Immunotherapy in lung cancer and mesothelioma:

A Renaissance

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Immunotherapy in Lung cancer and Mesothelioma: A Renaissance

Immunotherapie bij longkanker en mesothelioom: Een renaissance

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CHAPTER 1

General Introduction

In the last couple of decades, the world of clinical oncology has witnessed remarkable changes in the way cancer has been studied and treated. Starting from a period in which cancer was seen as the sum of genetic modifications and limitless replicative power in somatic cells, which brought to the identification of driver oncogenes or tumor-suppressor genes as key factors to guide the development of targeted chemotherapies, researchers have now learned to finally appreciate the crucial role that the immune system plays in controlling cancer growth.

In reality, immunology and oncology have a longer relationship, and interconnections between the two fields have been studied over the past century. The fact that thymectomized mice were more susceptible to developing cancer after exposure to carcinogens (e.g., 3,4-benzopyrene)[1] and polyoma virus[2,3] and the observed increased rate of tumors in immunosuppressed humans (e.g., following therapy for transplantation)[4], led to the theory of immunesurveillance that was initially conceived by Ehrlich[5] and supported by Burnet[6].

The question on how tumors trigger tumor immunity was addressed in the cancer immunoediting hypothesis[7]. According to this theory, in a first phase the immune system locates and eliminates (pre-) cancerous cells, yet if some cell variants escape this control, an equilibrium phase is reached with cells of the adaptive immune system restraining the growth of tumor cells. In a later phase, immune selective pressure on unstable tumor cells can lead to the onset of cell variants that escape immune control and ultimately become clinically apparent.

This phenomenon has been observed to occur not only in pre-clinical models but also in humans. This has been proven by multiple observations, spanning from the correlation between tumor infiltrating lymphocytes (TILs) and better prognosis[8] to the increased $CD8^+$ T cell frequency observed in regressing in-situ carcinomas[9].

Tumors display multiple mechanisms to evading the immune response, by downregulating antigen expression on their surface and in the context of MHC molecules, secreting immunosuppressive cytokines, or recruiting immune suppressive cell types[10].

Even when tumors are successfully invaded by anti-tumor T cells, shaping an immunogenic phenotype known as the 'inflamed' phenotype, some elusive mechanisms can come into play to impair activation of T cells and spontaneous tumor killing[11]. An effective T cell activation via the T cell receptor (TCR) normally requires not only the signal derived by antigen recognition but also a costimulatory signal. This response is also controlled by inhibitory checkpoint molecules on antigen-experienced effector T cells, such as CTLA-4 (CD152) and PD-1 (CD279), which by interacting with B7 proteins on antigen-presenting cells (APCs) or non-immune cells

(such as CD80, CD86, PD-L1, and PD-L2), prevent unrestrained T-cell activation[12]. Chronic antigen exposure, which occurs in viral infections but also in cancer, induce exhaustion of activated T cells and upregulation of the pathways regulated by CTLA-4 and PD-1 which in turn downregulates T cell activities[13].

These discoveries led to the pharmacological development of immune checkpoint inhibitors (ICIs), antagonistic antibodies directed towards known targets of exhaustion including PD-1 and CTLA-4 that enable T cells to proliferate and respond more vigorously against the tumor[14,15]. Immunotherapies that were developed in the past decades were primarily focused on broadly amplifying immune activation mechanisms thus resulting in rare objective responses and frequent toxicity. Contrarily, the use of these blocking antibodies aiming at selectively restoring the tumor-induced immune deficiencies has represented a breakthrough in the history of modern oncology, initiating a Renaissance for cancer immunotherapies.

Nowadays, ICIs represent the standard of care across a wide range of solid tumor types, and their use has resulted in unprecedented clinical responses in difficult-to-treat cancer histologies[16].

This introductory chapter will review recent advances in tumor immunology and immunotherapy, with a special focus on lung cancer and mesothelioma. An outline of the thesis, with the different research questions addressed in every chapter, will be also presented.

Rationale for immunotherapy in lung cancer and mesothelioma

Both lung cancer and mesothelioma include different biological entities, whose multi-faced genomic and phenotypic features cannot be fully encompassed by traditional histological classification. Immunotherapy has been one of the greatest advances in clinical research for these neoplasms, with ICIs reaching approval by health authorities and being authorized as standard treatment[17–19]. Albeit the use of this type of immunotherapy has been recently expanded beyond non-small cell lung cancer (NSCLC) to include treatment of small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM), every cancer entity deserves separate chapters to fully characterize the rationale upon this treatment and to further fuel its potential of action. As data in NSCLC are more mature than in SCLC and MPM, the following overview will focus on the differences among the three tumor types.

Non-small cell lung cancer

After decades of repeated failures, the treatment scenario in NSCLC has seen remarkable changes, making this cancer entity as one of the most interesting paradigms for the development of new-generation therapies. Starting from its approval in the recurrent metastatic setting in 2015, we have witnessed a Renaissance

of immunotherapy for NSCLC with ICIs (especially those targeting the PD-1/PD-L1 axis) making their way till the multimodal treatment of locoregional disease[20].

Despite the success of this strategy, which has led to a remarkable survival gain for some NSCLC patients, primary and acquired resistance occur in most of the cases[21], highlighting the need for further optimizing and fueling research in this setting. In addition, tumor responses often come at the price of new forms of toxicities, which should be properly managed to improve treatment benefit. Therefore, understanding the biological bases which prompted the investigation of immunotherapy in NSCLC is a crucial step to identify predictive biomarkers of response and toxicity.

By directly looking at immune cell population at tumor site or more indirectly measuring PD-L1 expression, interferon gamma-related signatures, NSCLC is considered an immunogenic tumor[22–25]. A crucial step for the induction of an antitumor immune responses is the recognition of cancer cells as foreign and NSCLC is largely caused by chronic exposure to mutagens such as carcinogens in cigarette smoke[26]. As such, it exhibits one of the highest prevalence of somatic mutations across cancer types[27]. This burden of nonsynonymous mutations usually observed in NSCLC[28], often translates in an elevated neoantigen expression, playing a central role in antitumor immunity. However, there is large variability in tumor mutational burden (TMB) within different NSCLC types, with tumors in never-smokers generally showing less somatic mutation compared with smoking counterparts[29]. This makes the pair with a marked heterogeneity in PD-L1 expression on tumor cells[30]. In fact, while PD-L1 expression has been reported to be greater than or equal to 1% of tumor cells in approximately 60% of advanced NSCLC, only 25% to 30% of cases have documented high levels (>50% of tumor cells) of expression. Moreover, while TMB levels have been associated with immune cell infiltration and an inflammatory T-cell–mediated response[31], only a weak association between TMB and PD-L1 expression has been found in NSCLC[32–34]. At first, this inter-tumor variation was thought to be related to the use of multiple PD-L1 immunohistochemistry (IHC) assays/platforms, yet recently many efforts have been done to harmonize their clinical use[30]. Nevertheless, PD-L1 expression has been observed to vary also intra-tumor, across different anatomical sites and to change during disease course[35]. This reveals the complexity of dissecting the mechanisms of antitumor immune response in NSCLC.

Compared to normal tissue, NSCLC tumors contain higher $CD3^+$ lymphocytes, $CD8^+$ cytotoxic cells, $CD8^+/CD45R0^+$ effector memory cells[36–40], and elevated levels of these T-cell subsets are correlated with better prognosis[41,42]. $CD4^+$ helper T cells, regulatory T cells (Tregs), $CD19^+/CD20^+$ B-lymphocytes (in a smaller percentage compared to T cells, often grouped in tertiary lymphoid structures) are also present in the tumor microenvironment (TME) of NSCLC tumors[36,38]. Moreover, T cells infiltrating the tumor often express immune inhibitory receptors, such as PD-1, LAG-3, and TIM-3,

supporting the presence of an ongoing immunoediting process[43]. Contrary, innate immune cells (encompassing macrophages, dendritic cells (DCs) and NK cells) are present at a lower level in NSCLC compared to normal lung tissues[37,40], yet their association with outcome is not fully elucidated[44,45]. These observations made by immunohistochemical staining are somehow mirrored by RNA sequencing data, showing that NSCLC tumors have a high calculated leukocyte fraction and often co-express CD8A and PD-L1 transcripts[46,47]. Taken globally, these data render NSCLC an immunologically active or "T-cell inflamed" tumor.

Differences in terms of immune composition and milieu have also been reported among histology variants [22,23,36,39,48,49] and among NSCLC harboring distinct mutational landscape[50] and should be taken into account to fully unleash the potential of immunotherapy in this malignancy. It is now well established that either the activation of driver oncogenes or the occurrence of genomic alterations in key tumor suppressor genes can promote the protumor phenotype of the TME[51–54]. For example, genomic alterations in STK11/LKB1 (present in approximately 18% of NSCLC adenocarcinomas (ADCs)) have been associated with recruitment of immunosuppressive myeloid cells (predominantly neutrophils) in mice models, T cell exclusion (low TILs) and reduced expression of PD-L1 on tumor cells of NSCLC patients, regardless of TMB levels[55]. Similarly, somatic mutations in KEAP1, PIK3CA and PTEN are associated with immunologically cold tumors, reduced recruitment and function of NK cells, and lower levels of PD-L1 expression[56–59]. On the contrary, inactivation of TP53 is associated with higher TMB and increased $CD8^+$ T-cell infiltrate in ADC[60,61]. As a result of the signaling pathway activation, most of the oncogene-addicted forms of NSCLC (main data concern EGFR-mutated and ALK-translocated NSCLC) are generally associated with high PD-L1 expression levels[62,63] yet still with impaired tumor immunogenicity[64,65] as witnessed by low TMB and reduced T-cell infiltration. Since most of the data on oncogenic pathways and co-mutations derive from retrospective studies, a better understanding of the exact mechanisms through which genotype influences immunophenotype in NSCLC is crucial to identify genomic correlates of response and resistance to immunotherapy.

So far, most of the developed therapeutic strategies that target NSCLC have been directed at the negative immune checkpoints signaling[66]. However, as learned from the benchmark study of Chen et al.[67], many steps of the cancer-immunity cycle need to be accomplished for the immune system to effectively kill cancer cells. These steps are controlled by a series of positive and negative regulators that can be impaired at different levels yet can be potentially regulated by new immunotherapeutic agents. In addition, host–tumor interactions at a systemic level have demonstrated to be associated with tumor development/progression and response to immunotherapy in NSCLC. Many host factors such as gender, body mass index (BMI), smoking habits, concomitant medications, and gut microbiota have shown correlation with

outcome under ICIs[68]. For this reason, research is currently ongoing dissecting the characteristics and dynamic interactions within the interface host–tumor. It still needs to be clarified whether targeting these systemic host-associated factors in NSCLC by changing lifestyle or restoring normal blood levels of proteins, metabolites, and electrolytes will ultimately improve the fitness of immune system and boost cancer immunotherapy.

Small cell lung cancer

SCLC accounts for about 15% of all new lung cancers. The carcinogenesis process is strongly associated with smoking, with specific molecular signatures (such as C:G > A:T transversions) found one-third of patients[69]. This pathogenesis related to smoking exposure might explain why SCLC is placed among the tumor types with the highest mutational loads[27,70]. This feature can lead to a release of tumor neoantigens capable to stimulate an anti-tumoral immune response. However, for many years, research has failed to identify new treatments in this neoplasm, including immunotherapy. In particular, chemotherapy-free ICI trials in SCLC have not generated solid evidence[71–73].

Interestingly, PD-L1 expression levels in SCLC are generally lower than NSCLC, with 17% to 31% of cases expressing PD-L1 above 1% and only a minority expressing very high levels (above 50%)[74,75]. The immunohistochemical classification performed on SCLC also revealed a lower TILs percentage, with 264.6 $CD8^+$ cells/mm2 in the peritumoral compartment of SCLC, compared to 1040.8 $CD8^+$ cells/mm2 reported in lung ADCs and 1365.6 $CD8^+$ cells/mm2 in squamous cell carcinomas (SCCs), confirming a less cytotoxic T-cell profile[76].

Due to the limited adaptive immune response, the relationship between TILs and outcome in SCLC has not been clarified, yet lower ratio between suppressive cells and $CD3^+$ cells has been demonstrated in tumors of long-term survivors[77]. Down-regulation of MHC antigens, immunosuppression induced by SCLC tumor cells, autocrine and paracrine regulation might also explain the negative influence on the antitumor immune response[78,79]. For this reason, finding the perfect companion for ICIs (moving beyond the standard platinum-based chemotherapy) is crucial in SCLC. The best would be to find an agent (either chemotherapy or other agents) able to enhance immune infiltration/activation and/or modulate the immunosuppressive TME, thereby promoting the effect of ICIs on effector T cells and leading to a synergistic more than just an additive effect.

Mesothelioma

MPM is an aggressive malignancy arising from mesothelial cells of the pleura whose pathogenesis is related to mineral fibers (such as asbestos and erionite) exposure. Following the success in lung cancer, immunotherapy have been actively investigated in the context of MPM[80].

The modest response rate observed with ICI monotherapy has been initially ascribed to the low TMB, unusual for a cancer derived by exposure to environmental carcinogens[81]. However, it is possible that currently available NGS approaches are not able to capture the full genomic complexity of MPM, being unable to identify recurrent inter- or intra-chromosomal structural rearrangements in patterns such as chromoplexy or chromothripsis[81]. In fact, these rearrangements can generate truncations or fusion transcript, which have neoantigenic potential similar to insertion and deletion.

Also looking at pathogenesis, MPM seems to be strictly linked to inflammatory and immune response (**Figure 1**). The interaction between asbestos fibers and immune cells initiates a process of chronic inflammation that last up to 30-40 years. However, when asbestos fibers are phagocytized by macrophages, this leads to the production of reactive oxygen species (ROS), inflammatory cytokines, growth factors and attracts a wide spectrum of immunosuppressive and stromal cells, which in turn enhance tumor progression and shape a TME which is predominantly immunosuppressive[82]. Continued exposure to asbestos has also a direct effect on both $CD8^+$ and $CD4^+$ T cells, dampening differentiation, cytokine production and impairing their anti-tumor activity[83,84]. A key role in shaping the immunosuppressive and anergic profile of the MPM TME is played by soluble factors, such as cytokines and chemokines. For example, C-C motif chemokine ligand 2 (CCL2) inflammatory chemokine and macrophage colony-stimulating factor (M-CSF) promote monocyte recruitment and skew them toward a pro-tumorigenic phenotype (M2 macrophages)[85–87]. Consequently, tumor associated macrophages (TAMs) become prominent in the TME, accounting for about 25-40% of total immune infiltrates and boosting the immunosuppressive phenotype of TME. Through secretory molecules, they lead to higher expression of cancer stem cell markers and promote infiltration of Tregs, rendering mesothelioma cells more treatment resistant[88–90]. According to a study by Cornelissen et al.[91], the $CD163^+/CD68^+$ ratio ($CD163^+$ representing M2 phenotype and $CD68^+$ positive cells representing all macrophages phenotypes) in epithelioid MPM tumor tissue would correlate with overall survival (OS) better than the total number of macrophages. Skewing macrophages phenotype rather than just depleting them could then be more efficient in eliciting the anticancer response[92,93]. Myeloid-derived suppressor cells (MDSCs), which consist in both polymorphonuclear (PMN-MDSC) and monocytic (M-MDSC), also detain a central role in shaping the MPM TME[94]. They produce ROS and secrete growth factors which in turn activate epithelial–mesenchymal transition (EMT) of cancer cells, angiogenesis[95,96], and suppress innate and adaptive immunity[97], promoting the state of anergy. At the same time, DCs inside MPM TME are highly dysfunctional, causing impaired antigen presentation and affecting cytotoxic T-cell action. When exposed to mesothelioma cells, immature human monocyte-derived DCs (MoDCs) accumulate a higher lipid content and this translates into a reduced antigen processing ability and production of the tolerogenic cytokines, such as IL-10[98].

As dendritic cell function is hampered in MPM, their generation ex vivo and usage as cancer vaccines is currently tested in patients. In pre-clinical models, a better outcome was achieved when DCs were injected early in tumor development[99,100]. A high tumor load is correlated with an increase of tumor-induced immunosuppression. Giving DC vaccination also after surgically reducing tumor load could therefore possibly improve clinical outcome and response to therapy.

MPM TME is also composed by a higher percentage of exhausted T cells, as demonstrated by many studies looking at PD-1, CTLA-4, T-cell immunoglobulin and mucin domain 3 (TIM-3), and T cell immunoreceptor with Ig and ITIM domains protein (TIGIT) expression on $CD8^+$ T cells[101]. However, the prognostic role of TILs quantitative infiltration and phenotype is more controversial than in other cancer types. While first observations suggested that $CD8^+$ TILs represented a significantly better prognostic factor for surgically resectable patients[102], new studies with larger sample size indicated that high TILs ($CD4^+$ and $CD8^+$ T cells) infiltration correlated with non-epithelioid histology (characterized by worse prognosis with standard or no treatment), higher PD-L1/PD-L2 expression and worse prognosis[103]. Accordingly, Pasello et al. showed that sarcomatoid and biphasic MPM samples were characterized by higher $CD8^+$ T cells, while epithelioid tumors had higher peritumoral $CD4^+$ T and $CD20^+$ B lymphocytes[104].

In conclusion, despite its anergic and immunosuppressive TME, MPM should not be confused for a "cold" cancer, but rather interpreted as a malignancy with "altered" TME, further categorizing it as "immunosuppressed" (with TILs infiltrating the tumors without carrying out any meaningful activity) or "excluded" (with TILs but not beyond the invasive margin)[105]. Along this line, two different gene expression studies have suggested to move further the traditional histological classification which entails three discrete entities: epithelioid, biphasic and sarcomatoid MPM. Alcala et al. have identified a continuum of expression profiles, with two bad-prognosis profiles at one extreme: a "hot" bad-prognosis profile, with high lymphocyte infiltration and high expression of immune check points and pro-angiogenic genes; a "cold" bad-prognosis profile, characterized by low lymphocyte infiltration[106]. At the other side of the spectrum, they identified another subgroup characterized by better prognosis, with high expression of VISTA (another immune checkpoint mainly expressed in epithelioid histotypes) and vascular endothelial growth factor receptor 2 (VEGFR2)[106]. Patil et al. have also recognized three MPM subgroups based on IHC and immune gene expression analysis: group 1 showing an immunologically ignorant or desert-like phenotype, group 2 having moderate expression of T-cell effector genes and high expression of B-cell genes, and group 3 being associated with higher PD-L1 expression levels and high expression of T effector cells[107]. This type of studies study paves the way for a rethinking of the concept of MPM as a low TMB tumor, underlining

the importance of dissecting its genetic but also pathophysiological vulnerabilities to deepen the role of immunotherapy in this neoplasm.

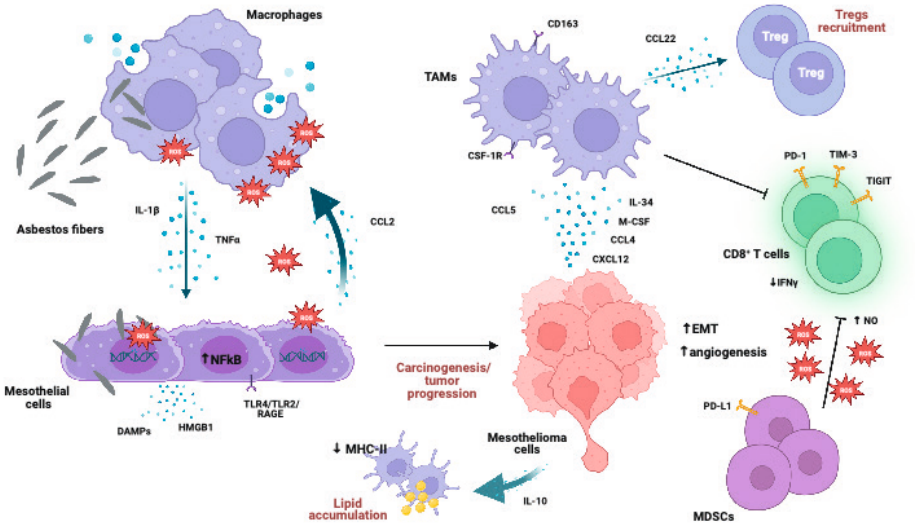

Figure 1. The role of immune cells in shaping the tumor microenvironment (TME) of mesothelioma. After penetrating the pleural space, asbestos fibers interact with both mesothelial cells and immune cells (macrophages), leading to tissue damage and local inflammation. Recruited macrophages are unable to digest these fibers, therefore they start producing reactive oxygen species (ROS) and proinflammatory mediators such as TNF-a and IL-1β, supporting carcinogenesis. In particular, IL-1β binds to IL-1R on mesothelial cells inducing cell survival and proliferation, while TNF-a promotes NF-kB pathway in mesothelial cells, supporting their survival to asbestos fibers. ROS also lead to DNA damage in mesothelial cells, inducing cell death and release of damage-associated molecular patterns (DAMPs) such as High Mobility Group Box 1 protein (HMGB1), which in turn binds to TLR2, TLR4 and Advanced Glycation End-products (RAGE), promoting carcinogenesis and tumor progression.

Once the tumor is established, tumor-associated macrophages (TAMs) play a key role in the TME. C–C chemokine ligand 4 (CCL4), C–C chemokine ligand 5 (CCL5) and C-X-C motif chemokine ligand 12 (CXCL12), are among the factors secreted by MPM cells that promote TAM recruitment. Similarly, IL-34 and macrophage colony-stimulating factor (M-CSF) boost TAM activation, by binding to colony stimulating factor-1 receptor (CSF- 1R) on TAM surface. TAMs induce an immunosuppressive TME, recruiting regulatory T cells (Tregs) via C-C motif chemokine ligand 22 (CCL22) and dampening the activity of $CD8^+$ T cells. The activity of $CD8^+$ T cells inside the TME is also inhibited by myeloid-derived suppressor cells (MDSCs) that, besides enhancing angiogenesis and epithelial–mesenchymal transition (EMT), produce ROS and nitric oxide (NO) that affect IFNγ synthesis by $CD8^+$ T cells. On the other hand, mesothelioma cells can suppress antigen-presentation activity of dendritic

cells (DCs), by releasing immunosuppressive cytokines, such as IL-10, which induce intracellular lipid accumulation and reduce major histocompatibility complex ii (MHC-II) expression on DC surface. Finally, in such an immunosuppressive milieu $CD8^+$ T cells, MDSCs and TAMs increase the expression on their surfaces of immune checkpoint molecules such as PD-1, PD-L1, TIM3, T-cell immunoreceptor with Ig and ITIM domains protein (TIGIT) that ultimately restrain the antitumor activity of the immune system. Created with https://biorender.com/

From pharmacokinetics to safety data

The complexity of the anticancer immune response provides enormous opportunities for different immuno-oncology agents. Among the different strategies being tested in the context of lung cancer and MPM, ICIs (especially the one targeting the PD-L1/PD-1 axis) have faced an unprecedented success, mainly attributable to their favorable response-to-toxicity profile. However, in some cases ICIs can lead to a broad and non-tumor specific activation of the immune system, which becomes supra supraphysiologic and translates in the onset of immune-related adverse events (irAEs)[108]. Since new agents entered the market, clinicians had to study the mechanism of action of immunotherapy and learn how to manage specific toxicities[109]. As documented by randomized trials and real-life reports in lung cancer[110–112], ICI monotherapy is generally well tolerated (few grade 3–5 irAEs), with only about 5% discontinuing treatment because of irAEs. A higher rate (up to 10%) of grade 3 to 5 toxicity has been observed with combinations of anti-PD1/PDL1 and anti-CTLA4, such as nivolumab–ipilimumab[113]. Unlike chemotherapy and targeted agents, irAEs may reach any organ at any time, although some preferential sites have been reported. Lung, colon, skin, and liver are the most affected organs, yet some more unusual irAEs, such as myocarditis or hypopituitarism are also observed. To note, ICI–chemotherapy combinations can lead to added toxicity in cancer patients, such as renal failure and interstitial nephritis[109]. Although most ICIs-related toxicities are self-limiting and readily manageable, a few may be severe and limit treatment, thus predicting the occurrence of ICIs-related toxicities can prevent interruption of continuum of care as well potential life-threatening consequences[114].

To this regard, studying pharmacokinetics and pharmacodynamics (PK/PD) may shed light on the mechanism of action of ICIs, yet researchers are now challenged by an increasing complexity of timing and dosing regimen. The first two anti-PD1 agents which entered the treatment landscape of solid cancers (NSCLC being one of the first indications), namely nivolumab and pembrolizumab, were initially registered with a recommended weight-based dosing (mg/kg) regimen. When evaluated in a dose-escalation study across multiple tumor types with doses ranging between 0.1 and 10 mg/kg every 2 weeks (Q2W), nivolumab showed similar receptor occupancy across all dose levels[115]. Neither a maximum tolerated dose (MTD) nor a relationship between dose and high-grade toxicity was identified, while a dose-response relationship

was observed, yet plateauing at levels greater than or equal to 3 mg/kg for NSCLC. This dose therefore became the recommended phase 2/3 dose across all tumors. Subsequent studies revealed a exposure–response (E-R) and a comparable safety profile between 240 mg Q2W to 3 mg/kg Q2W, leading to the approval of the flat-dosing regimen[116]. In fact, flat-dosing seems to represent a more suitable option for agents with a broad therapeutic index as it offers many practical advantages related to reduced risk of error in dose preparation and elimination of drug wastage[117].

Similar to nivolumab, no MTD was reached for pembrolizumab in phase I trials with adaptive dose escalation, where doses ranged from 0.005 to 10 mg/kg[118]. A quantitative E-R relationship and an association between dose exposure and AE rates were also not identified[119]. These assessments showed that the benefit-risk profile of pembrolizumab 200-mg every 6 weeks was comparable to 2 mg/kg every 3 weeks[119]. Most recently, the need of frequent patients accesses to oncology departments led to an increasing interest in alternative ICIs administration schedules able to offer longer dose intervals. Based on model-based approach and on patient data across multiple tumor types, extended-interval dosing (ED) of nivolumab (480 mg Q4W) and pembrolizumab (400 mg Q6W) was approved[120,121]. Nowadays, a wide percentage of patients in clinical practice have been shifted to (or treated upfront with) ED ICIs. However, incidence, clinical patterns, and survival implications for patients who develop irAEs across different dosing regimen need to be further elucidated.

Efficacy data: the evolution of immunotherapy in the clinical treatment of lung cancer and mesothelioma

At time of writing, three anti-PD1 monoclonal antibodies (mAbs; pembrolizumab, nivolumab, cemiplimab), two anti-PDL1 mAbs (atezolizumab and durvalumab) and one anti-CTLA4 mAb (ipilimumab) have been approved for immunotherapy treatment of NSCLC. The Renaissance of immunotherapy started from the recurrent metastatic setting, with nivolumab, pembrolizumab, and atezolizumab being approved by health authorities for second-line treatment based on survival and safety data from numerous clinical trials (CheckMate 078, CheckMate 017 and CheckMate 057, KEYNOTE 010, OAK)[110,111,122–124]. The full approval of pembrolizumab in this setting (after disease progression on or after platinum-containing chemotherapy or targeted therapy against ALK or EGFR) was initially conditioned to PD-L1 expression ≥1%, while nivolumab and atezolizumab could be used regardless of PD-L1 expression. Subsequentially, ICIs moved to the front-line setting for advanced NSCLC patients without druggable genomic alteration. Pembrolizumab in this case was the first-class agent to show a significant survival benefit over chemotherapy, as reported in the KEYNOTE 042[125] and in the KEYNOTE 024 trial[126]. Both studies were conducted in biomarker-selected patients and revealed an increased survival benefit in the subgroup of patients with high PD-L1 expression (≥50%). Similarly, the Impower 110 trial[127], which compared atezolizumab versus chemotherapy found a survival benefit

for patients in the PDL1-high categories according to the SP142 assay (PD-L1 staining on tumor cells (TCs) ≥50% or immune cells (Ics) ≥10%). Finally, cemiplimab has been recently approved in tumors with PD-L1 expression ≥50% based on data from the EMPOWER-lung1[128]. Altogether, data from these trials established ICIs as appropriate strategy for NSCLC with high PD-L1 expression.

For patients with PD-L1 expression ≥1%, an initial cross of the survival curves was observed at the beginning of the ICI treatment in these trials, suggesting that a relative proportion of them does not derive benefit from ICI monotherapy[125]. To this regard, the combination of nivolumab plus ipilimumab has been compared with chemotherapy in the CheckMate 227 trial, showing a survival benefit among patients with PD-L1 ≥1%, and leading to FDA approval of this strategy. Of note, the efficacy of this combination according to PD-L1 strata was not the primary endpoint of the trial[129].

