1985 in Son & Breugel, The Netherlands. She attended primary school at the *Vijverberg* and *Sonnewijzer*, followed by gymnasium at the *Lorentz Casimir Lyceum*. *After graduation in 2003*, she started medical studies at the University of Maastricht. After obtaining her medical degree in 2009, she began as a resident at the Department of Pulmonary Medicine at the Catharina Hospital in Eindhoven under the supervision of Prof. Dr. F. Smeenk. She completed the last year of her residency in 2017 in the field of thoracic oncology at the Erasmus Medical Center in Rotterdam under the supervision of Prof. Dr. H.C. Hoogsteden and Prof. Dr. J.G.J.V. Aerts. She continued her work as a pulmonologist in the field of thoracic oncology at the Erasmus Medical Center and became a permanent staff member in 2018. During her work, Daphne was involved in several preclinical and clinical research projects. Under the supervision of Prof. Dr. J.G.J.V. Aerts, she is leading the Comprehensive Cancer Network of the southwest of the Netherlands, comprising of 19 hospitals with close collaboration in thoracic cancer care. With this collaboration, cancer care could be improved, as well as the inclusion in clinical trials. Daphne developed a special interest in immunotherapy, which, through close collaborations, resulted in new research projects, multiple presentations, publications and subsequently this thesis.

Daphne lives in Barendrecht with Niels and their two daughters, Isabel and Emilie.

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