For patients unselected by PD-L1 expression, the combination of first-line pembrolizumab plus chemotherapy (carboplatin plus pemetrexed) proved to significantly improve survival outcomes compared to chemotherapy alone in non-squamous NSCLC with no EGFR/ALK genetic alterations in the context of the KEYNOTE 189 trial[130]. The benefit was observed across PD-L1 cutoff points and regardless of metastatic sites. The same survival advantage was also observed for the squamous histology in the KEYNOTE 407 trial in which pembrolizumab was combined with carboplatin and paclitaxel or nab-paclitaxel[131]. A shorter course of platinum doublet chemotherapy was used in the CheckMate 9LA trial, where 2 cycles of platinum-based chemotherapy combined with nivolumab and ipilimumab proved to be superior to chemotherapy alone in recurrent or metastatic NSCLC[132]. The FDA also approved atezolizumab in combination with chemotherapy (with or without bevacizumab) based on results from the IMPower 150[133] and IMPower 130 trials[134]. As recently presented long-term data[135,136] continue to support chemo-immunotherapy as best front-line treatment for metastatic NSCLC, this option should be also considered for patients with PD-L1 expression above 50%, yet with high tumor disease burden or worrisome symptoms[17].

Based on an unprecedented success in metastatic NSCLC, ICIs is currently being introduced in the earlier setting, with durvalumab being approved as consolidation therapy in unresectable stage III NSCLC following chemoradiation therapy[137]. Very recently, atezolizumab has been approved as adjuvant option after surgery and chemotherapy for patients with stage II to IIIA NSCLC whose tumors express PD-L1 on ≥ 1% of TCs[138], while 3 cycles of neoadjuvant nivolumab combined with chemotherapy have significantly increased pCR rates compared to chemotherapy alone (24% vs 2.2%) leading to its approval prior to definitive surgery in resectable NSCLC[20].

Despite a relatively high TMB and some pathological elements suggesting immunogenicity, the addition of ICIs to the treatment of extensive disease (ED) SCLC has not retraced the magnitude of benefit observed in advanced NSCLC. Addition of atezolizumab[139] or durvalumab[140] to first-line chemotherapy has proved additional benefit in terms of activity and efficacy, without relevant toxicities issues, and has become standard of care for patients with ED-SCLC regardless of PD-L1 expression in most countries. Of note, median survival values were only moderately higher than the ones observed with chemotherapy, yet long-term estimations of these trials indicate that ICI benefit may extend over time in a minority of patients[19].

After a recent phase III trial had failed to show any survival difference between second-line pembrolizumab and chemotherapy[141], the combination of nivolumab and ipilimumab has recently revealed an overall survival (OS) benefit over chemotherapy also in the context of unresectable MPM. Notably, the survival gain shown in the phase III Checkmate743 randomized trial differs remarkably among histological subtypes, revealing a statistically significant superiority of this ICI combination in non-epithelioid MPM only, with an almost twofold increase in median OS[142]. Nevertheless, results of these trials, with 28% of responding patients still showing an ongoing response at a 3-year update[143], clearly demonstrate that combining immunotherapies might be a successful strategy to overcome resistance in MPM.

How to improve outcomes and counteract resistance to immunotherapy in lung cancer and mesothelioma

Despite the advent of ICIs revamped the enthusiasm over highly treatment-resistant diseases such as NSCLC, SCLC, and MPM many issues remain unsolved. Only a subset of tumor histologies and a small percentage of the patients in each histology are responsive to these inhibitors. In NSCLC, for example, up to 30% still present with early progression and/or hyperprogression and less than 20% derive a very long-term benefit from ICI immunotherapy[18]. The percentage of long-term survivors is even inferior in SCLC and MPM. The difference in response and the limited durability of benefit highlights some crucial needs that need to be met.

Inferring data from real-world analyses

Randomized controlled trials represent the gold standard for evaluating new treatment strategies. However, outcomes observed within clinical trials are not always replicated in the real-world, mainly because of the more unselected and heterogeneous patient cohort usually treated in clinical practice[144]. Similarly, the efficacy of new emerging treatments such as immunotherapy in lung cancer and MPM as determined by clinical trials has not always been replicated by its effectiveness in the real world.

In a study assessing real world outcome of patients with advanced NSCLC treated with first-line pembrolizumab, the efficacy-effectiveness gap was 0.45, indicating a steep decrease of 55% in median survival in patients treated in clinical practice compared to those enrolled in clinical trials[145]. Similarly, the overall survival benefit is lower in patients with poor performance status (ECOG PS ≥2), as shown by a systematic real-world review of 35,103 patients treated with second-line immunotherapy[146].

Real-world analyses also enable investigators to deepen the role of immunotherapy in special populations. Because of that, ICIs are currently offered to patients initially excluded from clinical trials, such as elderly[147], those with non–life-threatening and quiescent autoimmune diseases[148,149], those with controlled viral infection (HBV, HCV, or HIV)[150,151], and those receiving steroid treatment at baseline[152].

Due to multiple comorbidities, cancer patients in the real-life context often receive different non-cancer medications at time of starting cancer therapy. Concomitant baseline medications such as statins but also systemic antibiotics (ATB) and proton pump inhibitors (PPIs)[153], have been differently associated with radiological response and survival following ICIs. This fact was further supported by pre-clinical models, for example supporting a role of statins in strengthening antigen presentation to T cells and synergizing with PD-1 inhibitors. However, data on the impact of concomitant medications are not consistent across malignancies and have not been confirmed in lung cancer and mesothelioma. Moreover, whether this association needs to be found in the connection with well-known baseline prognostic factors (such as disease burden, performance status, and PD-L1 expression) or in a drug-related immune-modulatory effect, is still a matter of debate.

Improving biomarkers-based selection: from the tumor to the patient

Due to the onset of primary resistance (defined as no objective tumor radiographic response and treatment duration < 6 months) and acquired resistance (an objective tumor response or treatment duration ≥ 6 months), identifying determinants of response and resistance to immunotherapy in lung cancer and MPM patients is needed[18]. In NSCLC (yet not in SCLC and MPM), PD-L1 expression represents the only validated predictive biomarker which is standardized and routinely available for ICI treatment[19]. Across many clinical trials[154–156] and retrospective analysis[157], its expression has been associated with progressively improved outcome, with a maximum benefit observed among tumors with PD-L1 expression ≥90%, corroborating its implication for treatment selection. However, PD-L1 is not perfect and in most cases, it is not sufficiently accurate for an individual prediction test. A subset of patients does not respond to ICIs despite having tumors with high PD-L1 expression; on the other hand, a few patients with tumors with absent PD-L1 get still benefit from ICI strategy. Besides, the heterogeneity of distinct IHC assays used to assess PD-L1 expression dampens the reproducibility of this biomarker across different indications[19]. Other

tumor-related factors, such as TMB, TILs infiltration, immune signatures, and specific molecular alterations are being investigated in NSCLC yet results have not always been replicated in prospective studies[158,159]. Moreover, some technical limitations often emerge in tumor site analyses, especially in patients with thoracic cancers. In addition, response and resistance to immunotherapy is related not only to the genetic and functional profile of tumor cells (intrinsic mechanisms) but also to the factors other than the tumor itself (extrinsic mechanisms)[68].

Recently, a more holistic approach has been adopted and different studies have suggested a potential impact of host-factors such as smoking history, gender, BMI, and microbiome diversity on the efficacy of ICI monotherapy[160–164]. Blood-derived parameters such as circulating tumor DNA[165], immune cell subsets[166], and inflammation markers[167] may represent a useful and easy-to-access predictor of immunotherapy response. Depicting the circulating immune profile is also an attractive strategy to infer immune-modulating off-target effects of chemotherapy. Finally, components of lipid profile have been assessed as determinants of several alterations occurring in tumor and immune cells[168–170].

In the era of high-throughput technologies, new computational tools and data sharing agreements will be needed to refine and integrate the plethora of available biomarkers and obtain an appropriate identification of patients who may derive benefit from immunotherapy.

Exploring new frontiers of cancer immunotherapy

Beside optimizing patient stratification through a more rational biomarker implementation, a further development of immunotherapy strategies is awaited to overcome resistance mechanisms. This new era of cancer immunotherapy involves different strategies[18,171] (**Figure 2**).

First strategy involves modulating co-stimulatory and/or co-inhibitory signaling pathways besides PD-1/PD-L1 axis to target the antitumor ability of effector T cells. Several novel ICIs targeting TIGIT, LAG-3, TIM-3 and VISTA are currently investigated in clinical trials to evaluate their efficacy in lung cancer and MPM[172–175]. Second strategy involves enhancing tumor antigenicity by using optimal agents to trigger immunogenic cell death and improve synergy with ICIs[176]. Third strategy also implies turning a cold tumor hot and enhancing the immune response directed towards tumor-associated antigens by using passive and active immunization approaches such as (DC-)vaccination[177], TCR-engineered or chimeric antigen receptor (CAR) T-cell therapy[178]. Fourth strategy involves integrating immunotherapy in the earlier setting as most of these agents demonstrated a better outcome when injected earlier in tumor development[99,100]. Fifth strategy involves targeting other actors in the context of the TME using targeted therapies but also exploiting off-target effects

of chemotherapy. As previously highlighted, TAMs, neutrophils, MDSCs and Tregs can exert an immunosuppressive activity in both lung cancer and MPM, thus strategies aimed at skewing or depleting these immune populations can help removing obstacles to the generation of an effective anti-tumor immunity[179]. Sixth strategy involves modulating the immune metabolism(s) both at the TME and systemically aiming to modulate different compounds (lipids, tryptophan, adenosine) which may limit antigen presentation and T-cell activity[180,181]. Seventh strategy involves overcoming the so-called host "immune breaking" factors. As tumors and immune system manipulate each other not only locally but also systemically, a multi-organ modulation which takes into account concomitant medications (statins, steroids, antibiotics) and microbiota composition before or during immunotherapy may unleash anti-tumor immune activity[164].

In conclusion, implementing one or more of these non-redundant strategies on a per patient basis will most probably alleviate both primary and secondary forms of immune resistance.

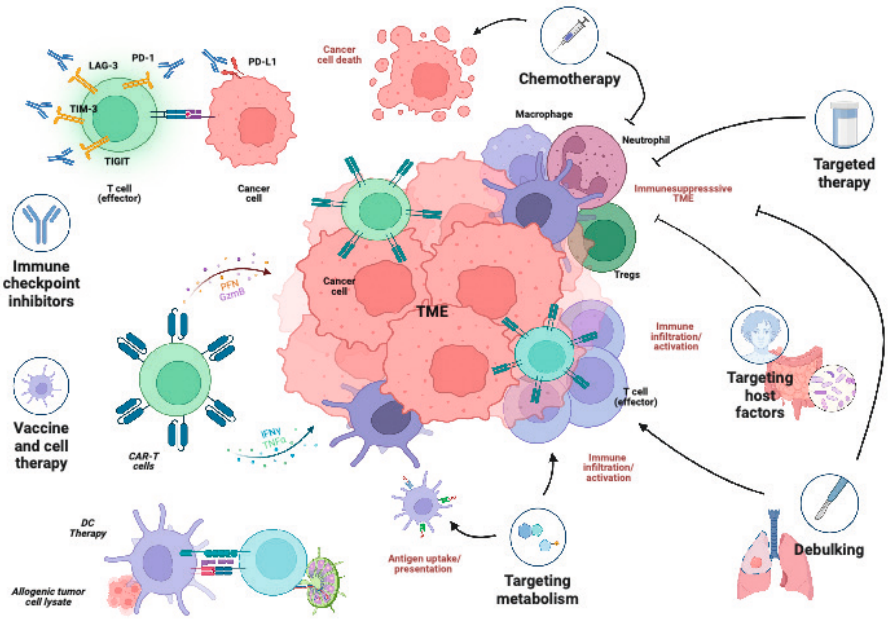

Figure 2. New frontiers of cancer immunotherapy for lung cancer and mesothelioma. In the context of lung cancer and mesothelioma, **m**ultiple strategies are investigated to overcome primary and acquired resistance to immune checkpoint inhibitor (ICI) monotherapy. Combinations of anti-PD1/PDL1 agents with other antibodies targeting co-inhibitory or co-stimulatory receptors (TIGIT, LAG-3, TIM-3 and VISTA) unleash the antitumor ability of effector T cells. Chemotherapy addition results in a synergistic effect by favoring an immunogenic type of cancer cell death and, similarly to some types of target therapy, is able alleviate immunosuppression thereby reshaping the tumor microenvironment (TME). Passive and active immunization approaches such as and chimeric antigen receptor (CAR) T-cell therapy DC therapy help turning a cold tumor hot thus making ICIs more active. Once T cells are primed by DC therapy and infiltrate the TME, usage of ICIs may prevent the onset of early exhaustion mechanisms. Modulating the immune metabolism(s) both at the TME and systemically as well as targeting host "immune breaking" factors also represent appealing strategies to obtain a more widespread reinvigoration of the anti-tumor immune response. Finally, translating immunotherapy to an earlier disease setting, when redundant pathways of immune escape are not activated yet, or simultaneously reducing tumor load to alleviate immunosuppression, would probably increase response rate to immunotherapy. Created with https://biorender.com/

Aims and outline of this thesis

Similar to Renaissance, which is a period that marked the transition from the Middle Ages to modernity, immunotherapy symbolized a paradigm shift for lung cancer and MPM. After an initial skepticism due to its lack of efficacy, inconsistency, and significant toxicity, immunotherapy (in the form of ICIs) nowadays represents the backbone upon other treatment are developed.

In MPM, despite a deeper appreciation of the pathobiology of this orphan disease, ICI monotherapy has not always retraced the magnitude of benefit observed in other cancers, with results of phase II studies not being replicated in larger, randomized, phase III trials. In **Chapter 2**, the most promising emerging therapies for the treatment of MPM are revised, discussing the biological rationale underlying their development as well as the issues surrounding clinical trial design and proper selection of patients for every treatment. In **Chapter 3**, we highlighted the potential of combining ICIs with other immunotherapies, as well as targeted agents and old-school chemotherapy to improve prognosis in MPM. Since the "one size fits all" approach is unlikely to adapt to MPM, focus should lie on the heterogeneity of the genetic and epigenetic landscape and of the composition of TME to take a step further single immune check point inhibition and increase the population of MPM patients who may derive clinical benefit from these approaches.

Due to the widespread use of ICIs, irAEs are now a cause for increasing concern regarding the comparative safety of these drugs in the treatment of cancer patients. Different ICI drugs and doses can lead to different toxicity features. As COVID-19 pandemic hit, physicians started feeling the need of modulating patients' access to oncology departments. This led to an increasing interest in alternative ICIs administration schedules able to offer longer dose intervals. In **Chapter 4**, we investigated the safety of switching ICI monotherapy from canonical interval dosing (CD) to ED to assess whether this regimen could represent a safe and feasible option also in solid cancer patients outside of clinical trials.

While randomized controlled trials remain the gold standard for evaluating the integration of immunotherapy in the clinical arena, their reported outcomes are not always replicated in everyday practice. Real-world analysis can therefore offer valuable insights into treatment practices by investigating understudied population or helping biomarker identification. In **Chapter 5**, we evaluated the outcome of nivolumab in a population of MPM patients pre-treated with chemotherapy. Furthermore, we analyzed the correlation between clinically important factors, baseline peripheral blood parameters and clinical outcomes. The impact of radiological response on outcome was also investigated.

In lung cancer and to a greater extent in MPM, responses to ICI monotherapy remain highly variable, with some patients experiencing durable partial or even complete tumor responses yet others responding temporarily or not at all. Combinations with other drugs are then needed to improve response. Since pre-clinical studies suggested a potential synergy between statins and PD-1 inhibitors, in **Chapter 6** we performed a multicenter study to assess the impact of baseline statin use on the clinical outcome of MPM and advanced NSCLC patients treated with PD-1 inhibitors. In **Chapter 7**, by looking at a real-world dataset SCLC and MPM patients treated with lurbinectedin chemotherapy, we explored the immune-modulating effect of this drug to provide novel insights into its mechanism of action and implement new data for a potential immunotherapy combination.

MPM is characterized by an anergic TME, in which both the effector arm (cytotoxic T cell response inside the tumor) and the inductive arm of the antitumoral immune response (antigen-presenting cells) are suppressed. Cancer vaccines, especially when used earlier in tumor development, might represent a valid immunotherapy strategy as they proved able to induce tumor-specific response without off-target toxicity. In **Chapter 8**, we presented the trial protocol of a phase I study with DC therapy (Mesopher) as (neo)adjuvant approach combined with extended pleurectomy/decortication (eP/D) surgery in patients with resectable epithelioid MPM. Such a study, with the availability of tumor tissue before and after DC therapy, may provide a platform to evaluate whether Mesopher is capable to establish and/or maintain a tumor-specific T cell response.

Finally, **Chapter 9** recapitulate the collected evidence and discuss it into the context of current literature to provide future directions in this new era of treatment for lung cancer and MPM.

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CHAPTER 2

Emerging treatments for Malignant Pleural Mesothelioma: where are we heading?

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Abstract

Malignant pleural mesothelioma (MPM) is an uncommon but aggressive and treatment resistant neoplasm with low survival rates. In the last years we assisted to an exponential growth in the appreciation of mesothelioma pathobiology, leading several new treatments to be investigated both in the early stage of the disease and in the advanced setting. In particular, expectations are now high that immunotherapy will have a leading role in the next years. However, caution is required as results from phase II studies in MPM were often not replicated in larger, randomized, phase III trials. In this review, we describe the most promising emerging therapies for the treatment of MPM, discussing the biological rationale underlying their development as well as the issues surrounding clinical trial design and proper selection of patients for every treatment.

Introduction

Malignant pleural mesothelioma (MPM) is an uncommon and highly lethal cancer. The annual incidence of MPM ranges between 10 cases per million to 29 cases per million depending on the country and, because of the long latency period, the peak is expected in the 2020s (1) in high-income countries. In addition, according to WHO prediction (2), developing countries where asbestos is still used, are likely to face a new epidemic of asbestos-related diseases, including MPM.

MPM pathogenesis is peculiar, as the direct causal relationship between exposure to airborne asbestos particles and the development of MPM is well established (3). The chronic exposure to asbestos fibers, which may enter the lung periphery and the pleura, leads to chronic inflammation of the mesothelium which sustains the carcinogenic processes (4). Individuals with germline BRCA1 associated protein-1 (BAP1) mutations may be predisposed to MPM, since they may develop it without any apparent asbestos exposure (5). Recent biological and preclinical studies provided further insights into MPM carcinogenesis, revealing the importance of tumor suppressor gene inactivation, through several mechanisms (single nucleotide variants (SNVs), copy number losses, gene fusions and splicing alterations). Tumor suppressor genes highly altered are cyclin-dependent kinase inhibitor 2A (CDKN2A, 60% of the cases), BAP1 (60% of the cases also in sporadic MPM) and neurofibromin 2 (NF2, 75% of the cases) (6–9).

The chronic inflammatory response to asbestos involved in the pathogenesis of MPM also causes a unique tumor environment. This microenvironment is mainly composed of immunosuppressive cells (regulatory T cells, macrophages and myeloid-derived suppressor cells (MDSCs)) and the number of these cells as determined by immunohistochemistry (IHC) represents a negative prognostic factor (10,11). On the other hand, immune-activating responses, such as the presence of $CD8^+$ T cells, are correlated with better outcome, although such links with prognosis are less important when compared with other cancer entities which are more immunogenic than MPM (12).

The management of MPM is complex and outcomes remain poor. For patients with early stage MPM the role of radical surgery is still a matter of debate and it should be considered only as part of a multimodal treatment (i.e., surgery combined with chemotherapy, radiotherapy, or both). Looking at unresectable MPM, no major breakthroughs have been made since the approval of antifolate and platinum combination chemotherapy (13,14). Median overall survival (OS) time with standard first-line options is about 13 months, with the best outcome for the epithelioid MPM subtype (14). Second-line treatment scenario is even more disappointing. With the only exception of a repeated course of pemetrexed-based chemotherapy for

previously responsive patients (15), limited options are available for relapsed MPM and new treatments are urgently needed.

Steps have been made towards a best appreciation of mesothelioma biology and have been essential to identify novel molecular therapeutic targets, representing the rationale for testing multiple targeted therapies in MPM (**Table 1**). Nevertheless, the potential to improve the potency and the specificity of the immune system, along with recent successes in other thoracic tumors, have attracted a growing interest in cancer immunotherapy. Continue efforts are necessary to further deepen our understanding of mesothelioma, taking into account biological and temporal heterogeneity of the disease in order to finally optimize the development of new treatment options in the context of well-designed clinical trials (**Figure 1**).

In this review, we describe last emerging therapies for mesothelioma, discussing the current status of knowledge in mesothelioma genetics and immune-biology, as well as the issues surrounding the conduction of high-quality trials in MPM and the selection of best patients for different treatments.

Table 1. Ongoing trials in malignant pleural mesothelioma patients (source: ClinicalTrials.gov).

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
Surgery	eP/D	NCT02040272 (MARS2)	III	Surgically resectable	Standard neoadjuvant chemotherapy before surgery	328	Multicentre randomised trial comparing eP/D versus no surgery
	eP/D - chemotherapy	NCT02436733	II	Surgically resectable	Neoadjuvant or adjuvant chemotherapy	64	Chemotherapy before or after P/D in patients with early stage MPM
Radiotherapy	Accelerated hypofractionated radiotherapy with tomotherapy	NCT03269227	I	Adjuvant (after eP/D)	N/A	30	
	Hemitoracic intensity modulated radiation therapy (IMPRINT)	NCT00715611	II	Adjuvant	Adjuvant chemotherapy	81	After enrolling 45 patients, hemithoracic IMPRINT was safe and had an acceptable rate of pneumonia
	Short neoadjuvant hemithoracic intensity-modulated radiation therapy	NCT00797719	I	Neoadjuvant	Adjuvant chemotherapy (+/-)	100	
Chemotherapy	Mithramycin (continuous 24-hours infusion)	NCT02859415	I/II	Relapsed	Single agent	100	Mithramycin is an antineoplastic antibiotic that inhibits cancer stem cell signaling
Antiangiogenic agents	Nintedanib	NCT02863055	II	Maintenance treatment after chemotherapy	Single agent	116	
PARP inhibitors	Olaparib	NCT03531840	II	Relapsed	Single agent	40	Recruitment is not limited to patients with germline/somatic mutations in DNA repair genes
	Niraparib	NCT03207347	II	Relapsed	Single agent	57	
EZH2 inhibitors	Tazemetostat	NCT02875548	Extension (rollover)	Relapsed	N/A	300	In multiple solid tumors

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
Base-excision repair inhibitors	TRC-102	NCT02535312	I/II	First line/Relapsed	Cisplatin and pemetrexed or only pemetrexed	58	
PI3K inhibitors	IPI-549	NCT02637531	I	Relapsed	Nivolumab (+/-)	220	In multiple solid tumors
FAK inhibitors	Defactinib	NCT02004028	Window-of-opportunity	Neoadjuvant	Single agent	38	
		NCT02758587	I/II	Relapsed	Pembrolizumab	59	
	APG-2449	NCT03917043	I	Relapsed	Single agent	40	APG-2449 is a novel, oral, multi-targeted tyrosine kinase inhibitor, which inhibits FAK, ALK, and ROS1
BCR/ABL pathway	Bosutinib	NCT03023319	I	N/A	Pemetrexed	24	In multiple solid tumors
Arginine deprivation	ADI PEG 20	NCT02709512 (ATOMIC)	II/III	First line	Cisplatin and pemetrexed	386	Double-blind, randomized (standard chemotherapy in the control group); only patients with biphasic or sarcomatoid histology are eligible; ASS1-deficiency is not required for study entry
Arginase inhibitors	INCB001158	NCT02903914	I	Relapsed	Pembrolizumab	424	In multiple solid tumors
Anti-CD30	Brentuximab vedotin	NCT03007030	II	Any line	Single agent	55	CD30 positive MPM
MDM2 antagonists (p53 pathway)	ASTX295	NCT03975387	I	Relapsed	Single agent	135	In multiple solid tumors (p53 wild type)
DR5 agonists	INBRX-109	NCT03715933	I	Relapsed	Single agent	80	INBRX-109 is a multivalent agonist of DR5

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
Tie2 inhibitors	Rebastinib (DCC-2036)	NCT03717415	I	First line/Relapsed	Carboplatin	117	Rebastinib acts on Tie2, a tyrosine kinase receptor that is expressed on endothelial cells and pro-tumoral macrophages
Immune check-point inhibitors	Pembrolizumab	NCT02707666	Window-of-opportunity	Neoadjuvant	Adjuvant pemetrexed and cisplatin	15	
		NCT02784171	II/III	First line	Cisplatin and pemetrexed	126	Randomized trial with both cisplatin/ pemetrexed and pembrolizumab alone (only in the phase II part) as active comparators
		NCT02959463	I	Adjuvant to radiotherapy	N/A	24	Primary goal is to determine the safety and tolerability of pembrolizumab administered after radiation therapy in patients with MPM who have not undergone EPP
		NCT03393858	II	Relapsed	DC-CIK immunotherapy combined with hyperthermia	40	
		NCT02628067 (KEYNOTE-158)	II	Relapsed	Single agent	1350	A trial of pembrolizumab (MK-3475) to evaluate predictive biomarkers in advanced cancers
	Nivolumab	NCT03063450 (CONFIRM)	III	Relapsed	Single agent	336	Double-blind, placebo controlled

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
		NCT03502746	II	Relapsed	Ramucirumab	35	
		NCT02834013	II	Relapsed	Ipilimumab	707	Anti-CTLA-4 and Anti-PD-1 combination in rare tumors
	MEDI4736	NCT02592551	Window-of-opportunity	Neoadjuvant	Tremelimumab (only 8 patients)	20	
	Atezolizumab	NCT03762018 (BEAT-meso)	III	First line	Bevacizumab and standard chemotherapy	320	Open-label, randomized (bevacizumab plus standard chemotherapy in the control group)
		NCT03074513	II	Relapsed	Bevacizumab	160	
		NCT03228537	I	Neoadjuvant	Cisplatin and Pemetrexed	28	Within 90 days after completition of surgery patients receive atezolizumab for up to 1 year
	Avelumab	NCT03399552	I	Adjuvant to radiotherapy (stereotactic body radiation therapy)	N/A	27	
	INCMGA00012	NCT03920839	I	First line	Cisplatin and pemetrexed	98	INCMGA00012 is a humanized IgG4 monoclonal antibody that targets human PD-1 and lacks antibody dependent cell-mediated cytotoxicity directed against effector lymphocytes

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
	XmAb20717	NCT03517488	I	Relapsed	Single agent	87	Phase I trial assessing the safety and tolerability of XmAb20717, a bispecific antibody that simultaneously targets immune checkpoint receptors PD-1 and CTLA-4, in multiple tumors
	Cosibelimab	NCT03212404	I	Relapsed	Single agent	500	In multiple solid tumors; CK-301 (cosibelimab) is a fully human monoclonal IgG1 antibody against PD-L1
	ABBV-181	NCT03000257	I	N/A	Single agent	221	In multiple solid tumors; ABBV-181 is an anti-PD1 monoclonal antibody
	TIM-3 inhibitor (INCAGN02390)	NCT03652077	I	Relapsed	Single agent	41	In multiple solid tumors
	LAG-3 inhibitor (INCAGN02385)	NCT03538028	I	Relapsed	Single agent	40	In multiple solid tumors
	GITR agonist (INCAGN01876)	NCT03126110	I/II	Relapsed	Nivolumab/ Ipilimumab	285	In multiple solid tumors
	OX40 agonist (ABBV-368)	NCT03071757	I	Relapsed	Single agent/ combination with anti-PD1 therapy	170	In multiple solid tumors
Mesothelin targeted therapy	Immunotoxin LMB-100	NCT03644550	II	Relapsed	Pembrolizumab	38	
		NCT04034238	I	Relapsed	Tofacitinib (inhibitor of Janus kinases)	45	

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
	Anetumab ravatansine	NCT03126630	I/II	Relapsed	Pembrolizumab	134	Open-label, randomized but not comparative (pembrolizumab alone in the non-experimental arm)
		NCT03926143	Extension (rollover)	Relapsed	N/A	20	
	Thorium-227 labeled antibody-chelator conjugate (BAY2287411)	NCT03507452	I	Relapsed	N/A	228	All tumors known to express mesothelin are eligible
Vaccines	Galinpepimut-S	NCT04040231	I	Relapsed	Nivolumab	10	
	Dendritic cell therapy (Mesopher)	NCT03610360 (DENIM)	II/III	Maintenance treatment after chemotherapy	Single agent	230	Dendritic cells are loaded with allogeneic tumour cell lysate (PheraLys)
		NCT02649829	I	Neoadjuvant	Standard concomitant chemotherapy and eP/D afterwards (in case of resectable disease)	20	Dendritic cells are loaded with the tumor antigen WT1
Adoptive cell therapy	iCasp9M28z CAR-T cells (targeting mesothelin)	NCT02414269	I	Relapsed	Cyclophosphamide prior to infusion +/- Pembrolizumab after infusion	66	After treating 20 patients, intrapleurally administered mesothelin-targeted CAR T cells were safe with encouraging antitumor activity
	TC-210 CAR-T cells (targeting mesothelin)	NCT03907852	I/II	Relapsed	Cyclophosphamide and fludarabine before treatment as lymphodepleting agents	70	

Table 1. Continued.

Class	Treatment	Trial name/ Identifier	Phase	Setting/Line of treatment	Single agent/ Combined therapy	Estimated enrollment	Notes
	CAR-T cells (targeting mesothelin)	NCT03638206	I	N/A	Cyclophosphamide and fludarabine	73	In multiple solid tumors
	TILs	NCT02414945	I/II	N/A	Cyclophosphamide and Fludarabine before treatment, low-dose IL-2 after cell infusion	10	
		NCT03935893	I	Relapsed	Cyclophosphamide and fludarabine	10	
Virotherapy	Intrapleural adenonovirus-deliveres interferon alpha-2b (rAd-IFN)	NCT03710876 (INFINITE)	III	Relapsed	Celecoxib and gemcitabine	300	Open-label, randomized with control group receiving only oral celecoxib plus intravenous gemcitabine
Other intrapleural therapies	Intrapleural Cryotherapy	NCT02464904	I	Neoadjuvant	N/A	15	
	Hyperthermic intraoperative chemotherapy (with pemetrexed and cisplatin)	NCT02838745	I	Adjuvant	N/A	36	
	Intracavitary cisplatin-fibrin localized chemotherapy	NCT01644994	I/II	Adjuvant	N/A	54	
	Intraoperative porfimer sodium -mediated photodynamic therapy	NCT02153229	II	Adjuvant	N/A	102	Open-label, randomized

N/A=data not available. ALK=anaplastic lymphoma kinase. ASSI=argininosuccinate synthase I. CAR=chimeric antigen receptor. CD30=cluster of differentiation 30. CTLA-4=cytotoxic T lymphocyte associated protein-4. DC-CIK=autologous dendritic cells-cytokine induced killer cell. DR5=death receptor 5. eP/D= extended pleurectomy and decortication. EPP=extrapleural pneumonectomy. EZH2=enhancer of zeste homolog 2. FAK=focal adhesion kinase. IgG=immunoglobulin G. MDM2=murine double minute 2. MPM=malignant pleural mesothelioma. PARP=poly ADP ribose polymerase. PD-1=programmed cell death-1. PI3K= phosphoinositide 3-kinase. ROS1= ROS proto-oncogene 1. TIE2= tyrosine kinase with immunoglobulin-like and EGF-like domains 1. WT1=Wilms' tumor.

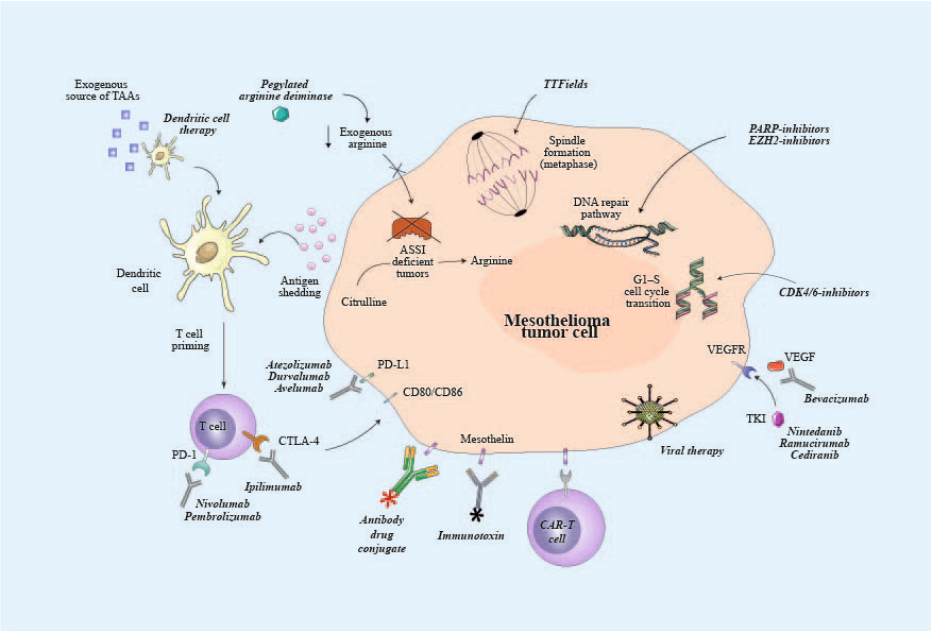

Figure 1. Potential targets of emerging therapies for malignant pleural mesothelioma. ASSI=argininosuccinate synthase I. CAR=chimeric antigen receptor. CD80=cluster of differentiation 80. CD86=cluster of differentiation 86. CDK4/6=cyclin-dependent kinase 4/6. CTLA-4=cytotoxic T lymphocyte associated protein-4. EZH2=enhancer of zeste homolog 2. PARP=poly ADP ribose polymerase. PD-1=programmed cell death-1. PD-L1=programmed death ligand-1. TAAs=tumor-associated antigens. TKI=tyrosine kinase inhibitor. TTF= tumor-treating fields. VEGFR=vascular endothelial growth factor receptor.

Neoadjuvant/adjuvant setting

Due to the anatomy, microscopically radical (R0) resection is not achievable in mesothelioma surgery and the goal of mesothelioma surgery is macroscopic complete resection (R1). Surgery alone is not curative; it is usually performed with chemotherapy and/or radiation therapy and reserved to a subset of patients with early tumor stage, epithelioid histology and good performance status.

Therapeutic surgery in mesothelioma has historically involved either an extended pleurectomy-decortication (eP/D) or an extrapleural pneumonectomy (EPP) (16,17). eP/D has been proven to offer better results in the context of multimodality treatment (18,19), and although the benefit of systemic therapy has been shown only in the advanced/unresectable disease, it is common practice to give four cycles of cisplatin or carboplatin with pemetrexed as adjuvant or neoadjuvant therapy. Two on-going trials, MARS 2 (NCT02040272) and EORTC1205-LCG (NCT02436733), are currently

evaluating the usefulness, the feasibility and the best timing for the combined approach of surgery and chemotherapy.

In order to improve local control and ideally survival, radiotherapy can be given. New approaches of radical hemithoracic radiation using intensity-modulated techniques are being tested. Rimner et al. showed that hemithoracic intensity-modulated pleural radiation therapy (IMPRINT) after chemotherapy and P/D was safe in 27 MPM patients as part of a multimodality lung-sparing treatment, with an acceptable rate of radiation pneumonitis (20). Larger clinical trials are awaited to confirm the effectiveness of this approach.

Recently, intrapleural therapies have been reported with the aim of improving loco-regional control of the disease by spreading drugs directly on the tumor surface. Several techniques with different rationale have been used with promising results: hypertermic intrapleural chemotherapy, photodynamic therapy (PDT), intrapleural immunotherapies (interferons (IFNs) and interleukin-2 (IL-2)) and gene therapy (21). However, available evidences are mainly based on retrospective, small and single-institution studies and controlled randomized trials are required.

If given as neoadjuvant therapy, novel agents should have the ability to induce tumor shrinkage, increasing the possibility of a complete microscopic resection and ultimately prolonging overall survival while maintaining a good safety profile. Designing studies in this setting remains a challenging effort that requires multidisciplinary involvement (22). Nevertheless, the neoadjuvant setting provides the unique possibility to conduct translational research in the context of window-of-opportunity trials, acquiring valuable information from blood and tissue collection. For example, the focal adhesion kinase (FAK)-inhibitor defactinib showed immunomodulatory effects when administered pre-operatively in a phase II window of opportunity trial (23) with a good tolerability profile, an objective response rate of 13% and 67% of stable disease, thus not altering resectability or mortality compared to historical controls. Final trial data are expected for 2020.

This approach has also paved the way for testing the properties of immune check-point inhibitors (CIs). There are several ongoing neoadjuvant trials which aim to assess the immunomodulatory and pharmacodynamics effect of CIs, as monotherapy (NCT02707666), as combination of anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and anti-programmed cell death protein (PD-1) agents (NCT02592551, NCT03918252) and as combination of anti-programmed death-ligand 1 (PDL1) with standard chemotherapy (NCT03228537).

By assessing translational surrogates of response, these trials may represent an opportunity to look into predictive biomarkers, improving selection of candidates to CIs-treatment.

CIs are also tested in the adjuvant setting (NCT02707666). From an immunological perspective, the main goal of combining surgery with adjuvant CIs is to reduce tumor induced immunosuppression (24). Increased tumor size correlates with major immune suppression and surgically shrinking tumor size may potentially reduce immune inhibition and T-cell exhaustion (25).

Another approach to increase immune activation in the adjuvant setting is represented by vaccines, either protein, bacteria or cell-based. An adjuvant Wilms tumor 1 (WT1) vaccine (galinpepimut-S), given with granulocyte-macrophage colony-stimulating factor (GM-CSF) and an immunologic adjuvant called montanide ISA 51 UFCH in MPM patients whose tumors expressed WT1 at IHC, had completed combined multimodality therapy and had no evidence of disease, showed a median progression-free survival (PFS) of 10.1 months (95% CI 5.5—20.8 months) and a median OS of 22.8 months (95% CI 9.1-37.6 months) with a favorable safety profile (26). Galinpepimut-S is currently being tested in the advanced setting combined with CI-treatment (NCT04040231).

In peritoneal mesothelioma, the feasibility of administering dendritic cells pulsed with an allogenic tumor cell lysate after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) is being assessed in the ongoing MESOPEC trial (NTR7060) (27). Secondary objectives of the study are to assess the safety of dendritic cells and determine whether this adjuvant treatment may induce a specific immunological response against the tumor (27). Pre-clinical evidences showed that dendritic cell therapy leads to better outcome when dendritic cells are injected in murine models with lower tumor volume (28,29). An efficient immune response is hampered by cytokines and regulatory T-cells induced by mesothelioma cells, showing that a low tumor load correlates with a better functioning immune system and higher anti-tumor responses. Giving dendritic cell therapy after surgically reducing tumor load might therefore improve response to therapy and clinical outcome.

To date, despite the neoadjuvant/adjuvant treatment represents a promising setting to test new therapeutic strategies, the global level of evidence is quite low and international guidelines (30) do not recommend either neoadjuvant or adjuvant radiotherapy/chemotherapy as standard options for resectable MPM.

Unresectable mesothelioma

Tumor treating fields

Based on the results of the prospective, single-arm, phase II STELLAR trial, the NovoTTF-100L System was approved by U.S. FDA in combination with pemetrexed plus platinum-based chemotherapy for the first-line treatment of unresectable locally advanced or metastatic MPM. NovoTTF-100L was approved under Humanitarian Device Exemption, an approval process guaranteed by the U.S. FDA which, taking into consideration the urgent need to identify more effective treatments for rare disease (such as MPM), allows medical devices to be marketed without requiring evidence of effectiveness.

NovoTTF-100L is a portable Tumor Treating Fields (TTFields) delivery system. TTFields represent a non-invasive, regional treatment modality by which alternating electric fields (at a frequency of 150 kHz) are continuously administer to the local site to arrest tumor cancer cell division. In human mesothelioma cell cultures, combining TTFields with cisplatin or pemetrexed led to reduction in cell count, induction of apoptosis and reduced clonogenic potential (31). These alternating electric fields act by disrupting spindle formation during metaphase and blocking the localization of intracellular organelles during telophase.

The 80 patients enrolled in the STELLAR trial (32) had a median OS of 18.2 months (95% CI 12.1-25.8), with 40.3% of partial responses and 97.2% of them obtaining a clinical benefit. Response rates were similar to the ones with standard chemotherapy but lasted longer by adding TTFields (median response duration was 5.7 months, ranging from 1.4 to 13 months). The rate of serious systemic adverse events remained the same when NovoTTF-100L was added to chemotherapy (either pemetrexed plus cisplatin or pemetrexed plus carboplatin, according to investigator choice). Expected TTFields-related skin toxicity was reported in 66% (53 patients) with only 5% of grade 3 skin toxicity. The planned duration was at least 18 hours/day along with up to 6 courses of chemotherapy and patients' compliance was 68% (16.3 hours/day).

To note, these results, although promising, should be considered in context of the randomized phase III MAPS trial (33), in which bevacizumab added to pemetrexed and cisplatin significantly improved median OS compared to pemetrexed plus cisplatin alone (median OS 18.8 versus 16.1 months, hazard ratio (HR) 0.77, p = 0.0167). The control arm of this trial performed 4 months better than the historical cohort analyzed by Ceresoli - the landmark study by Vogelzang et al. - (14) and should be considered while discussing STELLAR data. Also PFS (7.6 months) and response (40%) were similar when compared to control groups in the MAPS and the recent LUME-meso trials (34). Authors attributed the fact that the median OS in the STELLAR study was comparable to the control groups of the MAPS and the LUME-meso trials

to differences in prognostic factors (such as pathological subtypes). However, this fact, together with the potential sampling bias in single-arm studies and the effect of subsequent therapy, limits the interpretation of STELLAR data. Further investigation of TTFields in randomized trials is strongly encouraged.

Chemotherapy
There is no approved maintenance treatment for MPM patients who did not progress after first-line chemotherapy. NVALT19 was an open label, multicentric, randomized phase II trial, in which patients were assigned 1:1 to gemcitabine (1250 mg/m2 day 1 and 8 of 3 weekly schedule) or best supportive care (BSC) after 4-6 cycles of first-line platinum-pemetrexed without progression. Data presented at the last ESMO conference showed an improvement in PFS (median 6.2 months versus 3.2 months in the BSC arm (HR 0.42 (95% CI 0.28-0.63), $p < 0.0001$)), at the cost of an increased yet manageable toxicity (57% of patients experienced grade 3-4 adverse events versus 13% in the BSC-arm, with neutropenia, nausea and lung infection being the most frequent) (35). Since post-study treatments and OS data were not reported, the reported improvement in PFS could be simply due to an anticipation of second-line therapy.

Lurbinectedin is a new molecule that binds to the DNA minor groove in regulatory regions, inhibiting the function of oncogenic transcription factors. It also modulates the transcriptional program of monocytes and TAMs, hampering cytokine production (36). Investigator tested the role of lurbinectedin in the context of relapsed MPM, where no approved therapy exists. Recent data from the SAKK 17/16 multi-center, single-arm phase II trial, showed activity of lurbinectedin. Median PFS and median OS were 4.1 months (95% CI 2.6-5.5) and 11.9 months (95% CI 9.2-14.7), respectively. Lurbinectedin also worked independently of histology or prior immunotherapy (36).

These data support evaluation of the both gemcitabine as switch maintenance and lurbinectedin as second-line strategy in larger, randomized, phase III trials.

Anti-angiogenic agents
Activation of the vascular endothelial growth factor (VEGF) pathway, via its tyrosine kinase receptors, is crucial for mesothelioma cells growth (37), thus representing a rationale for antiangiogenic treatments in this neoplasm.

The addition of bevacizumab to pemetrexed and cisplatin chemotherapy as first-line treatment with bevacizumab maintenance therapy in patients who did not progress showed improved overall survival. However, bevacizumab remains currently unlicensed in this setting since the MAPS trial was not a registration trial (33). Moreover, results of Bevacizumab (an anti-VEGF monoclonal antibody (mAb)) as first-line option in combination with chemotherapy were not confirmed by other anti-

angiogenic agents, such as the tyrosine-kinase inhibitors (TKIs) axitinib (an anti-VEGFR TKI), sorafenib (anti-VEGFR2/3, platelet-derived growth factor receptor (PDGFR) and rapidly accelerated fibrosarcoma (RAF)/c-KIT), or imatinib mesylate (targeting BCR-ABL, c-KIT, and PDGFR) (38–41).

Since the benefit in the phase 2 trial (n=87 patients) (42) was higher in epithelioid MPM than in non-epithelioid subtypes, the multi-targeted anti-angiogenic kinase inhibitor, nintedanib (targeting VEGFR 1–3, PDGFR α or β, fibroblast growth factor receptor (FGFR) 1–3, SRC and ABL kinases pathways) was tested in conjunction with first-line cisplatin plus pemetrexed in a randomized phase III trial versus placebo only in patients with epithelioid histology. However, among the 458 randomized patients, the previous phase II efficacy findings were not confirmed and PFS did not differ between the nintedanib group (median 6.8 months (95% CI 6.1–7.0)) and the placebo group (7.0 months (95% CI 6.7–7.2); HR 1.01 (95% CI 0.79–1.30), p = 0.91). The interim analysis of OS also showed no difference between groups (34).

Nintedanib is also being currently investigated as only maintenance treatment for patients non-progressive after first line chemotherapy (NCT02863055).

Cediranib, a VEGFR and PDGFR inhibitor, added to first-line platinum-based chemotherapy, improved PFS in a randomized phase II trial (43). Primary end-point of the trial was to detect a PFS difference (by RECIST version 1.1) at the 1-sided 0.10 level and it was met. PFS was significantly higher in MPM patients who received cisplatin-pemetrexed chemotherapy with cediranib followed by maintenance cediranib, compared to the ones receiving cisplatin-pemetrexed with placebo. HR was 0.69 (median PFS 7.2 vs 5.6 months, p = 0.096). However, PFS was not different by modified RECIST and no significant difference in OS was reported. As with bevacizumab, cediranib is not approved as first-line treatment combined with chemotherapy.

Ramucirumab is a monoclonal antibody that binds the extracellular domain of human VEGFR-2. Due to VEGF-R2 expression on macrophages, ramucirumab also inhibits macrophages and their infiltration into mesothelioma microenvironment, thereby decreasing tumor growth and proliferation (44). One-hundred sixty-four patients are planned to be randomized in a multicenter, double-blind, placebo-controlled phase II trial comparing gemcitabine with or without ramucirumab in the second-line setting (NCT03560973 (RAMES)), whose completion is expected for 2020.

Targeted therapies

New studies have recently provided a comprehensive genomic profiling of mesothelioma. Genomic analysis may help in detecting actionable alterations and developing more tailored and effective therapies for MPM patients (6). Tumor suppressor inactivation (loss-of-function) represents one of the most frequent

mutational events in this tumor. In addition, multiple studies have pointed out frequent copy gains and copy losses involving different portions of the genome (6,7,45–48).

Carriers of inherited loss-of-function mutations in BAP1 are predisposed to mesothelioma (5,45,49,50). BAP1 encodes a deubiquitinase enzyme, a member of the ubiquitin carboxy (C)-terminal hydrolase (UCH) family, involved in different cellular pathways among which the cell cycle, cellular differentiation, cell death, metabolism, and the DNA damage response (51). In particular, BAP1 is thought to bind to the breast cancer type 1 susceptibility protein (BRCA1) and the BRCA1-associated RING domain protein 1 (BARD1) and enhance their tumor suppressor function (52). Besides germline mutations, recent analysis of the BAP1 locus by targeted next-generation sequencing identified homozygous inactivating mutations in approximately 60% of patients (53). This implies that the role of BAP1 in defective DNA repair and homologous recombination might be therapeutically exploited in a large number of MPM.

In a recent paper, among 385 patients treated with platinum chemotherapy, median OS was increased for MPM patients who had inherited mutations in DNA repair and/or other tumor suppressor genes (54). This is consistent with what already observed in ovarian and breast cancer patients with inherited mutations in BRCA1 and BRCA2 (55–58). Conversely, BAP1 mutant mesothelioma cell lines resulted significantly less sensitive than BAP1 wild type cells to gemcitabine (59). In addition, the role of somatic BAP1 expression in MPM patients receiving chemotherapy still represents a matter of debate, with retrospective studies showing contradictory evidences (60,61).

By inducing synthetic lethality of alternate DNA repair pathways, poly-ADP ribose polymerase (PARP) inhibitors have proved to be able to cause cell death in cell lines with loss of function of BAP1. This observation suggests that patients with mutations in BAP1 and in DNA repair genes might also benefit from treatment with PARP inhibitors (62). An enrolling clinical trial in MPM patients is examining the relationship between patient genotype and response to the PARP inhibitor olaparib (NCT03531840). Another PARP inhibitor, niraparib, is being tested in patients with BAP1 and other DNA damage response (DDR) pathway deficient neoplasms including mesothelioma (NCT03207347).

BAP1 inactivation also works as a putative epigenetic regulator involved in the polycomb repressive complex 2 (PRC2) and enhancer of zeste-homolog 2 (EZH2) pathway. Mesotheliomas with BAP1 loss proved to be responsive to EZH2 inhibition in vitro and in vivo (63). EZH inhibition may then represent a promising strategy, with tazemetostat showing a promising disease control rate of 51% at 12 weeks in a multicenter phase 2 trial (64).

CDKN2A is a tumour suppressor gene frequently inactivated in mesothelioma. CDKN2A encodes the ADP-ribosylation factor (ARF, also known as p14) and INK4A

(also known as p16) via alternative reading frames (65). By inhibiting cyclin-dependent kinase 4 (CDK4) and CDK6, INK4A decelerates the G1–S cell cycle transition. Small molecules CDK4 and CDK6 inhibitors induce apoptosis in CDKN2A-mutated tumors (66–69) and MPM cell lines viability was inhibited in a dose-dependent manner by the CDK4/CDK6 inhibitor abemaciclib (70). Combined with radiotherapy, this agent also completely suppressed tumor growth in a mouse model of MPM (70). These finding led to the investigation of abemaciclib in p16INK4A negative MPM patients (NCT03654833 (MiST)).

The hepatocyte growth factor (HGF), by binding to the MET receptor and activating its downstream target PI3K has been shown to enhance MPM cell proliferation, migration and invasiveness. Therefore, this pathway represents a compelling therapeutic target in this disease (71). However, the modest response rate observed in the early phase trials assessing agents targeting this pathway (72), indicates that combination regimens with other classes of antitumor agents with a sufficiently wide therapeutic window, will be necessary.

The enzyme argininosuccinate synthetase 1 (ASS1) leads to arginine biosynthesis from citrulline and is epigenetically suppressed in a high proportion of mesothelioma cell lines (73). Loss of ASS1 renders mesothelioma cells addicted to exogenous arginine (74), and this defect may be therapeutically exploited by pegylated arginine deiminase (ADI-PEG20), which works by clearing circulating arginine (73). Non-epithelioid (biphasic and sarcomatoid) MPM subtypes are characterized by a 75% rate of ASS1 loss and disease control rate (DCR) of this subgroup resulted 94% in the TRAP Phase I trial (75) of ADI-PEG 20 combined with 1st-line pemetrexed and cisplatin chemotherapy. Results from the randomized, placebo-controlled, double-blind phase 2/3 global ATOMIC-meso trial (NCT02709512) in non-epithelioid MPM are awaited.

In conclusion, despite our improved understanding of the biology of MPM, response to targeted therapies is hampered by intra-tumor heterogeneity and it is still unclear whether most of the actionable mutations constitute clonal or sub-clonal driver events. Longitudinal prospective studies, such as the TRACERx study in lung cancer (76), aiming at elucidating mechanism of resistance to treatment, are still missing in MPM. Properly designed clinical trials, which stratify patients for predictive biomarkers, are warranted. To this regard, patients enrolled in the MiST trial (NCT03654833) are currently offered a specific study treatment (either the parp-inhibitor rucaparib, the CDK4/6 inhibitor abemaciclib, the combination of the PD-1 inhibitor pembrolizumab and the AXL inhibitor bemcentinib or the combination of the PD-L1 inhibitor atezolizumab and the anti-angiogenic agent bevacizumab) determined by the results of the molecular panel testing of their diagnostic tumor block. The ones who exhibit positive testing in more than one biomarker, will potentially be eligible

for a subsequent protocol upon disease progression. This trial design is aimed at providing a more tailored approach for MPM patients.

Mesothelin targeted therapies

Mesothelin (MSLN) is a glycoprotein with high expression in epithelioid mesothelioma and low expression in normal tissues, thereby it represents an attractive target for several therapies. A phase II trial comparing amatuximab (an anti-MSLN chimeric monoclonal antibody) plus first-line chemotherapy versus chemotherapy alone was prematurely stopped in January 2017, not because of unacceptable toxicity but because of business reasons (NCT02357147).

According to a public announcement, anetumab ravtansine (an antibody-drug conjugate made by combining a human anti-MSLN antibody and the maytansinoid tubulin inhibitor DM4) also failed to improve PFS compared to vinorelbine in a randomized phase II trial for patients progressing after first-line (NCT02610140) (77).

CRS-207 is a live, attenuated, non-virulent, Listeria monocytogenes (LADD) encoding human MSLN. After receiving two priming infusions of CRS-207, followed by pemetrexed/cisplatin chemotherapy, and CRS-207 booster infusions in a phase Ib trial, 89% (31/35) of patients had disease control; one complete response (3%) and 19 partial responses (54%) were reported. Reduction of tumor size was also observed post-CRS-207 infusion prior to chemotherapy in 11 patients and no treatment-related serious adverse events or deaths were observed. These results suggested that combining CRS-207 with traditional chemotherapy might potentially result in increased anti-tumor activity (78). However, after a phase II trial had showed no clinical activity of the combination of CRS-207 with PD-1 inhibition (NCT03175172), clinical development of this therapy was discontinued.

LMB-100 is a next generation immunotoxin against MSLN that consists of a humanized fragment of the anti-MSLN Fab bound to a de-immunized Pseudomonas exotoxin (PE). This PE-fusion protein has been engineered to decrease its immunogenicity. A Phase I, open-label study to investigate the safety, pharmacokinetics, and activity of LMB-100 in relapsed MPM patients is planned to complete accrual this year (NCT02798536).

Evaluating new combinations of MSLN directed therapies with checkpoint inhibitors and integrating MSLN targeting into new approaches such as adoptive T cell transfer might constitute the next step in the field, as first results have been promising (79).

Immunotherapy

Immune checkpoint inhibitors. The immune system is known to play a key role in MPM. Immune suppression locally induced by the tumor is high (80). Survival of

patients with MPM is longer when tumors are highly infiltrated by cytotoxic $CD8^+$ T cells (tumor-infiltrating lymphocytes), whereas PD-L1 expression is associated with shorter survival (median OS 5.0 in patients who are PDL1-positive vs 14·5 months PDL1-negative patients; $p < 0{\cdot}0001$) (81,82). Due to their ability to restore the capacity of immune system to counterattack tumor growth, CIs (directed towards CTLA4, PD1, PDL1 or their combinations) started to be investigated in MPM patients. A large randomized phase IIb trial, assessing tremelimumab, an anti-CTLA4 mAb, versus placebo in a second or third-line setting did not show superiority of the immunotherapy in terms of OS (83). Looking at agents targeting the PD-1/PD-L1 pathway, interesting results were reported in the first early phase trials with overall response rates (ORR) ranging from 9 to 29% in patients previously treated with chemotherapy (84).

As shown in other types of cancer (85), combining CTLA-4 and PD-(L)1 mAb might further improve outcomes. In a single-center, single-arm, phase II trial (INITIATE) (86), the combination of ipilimumab and nivolumab for the treatment of recurrent MPM was assessed. Of the 34 patients evaluated for radiological response at 12 weeks, ten (29%) patients were partial responder and 13 (38%) had stable disease; adverse events were quite frequent (94% of patients) with 12 (34%) patients reporting grade 3 toxicity. Another randomized, non-comparative, open-label, phase 2 trial (MAPS2), conducted in 21 hospitals in France (87), met its primary endpoint of DCR after randomization in the first 108 patients. This trial aimed to assess the anti-PD1 mAb alone (nivolumab) or in combination with anti-CTLA4 (ipilimumab) mAb in MPM patients who progressed to first-line chemotherapy. Twenty-four (DCR 44%) of 54 patients treated with nivolumab and 27 (DCR 50%) of 54 patients treated with nivolumab plus ipilimumab achieved disease control at 12 weeks. Objective responses were ten (19%) with nivolumab and 15 (28%) with nivolumab plus ipilimumab. Again, the safety profile was consistent with previous data on the combination. To note, three (5%) treatment-related death were reported with the combination (one fulminant hepatitis, one encephalitis, and one acute kidney failure).

These findings confirm the promising activity of both single and double check-point blockade in MPM patients who have relapsed. However, data presented at 2019 ESMO conference from the European Thoracic Oncology Platform (ETOP 9-15) PROMISE-meso randomized phase III trial (NCT02991482) comparing PD-1 inhibition with pembrolizumab to institutional choice single agent CT (gemcitabine or vinorelbine) as second line treatment failed to show superiority of PD-1 treatment (88). Nearly four times more patients responded to immunotherapy (ORRs were 22% with pembrolizumab versus 6% in CT, $p = 0.004$), but these responses were not translated into delayed progression or improved survival (median PFS was 2.5 months (95% CI 2.1-4.2) with pembrolizumab and 3.4 months (95% CI 2.2-4.3) with chemotherapy, HR = 1.06 (95% CI 0.73–1.53), $p = 0.76$). In this study long term responders to pembrolizumab were

also found, again underlining the importance of understanding which patients should receive this treatment instead of chemotherapy (88). Data from another randomized trial comparing nivolumab versus placebo in patients pre-treated with at least two lines of chemotherapy (NCT03063450 (CONFIRM)), are also warranted in order to select the best strategy. At the current time, results from the MAPS2 trial supported the National Comprehensive Cancer Network (NCCN) panel decision to introduce either nivolumab or nivolumab plus ipilimumab as treatment options in relapsed MPM patients and nivolumab was approved in Japan as second-line treatment after results from a multicenter, open-label, single-arm, Japanese phase II study in MPM (MERIT) were reported, with ten (29%) patients showing an objective response (89).

Similar to other cancers, there might be a subgroup of MPM patients who might obtain a larger benefit from CIs, but relevant biomarkers have not been determined yet. Tumor PD-L1 IHC expression (with a cut-off of 1%) was correlated to ORR in both groups of MAPS-2 trial (nivolumab alone or nivolumab combined with ipilimumab) (87) but resulted in a better OS only in the nivolumab group. These correlations were not consistent in another phase II trial with nivolumab (90) and, although PD-L1 status may be associated with sensitivity to CIs, also patients with low PD-L1 expression benefit from this treatment, with a reported ORR of 11.1% (91). Intra-patient heterogeneity, different cut-points for PD-L1 positivity and lack of assay standardization also prevent PD-L1 from being used as the only selection criteria for CIs-treatment in MPM. This should lead researchers to investigate other tumor and patients' characteristics (histological subtype, performance status, blood-derived tests) to get an upfront identification of patients who are likely to respond to CIs and integration of multiple parameters (infiltration of CD8 and other subpopulations of T-cells (92), genomic signatures, specific mutations, expression of different checkpoint inhibitors) beyond PD-L1 status will be crucial.

To improve response rate to CIs in MPM patients, two options may be pursued. The first one is to move CIs towards the first-line setting, where the reinvigoration of the immune system may be stronger and more efficient, and to combine them with chemotherapy, similar to what happened in non-small cell lung cancer. Results of the addition of the PD-L1 inhibitor durvalumab to cisplatin and pemetrexed were presented in form of an abstract at the 2018 World Conference on Lung Cancer (93), showing a PFS of 6.2 months with a 48% ORR in the context of a non-randomized phase II trial - ORR is 41.3% with first-line chemotherapy alone, as historically reported (14). In the United States, a similar phase II trial investigating durvalumab (MEDI4736) in combination with chemotherapy for first-line treatment of MPM is currently in the analysis phase (NCT02899195). The addition of either pembrolizumab (NCT02784171) or nivolumab (in a Japanese population) (94) to chemotherapy is also being studied. The combination of ipilimumab and nivolumab is being compared with the cytotoxic

chemotherapy standard in the first-line setting as well, with about 600 patients expected to be enrolled in a phase III trial (95).

The second option may be to combine CIs with either different immune-modulatory molecules, targeted therapies, antiangiogenic agents, or radiotherapy. Additional co-inhibitory and co-stimulatory molecules such as T-cell immunoglobulin and mucin-domain containing-3 (TIM3, also known as HAVCR2), lymphocyte activation gene 3 (LAG3) and inducible T cell co-stimulator (ICOS) are being investigated in mesothelioma (96–98). Inhibiting FAK together with PD-1, may enhance immune cell-associated antitumor cytotoxicity in vivo, which is hampered by expression of PD-L1 (99) and this represented the rationale for a phase I/IIa currently ongoing (NCT02758587). Similarly, in addition to the direct anti-tumor effects, pegylated arginine deiminase (ADI-PEG 20) may boost tumor immune surveillance and might be a good primer for an additional anti-tumor immune therapy (100), raising the question whether combining ADI-PEG 20 with PD-1/PD-L1 blockers may further enhance these drugs' anti-tumor efficacy (101).

Early phase trials also assessed the combination of anti-PD1/PDL1 agents and MSLN-directed therapies (in MSLN-positive patients). After results from a pre-clinical murine lung tumor model (CT26hMeso) demonstrated anti-PD1 enhanced LADD-induced tumor response (102), a phase 2 single-arm study of CRS-207 with pembrolizumab in relapsed MPM was started but no responses were showed, and the study was discontinued (102). Two other phase 2 trials (NCT03644550, NCT03126630) assessing the combination of pembrolizumab with the anti-MSLN Immunotoxin LMB-100 and with the antibody-drug conjugate anetumab ravtansine are currently enrolling patients, with the latter one also randomizing patients to pembrolizumab alone as active comparator.

Growing evidence that pro-angiogenesis factors have immunosuppressive activity has led researchers to evaluate the potentially synergistic combination of antiangiogenic agents and immunotherapy also in the treatment of MPM. VEGF signaling has been shown to attenuate the immune antitumor response by either influencing lymphocyte trafficking across endothelia to the tumor or directly inducing inhibitory immune cell subsets (103). Several trials are aiming to address whether the combination of CIs and antiangiogenic agents (either mAbs as bevacizumab and ramcirumab or TKIs as nintedanib) is able to improve outcomes in MPM patients (NCT03762018, NCT02856425, NCT03502746).

Finally, similarly to certain types of chemotherapy, radiotherapy can be exploited for its ability to cause immunogenic cell death (ICD), thus priming the release of damage-associated molecular patterns (DAMPs) and tumor-associated antigens (TAAs) and

inducing a systemic anti-tumor immune response, that may be further enhanced by PD-1 (pembrolizumab) or PD-L1 (avelumab) blockade (NCT02959463, NCT03399552).

Vaccines. Vaccines represent another way to boost the immune system activation against the tumor. Both protein, vector and cell-based vaccines have been tested in MPM.

Galinpepimut-S is a WT-1 synthetic peptide vaccine made out of molecules similar to those in the WT1 protein. After a phase II trial confirmed vaccine's safety when administered in the adjuvant setting, researchers' efforts are currently directed towards the assessment of the combination of galinpepimut-S and nivolumab (NCT04040231). It has been hypothesized that the negative influence of tumor microenvironment factors on the immune response might be mitigated by nivolumab, thus providing the opportunity for the reinvigorated immune cells, specifically sensitized against WT1 by the vaccine, to invade and destroy cancerous growth deposits.

Dendritic cells are antigen-presenting cells that present tumor-associated antigens (TAAs) to the immune system by trafficking from tumors to lymph nodes. They are essential in priming proliferation and activation of $CD8^+$ cytotoxic T–lymphocytes and $CD4^+$ helper T-lymphocytes resulting in a potent and specific anti-tumor response (104). Dendritic cell function is hampered in cancer patients by tumor-derived soluble factors that suppress their immune-stimulatory ability (105,106). However, dendritic cells can be generated in large amounts ex vivo and loaded with TAAs, prompting their recent usage as cancer vaccines in several neoplasms, including MPM. Several sources of tumor antigens (mRNA, peptides, proteins or whole tumor cell lysate) can be used to load DCs (107). Because TAAs are difficult to identify in mesothelioma (thus excluding peptides as best source), and adequate tumor tissue is rarely obtained from mesothelioma patients (108,109), an allogenic tumor lysate has been developed (110). Results from a first-in-human clinical trial involving nine MPM (non-progressive after at least 4 cycles of chemotherapy) showed that this approach is safe (no dose-limiting toxicities were established) and led to radiological responses and promising survival data, with median PFS of 8.8 months and median OS not reached (110). A large multicentric phase II/III randomized trial with allogeneic-lysate pulsed dendritic cell immunotherapy as maintenance treatment after platinum-based chemotherapy is currently enrolling in Europe (NCT03610360 (DENIM)) (111).

T cell therapies. Another promising cell-based strategy in mesothelioma is represented by adoptive T cell therapy. Data from a phase I trial investigating chimeric antigen receptor (CAR) T cell therapy targeted to the MSLN protein in 19 MPM patients progressed following standard platinum-based chemotherapy were recently reported (79). A single-dose of second-generation CD28-costimulated MSLN-CAR T cells with the Icaspase-9 safety gene (IcasM28z) was given intrapleurally (as recommended

by previous observations in murine models, in which intrapleural administration vastly outperformed intravenous infusion) (112) with or without cyclophosphamide preconditioning. No evidence of on-target, off-tumor or therapy related toxicity was seen, and CAR T-cell persistence was associated with decreased levels of serum soluble MSLN-related peptide (SMRP) levels (>50% compared to pretreatment) and evidence of tumor response. Of the 14 patients who received anti-PD1 agents, off-protocol, after the CAR T-cell therapy, 2 achieved a complete metabolic response, 5 obtained a partial response, and 4 had stable disease. Combining anti-PD1 therapy with CAR T cells is also supported by prior preclinical data showing that CAR T cells become functionally exhausted in the presence of a large tumor burden and that anti-PD-1 therapy can reactivate these exhausted cells (113).

Virotherapy. Oncolytic viral therapy represented in the last decades an emerging field of immunotherapy and a promising experimental strategy. Viruses can act by infecting cancer cells and leading to cell lysis after replication. This renders tumor-associated and viral antigens recognizable to the immune system, thus triggering antitumor immune responses (viroimmunotherapy) (114,115). Oncolytic viruses need also to be tumor selective, and although malignant cell-specific oncolysis naturally occurs because of the impairment of the type I interferon pathway in many tumor cells, viruses may be engineered in order to increase their selectivity. Viruses may be used also for gene therapy, thereby therapeutically changing the infected tumor cells by gene transfer (116).

The pleural location and the peculiar pattern of growth (mostly localized), which provide access to direct intratumoral injection of virus, make MPM an ideal candidate for assessing the efficacy of oncolysis (116). The safety of virotherapy has been assessed and some clinical response have been reported (114). Among the many viral vectors that have been investigated, the recombinant replication incompetent adenoviral (ADV) vector encoding human interferon-α (IFNα, a naturally-occurring protein with anti-cancer properties) administered 'in situ' (intrapleurally) with celecoxib (to reduce the number of immunosuppressive MDSCs) before chemotherapy, was well tolerated and appeared to improve overall survival rates (117). Combinations of virotherapy with CIs, chemotherapy, and radiation are expected to further boost the effects on antitumor immunity and represent the object of ongoing trials (118–120), such as the phase III INFINITE trial (NCT03710876), in which about 300 patients will receive gemcitabine and celecoxib with or without the ADV-delivered IFNα-2b (rAd-IFN).

Conclusion

In the past two decades there was limited success in the development of novel therapies for MPM. Multiple biases in the design of clinical trials and the peculiar

biological features of MPM were most probably responsible for delaying the discovery of effective therapeutic agents. Most of the previous trials attempted to readapt drugs that succeeded in other cancer types to MPM. However, they were either too small or not stratified for predictive biomarkers. Results from phase II studies were often not replicated in larger, randomized, phase III trials, pointing out that well controlled trials with appropriate size and duration are crucial to confirm the efficacy of a new agent (121).

In the last few years, mesothelioma genetics, epigenetics and the tumor microenvironment (especially immune-biology) have been studied more deeply and this knowledge has started to be properly applied to discover new therapies. In particular, expectations are now high that CIs and other immunotherapies will have a leading role in the future therapeutic armamentarium of MPM. Noteworthy, scientific evidence supporting the use of CIs in MPM are still incomplete, mainly based on non-randomized studies with surrogate end-points and they have not been always replicated in the real-life context. Because of the risk of cumulative toxicities and of the high cost of these drugs (especially of combinations), validated biomarkers are urgently needed to select MPM patients who may benefit from immunotherapies. Since the 'one-size fits all' approach is not recommended for immunotherapy and MPM and the efficacy of CIs is still to be established in a larger population, there is still a need for new treatments in MPM and the implementation of other targeted agents is eagerly awaited.

Only a close collaboration between medical centers and industry may lead to the conduction of well-designed, biomarker-driven clinical trials. New trials should always include translational and quality of life components, in order to clarify the molecular basis of response or progression to treatments and to finally improve the degree of reliability of the possible benefit of new therapies for MPM.

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CHAPTER 3

Immunotherapy for mesothelioma: moving beyond single immune check point inhibition

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Abstract

Malignant pleural mesothelioma (MPM) is an aggressive neoplasm with low survival rates. Platinum-based chemotherapy has represented the cornerstone of treatment for over a decade, prompting the investigation of new therapeutic strategies both in the early stage of the disease and in the advanced setting. The advent of immune check-point inhibitors (ICIs) has recently revamped the enthusiasm for using immunotherapy also in MPM. However, results from first clinical trials using single immune check-point inhibition have been conflicting, and this may be mainly attributed to the lack of specific biomarkers as well as to intra- and inter- patient heterogeneity. The phase III Checkmate743 firstly demonstrated the superiority of an ICI combination (nivolumab plus ipilimumab) over chemotherapy in the first-line treatment of unresectable MPM, leading to FDA approval of this regimen and showing that moving beyond single immune check point inhibition might be a successful strategy to overcome resistance in the majority of MPM patients. In this review, we describe the emerging immunotherapy strategies for the treatment of MPM. We also discuss how refining the approach in pre-clinical studies towards a more holistic perspective (which takes into account not only genetic but also pathophysiological vulnerabilities) and strengthening multi-institutional collaboration in clinical trials is finally helping the clinical development of immunotherapy in MPM.

Introduction

Malignant pleural mesothelioma (MPM) represents an uncommon and orphan thoracic malignancy, whose incidence is peaking worldwide. The prognosis of this disease has not been improving since the addition of pemetrexed to platinum-based chemotherapy in 2004 [1], with a median survival of about 1-year post-diagnosis for unresectable patients [2]. The advent of immunotherapy across multiple cancer types along with a better appreciation of mesothelioma biology has revamped the enthusiasm for improving outcomes of this extremely resistant neoplasm [3]. Expectations were high that immune check-point inhibitors (ICIs) might have entered the treatment landscape, mirroring what had already happened in other thoracic cancer [4]. However, findings from both clinical trials [5, 6] and real-life observations [7, 8] with single ICIs were conflicting, with tumor responses being variable and so far unpredictable. The CONFIRM (Checkpoint Blockade for Inhibition of Relapsed Mesothelioma) randomized phase III trial showed the superiority of the anti-programmed death-1 (PD-1) agent nivolumab versus placebo in relapsed MPM [9]. However, investigators of the PROMISE-MESO trial did not find any difference in terms of progression free survival (PFS) when pembrolizumab, another anti-PD1 agent, was compared to investigator's choice chemotherapy, despite an increase in response rate [10]. More recently, the CheckMate 743 trial combined ICIs by evaluating the combination of nivolumab and ipilimumab (anti-PD-1 and anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4), respectively) reporting a significant improvement in overall survival (OS) versus platinum-based chemotherapy (to a greater extent in non-epithelioid tumors), leading to a new standard of care in the first-line setting [11].

These results highlighted the potential of combining ICIs with other immunotherapies, as well as targeted agents and old-school chemotherapy to improve prognosis in MPM. Since the "one size fits all" approach is unlikely to be satisfying in MPM, focus should now lie on the heterogeneity of the genetic and epigenetic landscape and of the composition of the tumor immune microenvironment of MPM.

Herein, we discuss the rationale behind and the preclinical development of new immunotherapy strategies, taking a step further single immune check point inhibition to increase the population of MPM patients who may derive clinical benefit from these approaches.

Rationale of immune-modulation in mesothelioma patients

Studying mesothelioma pathogenesis is crucial to understand how its unique tumor immune microenvironment (TIME) is shaped and to unleash the potential of immune-modulation in treating patients. By migrating to pleural space and interacting with mesothelial cells and immune cells, asbestos fibers are known to establish a process of chronic inflammation [12]. Asbestos fibers are phagocytized inside the pleural space by macrophages, which are in turn unable to totally digest them, leading to the production of reactive oxygen species (ROS). Asbestos has also a direct effect on mesothelial cells through DNA damage and strand breaks, which adds to the indirect effect caused by ROS release [13]. The release of inflammatory cytokines and growth factors by both mesothelial cells and macrophages attracts a wide spectrum of immune and stromal cells, leading to carcinogenesis (through genetic and epigenetic mechanisms) [14] and progression with a latency of up to 30-40 years from exposure to MPM diagnosis [3].

This long carcinogenesis process, combined with tumor localization, might explain the resistance of MPM tumors to treatments. In particular, two main steps of the anti-tumor immune response machinery contribute to the conflicting results of ICI monotherapy in mesothelioma: tumor antigen presentation and T cell activation. While the second may be restored by the use of anti-PD(L)1 agents, it would not translate into an effective and durable tumor response in the absence of the first factor [15] [16].

Unlike other tumors associated with carcinogenic exposures such as lung cancer and malignant melanoma, next generation sequencing (NGS) reported very low tumor mutation burdens (TMB) in mesothelioma [17]. Noteworthy, besides events like alternative splicing which seems *per se* a source of tumor-specific neoantigens and shapes the TIME in MPM [18], currently available NGS approaches might be not able to capture the full genomic complexity of this tumor, being unable to identify recurrent inter- or intra-chromosomal structural rearrangements in patterns such as chromoplexy or chromothripsis. Chromosomal rearrangements generate truncations or fusion transcript which, similarly to insertion and deletion, have neoantigenic potential [17]. Kosari and colleagues used a new genomic approach to detect structural variants (as defined by tumor junction burdens) resulting from chromosomal rearrangements and combined them with transcriptomic data to refine selection of patients receiving ICIs. They showed that genomic structural variants were associated with improved survival, but only in the context of antigen processing and presentation gene set expression. In contrast, tumor junction burdens in the absence of antigen processing and presentation gene set expression were predictive of reduced survival [19]. These findings were specific to patients treated with immune checkpoint inhibitors, highlighting that expression of neoantigens and aberrant self-

antigens, as well as antigen processing and presentation are crucial hallmarks of response to ICB also in the context of mesothelioma [20].

Since T-cell receptor (TCR) repertoire is driven by the intratumoral neoantigen landscape, in turn modeled by focal HLA loss or antigen processing defects, it was questioned whether TCR clonality/diversity might be correlated with clinical outcome for ICI therapy in MPM.

In contrast to TMB-high tumors such as melanoma [21] or non-small cell lung cancer (NSCLC) [22] in which the anti-tumor immune responses is driven by a clonal TCR, Forde and colleagues showed that immune cell repertoire diversity is required to mount an effective anti-tumor immune response in MPM. Noteworthy, these data were observed in MPM patients treated with chemoimmunotherapy and chemotherapy-induced cell death might have impacted on the TCR repertoire landscape leading to efficient antigen presentation to T cells and expansion of specific clones [23]. On one hand, this fact prevents the authors from extrapolating the results in the context of single-agent ICI, on the other it paves the way for new mechanistic insights on the way chemoimmunotherapy might help overcoming primary resistance to check-point inhibition alone [24] (**Figure**).

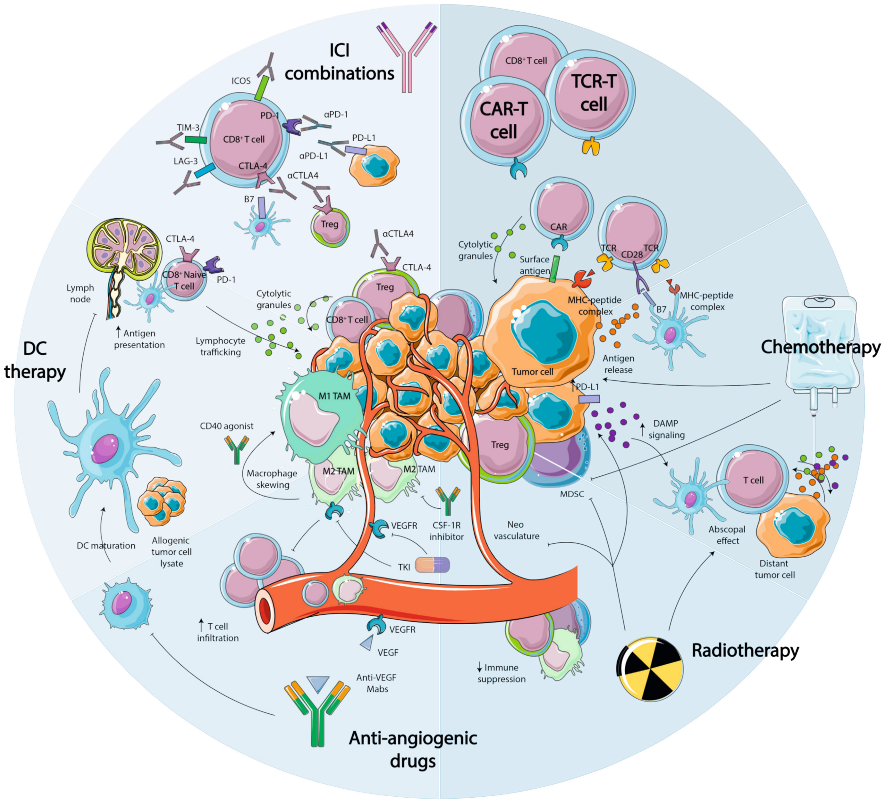

Figure 1. Emerging immunotherapy strategies in malignant pleural mesothelioma (MPM) beyond single immune check-point inhibitors (ICI). Multiple strategies are currently investigated in the context of MPM to overcome primary and acquired resistance to ICI monotherapy. Combination with platinum-based chemotherapy results in synergistic effect by favoring an induction of an immunogenic type of cancer cell death through exposure of calreticulin and release of ATP and high-mobility group protein box-1 (HMGB-1). As epithelioid MPM is more chemosensitive while ICIs conferred a striking survival advantage especially in non-epithelioid MPM, it is possible that the synergistic effect of chemo-immunotherapy confers a particular advantage for patients with epithelioid MPM. Radiotherapy facilitates recruitment of anti-tumor immune cells and cause tumor-specific activated T lymphocytes to mediate regression of distant tumors not being irradiated themselves (abscopal effect), although this effect might have a lower impact in mesothelioma as most tumors spread locally. Anti-angiogenic agents can also alleviate immunosuppression and, when combined to ICIs, reshape the tumor immune microenvironment (TIME) through vascular remodeling. DC therapy, namely DCs which are ex vivo loaded with tumor-associated antigens (TAAs), acts as adjuvant in those first critical steps of tumor-specific immune responses which are critically impaired in MPM because of its "cold" phenotype. Once T cells are primed and activated by DC therapy, usage of ICIs may prevent the onset of early exhaustion mechanisms. Combinations of anti-PD1/PD-L1 agents with other antibodies targeting co-inhibitory or co-stimulatory receptors (T cell immunoglobulin and mucin-domain containing-3 (TIM3) receptor, lymphocyte activation gene 3 (LAG3) receptor, or inducible T cell COStimulator (ICOS)) confer a more widespread reinvigoration of the anti-tumor immune response. Albeit adoptive T-cell therapy in MPM is usually limited by

heterogeneity in tumor antigen expression, immunosuppressive TIME, and inhibition of immune cell trafficking, it may optimally synergize with ICIs blocking inhibitory signals and therefore allowing transferred T cells to function effectively. Other abbreviations: B7, B7 protein (CD80/CD86); CAR, chimeric antigen receptor; CD28, cluster of differentiation 28; CD40, cluster of differentiation 40; CSF-1R, colony stimulating factor 1 receptor; CTLA-4, cytotoxic T lymphocyte associated protein-4; DAMP, damage-associated molecular pattern; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility complex; PD-1, programmed cell death-1; PD-L1, programmed death ligand-1; TAM, tumor-associated macrophages; TCR, T-cell receptor; TCR-T cell, TCR engineered T cell; TKI, tyrosine kinase inhibitor; Treg, regulatory T cells; VEGFR, vascular endothelial growth factor receptor.

Considering intra and inter-patient heterogeneity, Zhang and colleagues unveiled that the genomic clonal architecture modulates immune surveillance, with MPM harboring higher subclonal neoantigen burden being associated with higher T cell infiltration, but also with HLA loss of heterozygosity (HLA LOH) consistent with immune escape, probably as a consequence of heightened tumor surveillance [25]. This is paralleled by a substantial changes in the expression of immune-related genes in different tumor samples from the same patient, as well as by differential infiltration of immune populations in the TIME, supporting the necessity of multi-sampling for the implementation of a tailored immunotherapy approach [26, 27].

By performing an unsupervised analysis of gene expression in 284 MPMs, Alcala and colleagues showed that samples did not form discrete clusters, and rather conformed to a continuum of expression profiles, suggesting to move further the traditional histological classification which foresees three discrete entities: epithelioid, biphasic and sarcomatoid MPM. At one extreme of this continuum two bad-prognosis specific molecular profiles were identified: a "hot" bad-prognosis profile, constituted by high lymphocyte infiltration and high expression of immune check points and pro-angiogenic genes; a "cold" bad-prognosis profile, with low lymphocyte infiltration [28]. These evidences, together with the fact that antiangiogenic agents encourage the differentiation and activity of immune cells [29], targeting both the immune and vascular systems in MPM might represents an alternative therapeutic strategy in the context of proper patients' stratification (**Figure**).

Due to its relatively modest response rates with immune check-point inhibition alone, MPM might generally be considered a "cold" cancer. However, MPM TIME profile might be more commonly defined as "altered" and further categorized as "immunosuppressed" (with cytotoxic T-ymphocytes [CTLs] infiltrating the tumors without carrying out any meaningful activity) or "excluded" (with CTL infiltration but not beyond the invasive margin) [30]. In fact, the MPM TIME tends to acquire an anergic profile, sculpted by the presence of different immunosuppressive cells, which in turn suppresses not only the effector arm (CTL response inside the tumor) but also the inductive arm of the anti-tumoral immune response (antigen-presenting cells) [31].

Awad and colleagues found frequent co-expression of PD-1 and T-cell immunoglobulin and mucin domain 3 (TIM-3) on $CD8^+$ T cells in the TIME, suggesting that combined immune check-point inhibition might improve tumor responses in specific MPM subtypes such as PD-L1 positive and those with a sarcomatoid component [32]. Noteworthy, the tumor draining lymph node (TDLN) might also play a crucial role in generating primary anti-tumor immune responses following ICI therapy. In fact, recent data derived from a mesothelioma mouse model suggest that anti-tumor T cell immunity can also be boosted by selectively alleviating immune suppression in the TDLN leading to effectively control distant tumor sites [33]. This follows recent insights into tumor biology showing that PD-1 inhibition takes place mostly in B7-rich environment such as LNs, and is less likely in the immune-suppressive TIME such as MPM [34].

A strong expression of the immune-checkpoint gene V-domain Ig suppressor of T cell activation (VISTA), strikingly higher than in other solid cancers, was also reported in epithelioid MPM. VISTA is a member of the B7 family of and is thought to act as negative checkpoint regulator primarily expressed on infiltrating tumor macrophages. Because in the context of MPM, VISTA was found to be highly expressed mainly on MPM cells (unlike other cancer types where it is more often expressed on immune cells) [35], and was not related with overall mutation load, it was speculated that VISTA may restrain antitumor immune responses in a subset of MPM cases [36].

Using gene set variation analysis to infer the abundance of immune cell fractions out of 516 MPM samples, Alay and colleagues found three prognostic immune clusters. IG1 (54.5% of the samples) was characterized by high T-helper 2 and low cytotoxic T cell levels, while on the opposite side of the spectrum IG3 (8.5%) was defined by low T-helper 2 and high cytotoxic T cell levels. Authors also stated that patients belonging to IG3 may derive a larger benefit from immunotherapy [37].

Tumor response can also be promoted or hindered by the dynamic cross talk between tumor cells and immune cells other than helper and/or cytotoxic T cells. Regulatory T cells (Tregs), as well as tumor associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) play a key role in shaping the MPM TME and their presence should be regarded not only as prognostic biomarker but also as critical regulator of resistance to ICI monotherapy as these cells are able to attenuate the activity of effector and helper T-cells [38, 39]. Higher Treg abundance was found in MPMs with lower neoantigen diversity [25]. In a mice mesothelioma model, tumor growth was significantly reduced and survival increased after depleting Treg cells by a CD25-depleting antibody prior to tumor implantation or by using cyclophosphamide [40, 41].

The size of the myeloid suppressor compartment is considered to be an important factor in the clinical success or failure of cancer immunotherapy [42]. When co-cultured with $CD8^+$ T-cells from the same MPM tissue, both granulocytic and monocytic myeloid-derived suppressor cells (Gr-MDSC/Mo-MDSC) reduced $CD8^+$ T-cell interferon gamma (IFNγ) production and proliferation. Moreover, they produced ROS, nitric oxide (NO) and kynurenine, further boosting the immunosuppressive effect on T-cells [43].

TAMs are also numerous in MPM tissue, with about 25-40% of total immune infiltrates [31, 44]. Their phenotype is plastic and regulated by the local microenvironment, yet they are held responsible for tumor development and for boosting the immunosuppressive TIME of MPM [45, 46]. TAMs can secrete chemokines and cytokines that promote the development of tumors such as IL-6, IL-8, IL-10, and CCL22 [47], which further promotes the infiltration of Tregs inside TIME. Albeit too simplistic, a binary classification of TAMs has been proposed, with M1 macrophages displaying antitumorigenic function and type M2 macrophages promoting tumor development [48]. Using multiplexed fluorescence, Ollila and colleagues recently confirmed the association between type M2 pro-tumorigenic macrophages ($CD163^+$ $CMAF^+$ $HLA\text{-}DRA1^-$) in the TIME and shorter survival [49]. Colony-Stimulating Factor 1 (CSF1)/Colony-Stimulating Factor Receptor (CSF1R) signaling also detains a key role in the differentiation of monocytes into specific TAM phenotypes and CSF1R inhibition, combined with PD-L1 inhibitors, might limit mesothelioma growth [50].

However, effectively targeting and reducing TAMs by M-CSFR inhibition led to lower neo-angiogenesis and ascites in mesothelioma mouse models, but did not increase local infiltration of $CD8^+$ T-cells [51]. When targeting TAMs was combined with dendritic cell (DC) vaccination, longer survival was achieved in the same mouse models with a concomitant increase in $CD8^+$ T-cell numbers and functionality [51]. This may be ascribed to the fact that by depleting TAMs, not only the immunosuppressive but also the immunoactivating ones were depleted. Other efforts have been done to shift the macrophage fenotype in vivo [52], but results in murine models have not be replicated in the clinic so far. Again, this heterogeneity and granularity of results highlight the importance of understanding how best to harness the immune response to MPM to improve prognosis (**Figure**).

Another complicating factor in mesothelioma is the lack of a common tumor associated antigen (TAA) [53-55]. Another issue is the fact that a tumor might be able to express different tumor antigens in different amounts depending on the environmental circumstances. When the cytotoxic T-cell reaction is directed against a certain antigen this antigen may be downregulated by the tumor cell or clonal selection might occur followed by resistance to antigen-targeting immunotherapy [15]. This might impair the multiple tumor-associated antigen-targeting immunotherapy

approaches (e.g. antibody based therapy, chimeric antigen receptor [CAR] and TCR T-cell therapies) that are currently tested in MPM [56].

In this context, vaccines with tumor lysate priming strategies may be advantageous in providing the full antigenic repertoire of the tumor, reducing the possibility of tumor escape and inducing a broader immune response. Therefore, both priming (through vaccines) and activating (through active and passive immunotherapy) the anti-tumoral immune response, thus moving beyond single immune check point inhibition, is key to obtain significant and durable tumor responses and is where most of the research on MPM is moving in the last two years [56] (**Figure**). As already done for other cancer types, the impact of host-intrinsic factors should also be taken into account, as it might help researchers to better understand MPM and immune co-evolution. To this extent, evidence is now accumulating on the role of glucose and lipid metabolism in the competition between tumor and immune cells [57, 58] and on the susceptibility to nutrient stress in MPM cell lines [2, 59], yet success in targeting cancer metabolism therapeutically has been limited so far [60, 61].

In conclusion, adopting a more holistic perspective and identifying not only genetic but also pathophysiological vulnerabilities, may lead to the development of new therapeutic combinations and ultimately increase the percentage of MPM patients who benefit from immunotherapy.

Combination of immune check point inhibitors with conventional therapies

Combination of multiple immune check point inhibitors

A combination of ICIs may amplify their antitumoral effect and help overcome frequently observed therapeutic resistance since immune checkpoint proteins are involved in different stages of T-lymphocyte activation. Interest has emerged in using ICI combinations as both initial and rescue therapies. Positive clinical effects have been previously documented with the combination of anti-CTLA-4 monoclonal antibodies (Mabs) with anti-PD-L1 Mabs in other cancers by limiting the ability of malignancies like melanoma and NSCLC from exploiting negative feedback mechanisms leading to immune exhaustion and tolerance [62]. Several trials now support the use of this and of other immune checkpoint combination strategies in MPM.

In NIBIT-MESO-1, an open-label, single center, phase 2 study, Calabro' and colleagues investigated the use of tremelimumab (anti-CTLA-4 MAb) combined with durvalumab (anti-PD-L1 MAb) in patients with unresectable pleural (or peritoneal mesothelioma) that had declined or progressed after first-line chemotherapy. Of the 40 patients recruited, the majority of whom had pleural mesothelioma, 28% had partial response to therapy with a duration of 16.1 months and 65% had disease control with a median

duration of 10.6 months. Median PFS was 8 months and mOS was 16.6 months. Notably, 75% of participants had immune-related adverse events (irAEs), mainly involving the skin and gastrointestinal tract, with a smaller proportion affecting the endocrine, hematological, neurological, and renal systems [63]. Subsequently, another single center, phase 2 study by Venkatraman and colleagues, assessed again the combination of tremelimumab and durvalumab for patients that had previously received pemetrexed-based chemotherapy. Despite enrollment of 19 participants, they were unable to meet their primary endpoint of overall response rate (complete and partial response to therapy) as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, but they showed that the treatment combination was well-tolerated [64].

Disselhorst and colleagues demonstrated in the INITIATE trial – a prospective, single center, phase 2 trial – that the combination of ipilimumab and nivolumab, in patients with previously treated and/or recurrent MPM, led to disease control (either stable disease or partial response) at 12 weeks in 68% of the study participants with a median duration of response (time from start of response to progression) of 14.3 months. At 6-month follow-up, 50% of patients enrolled had continued disease control. As with previous combination trials, however, a large proportion of enrollees (94% of participants) experienced some irAEs with 23% requiring systemic corticosteroids [65]. Published in the same year, the MAPS2 trial by Scherpereel and colleagues - a multicenter, randomized, non-comparative, open label phase 2 trial - evaluated the role of nivolumab as monotherapy and of the combination of nivolumab and ipilimumab in patients with MPM progressing after first-line chemotherapy. The investigators showed a disease control rate at 12 weeks of 50% of those in the combined therapy group and of 44% in the monotherapy group, with a 1-year estimated survival of 79.2% and 58.1%, respectively. Most participants in the combination arm (94%) had mild treatment related AEs, but importantly three (5%) resulted in death, specifically from encephalitis, hepatitis, and acute renal failure in an end-of-life patient with progressive disease [6].

More recently, the open-label, multicenter, randomized, phase 3 study (CheckMate 743) compared the effect on OS of the combination of nivolumab and ipilimumab versus platinum-based chemotherapy in patients with previously untreated MPM. The combination immunotherapy group had a median OS of 18.1 months versus 14.1 months in the chemotherapy group (96.6% CI 0.60-0.91 p=0.0020) and an impressive 2-year survival rate of 41% versus 27%. The benefit also extended to 3 years with an OS rate of 23% with nivolumab plus ipilimumab versus 15% with chemotherapy. Patients with non-epithelioid histology (HR 0.46 [95% CI 0.31–0.68]) or PD-L1 tumor expression of 1% or higher (HR 0.69 [95% CI 0.55–0.87]) benefited more by the combination of nivolumab and ipilimumab in terms of OS than those with epithelioid subtype (HR 0.86 [95% CI 0.69–1.08]) or PD-L1 expression of less than 1% (HR 0.94 [95% CI 0.62–1.40]) [11].

Noteworthy, this regimen was generally well tolerated, with three treatment related deaths occurring secondary to pneumonitis, encephalitis, and heart failure, showing that moving the combinational treatment as front-line may also result in a better patients' compliance [11]. These studies paved the way for FDA approval as first-line treatment of unresectable MPM for this combination in October 2020. The treatment has been endorsed as first-line approach also in the recently published ESMO guidelines [66].

The large differences observed in patient responses across different trials still remains difficult to interpret by using the traditional biomarkers. In an attempt to solve this research question, Mankor and colleague evaluated participants' peripheral blood samples from the INITIATE and NivoMes trials (ClinicalTrial.gov identifier: NCT02497508) to characterize the immune cell response resulting from nivolumab monotherapy compared to nivolumab/ipilimumab combination ICI therapy. Unlike nivolumab alone, combination therapy resulted in proliferation and activation of more memory T-cell subsets independent of clinical response, and study participants who achieved better outcomes had a different T-cell distribution at baseline with more cytokine expressing terminally differentiated effector memory cells re-expressing CD45RA (EMRA) $CD8^+$ T cells and less naïve $CD8^+$ T cells [67]. Understanding this difference in immune response with single versus combined ICIs further supports additional study of combined immune pathway as targets for the treatment of MPM.

In addition to CTLA-4 blockade, animal models exploring PD-L1 ICI in combination with TIM-3 or lymphocyte activation gene product (LAG-3) blockade showed in-vivo survival advantage by targeting these tumor infiltrating lymphocyte receptors [68, 69], and safety studies are ongoing exploring these target combinations in the clinic in a variety of malignancies including mesothelioma (ClinicalTrial.gov identifier: NCT03219268). CA-170, an oral PD-L1, PD-L2 and VISTA checkpoint blocker recently completed a phase I trial (ClinicalTrial.gov identifier: NCT02812875) with a good safety profile, and phase 2 trials are pending to assess clinical efficacy of this drug [70].

Combination of immune check-point inhibitors with chemotherapy

Despite the advances with combinations of ICIs, chemotherapy is expected to remain the backbone of therapy for years to come especially in patients with epithelioid MPM, with cisplatin and pemetrexed being the drugs more commonly used and the only approved therapy for over a decade [1]. One of the problems with combined ICI-strategies (such as the combination of nivolumab and ipilimumab investigated in the Checkmate 743 trial) is the rapid drop-off in PFS in patients receiving only ICIs. A similar problem has been already observed in NSCLC and it has been circumvented by adding a few cycles of cytotoxic chemotherapy that may further induce immune activation [71]. Therefore, clinical development of the treatment strategies in first-line treatment of MPM is likely to follow the same trajectory.

As described previously, anti-PD-1 ICIs are safe and well tolerated when used in MPM, including patients previously treated with chemotherapy [72]. Durvalumab, an ICI against PD-L1, besides being safe, also showed signs of activity when used in combination with chemotherapy in the context of the DREAM trial [24]. In this study conducted in Australia, 54 MPM patients received cisplatin, pemetrexed, and durvalumab for a maximum of six cycles, followed by durvalumab maintenance for up to 12 months as first-line treatment. Six-month PFS resulted 57%, while objective response rate (ORR) and disease control rate were 48% and 87%, respectively. The recently published PrE0505 trial reported the effects of the same ICI-chemotherapy combination on OS. This phase 2 single arm study enrolled 55 patients of either MPM histology and at an average 24-month follow-up the median OS for participants was 20.4 months (compared to the historical 12-month median OS) with 44.2% of patients being alive at the end of the study. Those with epithelioid histology and with a higher tumor mutation burden were more responsive, both radiologically and in terms of OS [23]. Very recently, data from 18 MPM patients enrolled in the phase II JME-001 trial of first-line combination chemotherapy with nivolumab were also reported, showing an impressive rate of objective responses (77.8%; 95%CI 52.4%-93.6%) and disease control (94.4%; 95% CI 72.7%-99.9%). Grade 3 or worse AEs were experienced by 10 (55.6%) patients [73]. **Table 1** summarizes clinical outcomes and safety of clinical trials assessing the role of chemo-immunotherapy in first-line treated MPM patients. The ongoing DREAM3R (ClinicalTrial.gov identifier: NCT04334759) phase III trial will compare durvalumab in combination with standard chemotherapy versus chemotherapy alone helping to definitely clarify the role of this chemo-immunotherapy combination.

Table 1. Phase II clinical trials assessing the role of chemo-immunotherapy as first-line treatment in malignant pleural mesothelioma patients.

Trial	Drug	Chemotherapy backbone	Primary outcome	N	RR (%)	DCR (%)	PFS (mo)	OS (mo)	1-year OS	Grade ≥ 3 AEs
DREAM [24]	Durvalumab	Cisplatin/carboplatin - pemetrexed	6-mo PFS	54	48	87	6.9	18.4	65	53
PrE0505 [23]	Durvalumab	Cisplatin/carboplatin - pemetrexed	OS	55	56	93	6.7	20.4	70	65
JME-001 [73]	Nivolumab	Cisplatin - pemetrexed	RR	18	78	94	8.0	20.8	92	56

Abbreviations: RR, response rate; DCR, disease control rate; PFS, progression-free survival, OS, overall survival, AEs, adverse events.

3.3. Combination of immune check-point inhibitors with other biological targets

Therefore, combination of multiple ICIs (and probably with chemotherapy) can further improve survival in MPM with generally manageable safety profiles. However, the most effective combination of ICI for each patient remains probably to be determined, and expanding our pool of drugs beyond targeting T-cell function to include other mechanisms such as angiogenesis, cell adhesion, mesenchymal transition, or combining immune-stimulation or microbiome manipulation may prove to be beneficial [74].

To this regard, several trials are ongoing exploring non-immune checkpoint mAb with potential to aid ICIs in disease control. For example, the BEAT-meso (ClinicalTrial.gov identifier: NCT03762018) a multicenter, randomized phase 3 trial, is looking to enroll 400 participants to compare the OS using a combination of atezolizumab (anti-PDL1) with bevacizumab (anti-vascular endothelial growth factor [VEGF]) and chemotherapy versus chemotherapy and bevacizumab as first-line therapy. The combination of the antiangiogenic mABs (bevacizumab) with cisplatin and pemetrexed chemotherapy in treatment naïve MPM patients already proved to be effective in a phase 3 trial (MAPS study) done by Zalcman and colleagues that demonstrated a statistically significant increase in OS (18.8 months in the treatment group versus 16.1) [75]. However, bevacizumab has never been submitted for regulatory approval and other anti-angiogenic agents such as nintedanib and cediranib, both targeting VEGF, failed to produce a significant difference in OS when combined with standard chemotherapy and compared to placebo [76, 77]. Therefore, the BEAT-meso will finally investigate the potential of a unique combination of ICI with an anti-angiogenetic agents and standard chemotherapy, mixing T-cell and angiogenesis and lymphangiogenesis manipulation, all important elements in MPM carcinogenesis [78].

Clinical trials studying the combination of ICIs with additional targets relevant for MPM (mesothelin, VEGFR-2, ILT2/ILT4), bispecific antibodies (i.e, vudalimab), and T-cell activating proteins are ongoing (**Table 2**).

Combination of immune check-point inhibitors with local approaches

The evaluation of combined ICI therapy is not limited to additional biological targets, and combination with local approaches such as surgical resection is another avenue of active research. At the 2021 World Lung Cancer Conference (WCLC), Tsao and colleagues showed that neoadjuvant atezolizumab combined with cisplatin-pemetrexed and followed by either pleurectomy/decortication (P/D) or extrapleural pneumonectomy (EPP) is a safe strategy for resectable MPM, with sixty percent of eligible patients being able to proceed to maintenance atezolizumab [79]. A currently enrolling Phase I/II trial is looking to use nivolumab with or without ipilimumab as neoadjuvant therapy for surgically resectable MPM and determine its safety, feasibility as well as disease response (ClinicalTrial.gov identifier: NCT03918252).

In addition to direct tumor cell destruction via DNA injury, radiation therapy improves antigen presentation, upregulates inflammatory mediators and immunomodulatory cytokines with local and abscopal (i.e., distant from the irradiated field) antitumoral effects [80-82], thus an additive therapeutic effect may be seen when combined with ICI. Animal models using CTLA4 and PD-1 checkpoint blockade combined with radiotherapy have shown promising preclinical results with evidence of effector T cell activation and downregulation of Tregs [83-85]. While clinical data is very limited, local and abscopal anti-tumoral response with sequential radiation therapy and pembrolizumab has been described. However, there is reasonable concern that the immune potentiating effects of these therapies may increase the incidence of irAEs [86, 87]. Early phase trials are ongoing to evaluate stereotactic body radiation therapy (SBRT) in combination with ICI (ClinicalTrial.gov identifiers: NCT04926948, NCT03399552) also in mesothelioma.

Table 2. Ongoing interventional studies involving combination of immune checkpoint inhibitors, local approaches, cellular therapy, and vaccines.

ClinicalTrial.gov identifier	Drug or intervention combination	Phase	Design	Center	Planned participant enrollment	Expected completion date
Combined ICI trials						
NCT03762018	Bevacizumab (anti-VEGF) Atezolizumab (anti-PD-L1).	Phase 3	Randomized, parallel assignment, open label.	Multicenter, 42 sites.	400	January 31, 2024
NCT03126630	Pembrolizumab (anti-PD1) Anetumab ravtansine (anti-mesothelin conjugated to maytansinoid DM4).	Phase 1	Randomized, crossover assignment, open label.	Multicenter, 33 sites.	110	February 2, 2022
NCT03517488	Vudalimab XmAb®20717 (anti PD-1 and anti-CTLA-4).	Phase 1	Sequential assignment, open label.	Multicenter, 17 sites.	154	February 28, 2023
NCT03872206	HPN536 (T-cell–activating protein-based construct, which binds to MSLN-expressing tumor cells, CD3ε on T cells, and to serum albumin).	Phase 1/2a	Non-randomized, sequential assignment, open label.	Multicenter, 14 sites.	180	May 1, 2022
NCT03918252	Neoadjuvant nivolumab (anti-PD-1) Ipilimumab (anti- CTLA-4).	Phase 1/2	Non-randomized sequential assignment, open label.	Multicenter, 3 sites.	30	June 2026
NCT03074513	Atezolizumab (anti-PD-L1) Bevacizumab (anti-VEGF).	Phase 2	Single group assignment, open label.	Single center.	164	March 31, 2021
NCT03502746	Nivolumab (anti-PD-1) Ramucirumab (anti- VEGFR-2).	Phase 2	Single group assignment, open label.	Multicenter, 4 sites.	35	June 2023
NCT04840615	lmb-100 (Anti-mesothelin immunotoxin) Ipilimumab (anti- CTLA-4).	Phase 1	Single group assignment, open label.	Single center.	20	November 30, 2025
NCT04913337	NGM707 (ILT2/ILT4 dual antagonistic antibody) as monotherapy and combined with pembrolizumab (anti-PD-1).	Phase 1/2	Non-randomized, factorial assignment, open label.	Multicenter, 5 sites.	179	July 2025
NCT04013334	Nivolumab (anti-PD-1) and MTG201 (adenoviral therapy).	Phase 2	Single group assignment, open label.	Single site	12	December 20, 2021

Table 2. Continued.

ClinicalTrial.gov identifier	Drug or intervention combination	Phase	Design	Center	Planned participant enrollment	Expected completion date
NCT03219268	MGD012 (Bispecific DART® Protein Binding PD-1 and LAG-3).	Phase 1	Single group assignment, dose escalation followed by cohort expansion at the maximum tolerated dose.	Multicenter, 38 sites.	353	July 2022
NCT04040231	Galinpepimut-S and Nivolumab (anti-PD-1).	Phase 1	Single group assignment, open label.	Multicenter, 6 sites.	10	July 2023
NCT04914897	SAR444245 (THOR-707, pegylated IL-2) with other therapies (including pembrolizumab anti-PD-1).	Phase 2	Non-randomized, parallel assignment, open label.	Multicenter, 21 sites.	354	July 2025
Radiation and surgery trials						
NCT03918252	Nivolumab (anti-PD-1) with or without ipilimumab (anti-CTLA-4) prior to surgery.	Phase 1 /2	Non-randomized, sequential assignment, open label.	Multicenter, 3 sites.	30	June 2026
NCT04162015	Nivolumab (anti-PD-1) with pemetrexed and cisplatin or carboplatin prior to surgery.	Phase 1	Single group assignment, open label.	Multicenter, 7 sites.	35	November 2023
NCT02707666	Pembrolizumab (anti-PD-1) in surgically resectable MPM.	Phase 1	Single group assignment, open label.	Single center.	15	December 2025
NCT04926948	Focal SBRT with ICI (specific immunotherapy up to oncologist).	Phase 1	Single group assignment, open label.	Single center.	20	May 2026
NCT03399552	SBRT with avelumab (anti-PD-L1).	Phase 1/2	Single group assignment, two stage study.	Multicenter, 7 participating sites.	15	December 2022
NCT02959463	Pembrolizumab (anti-PD-1) after radiation therapy..	Phase 1	Non-randomized, parallel assignment, open label.	Single center	24	May 2021 (ongoing recruitment)

Table 2. Continued.

ClinicalTrial.gov identifier	Drug or intervention combination	Phase	Design	Center	Planned participant enrollment	Expected completion date
Cell therapy trials						
NCT03610360	MesoPher (dendritic cells loaded with allogeneic tumor cell lysate).	Phase 2/3	Randomized, parallel assignment, open label.	Multicenter, 6 sites.	230	February 2023
NCT04577326	MSLN targeted CAR-T cells.	Phase 1	Single group assignment, dose escalation trial.	Single center	30	September 2023
NCT03054298	Lentiviral transduced huCART-meso cells.	Phase 1	Non-randomized, parallel assignment, open label.	Single center	27	March 2025
NCT03907852	TC-210 (Gavocabtagene autoleucel, autologous genetically engineered T cells with single-domain antibody fused to the CD3ε subunit against MSLN).	Phase 1/2	Non-randomized, single group assignment, open label.	Multicenter, 7 sites.	70	January 2023
NCT03935893	Adoptive transfer of TIL.	Phase 2	Single group assignment, open label.	Single center	10	June 2030
Vaccine trials						
NCT04525859	poly-ICLC (Hiltonol®).	Phase 1	Non-randomized, parallel assignment, open label.	Single center	19	August 2024
NCT01503177	MV-NIS (modified vaccine strain measles virus genetically engineered to produce human thyroidal sodium iodine symporter).	Phase 1	Single group assignment, open label.	Single center	15	April 2019
NCT03710876	Intrapleural administration of rAd-IFN in combination with celecoxib and gemcitabine.	Phase 3	Randomized, parallel assignment, open label.	Multicenter, 42 participating sites.	53	November 2024

Abbreviations: *CAR-T* – chimeric antigen receptor T-cell, *CTLA-4* – cytotoxic T-lymphocyte associated protein 4, *huCART* – human chimeric antigen receptor modified T-cells, *ICI* – immune checkpoint inhibitor, *IL* – interleukin, *ILT* – Ig-like transcript, *LAG-3* – lymphocyte activation gene 3, *MPM* – malignant pleural mesothelioma, *MSLN* – mesothelin, *PD-1* – programmed cell death protein 1, *PD-L1* – programmed death-ligand 1, *rAd-IFN* - adenovirus-delivered Interferon Alpha-2b, *SBRT* – stereotactic body radiation therapy, *TIL* – tumor infiltrating lymphocytes, *VEGF* – vascular endothelial growth factor, *VEGFR* – vascular endothelial growth factor receptor.

New avenues: vaccines and cellular immunotherapies

Therapeutic cancer vaccines utilize TAAs as targets for T-cell activation to overcome the immune suppressant TIME through diverse platforms and with multiple potential targets, some potentially useful in MPM. This may complement not only traditional chemotherapy but also ICIs (**Table 2**) [88, 89].

Dendritic cell therapy

One platform utilizes autologous DCs, by exposing them to tumor lysates and generating a cytotoxic T-cell response to the target cancer using these antigen presenting cells (APCs). In 2010, Hegmans and colleagues published the first DC therapy use in humans for MPM. Participants received three immunizations with mature DCs that were previously exposed to autologous tumor lysate. Three out of the ten participants showed a partial response to immunotherapy while one had disease stabilization. Overall, therapy was well tolerated [54]. Since ICIs lead to a clinical response in a small proportion of patient partly due to a low tumor-infiltrating $CD8^+$ T-cell population, a population that can be induced by DCs sensitized to TAAs, DC immunotherapy is a promising companion/alternative to ICIs for MPM [4, 90, 91]. The subsequent study was published in 2015, this time combining DC immunotherapy with cyclophosphamide to reduce tumor induced immune suppression: out of ten participants, 8 had disease control and 7 out of 10 had survived for at least two years [92]. In 2018, unlike the prior two studies, DCs sensitized with allogeneic tumor lysate were used in 9 MPM patients, resulting in a median PFS of 8.8 months with OS approaching 2 years [55]. If these results are supported in the ongoing DENIM (ClinicalTrial.gov identifier: NCT03610360) trial, it would make DC therapy more accessible, as it would likely eliminate the need to obtain an autologous tumor lysate to generate APC useful for a cytotoxic response. The DENIM trial is a multicenter phase II/III study that will compare allogenic tumor lysate sensitized DCs as maintenance treatment after first-line chemotherapy versus chemotherapy alone, at the same time analyzing safety and tolerability [93].

Adoptive T cell transfer

Using CAR T-cells may be also a therapeutic option for MPM and other malignancies. These T cells, following introduction of CARs, bind specific TAAs tailored to a specific malignancy. Combining this therapy with ICI may circumvent the issue of T-cell exhaustion limiting efficacy of CAR T-cells in solid malignancies like MPM [94]. While several CAR T-cell targets have been proposed (e.g., ErbB2, 5T4, CSPG4) [56, 95-101], the majority of clinical trials have focused on targeting mesothelin. Adusumilli and colleagues administered intrapleural mesothelin-targeted CAR T-cells in addition to the anti-PD1 agent pembrolizumab to 18 patients with MPM; this resulted in a median OS of 23.9 months, with 8 participants achieving disease stability for over 6 months and 2 participants a complete response on positron emission tomography (PET)

imaging [102]. A phase I trial, currently recruiting participants, aims to assess the safety of genetically engineered autologous T-cells targeting mesothelin that also have a cell-intrinsic anti-PD1 component (ClinicalTrial.gov identifier: NCT04577326). A different phase I trial is exploring the safety of both local and intravenous administration of lentiviral transduced huCART-meso cells (human CAR T-cells targeting mesothelin) for MPM and other mesothelin expressing malignancies like lung adenocarcinoma, sierous epithelial ovarian cancer, among others; recruitment is ongoing (ClinicalTrial.gov identifier: NCT03054298). In addition to mesothelin, fibroblast activation protein (FAP) as a target for this therapeutic modality is also promising, with locally delivered anti-FAP CAR T-cells being well tolerated after first line chemotherapy and with ICI blockade in a small safety study [103].

Other approaches

In addition to ICI combinations and the previously described cell-based therapy, other therapeutic approaches, e.g., vaccine-based therapies to enhance the cellular immune response to MPM are being evaluated. Locally delivered (intrapleural) viral based therapy is proving to be a safe therapeutic option. HSV1716 is an oncolytic herpes simplex virus that in a phase I/IIa trial was associated with disease stability in half of the MPM patients 8 weeks after therapy meeting the primary objective of safety with only mild AEs reported (mainly fatigue and fever) [104]. A phase I trial assessing dosing and safety of a oncolytic measles virus MV-NIS was recently completed (ClinicalTrial.gov identifier: NCT01503177) and published data reports an adequate safety profile and disease stability in 67% of the 12 participants [105]. The effect on OS in patients with MPM after local administration of adenovirus-delivered IFN-α-2b with celecoxib and chemotherapy is being evaluated in the INFINITE trial, a phase 3 randomized clinical trial (ClinicalTrial.gov identifier: NCT03710876), building on prior pilot studies showing an excellent safety profile and anti-tumoral effect [106, 107]. Poly-ICLC (Hiltonol®) is a syntetic dsRNA viral mimic that increases antitumoral cellular response by inducing IFNγ production leading to cytotoxic cytokine release by $CD8^+$ T-cells and circumventing resistance ICIs as IFNγ also induces expression of PD-1 and PD-L1 on T cells and cancer cells. [108, 109]. The study of poly-ICLC use in MPM is currently ongoing (ClinicalTrial.gov identifier: NCT04525859).

Wilms tumor-1 (WT1) protein is overexpressed in MPM, unlike in normal tissues. Synthetic immunogenic peptides eliciting a heteroclitic response (i.e. variable antibody binding) have been developed and are undergoing study as therapeutic options for cancer. Galinpepimut-S is one of those peptides and it is made out of molecules similar to those in the WT1 protein. When used in MPM, it showed a median PFS of 10.1 months (95% CI, 5.5–20.8 months) and median OS of 22.8 months (95% CI, 9.1–37.6 months) [110]. Currently, this vaccine is being studied in combination with nivolumab (ClinicalTrial.gov identifier: NCT04040231).

Isolating autologous tumor-infiltrating lymphocytes (TILs), expanding them ex-vivo, and later infusing them back to the patient following lympho-depleting chemotherapy is another way to boost the immune response by avoiding $CD8^+$ T-cell exhaustion [111]. With evidence primarily for melanoma, autologous TIL may play a role in patients that fail ICI therapy [112], and a phase II study of adoptive cell transfer (ACT), is recruiting participants to evaluate its use in solid tumors, including MPM (ClinicalTrial.gov identifier: NCT03935893).

Conclusion

After two decades of disappointing results, a new era in the treatment of mesothelioma has just started in which clinical scientists will be finally able to talk about treatment sequences with their patients. The potential to combine ICIs with other immunotherapies, as well as targeted agents and old-school chemotherapy needs to be fully unleashed and expectations are now high that this will lead to a plethora of new treatment options and eventually to cure some mesothelioma patients. In order to achieve that, it is critical to couple clinical research with translational investigations and to strengthen multi-institutional collaboration in the conduction of well-designed, biomarker-driven clinical trials, finally tackling disparities and delays that are usually encountered in cancer of rare incidence. The adoption of more holistic approach, which looks at different facets of MPM patients and takes into account spatial and temporal heterogeneity of this disease, is the final needed step to move further the clinical development of immunotherapy in MPM.

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CHAPTER 4

Safety of Extended interval Dosing Immune Checkpoint Inhibitors: a multicentre cohort study

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Abstract

Background: Real-life spectrum and survival implications of immune-related adverse events (irAEs) in patients treated with extended interval dosing (ED) immune checkpoint inhibitors (ICIs) are unknown.

Methods: Characteristics of 812 consecutive solid cancer patients who received at least one cycle of ED monotherapy (pembrolizumab 400 mg Q6W or nivolumab 480 mg Q4W) after switching from canonical interval dosing (CD, pembrolizumab 200 mg Q3W or nivolumab 240 mg Q2W) or *upfront* were retrieved. Primary objective was to compare irAEs patterns within the same population (before and after switch to ED). irAEs spectrum in patients treated *upfront* with ED and association between irAEs and overall survival (OS) were also described.

Results: 550 (68%) patients started ICIs with CD and switched to ED. During CD, 225 pts (41%) developed any grade and 17 (3%) G3/G4 irAEs; after switching to ED, any grade and G3/G4 irAEs were experienced by 155 (36%) and 20 (5%) patients. Switching to ED was associated with a lower probability of any grade irAEs (adjusted odds ratio [aOR]: 0.83; 95% CI: 0.64-0.99; p=.047), while no difference for G3/G4 events was noted (aOR: 1.55; 95% CI: 0.81-2.94; p=.18). Among patients who started *upfront* with ED (n=232, 32%), 107 (41%) developed any grade and 14 (5%) G3/G4 irAEs during ED. Patients with irAEs during ED had improved OS (aHR: 0.53; 95% CI 0.34-0.82; p=.004 after switching; aHR: 0.57; 95% CI 0.35-0.93; p=.025 *upfront*).

Conclusions: Switching ICI treatment from CD and ED did not increase the incidence of irAEs and represents a safe option also outside clinical trials.

Background

Immune checkpoint inhibitors (ICIs) have deeply changed clinical practice in the field of medical oncology. Despite their first introduction as traditional body weight-based dosing regimens, simulation pharmacokinetics studies demonstrated that weight provides only a marginal contribution to ICIs physiological distribution, therefore ICI flat doses became the standard (1–3).

Recently, long life expectancy of patients treated with ICIs, high healthcare costs, and the need to reduce avoidable hospital admissions during COVID-19 crises, led to an increasing interest in alternative longer dosing schedules. According to clinical trials data, adoption of extended interval dosing (ED) ICIs, pembrolizumab 400 mg Q6W and nivolumab 480 mg Q4W, offers similar outcomes and safety compared with canonical interval dosing (CD) schedules (200 mg Q3W and 240 mg Q2W, respectively) (4–7). This makes the pair with economic and logistic advantages provided by ED ICIs which seem to be unquestionable. Although in the real-life setting an increasingly wide percentage of patients have been shifted to (or treated *upfront* with) ED ICIs, incidence, clinical patterns, and survival implications for patients who develop immune-related adverse events (irAEs) during ED ICIs, is unknown. In a recent study involving 45 patients with advanced non-small cell lung cancer (NSCLC), the switching of pembrolizumab from CD to ED resulted in the manifestation of different and worsening irAEs (8). In this multicenter cohort study, we aim to provide further insights on this topic by (1) investigating the safety of switching the ICI interval dosing from CD to ED across multiple cancer types and different indications (2) characterizing the spectrum of irAEs in cancer patients treated *upfront* with ED ICIs and (3) describing the association between irAEs and overall survival (OS) in ED treated patients.

Patients and methods

Study design and population

To investigate the primary objective of our study, which was to characterize incidence and spectrum of irAEs in patients switched to ED ICIs and compare them with those before switching (during CD ICI treatment), we designed the multicentre EDICI ("Extended interval Dosing in patients receiving Immune Checkpoint Inhibitors") study. Patients with a diagnosis of malignancy undergoing treatment with ICIs as monotherapy (namely pembrolizumab and nivolumab) for an approved oncological indication between April 2015 and December 2021 were retrospectively identified from electronic medical records at 30 European Oncological Departments (**Supplementary Table 1**) and entered into a prospectively maintained database. Patients were included if they were ≥ 18 years of age, if they were switched from the CD (pembrolizumab 2 mg/Kg or 200 mg Q3W and nivolumab 3 mg/Kg or 240 mg Q2W) to the ED (pembrolizumab 400 mg Q6W and nivolumab 480 mg Q4W) of the same ICI (first switch reported in May 2018), or if they had started upfront with ED (first *upfront* ED treatment reported in May 2018). This allowed us to compare irAEs patterns within the same population (before and after switch to ED) but also to describe the irAEs spectrum in cancer patients treated *upfront* with ED ICIs.

irAEs were evaluated according to Common Terminology Criteria for Adverse Events (version 5) and further defined according to the organ or system involved as follows based on previous retrospective studies (9,10) and Society for Immunotherapy of Cancer (SITC) guidelines (11): thyroiditis, diarrhea/colitis, endocrine (excluding thyroid disorders), hepatitis, neurologic, arthralgia, asthenia (or fatigue), dermatitis, pneumonitis, others (cardiac, pyrexia, anorexia, renal, hematologic, rheumatic other than arthralgia/arthritis, pulmonary other than pneumonitis, gastrointestinal other than diarrhea/colitis). Investigators assigned the respective irAE to the patient after excluding other alternative diagnosis, based on multidisciplinary evaluation, clinical benefit after ICI discontinuation and/or immunosuppressive treatment, or pathologic evidence of irAE. Multi-system irAEs were defined as irAEs involving more than one organ system. irAEs data were collected until death or date of last contact if patients were still alive or lost at follow-up. The data cutoff period was March 2022.

The following clinicopathological and treatment characteristics were also collected at start of upfront CD/ED: age, gender, weight, height, smoking status, past medical and family history, Eastern Cooperative Oncology Group Performance Score (ECOG PS), concomitant medications, tumor type/histology, driver mutations, treatment setting, number and site of metastasis, previous local and systemic treatments.

To switch from CD to ED, a patient must have survived until that point, and no events ("deaths") can be expected before. Therefore, OS was calculated as time from ED

ICI start (after switching for patients who received upfront CD) until death from any cause; patients still alive at the time of data cut-off (March 2022) were censored at the date of last contact. Ethical approval to conduct this study was obtained by the respective local ethical committees on human experimentation of each participating center, after previous approval by the coordinating center ('Comitato Etico Regionale delle Marche - C.E.R.M.', Reference Number 2021 389). All study related procedures and data collection were conducted in accordance with the Declaration of Helsinki and in accordance with Good Clinical Practice.

Statistical analysis

Clinicopathological characteristics were presented using count and percentage for categorical variables, median, and range for continuous variables. McNemar test was used to compare irAEs onset before and after switch to ED. In order to adjust for exposure time (represented by number of cycles) that may affect the chance of irAEs onset, nested logistic regressions with intraclass correlation correction between different ICI interval dosing on the same patient was used. More precisely, since different treatment schedules of the same patients become part of the model, a nested model has been implemented to avoid the risks associated with non-independence. In other words, this approach avoids the bias in direct comparisons of coefficients across models related to the scale changes that accompany changes in the set of explanatory variables. A sensitivity analysis was also performed stratifying patients by tumor type.

OS curves were plotted using the Kaplan-Meier method and differences in probability of surviving between the strata were evaluated by log-rank (Mantel-Cox) test. As the incidence of irAEs is "time-dependent" (12,13), those patients interrupting ICI treatment quickly were exposed to the potential "triggering effect" for a shorter time, and had a lower risk of experiencing irAEs. For minimizing the immortal time bias a landmark method was then used, and all patients who died before 3 months were excluded from the OS analysis. The cut-off point of 3 months was chosen to evaluate the impact of both early and late onset irAEs, as median time to onset of irAEs usually ranges between 2 and 16 weeks from ICIs start. Among patients who switched to ED, 39 were excluded from the 12-week landmark analysis because of death before the 3-month cut-off; 39 patients among those who started *upfront* with ED were also excluded from the 12-week landmark analysis. To evaluate the association of irAEs onset with OS independent of other clinicopathological factors, a multivariable proportional hazard regression model was built.

Data for this study were collected in a REDCap® (Research Electronic Data Capture) database, and analyses were conducted using R (version 4.0.3; R Foundation). All P values are two-sided and confidence intervals (Cis) are at the 95% level, with statistical significance defined as $P \leq .05$.

Results

Patient characteristics

A total of 835 patients were enrolled in the EDICI study. Among these, 812 were included in the final safety analysis (**Figure 1**). ICI treatment was represented by nivolumab in 540 (66.5%) cases and pembrolizumab in 272 (33.5%). The most common tumor types were melanoma (n=456, 56.2%) and NSCLC (n=204, 25.1%), with 663 (81.6%) patients being treated in the advanced/metastatic setting.

Among the enrolled patients, 550 (67.7%) started ICIs with CD and subsequentially switched to ED. The exposure time was similar, with a median number of 13 CD cycles and 7 ED cycles (1 cycle of ED corresponding to 2 cycles of CD in terms of exposure time). Main reason for switching to ED was physicians' choice (n=465, 84.6%), while 73 patients (13.2%) requested to switch. The remaining patients (n=262, 32.3%) started *upfront* with ED and were exposed to the drug for a median of 7 cycles.

At a median follow up of 24.8 (95% CI: 23.0-26.4) months, median OS was 67.2 (95% CI: 56.2-not reached [NR]) months in the whole cohort. Among all 812 patients, 368 (45.3%) experienced 1 or more irAEs regardless of the treatment schedule, including 52 (6.4%) G3 to G4 irAEs.

The clinical baseline characteristics of the whole cohort, stratified by tumor type, treatment initiation (*upfront* CD vs *upfront* ED), and irAEs onset are outlined in **Table 1**, **Supplementary Table 2 and 3**.

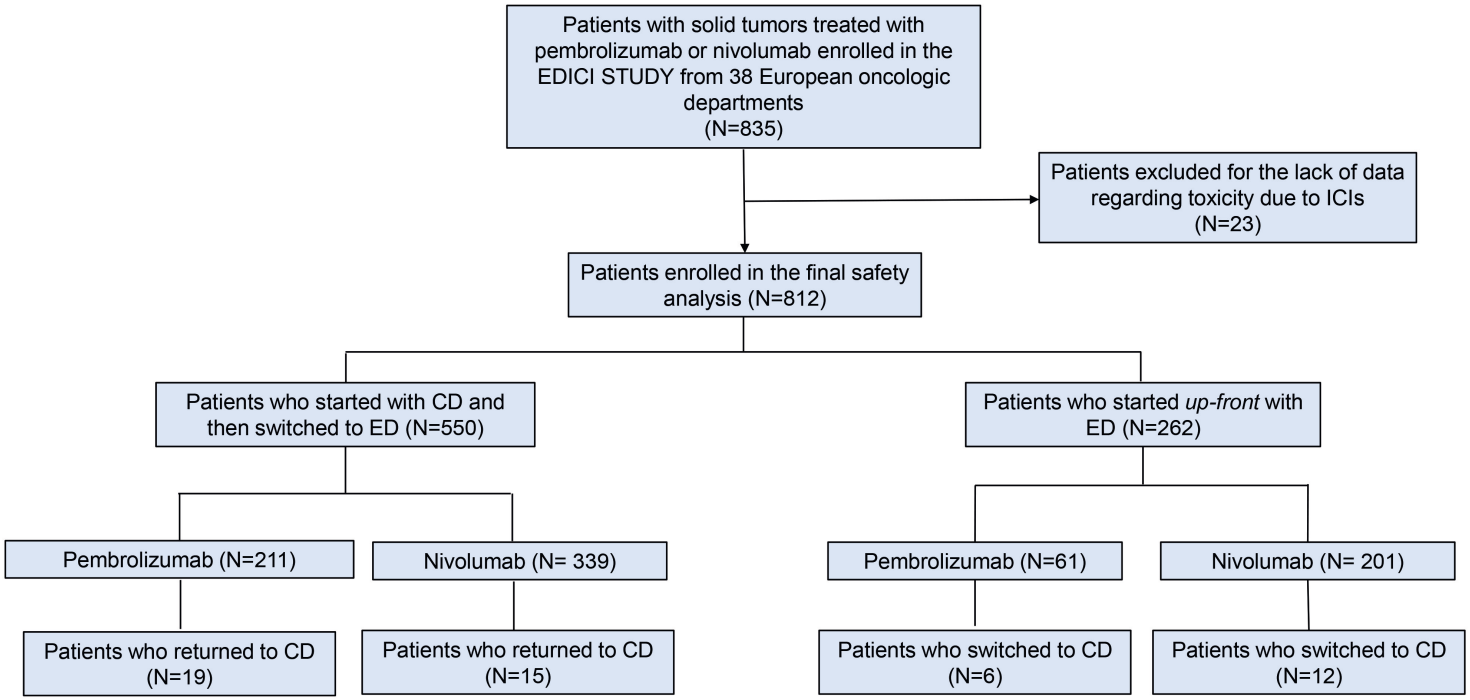

Figure 1. STROBE diagram of the EDICI study. ICIs, immune checkpoint inhibitors.

Table 1. Baseline clinical characteristics by tumor type.

Characteristic	NSCLC (%)	Melanoma (%)	Renal (%)	Other (%)
No. (n = 812)	204 (25.1)	456 (56.2)	141 (17.4)	11 (1.3)
Age, median (range), y	68 (43-85)	67 (26-94)	67 (43-86)	68 (61-81)
Gender				
Female	73 (35.8)	184 (40.4)	31 (22)	1 (9.1)
Male	131 (64.2)	272 (59.6)	110 (78)	10 (90.9)
ECOG-PS				
0-1	181 (88.7)	439 (96.3)	134 (95)	11 (100)
≥ 2	22 (10.8)	16 (3.5)	7 (5)	0
Unknown	1 (0.5)	1 (0.2)	0	0
Smoking status				
Current	61 (29.9)	59 (12.9)	15 (10.6)	3 (27.3)
Former	128 (62.7)	119 (26.1)	72 (51.1)	6 (54.5)
Never	15 (7.4)	278 (61)	54 (38.3)	2 (18.2)
Treatment setting				
First line	138 (67.6)	232 (50.9)	8 (5.7)	2 (18.2)
≥ 2nd line	66 (32.4)	75 (16.4)	133 (94.3)	9 (81.8)
Adjuvant	0	149 (32.7)	0	0
Number of metastatic sites[a]				
< 2	61 (29.9)	86 (28)	23 (16.3)	1 (9.1)
≥ 2	119 (58.3)	180 (58.6)	111 (78.7)	9 (81.8)
Unknown	24 (11.8)	41 (13.4)	7 (5)	1 (9.1)
Surgery[b]				
Yes	34 (16.7)	393 (86.2)	121 (85.8)	7 (63.6)
No	170 (83.3)	63 (13.8)	20 (14.2)	4 (36.4)
Concomitant radiotherapy[c]				
Yes	37 (18.1)	85 (18.6)	33 (23.4)	1 (9.1)
No	166 (81.4)	370 (81.1)	107 (75.9)	10 (90.9)
Unknown	1 (0.5)	1 (0.3)	1 (0.7)	0
Type of ICI				
Pembrolizumab	169 (82.8)	87 (19.1)	5 (3.5)	11 (100)
Upfront CD	138 (67.6)	62 (13.6)	3 (2.1)	8 (72.7)
Upfront ED	31 (15.2)	25 (5.5)	2 (1.4)	3 (27.3)
Nivolumab	35 (17.2)	369 (80.9)	136 (96.5)	0
Upfront CD	34 (16.7)	208 (45.6)	97 (68.8)	0
Upfront ED	1 (0.5)	161 (35.3)	39 (27.7)	0
irAEs onset				
Yes	95 (46.6)	252 (55.3)	60 (42.6)	4 (36.4)
No	109 (53.4)	204 (44.7)	81 (57.4)	7 (63.6)

[a]Percentage calculated on the number of patients with metastatic cancer. [b]Surgery refers to resection of primitive tumor or metastatic site or both. [c]Radiotherapy concomitant to ICIs refers to primitive tumor or metastatic site or both.
CD, canonical interval dosing; ECOG-PS, Eastern Operative Oncology Group-Performance status; ED, extended interval dosing; ICI, immune checkpoint inhibitor; irAEs, immune related adverse events, NSCLC, non-small cell lung cancer.

Spectrum and comparison of irAEs in patients who switched from CD to ED

Among patients who started with CD ICI and subsequentially switched to ED (n=550), 225 pts (40.9%) developed irAEs of any grade and 17 patients (3.1%) had G3/G4 events during CD; once they switched to ED-ICI, irAEs of any grade and G3/G4 events were experienced by 179 (37.1%) and 23 (4.8%) patients, respectively (p=.09 for any grade irAEs and p=.11 for G3/G4 irAEs). After adjusting for exposure time in a multivariable nested logistic regression model, ED treatment was associated with a lower probability of irAEs of any grade (adjusted odds ratio [aOR]: 0.83; 95% CI: 0.64-0.99; p=.047), while no difference in the likelihood of experiencing G3/G4 events was noted (aOR: 1.55; 95% CI: 0.81-2.94; p=.18). Sensitivity analysis stratified by tumor type showed that melanoma patients had lower risk of any grade irAEs after switching to ED (aOR: 0.59; 95% CI: 0.41-0.85; p=.005) and similar risk of G3/G4 irAEs (aOR: 1.06; 95% CI: 0.43-2.60; p=.89). No difference between CD and ED was noted in NSCLC patients, either in terms of any grade (aOR; 1.31; 95% CI: 0.80-2.12; p=.27) or G3/G4 irAEs (aOR: 2.97; 95% CI: 0.73-11.98; p=.12).

Noteworthy, 78 out of 179 (44.6%) cases of any grade irAEs and 12 (52.2%) of G3/G4 irAEs during ED represented *de novo* toxicity, meaning that patients had not experienced any irAEs during CD. In a subgroup of patients, any grade (n=77 out of 179, 43%) or G3/G4 (n=7 out of 23, 30.4%) irAEs arised after only one ED administration. Thirty-four (6.2%) patients switched back to CD, and main reason for returning to CD was toxicity (n=15, 44.1%).

The most common irAEs (any grade) in patients during CD ICI were dermatitis (n=77, 14%), thyroiditis (n=69, 12.6%), and asthenia (n=57, 10.4%) (**Figure 2** and **Supplementary Table 4** for stratification by tumor type); the spectrum of irAEs did not change after switching to ED, either at time of first switching (after first ED administration, **Supplementary Table 5** for stratification by tumor type) or long-term, with dermatitis (n=62, 12.8%, p=.24), asthenia (n=53, 10.9%, p=.71) and diarrhea/colitis (n=46, 9.5%, p=.77) being the most common irAEs (any grade) (**Figure 2** and **Supplementary Table 6** for stratification by tumor type); also looking at more worrisome toxicities such as pneumonitis (p=.45) and hepatitis (p=.12), no statistically significant differences were noted after switching; 104 (18.9%) patients developed multisystem irAEs during CD, and 79 (16.4%) after switching to ED (p=.21), with the difference being statistically significant after adjusting for the number of cycles administered (ED vs CD, aOR: 0.80; 95% CI: 0.58-0.99; p=.049).

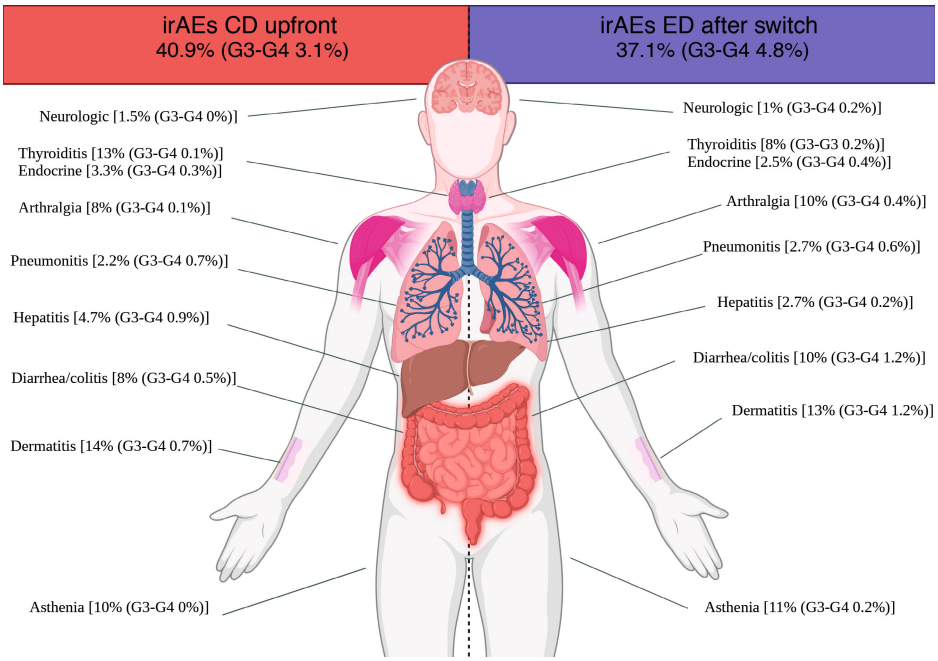

Figure 2. Spectrum of irAEs for cancer patients before (during *upfront* CD) and after switch to ED, overall and per organ/system involved. irAEs, Immune-related adverse events; CD, canonical interval dosing; ED, extended interval dosing.

Spectrum of irAEs in patients who started *upfront* with ED

Among patients who started *upfront* with ED (262), 107 of them (40.8%) developed irAEs of any grade and 14 (5.3%) G3/G4 irAEs during ED. Only 18 (6.8%) switched to CD, mainly due to toxicity (7 [38.8%]).

Patients who started *upfront* with ED experienced dermatitis (32 [12.2%]), diarrhea/colitis (32 [12.2%]) and thyroiditis (26 [9.9%]) as most common irAEs (any grade) (**Supplementary Table 7** for stratification by tumor type). Any-grade pneumonitis and hepatitis were observed in 16 (6.1%) and 12 (4.6%) cases during upfront ED, while multisystem irAEs were registered in 30 cases (11.4%).

Association between irAEs onset and survival

Patients who developed irAEs during ED also had longer OS compared to the no irAEs group. Among patients who switched to ED and were included in the landmark analysis (n=444), median OS was NR (95% CI: NR-NR) in the irAEs group versus 40.4 months (95% CI: 26.4-NR) in the no irAEs group (p=.005). Among patients who started *upfront* with ED and were included in the landmark analysis (n=223), median OS was 34.2 months (95% CI: 19.1-NR) in the irAEs group versus 23.4 months (95% CI: 17.4-27.1) in the no irAEs group (p=.01) (**Figure 3**). This association between irAEs onset and OS

was confirmed in a multivariable model which included tumor type, treatment setting and ECOG PS as other variables (adjusted hazard ratios [aHR]: 0.53; 95% CI: 0.34-0.82; p=.004 and aHR: 0.57; 95% CI; 0.35-0.93; p=.02, respectively, **Table 2 and Table 3**).

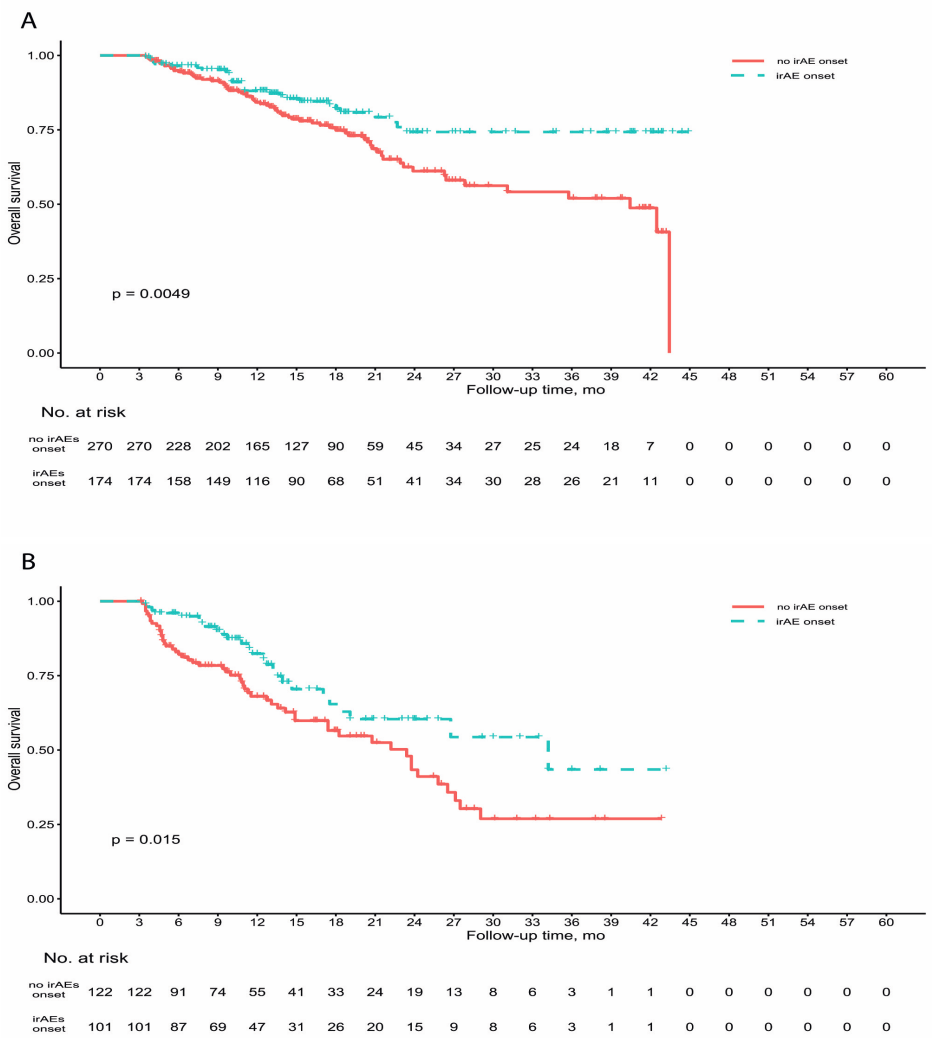

Figure 3. Overall survival stratified by irAEs onset in patients who switched from CD to ED (A) and in patients treated *upfront* with ED ICIs (B). Overall survival in Figure 3 was calculated since start of ED treatment. Landmark method was used to correct for immortal time bias (all patients who died before 3 months were excluded from the analysis). CD, canonical interval dosing; ED, extended interval dosing; ICI, Immune checkpoint inhibitors; irAEs, Immune-related adverse events.

Table 2. Multivariable Cox regression model of overall survival in patients who switched from CD to ED ICIs.

Characteristic	Hazard ratio (95% CI)	p value
irAEs onset		
No	Ref	
Yes	0.52 (0.33 - 0.81)	.004
Tumor type		
NSCLC	Ref	
Melanoma	1.24 (0.71 - 2.18)	.43
Renal	0.99 (0.55 - 1.77)	.97
Other	1.49 (0.34 - 6.45)	.58
Treatment setting		
Adjuvant	Ref	
Advanced/metastatic	1.03 (0.50 - 2.11)	.92
ECOG PS		
0	Ref	
≥1	3.29 (2.14 - 5.06)	<.001

Overall survival was calculated since start of ED treatment. Landmark method was used to correct for immortal time bias (all patients who died before 3 months were excluded from the analysis).
CD, canonical interval dosing; ED, extended interval dosing; ICIs, immune checkpoint inhibitors; irAEs, immune-related adverse events; NSCLC, non-small cell lung cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Score.

Table 3. Multivariable Cox regression model of overall survival in patients treated *upfront* with ED ICIs.

Characteristic	Hazard ratio (95% CI)	p value
irAEs onset		
No	Ref	
Yes	0.57 (0.35 – 0.93)	.02
Tumor type		
NSCLC	Ref	
Melanoma	1.16 (0.58 – 2.33)	.66
Renal	0.51 (0.22 – 1.14)	.10
Treatment setting		
Adjuvant	Ref	
Advanced/metastatic	7.66 (1.84 – 31.89)	.005
ECOG PS		
0	Ref	
≥1	1.74 (1.08 – 2.79)	.02

Landmark method was used to correct for immortal time bias (all patients who died before 3 months were excluded from the analysis).
ED, extended interval dosing; ICIs, immune checkpoint inhibitors; irAEs, immune-related adverse events; NSCLC, non-small cell lung cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Score.

Discussion

The findings of this international multicenter pan-cancer cohort study suggest that switching from CD to ED during ICI treatment did not worsen the safety profile. After switching to ED, any grade and G3/G4 irAEs occurred in 37.1% and 4.8% of patients respectively, and only 6.2% of patients returned to CD it. Dermatitis, asthenia, and diarrhea/colitis were the most common irAEs after swiching to ED treatment. The progressive reduction of toxicities observed after switching to ED in our study, with a incidence even lower than that reported in clinical trials, somehow corroborates previous observations (particularly in the real-life context) indicating that prolonged ICI treatment does not lead to an increased cumulative incidence of irAEs (14–16).

Nevertheless, some irAEs cases after switch to ED represented *de novo* toxicity, revealing that the pathobiology of immune-related toxicity might differ between the two schedules; moreover, 43% of any grade and 30.4% of G3/G4 irAEs occurred after only one ED administration. This phenomenon was already observed in a recently published retrospective study limited to NSCLC patients (8); on one hand, this might reflect the increase in peak concentrations (Cmax) observed with ED compared to CD and the peak proliferative response of CD8+ T cells occurring in the first weeks after switching (4,17,18); on the other hand, it suggests that surveillance should be more intensive during the first ED cycles and that biomarkers of toxicity should be found to support the decision making (19).

To this extent, investigating how the two schedules differently affect the abundance of specific circulating immune cell types and/or T cell receptor (TCR) diversity might help predicting irAEs onset and improve clinical management (20).

The study also investigated a separate cohort of pan-cancer patients treated *upfront* with ED, showing a real-life incidence and a spectrum of irAEs in line with those observed in historical cohort of patients treated with CD and with those reported by clinical trials with ED ICIs (7,10,13,15). The lack of a control cohort treated with *upfront* CD with similar baseline characteristics and follow-up time prevented us from making comparisons to avoid selection bias; in fact, the cohort included in our study was skewed towards long survival and good tolerability as, to transition from CD to ED, a patient must have survived and tolerated CD ICI well enough. However, a recent study which shown no differences in time-to-treatment discontinuation (a measure of real-world effectiveness) between upfront CD and upfront ED, also tried to infer irAEs incidence using incident levothyroxine and prednisone prescription and found no discrepancies between the two groups (21).

Finally, this analysis revealed that irAEs onset during ED was associated with improved OS. While results in melanoma and NSCLC patients treated with CD ICIs are

contradictory, due in part to methodological limits (10,13,22,23), our results suggest that irAEs might be considered a surrogate of clinical activity in the setting of ED ICIs. External validation among more homogeneous patient populations will be needed to confirm this observation.

Besides the retrospective nature which may have led to underreporting of irAEs (in particular grade 1 to 2), another limitation of this study is represented by missing data about treatment discontinuation. Nevertheless, taking into account the occurrence of *de novo* toxicity that may develop in a subset of patients, these findings demonstrate that switching ICI treatment from CD and ED did not increase the incidence of irAEs across different indications. Since the need of remodulating patients accesses to oncology departments is increasing, this treatment schedule represents an important alternative for treating physicians. Further prospective studies with proper comparison should look at the safety of this approach when used *upfront*, investigate ED efficacy data outside of clinical trials, and deepen the potential economic impact of this strategy.

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Supplementary Material

Supplementary Table 1. Contributing centers.

Center	Location	No. patients enrolled	% Total cohort
Ospedali Riuniti di Ancona	Ancona	63	7.6
Azienda Ospedaliera Santi Antonio e Biagio e Cesare Arrigo	Alessandria	10	1.2
Azienda Ospedaliera Universitaria Policlinico di Bari	Bari	33	4
Policlinico Universitario Monserrato Casula	Cagliari	14	1.7
Policlinico Campus Biomedico	Roma	41	4.9
Azienda Ospedaliera Universitaria Careggi	Firenze	50	6
Ospedale SS Annunziata	Chieti	9	1.1
Centro di Riferimento Oncologico di Aviano	Aviano	34	4.1
Geneva University Hospital	Geneva	2	0.2
IRCCS Ospedale Policlinico San Martino	Genova	149	17.9
INT IRCCS SS Oncologia Medica Toraco-Polmonare	Milano	42	5
INT IRCCS SS Oncologia Medica GenitoUrinaria	Milano	20	2.4
INT IRCCS Fondazione Pascale	Napoli	30	3.6
INT IRCCS Istituto Regina Elena	Roma	10	1.2
Ospedale San Salvatore	L'Aquila	10	1.2
Ospedale S.M. Goretti	Latina	12	1.4
Ospedale Generale Provinciale	Macerata	7	0.8
Ospedale San Gerardo	Monza	30	3.6
AOU Luigi Vanvitelli	Napoli	3	0.4
Ospedale Michele e Pietro Ferrero ASL CN2	Verduno	2	0.2
Policlinico Fondazione IRCCS Ca' Granda	Milano	10	1.2
Ospedale Santa Maria delle Croci	Ravenna	8	1
AOU Sant'Andrea	Roma	26	3.1
Erasmus University Medical Center	Rotterdam	78	9.4
Policlinico Le Scotte UOC Immunoterapia Oncologica	Siena	22	2.6
Ospedale Santa Chiara	Trento	22	2.6
Policlinico Umberto I	Roma	15	1.8
Azienda Ospedaliera Universitaria di Verona	Verona	16	1.9
Ospedale di Circolo	Varese	66	7.9

Supplementary Table 2. Baseline clinical characteristics by treatment starting (canonical vs extended).

Characteristic	Upfront CD (%)	Upfront ED (%)
No. (n = 812)	550 (67.7)	262 (32.3)
Age, median (range), y	67 (26-93)	67 (28-94)
Gender		
Female	189 (34.4)	100 (38.2)
Male	361 (65.6)	162 (61.8)
ECOG-PS		
0-1	524 (95.3)	241 (92)
≥ 2	24 (4.4)	21 (8)
Unknown	2 (0.3)	0
Smoking status		
Current	98 (17.8)	40 (15.3)
Former	241 (43.8)	84 (32)
Never	211 (38.4)	138 (52.7)
Treatment setting		
First line	264 (48)	116 (44.3)
≥ 2nd line	198 (36)	85 (32.4)
Adjuvant	88 (16)	61 (23.3)
Number of metastatic sites[a]		
<2	129 (27.9)	42 (20.9)
≥ 2	287 (62.1)	132 (65.7)
Unknown	46 (10)	27 (13.4)
Surgery[b]		
Yes	357 (64.9)	198 (75.6)
No	193 (35.1)	64 (24.4)
Concomitant radiotherapy[c]		
Yes	107 (19.5)	49 (18.7)
No	440 (80)	213 (81.3)
Unknown	3 (0.5)	0
Type of ICI		
Pembrolizumab	211 (38.4)	61 (23.3)
Nivolumab	339 (61.6)	201 (76.7)
Tumor type		
NSCLC	172 (31.3)	32 (12.2)
Melanoma	270 (49.1)	41 (15.6)
Renal	100 (18.2)	186 (71.1)
Other	8 (1.4)	3 (1.1)
irAEs onset		
Yes	303 (55.1)	108 (41.2)
No	247 (44.9)	154 (58.8)
Treatment cycles, median (range), No.[d]	13 (1-121)	7 (1-44)

[a]Percentage calculated on the number of patients with metastatic cancer. [b]Surgery refers to resection of primitive tumor or metastatic site or both. [c]Radiotherapy concomitant to ICIs refers to primitive tumor or metastatic site or both. [d]Upfront CD treatment is Q3W (pembrolizumab) or Q2W (nivolumab), upfront ED treatment is Q6W (pembrolizumab) or Q4W (nivolumab).
CD, canonical interval dosing; ECOG-PS, Eastern Operative Oncology Group-Performance status; ED, extended interval dosing; ICI, immune checkpoint inhibitor; irAEs, immune related adverse events, NSCLC, non-small cell lung cancer.

Supplementary Table 3. Baseline clinical characteristics by irAEs onset.

Characteristics	irAEs (%)	No irAEs (%)
No. (n = 812)	411 (50.6)	401 (49.4)
Age, median (range), y	67 (33-90)	67 (26-94)
Gender		
Female	149 (36.3)	140 (34.9)
Male	262 (63.7)	261 (65.1)
ECOG-PS		
0-1	393 (95.6)	372 (93)
≥ 2	17 (4.1)	23 (6)
Smoking Status		
Current	82(20)	56 (14)
Former	152 (37)	173 (43.1)
Never	177 (43)	172 (42.9)
Treatment setting		
First line	208 (50.6)	172 (42.9)
≥ 2nd line	124 (30.2)	159 (39.7)
Adjuvant	79 (19.2)	70 (17.4)
Number of metastatic sites[a]		
< 2	86 (26)	85 (25.7)
≥ 2	209 (63)	210 (63.4)
Unknown	37 (11)	36 (10.9)
Surgery[b]		
Yes	288 (70.1)	267 (66.6)
No	123 (29.9)	134 (33.4)
Concomitant radiotherapy[c]		
Yes	79 (19.2)	77 (19)
No	329 (80)	324 (81)
Unknown	3 (0.8)	0
Type of ICI		
Pembrolizumab	128 (31.1)	144 (35.9)
CD	115 (28)	96 (23.9)
ED	13 (3.1)	48 (12)
Nivolumab	283 (68.9)	257 (64.1)
CD	188 (45.8)	151 (37.7)
ED	95 (23.1)	106 (26.4)

[a]Percentage calculated on the number of patients with metastatic cancer. [b]Surgery refers to resection of primitive tumor or metastatic site or both. [c]Radiotherapy concomitant to ICIs refers to primitive tumor or metastatic site or both.
CD, canonical interval dosing; ECOG-PS, Eastern Operative Oncology Group-Performance status; ED, extended interval dosing; ICI, immune checkpoint inhibitor; irAEs, immune related adverse events.

Supplementary Table 4. Toxicity of *upfront* canonical interval dosing ICI, by tumor type.

	All (%) n=550		NSCLC (%) n=172		Melanoma (%) n=270		Renal (%) n=100		Other (%) n=8	
	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4
Patients with irAEs	225 (40.9)	17 (3.1)	67 (38.9)	3 (1.7)	121 (44.8)	10 (3.7)	33 (33)	2 (2)	4 (50)	2 (25)
Spectrum of irAEs										
Thyroiditis	69 (12.6)	1 (0.1)	18 (10.5)	0 (0)	39 (14.5)	0 (0)	9 (9)	1 (1)	3 (37.5)	0 (0)
Diarrhea/colitis	46 (8.4)	3 (0.5)	15 (8.7)	1 (0.6)	26 (9.7)	2 (0.7)	4 (4)	0 (0)	1 (12.5)	0 (0)
Endocrine	18 (3.3)	2 (0.3)	5 (2.9)	0 (0)	11 (4.1)	1 (0.4)	0 (0)	0 (0)	2 (25)	1 (12.5)
Hepatitis	26 (4.7)	5 (0.9)	8 (4.6)	2 (1.2)	17 (6.3)	3 (1.1)	1 (1)	0 (0)	0 (0)	0 (0)
Neural	8 (1.5)	0 (0)	6 (3.5)	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Arthralgia	45 (8.2)	1 (0.1)	15 (8.7)	0 (0)	25 (9.3)	1 (0.4)	5 (5)	0 (0)	0 (0)	0 (0)
Asthenia	57 (10.4)	0 (0)	17 (9.9)	0 (0)	32 (11.9)	0 (0)	7 (7)	0 (0)	1 (12.5)	0 (0)
Dermatitis	77 (14)	4 (0.7)	19 (11)	0 (0)	45 (16.7)	3 (1.1)	12 (12)	1 (1)	1 (12.5)	1 (12.5)
Pneumonitis	12 (2.2)	0 (0)	7 (4.1)	0 (0)	2 (0.7)	0 (0)	3 (3)	0 (0)	0 (0)	0 (0)
Other	23 (4.2)	1 (0.1)	6 (3.5)	0 (0)	11 (4.1)	0 (0)	3 (3)	0 (0)	3 (37.5)	1 (12.5)

ICI, Immune checkpoint inhibitor; irAEs, Immune-related adverse events; NSCLC, non-small cell lung cancer.

Supplementary Table 5. Toxicity of extended interval dosing ICI at time of first switching, by tumor type.

	All (%) n=550		NSCLC (%) n=172		Melanoma (%) n=270		Renal (%) n=100		Other (%) n=8	
	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4
Patients with irAEs[a]	77 (15)	7 (1.4)	22 (14.9)	3 (2)	35 (13.4)	2 (0.8)	20 (20.7)	2 (2)	0 (0)	0 (0)
Spectrum of irAEs										
Thyroiditis	16 (3.1)	1 (0.2)	7 (4.7)	0 (0)	7 (2.7)	1 (0.4)	2 (2.1)	0 (0)	0 (0)	0 (0)
Diarrhea/colitis	17 (3.3)	3 (0.6)	7 (4.7)	2 (1.4)	7 (2.7)	0 (0)	3 (3.1)	1 (1)	0 (0)	0 (0)
Endocrine	2 (0.4)	0 (0)	1 (0.7)	0 (0)	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hepatitis	6 (1.2)	0 (0)	1 (0.7)	0 (0)	3 (1.2)	0 (0)	2 (2.1)	0 (0)	0 (0)	0 (0)
Neural	2 (0.4)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Arthralgia	12 (2.3)	1 (0.2)	2 (1.4)	0 (0)	8 (3.1)	1 (0.4)	2 (2.1)	0 (0)	0 (0)	0 (0)
Asthenia	26 (5.1)	1 (0.2)	7 (4.7)	0 (0)	11 (4.2)	0 (0)	8 (8.2)	1 (1)	0 (0)	0 (0)
Dermatitis	17 (3.3)	0 (0)	1 (0.7)	0 (0)	10 (3.8)	0 (0)	6 (6.2)	0 (0)	0 (0)	0 (0)
Pneumonitis	3 (0.6)	1 (0.2)	1 (0.7)	1 (0.6)	0 (0)	0 (0)	2 (2.1)	0 (0)	0 (0)	0 (0)
Other	5 (1)	0 (0)	0 (0)	0 (0)	4 (1.5)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)

[a]irAE data (at time of first switch) missing for 40 patients (24 NSCLC, 11 melanoma, 3 renal, 2 other).
ICI, Immune checkpoint inhibitor; irAEs, Immune-related adverse events; NSCLC, non-small cell lung cancer.

Supplementary Table 6. Long-term toxicity of extended interval dosing ICI (including all cycles after switching), by tumor type.

	All (%) n=550		NSCLC (%) n=172		Melanoma (%) n=270		Renal (%) n=100		Other (%) n=8	
	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4
Patients with irAEs[a]	179 (37.1)	23 (4.8)	54 (41.5)	7 (5.4)	87 (34.7)	11 (4.4)	38 (39.6)	5 (5.2)	0 (0)	0 (0)
Spectrum of irAEs										
Thyroiditis	40 (8.3)	1 (0.2)	18 (13.8)	0 (0)	18 (7.2)	1 (0.4)	4 (4.2)	0 (0)	0 (0)	0 (0)
Diarrhea/colitis	46 (9.5)	6 (1.2)	15 (11.4)	3 (2.3)	21 (8.4)	2 (0.8)	10 (10.4)	1 (1)	0 (0)	0 (0)
Endocrine	12 (2.5)	2 (0.4)	2 (1.5)	0 (0)	10 (4)	2 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)
Hepatitis	13 (2.7)	1 (0.2)	1 (0.8)	0 (0)	10 (4)	1 (0.4)	2 (2.1)	0 (0)	0 (0)	0 (0)
Neural	5 (1)	1 (0.2)	1 (0.8)	0 (0)	3 (1.2)	1 (0.4)	1 (1)	0 (0)	0 (0)	0 (0)
Arthralgia	46 (9.5)	2 (0.4)	9 (6.9)	0 (0)	25 (10)	2 (0.8)	12 (12.5)	0 (0)	0 (0)	0 (0)
Asthenia	53 (10.9)	1 (0.2)	16 (12.2)	0 (0)	24 (9.6)	0 (0)	13 (13.5)	1 (1)	0 (0)	0 (0)
Dermatitis	62 (12.8)	6 (1.2)	15 (11.5)	2 (1.5)	29 (11.6)	2 (0.8)	18 (18.7)	3 (3.2)	0 (0)	0 (0)
Pneumonitis	13 (2.7)	3 (0.6)	4 (3.1)	3 (2.3)	5 (2)	0 (0)	4 (4.2)	0 (0)	0 (0)	0 (0)
Other	22 (4.5)	1 (0.2)	5 (3.8)	0 (0)	11 (4.4)	1 (0.4)	6 (6.2)	0 (0)	0 (0)	0 (0)

[a]irAE data (at time of first switch and after) missing for 67 patients (42 NSCLC, 19 melanoma, 4 renal, 2 other).
ICI, Immune checkpoint inhibitor; irAEs, Immune-related adverse events; NSCLC, non-small cell lung cancer.

Supplementary Table 7. Toxicity of *upfront* extended interval dosing ICI, by tumor type.

	All (%) n=262		NSCLC (%) n=32		Melanoma (%) n=186		Renal (%) n=41		Other (%) n=3	
	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4	any	G3-G4
Patients with irAEs	107 (40.8)	14 (5.3)	6 (18.7)	1 (3.1)	91 (48.9)	8 (4.3)	10 (24.4)	5 (12.2)	0 (0)	0 (0)
Spectrum of irAEs										
Thyroiditis	26 (9.9)	3 (1.1)	1 (3.1)	0 (0)	22 (11.8)	2 (1.1)	3 (7.3)	1 (2.4)	0 (0)	0 (0)
Diarrhea/colitis	32 (12.2)	2 (0.8)	2 (6.2)	0 (0)	26 (14)	1 (0.5)	4 (9.8)	1 (2.4)	0 (0)	0 (0)
Endocrine	4 (1.5)	0 (0)	0 (0)	0 (0)	4 (2.2)	2 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Hepatitis	12 (4.6)	4 (1.5)	1 (3.1)	1 (3.1)	8 (4.3)	2 (1.1)	3 (7.3)	1 (2.4)	0 (0)	0 (0)
Neural	3 (1.1)	1 (0.4)	0 (0)	0 (0)	3 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Arthralgia	2 (0.8)	0 (0)	0 (0)	0 (0)	19 (10.2)	1 (0.5)	1 (2.4)	0 (0)	0 (0)	0 (0)
Asthenia	24 (9.2)	0 (0)	0 (0)	0 (0)	21 (11.3)	0 (0)	3 (7.3)	0 (0)	0 (0)	0 (0)
Dermatitis	32 (12.2)	0 (0)	1 (3.1)	0 (0)	30 (16.1)	0 (0)	1 (2.4)	0 (0)	0 (0)	0 (0)
Pneumonitis	16 (6.1)	3 (1.1)	2 (6.2)	0 (0)	11 (5.9)	1 (0.5)	3 (7.3)	2 (4.9)	0 (0)	0 (0)
Other	12 (4.6)	1 (0.4)	1 (3.1)	0 (0)	11 (5.9)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)

ICI, Immune checkpoint inhibitor; irAEs, Immune-related adverse events; NSCLC, non-small cell lung cancer.

CHAPTER 5

Nivolumab in pre-treated Malignant Pleural Mesothelioma: real-world data from the Dutch Expanded Access Program

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Abstract

Background: Randomized phase III trials are ongoing to investigate the efficacy of nivolumab in malignant pleural mesothelioma (MPM), but real-world data are still scarce. In this real-world study, we investigated the clinical outcomes of nivolumab treatment in pre-treated MPM patients.

Methods: Data from 107 nivolumab treated MPM patients within the Dutch expanded access program were retrospectively analyzed. Treatment was independent of programmed death ligand 1 (PD-L1) expression on tumor samples. Univariable and multivariable analyses were performed to evaluate the relationship between clinically important factors, baseline peripheral blood parameters and survival. The landmark method was used to compare the outcome of patients according to their radiological response.

Results: In the full cohort, the median progression-free survival (mPFS) was 2.3 months (95% CI: 1.6-2.9) and the median overall survival (mOS) was 6.7 months (95% CI: 6.2-10.0). After 12 weeks, the disease control rate (DCR) was 37% and the objective response rate (ORR) was 10%. PD-L1 status was determined in 33 patients (30%) and PD-L1 positivity (≥1%) was associated with an improved ORR (36% vs 9%, p-value 0.05), but not with PFS or OS. Low albumin was associated with worse OS (p-value 0.002). Median OS was significantly longer for patients who had partial response to treatment (p-value 0.0002).

Conclusions: In this real-world analysis, ORR and mOS were lower compared to those obtained in phase II trials. However, exceptional survival rates were observed in patients who had a radiological response. Although we cannot determine whether prognostic or predictive, PD-L1 expression and albumin were associated with greater response rate and may represent useful biomarkers for nivolumab treatment in MPM.

Introduction

Malignant pleural mesothelioma (MPM) is an uncommon but aggressive neoplasm with low survival rates (1,2). Current first-line treatment consists of combination chemotherapy with platinum and anti-folate agents (1,3), with the possible addition of bevacizumab (2). Historically, no therapeutic agent has shown strong activity against mesothelioma in second or third-line treatment (4). The breakthrough of checkpoint inhibitors (CIs) in solid tumors has led to their investigation in MPM patients as well (5). Despite promising results in phase I/II trails with CIs, phase III trials investigating both single agent anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and anti-programmed cell death 1 (PD-1) treatments failed to show efficacy (6,7). Recently, the PROMISE-meso, a phase III randomized clinical trial (RCTs), comparing the PD1 CI pembrolizumab to chemotherapy (gemcitabine or vinorelbine) as second-line treatment, failed to show superiority of the anti-PD-1 treatment for the primary endpoint progression free survival (PFS) (7). The objective response rate (ORR) was significantly higher in the pembrolizumab arm (22%) than in the chemotherapy arm (6%), but duration of response (DoR) and overall survival (OS) were equal. Nivolumab, another PD-1 inhibitor, showed promising results in phase II trials in pre-treated MPM patients (with ORR up to 29%) (8–11) and is currently being tested in the context of phase III RCTs (NCT03063450, NCT02899299).

Only one study has reported real-world data on second or third-line PD-1 inhibition (pembrolizumab) in MPM (12). In this study, both PFS and OS did not match phase II trial results which could be explained by the use of strict inclusion criteria in the clinical trials (9,11). Outside of clinical trials, there are no reports on the role of nivolumab in pre-treated MPM patients. Most probably, as already observed in phase II/III trials, a small group of MPM patients might benefit from CI treatment.

Relevant biomarkers for response have not yet been determined in this specific setting of MPM. Programmed death-ligand 1 (PD-L1) expression on tumor cells has a controversial role in predicting outcome in MPM (8,12). The low predictive value of PD-L1 expression in MPM has been explained by intra-patient heterogeneity, different cut-off points for PD-L1 positivity and the use of different immunohistochemistry (IHC) markers (8,12). Likewise different cancer types (13,14), other tumor and patient characteristics, as well as peripheral blood values should then be investigated in MPM patients treated with nivolumab, to identify biomarkers for response.

Since February 2018, nivolumab has been provided to MPM patients in the Netherlands through an expanded access program (EAP). This program has offered the unique opportunity to conduct a real-world analysis to investigate the outcome of nivolumab in a population of MPM patients pre-treated with antifolate and platinum-based chemotherapy. Furthermore, we extensively analyzed the correlation between

clinically important factors, baseline peripheral blood parameters and clinical outcomes. The impact of radiological response on outcome was also investigated. We present the following article/case in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Guidelines.

Methods

Patients

We retrospectively reviewed data from all 135 MPM patients enrolled at the Erasmus Medical Center (Rotterdam, NL) and The Netherlands Cancer Institute (Amsterdam, NL) in the EAP for nivolumab. Patients had a cytological and/or histological proven MPM and progression after at least one previous line of chemotherapy. Inclusion in the program was independent of PD-L1 expression on tumor samples, which was assessed by IHC using the Ventana SP263 or the Dako 22C3 assays. A recent tumor biopsy was not mandatory. Patients were excluded if they had received any immunotherapy as first-line or maintenance treatment. Patients with a follow-up shorter than 3 months were also excluded from the analysis, unless they progressed or died earlier. Nivolumab was given intravenously at a dose of 3 mg/kg every 2 weeks. Radiological tumor assessment was carried out 6 weeks (±1) after start of treatment and every 6 weeks (±1) until progression depending on previous computed tomography (CT) evaluation.

Data collection

Patient and tumor characteristics, as well as radiological response data and blood count parameters within 14 days before the initiation of nivolumab treatment were collected from the digital patient register. The following variables were collected and investigated in statistical analyses: age, gender (male vs female), histologic subtype (non-epithelioid vs epithelioid), Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) at start of nivolumab (0 vs ≥1), clinical TNM stage (stage III/IV vs I/II [VIII edition]) (15), line of treatment (later-lines vs second-line), PD-L1 status (considered as positive if tumor cell expression levels were ≥1%, negative if <1%), time to progression (TTP) to previous line of chemotherapy (<6 months vs ≥6 months), time interval (TI) from diagnosis to start of nivolumab, body mass index (BMI). Albumin values (as continuous variable), platelet count (as continuous variable), and absolute counts for neutrophils, monocytes, eosinophils and lymphocytes were also collected.

Tumor response was assessed using a combination of modified Response Evaluation Criteria In Solid Tumors (mRECIST) for mesothelioma version 1.0 and RECIST modified for immunotherapeutic agents (iRECIST) (16,17). Per iRECIST, if tumor imaging shows initial progression of disease (PD), tumor assessment should be repeated 4 to 8 weeks later in order to confirm PD with the option of continuing treatment if the patient is clinically stable. Patients who had confirmed disease progression by iRECIST

discontinued treatment, and the date of the initial CT scan was taken as the time of progression. OS was defined as the time from first CI administration to death from any cause, censored at the last tumor assessment date for patients who were alive at the time of data cutoff. PFS was measured from the time of nivolumab initiation to clinical or radiological progression or death from any cause. ORR was defined as the proportion of patients who had a partial (PR) or complete response (CR) to therapy and DCR as the percentage of patients who achieved complete response, partial response and stable disease (SD). A cut-off of 12 weeks ***(±2)*** was selected for both ORR and DCR, according to the majority of RCTs investigating CIs in MPM. DoR was defined as the time from documentation of tumor response to disease progression.

Statistical analysis

Patient and disease characteristics were reported using count and percentage for categorical variables, median and range for continuous variables. Median PFS and OS were estimated by the Kaplan–Meier method. Differences in probability of surviving between the strata were evaluated by log-rank (Mantel-Cox) test and Bonferroni's correction was used for comparison between more than two groups. The landmark method was used for handling immortal time bias when comparing the outcome of patients according to their radiological response (18). For this specific analysis, all the patients who died before 12 weeks were excluded. A landmark of 12 weeks was chosen because at that time ORR was also calculated.

The hazard ratios (HR) of progression and death, the odds ratios (OR) of response and their associated 95% confidence intervals (95% CI) for clinically important factors (including PS, histology, stage, gender, age, line of treatment, TTP to previous line of chemotherapy, PD-L1 status) were calculated using a univariable Cox proportional hazard model or a univariable logistic regression.

Missing data in blood-derived parameters analyzed in the multivariable analysis were imputed ten times. In order to determine a subset of variables with the strongest impact on PFS, OS and ORR, blood-derived biomarkers (including albumin, platelets, absolute neutrophils, monocytes, eosinophils and lymphocytes) were combined with clinically important factors and a Cox multivariable proportional hazard regression model or a multivariable logistic regression were performed on the imputed datasets. Since the number of candidate variables exceeded the number of events divided by 10, a ridge version of the models was used for variable selection. Variables were selected in the final model if they were included 5 times of more in the models on the imputed data sets. The final model was fitted on the imputed data sets and the results were pooled using Rubin's rules (19). As a sensitivity analysis, the final model was also estimated on the complete case data (without imputed data).

Associations between categorical variables were assessed by Pearson's Chi-Square or Fisher exact tests.

A significance level of 0.05 was chosen to assess the statistical significance. All reported p-values were two sided. Statistical analyses were performed using R 3.6.0 (R Foundation for Statistical Computing). Multiple imputation was performed using the "smcfcs" package and pooling was conducted with the "mice" and "mitools" packages in R.

Results

Patient characteristics

At the data cut-off of November 2019, 135 patients were treated with at least one cycle of Nivolumab. Among them, 107 patients were eligible for the analysis (**Supplementary Figure S1**). Eighty-eight patients (93%) had a PS of 0 or 1 at start of treatment. Ninety-seven (90%) were treated in second-line. PD-L1 expression was determined in 33 patients: 22 biopsies (66%) were PD-L1 negative and 11 (33%) were PD-L1 positive. PD-L1 positive status was associated with non-epithelioid histology (Fisher's exact test p-value 0.004). The majority of patients (69%) had an advanced clinical stage of disease (stage III/IV). Other baseline patient characteristics are summarized in **Table 1**.

At a median follow-up time of 10.1 months, 85 patients had progression of disease of whom 59 died. The median PFS (mPFS) was 2.3 months (95% CI: 1.6-2.9) and median OS (mOS) was 6.7 months (95% CI: 6.2-10.0) (**Figure 1A and 1B**). The disease control rate (DCR) was 37% (40 out of 107) after 12 weeks and 11 patients (10%) had an objective radiological response (all partial responders, no complete responses were registered). The 6-month PFS rate was 23% (95% CI: 16%–33%). The 6-month OS rate was 60% (95% CI: 51%–71%) and the 1-year OS rate was 31% (95% CI: 22%–45%).

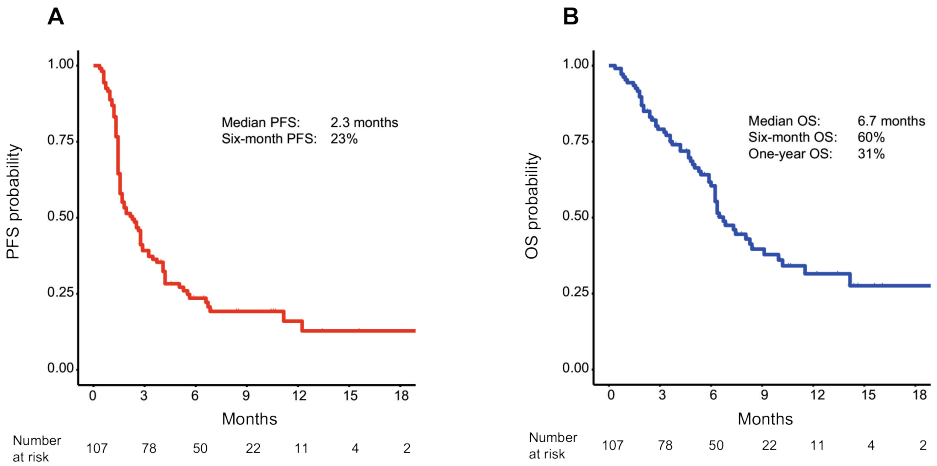

Figure 1. Kaplan-Meier curves of survival in the entire cohort of nivolumab treated MPM patients (median follow-up time of 10.1 months). (A) Overall survival in the entire cohort. (B) Progression-free survival in the entire cohort PFS, progression-free survival; OS, overall survival.

5

Association of clinically important factors with survival outcomes

Univariable Cox proportional hazard regression analysis of clinically important factors revealed that patients with advanced clinical stage (stage III/IV) had a shorter PFS (mPFS 1.6 vs 3.6 months [HR 1.82, 95% CI 1.11-3.01, log-rank p-value 0.02, **Figure 2A**]) but similar OS (mOS 6.5 vs 6.8 months [HR 1.27, 95% CI 0.71-2.28, log-rank p-value 0.40], **Figure 2B**) compared to those with early stage (I/II). All other clinical factors were not significantly associated with PFS or OS (**Table 2**).

In particular, PS was not significantly correlated with PFS or OS, although patients with a PS of 0 had a trend towards a longer mOS compared to patients with PS ≥1 (mPFS 2.9 vs 1.8 months [HR 0.64, 95% CI: 0.36-1.16, log-rank p-value 0.14]; mOS 10.2 vs 6.2 months [HR 0.51, 95% CI: 0.25-1.05, log-rank p-value 0.06]). PFS was also similar among patients with non-epithelioid and epithelioid histology (log-rank p-value 0.89, **Figure 2C**), yet patients with non-epithelioid histology had a non-significant trend towards worse OS (mOS 4.8 vs 7.4 months [HR 1.71, 95% CI: 0.92-3.16, log-rank p-value 0.08], **Figure 2D**). Patients with positive PD-L1 status showed a longer, albeit non-significant, mPFS (4.2 vs 1.7 months [HR 0.52, 95% CI: 0.23-1.20, log-rank p-value 0.11], **Figure 2E**) while no difference in terms of OS was observed (mOS 5.4 vs 6.1 months [HR 0.67, 95% CI 0.27-1.64, log-rank p-value 0.39], **Figure 2F**).

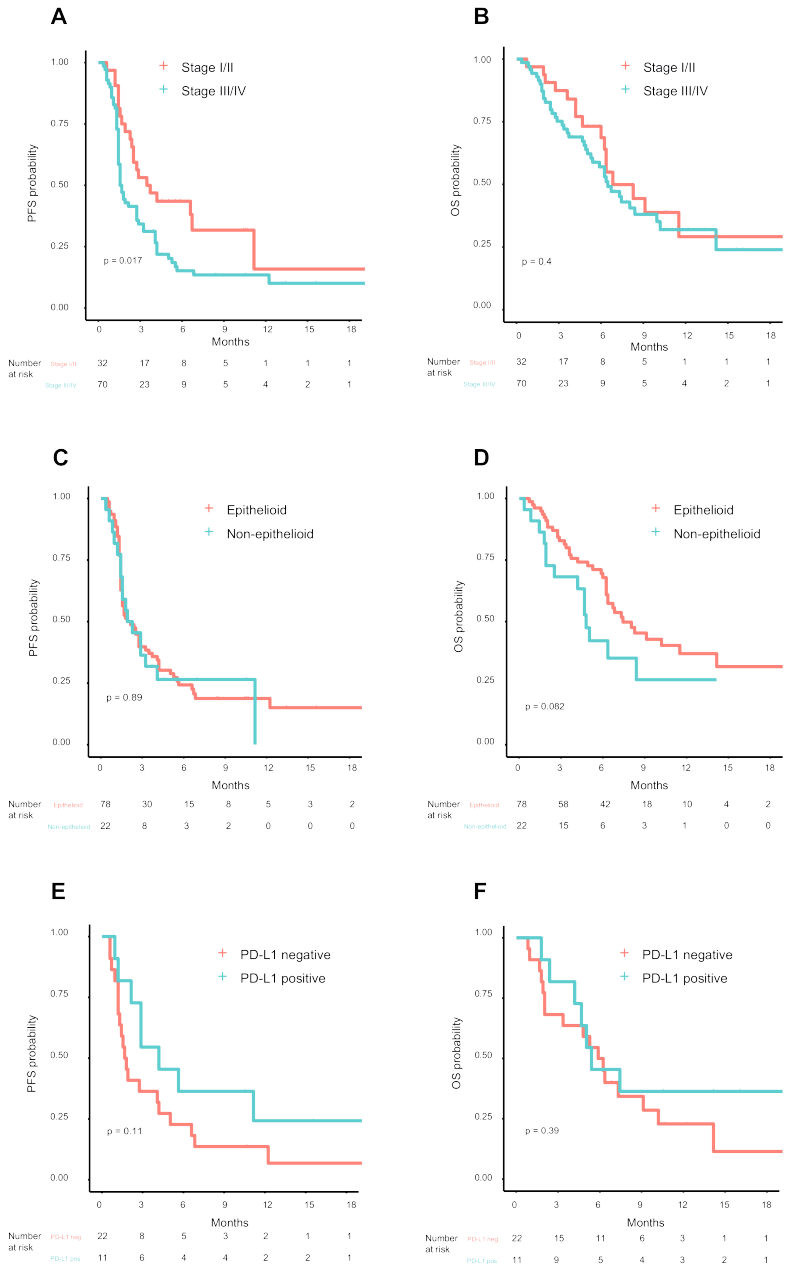

Figure 2. Kaplan-Meier curves of survival of subgroups based on stage of disease, histological subtype and programmed death ligand 1 (PD-L1) status. (A) Progression-free survival and overall survival by stage of disease as determined by IASLC 8th edition of TNM for pleural mesothelioma. (B) Progression-free survival and overall survival by histology. (C) Progression-free survival and overall survival in patients with a PD-L1 expression ≥1% versus in those with a PD-L1 expression <1%. PFS, progression-free survival; OS, overall survival; PD-L1, programmed death ligand 1.

Impact of radiological response to nivolumab on outcome and association of clinically important factors with response

To better elucidate the importance of response to nivolumab, we compared PFS and OS of patients according to ORR. To avoid an immortal time bias, only patients who were still alive at 12 weeks and underwent radiological assessment at that time point were taken into account for the analysis. Remarkably, with a median follow up of 14.1 months in the group of patients with PR, no deaths were reported and only 2 patients progressed (median DoR not reached, **Figure 3A**). Median OS was not reached for patients with a PR. Median OS was 10.2 months for patients with SD and 6.4 months for those with PD (log-rank p-value 0.0002, **Figure 3B**). Among the clinically relevant factors, the only one which seemed to predict ORR in univariable logistic regression was PD-L1 status (**Table 2**). To note, data about PD-L1 expression were only available in 6/11 PR, 8/29 SD and 19/67 PD patients (**Figure 4**). Four of the responders had PD-L1 positive tumors and two had PD-L1 negative tumors (**Figure 4**). ORR was 36% in the PD-L1 positive group vs 9% in the PD-L1 negative group (OR 1.31, 95% CI 1.00-1.72, p-value 0.05, **Table 2**).

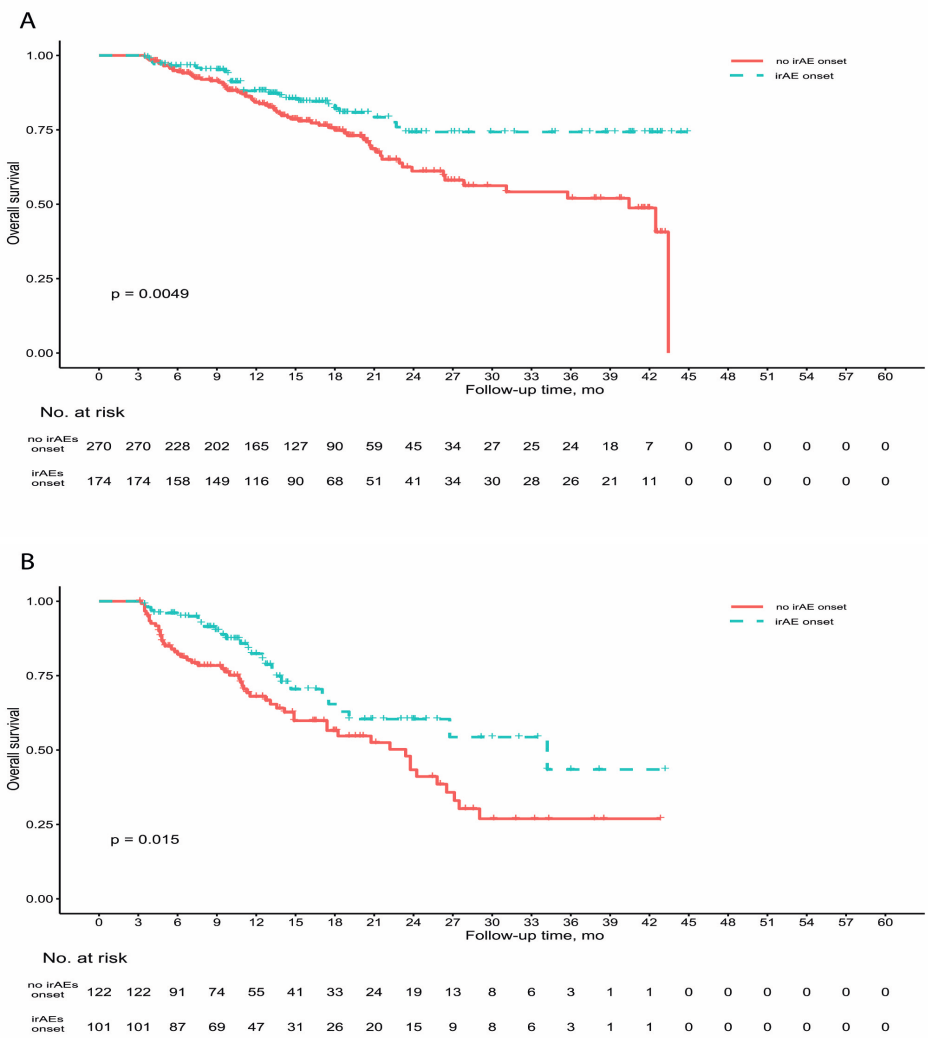

Figure 3. Kaplan-Meier curves of survival according to best overall radiological response. (A) Progression-free survival in patients with a partial response and stable disease as objective response to nivolumab treatment (B) Overall survival in patients with a partial response, stable disease and progressive disease as objective response to nivolumab treatment. PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease.

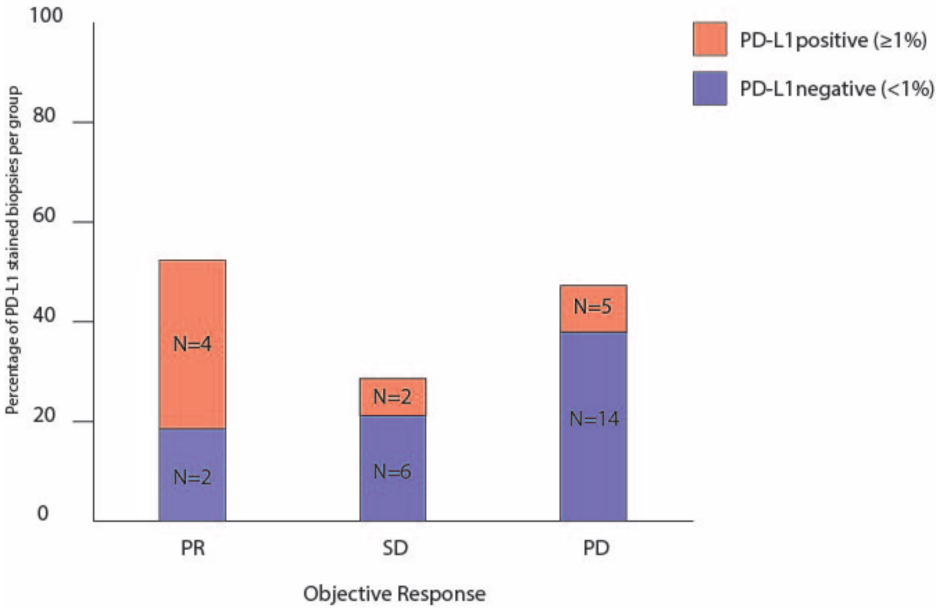

Figure 4. Expression of programmed death ligand 1 (PD-L1) according to objective response to nivolumab treatment. PR, partial response; SD, stable disease; PD, progressive disease; PD-L1, programmed death ligand 1.

Association of peripheral blood biomarkers with survival outcomes and response to nivolumab

After imputation for missing values (refer to **Supplementary Figure S1** for the number of available blood samples at baseline), peripheral blood-derived parameters (albumin, platelets, absolute neutrophils, monocytes, eosinophils and lymphocytes) and clinically important factors (including PS, histology, clinical stage, gender and age) were used as covariates in multivariable analysis to identify independent factors related to the efficacy of nivolumab in terms of PFS and OS. Regarding PFS, only high absolute monocyte count was significantly associated with worse PFS after ridge regression (HR 3.16, 95% CI: 1.56-6.37, p-value 0.001, **Table 3**). The role of monocytes was confirmed also by using non-imputed data (HR 3.78, 95% CI: 1.84-7.76, p-value 0.0002).

The ridge regression for OS showed that albumin, thrombocytes, neutrophils had the strongest association with OS. Subsequent multivariable Cox proportional hazard regression analysis with these variables (**Table 3**) showed that only albumin retained its prognostic value revealing that patients with a high albumin had a lower change of dying (HR 0.87, 95% CI 0.81-0.95, p-value 0.002). The role of albumin was confirmed by the sensitivity analysis with non-imputed data (HR 0.88, 95% CI: 0.80-0.96, p-value 0.005).

A multivariable analysis for ORR with peripheral blood-derived parameters was not performed because of the low number of events (only 11 responder patients). At univariable analysis with imputed data, again only albumin resulted significantly associated with ORR (OR 1.02, 95% CI 1.00-1.03, p-value 0.03, **Supplementary Table S1**).

Since albumin was the only significant prognostic factor for OS and was also associated with ORR in univariable analysis, patients were further divided in quartiles according to their baseline albumin values and their outcomes were analyzed. Patients in the lower quartile (< 38 mg/dL) revealed a significantly shorter OS compared to patients in the other quartiles (HR 3.76, 95% CI: 1.93-7.31, log-rank p-value 0.003 with Bonferroni's correction, **Figure 5**). The median OS for patient with baseline albumin levels below 38 was 2.5 months (95% CI: 1.9 - not reached) compared to 8.0 months (95% CI: 6.4-not reached) for patients with albumin levels above 38. Six-month OS rates were 34% (95% CI: 18%-65%) and 74% (95% CI: 62%-86%), respectively. In addition, 4 out of 20 (20%) patients in the higher quartile (> 43 mg/dL) had a partial response, compared to 3/65 (4%) in the other three quartiles, with a 16% increase in the chance of getting a response to nivolumab (OR 1.16, 95% CI: 1.02-1.33, p-value 0.02).

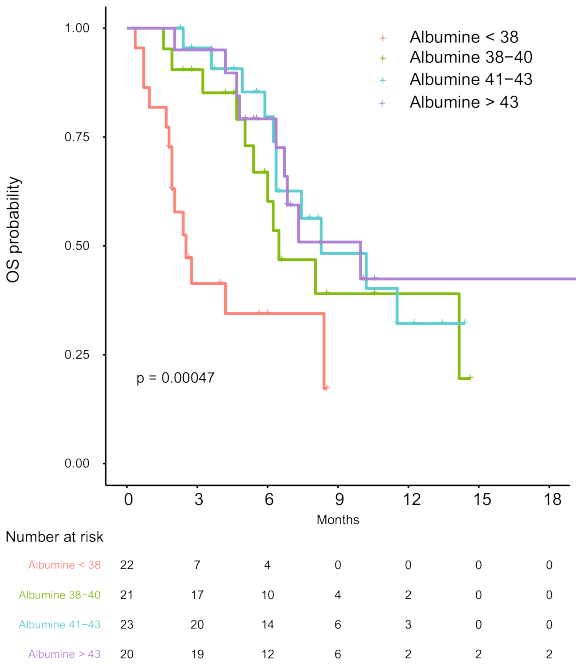

Figure 5. Kaplan-Meier curves of survival in patient groups per quartile of albumin level. OS, overall survival.

Discussion

This is the largest real-world analysis of nivolumab treatment in pre-treated MPM patients. We observed an ORR of 10%, a mPFS of 2.3 months and a mOS of 6.7 months. The PFS and OS did not significantly differ per histological subtype or PD-L1 expression. Patients with PD-L1 positive tumors had a higher ORR than patients with PD-L1 negative tumors. We did not observe an association between time from diagnosis or response to chemotherapy and response to nivolumab. Strikingly, there seemed to be an incremental impact on OS for patients with a PR to nivolumab as we did not observe any deaths in these patients during a median follow-up time of 14.1 months.

By comparing our data with the real-world study of MPM patients treated with pembrolizumab, we observed a similar OS but a worse PFS, which could be explained by the type of radiological assessment used. In the study of Metaxas et al. (12), the type of radiological assessment was not described. In our study, we retrospectively analyzed all CT scans according to a combination of mRECIST for mesothelioma and iRECIST (16,17). Per iRECIST, tumor assessment had to be repeated 4 to 8 weeks after first evidence of PD with the option of continuing treatment if the patient was clinically stable. In case of confirmed progression, the date of the initial CT scan was taken as the time of progression.

By comparing our data with those of clinical trials (7–11), our ORR and mOS were inferior, which could be explained by the fact that there were no strict inclusion criteria in our analysis, leading to a less selected patient population. In the PROMISE-meso trial an ORR of 22% was reported for the pembrolizumab group and an ORR of 6% for the second-line chemotherapy treated patients. However, this difference in ORR was not translated into a difference in mPFS (pembrolizumab: 2.5 months vs chemotherapy: 3.4 months) or mOS (pembrolizumab: 10.7 months vs chemotherapy: 11.7 months) (7). Conversely, long survival for patients with a PR in our analysis does suggest a clinical benefit that is correlated with ORR. The lack of significant benefit in terms of mPFS and mOS, despite a higher ORR, in the pembrolizumab arm of the PROMISE-meso might be due to the low ORR combined with the short time to progression in patients where therapy is not effective. For example, if only a minority of patients (10-20%) respond to therapy, mPFS and mOS will not be influenced, because more than 50% of the patients will progress or die earlier according to the natural course of disease. Six-months PFS and one-year OS might be more reliable endpoints for (immune) therapies with low response rates. Analysis of those patients who achieved a PR to pembrolizumab in the PROMISE-meso study has not yet been published but could be explanatory.

Since retrospective data may be biased by underreporting of adverse events and misleading, we decided not to report safety data. Nevertheless, to avoid a potentially harmful treatment, identifying a subgroup of MPM patients that benefit from nivolumab becomes crucial. This patient selection should probably be based on multiple parameters.

MPM patients with epithelioid histology have usually a better natural disease course than patients with non-epithelioid tumors (20). However, in our retrospective analysis we did not see any significant difference in mPFS and mOS according to histological subtypes, suggesting that nivolumab might have had an impact on prognosis of non-epithelioid patients. Moreover, PD-L1 expression was associated with non-epithelioid histology and higher ORR in our study. These results are consistent with the exploratory analysis of the MAPS2 trial, where PD-L1 expression of ≥1% was found to be significantly associated with objective response to immunotherapy (8). Unfortunately, our analysis on PD-L1 expression was limited because only 30% of biopsies were stained for PD-L1. Another limitation is that PD-L1 expression was often determined on the biopsy from diagnosis, because in most cases there was no biopsy taken prior to nivolumab treatment.

Looking at the role of baseline peripheral blood biomarkers, our study showed that baseline albumin was the only significant prognostic factor for mOS. In addition, patients with an albumin level higher than 43 mg/dL had a 16% higher chance of responding to therapy than patients with albumin levels below 38 mg/dL. Albumine is known to reflect the nutritional status of cachectic patients and is described as a prognostic factor for many cancer types, including mesothelioma (21–23). Due to the lack of a control group, we cannot draw definitive conclusions about the predictive role of albumin from our analysis. However, we showed that low levels of albumin might identify patients who are unlikely to benefit from the treatment.

Our analysis also showed that baseline absolute monocyte count represents an optimal predictor of PFS in MPM patients (HR 3.16, 95% CI: 1.56-6.37, p-value 0.001). This negative association between the number of monocytes and outcome in MPM is consistent with previous studies (24,25). Burt et al. reported that pre-operative peripheral absolute monocyte count was associated with poor OS in patients with MPM, regardless of tumor histology (HR 3.98, 95% CI: 2.64-5.93, p-value <0.0001) (25).

Conclusions

In conclusion, our study showed that ORR and mOS were lower in our real-world database compared to those of clinical trials, which could be due to a less selected population. However, we identified a subgroup of MPM patients with a radiological response to nivolumab that had a significant benefit in terms of PFS and OS compared

to patients without a radiological response to nivolumab treatment. We also showed that PD-L1 expression and albumin were associated with higher response rate, yet the retrospective nature of our study and the lack of a control group prevent us from drawing definitive conclusions on their role as potential predictive biomarkers. Future phase III RCTs on CI treatment in MPM should not be conducted without an extensive exploratory analysis plan based on the evaluation of peripheral blood parameters and tumor samples in order to deeply characterize the small group of patients that benefit from CI treatment.

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Supplementary material

Table S1. Univariable analysis of ORR for peripheral blood derived parameters.

Parameter	ORR		
	OR	95% CI	p-value
Albumin (mg/dL)	1.02	1.00-1.03	0.03
Platelet count (/µL)	0.99	0.99-1.00	0.42
Neutrophils (/µL)	0.98	0.96-1.00	0.28
Lymphocytes (/µL)	1.00	0.93-1.06	0.99
Monocytes (/µL)	0.98	0.78-1.22	0.89
Eosinophils (/µL)	0.75	0.44-1.28	0.30

The univariable logistic regression was used to calculate the ORs of response for peripheral blood derived parameters (with imputed data).
ORR, objective response rate; OR, odds ratio; CI, confidence interval.

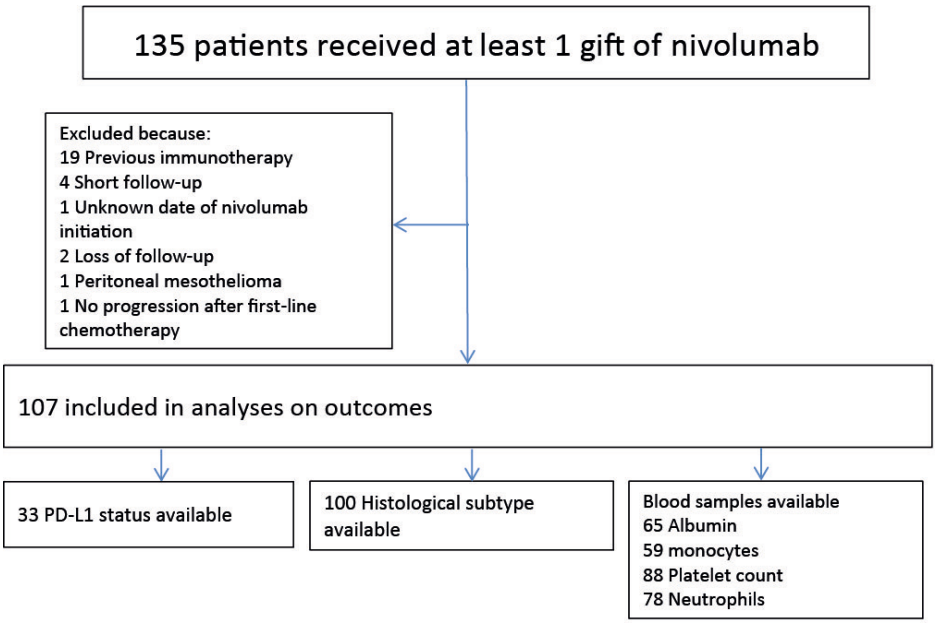

Figure S1. Flow diagram of study population.
PD-L1, programmed death ligand 1.

CHAPTER 6

High-intensity statins are associated with improved clinical activity of PD-1 inhibitors in malignant pleural mesothelioma and advanced non-small cell lung cancer patients

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Abstract

Background: In pre-clinical models, statins showed vaccine adjuvant activities and synergized with PD-1 inhibitors. We analyzed the impact of statin treatment on clinical outcome in thoracic cancer patients treated with PD-1 inhibitors.

Methods: A total of 82 malignant pleural mesothelioma (MPM) and 179 advanced non-small cell lung cancer (aNSCLC) patients treated with PD-1 inhibitors as second or further-line treatment were examined. Seventy-seven MPM patients treated with standard chemotherapy were analyzed as control cohort. Objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were calculated.

Results: Among 253 patients with available data, statin use was associated with increased ORR (32% versus 18%, $P = .02$), PFS (median 6.7 versus 2.9 months, hazard ratio [HR] 0.57, 95% CI 0.39–0.83, $P < .01$), and OS (median 13.1 versus 8.7 months, HR 0.67, 95% CI 0.45–1.00, $P = .05$). In the control MPM cohort treated with chemotherapy (n=77), no association was found. MPM patients who used statins showed improved ORR (22% versus 6%, $P = .05$), PFS (median 6.7 versus 2.4 months, $P < .01$) and OS (median not reached versus 6.0 months, $P = .01$). In aNSCLC patients, statin use was associated with improved ORR (40% versus 22%, $P = .04$) and PFS (median 7.8 versus 3.6 months, $P = .03$), but no significant difference in OS was found (median 13.1 versus 10.1 months, $P = .30$). Multivariable analysis confirmed the correlation between statin use and better PFS and OS in MPM and better PFS in aNSCLC. In the whole cohort, high but not low/moderate-intensity statins were associated with better OS compared to no user ($P = .02$ and $P = .59$, respectively).

Conclusions: Our study showed that statins are associated with better clinical outcome in MPM and aNSCLC patients treated with PD-1 inhibitors in an intensity-dependent manner.

Introduction

Immune checkpoint inhibitors targeting the programmed cell death protein 1 (PD-1) axis represent a novel therapeutic option for advanced non-small cell lung cancer (aNSCLC) [1,2] and an attractive experimental strategy for malignant pleural mesothelioma (MPM) [3–5]. However, clinical benefit from this treatment modality is restricted to a proportion of patients and combinations with other drugs are needed to improve response [6,7].

Statins act by inhibiting the mevalonate (MVA) pathway, involved in the production of cholesterol [8]. Pre-clinical works demonstrated that statins also interfere with prenylation, a post-translational modification, of small GTPases proteins, altering their internal cell membrane anchorage and, therefore, arresting endocytic vesicles trafficking [9]. By this mechanism, statins may lead to prolonged antigen retention on cell membrane and strengthen antigen presentation to T cells, thus suggesting a potential synergy with PD-1 inhibitors. In our multicentre study, we investigated the impact of baseline statin use on the clinical outcome of MPM and advanced aNSCLC patients treated with PD-1 inhibitors.

Methods

Patients.
Patients with a diagnosis of aNSCLC undergoing treatment with PD-1 inhibitors (either nivolumab or pembrolizumab) for an approved oncological indication between January 2016 and December 2019 were identified from patient electronic records of the Università Politecnica delle Marche (Ancona, IT) and the Erasmus Medical Center (Rotterdam, NL) and entered into a prospectively maintained database. We also reviewed data from malignant pleural mesothelioma (MPM) patients enrolled at the Erasmus Medical Center in the expanded access program (EAP) for the anti-PD1 agent nivolumab. All enrolled patients received PD-1 inhibitors alone as second or further-line treatment. Patients were excluded if they had EGFR mutation or ALK rearrangement and if they had received any immunotherapy as first-line or maintenance treatment. Patients with a follow-up shorter than 3 months were also excluded from the study, unless they progressed or died earlier. As control cohort, data from MPM patients treated at Università Politecnica delle Marche with standard first-line chemotherapy were also collected.

Clinicopathological variables including survival were derived from electronic medical records. Patient characteristics were described and compared according to statin use (statins versus no statins). Type, lipophilicity and intensity of statins were collected. Intensity of statins was defined according to the 2018 American College of Cardiology/American Heart Association Guideline on the Management of Blood Cholesterol (**Supplementary Table 1**) [10]. Response to PD-1 inhibitors was evaluated according to RECIST criteria (version 1.1) for aNSCLC patients and modified RECIST for mesothelioma for MPM patients. Objective response rate (ORR) was defined as the proportion of patients with radiological evidence of tumor size reduction (either complete response (CR) or partial response (PR)). Progression free survival (PFS) and overall survival (OS) were calculated from the time of PD-1 inhibitors/chemotherapy commencement until radiological progression or death/last follow up for PFS and until death/last follow up for OS. All patients were followed-up until death or data lock (December 2019).

Ethical approval to conduct this study was granted by Università Politecnica delle Marche (Reference Number 208128) under a broader protocol to investigate tissue and clinical predictors of outcome in MPM patients and by the Erasmus Medical Center (Dutch Trial Register number NTR7015/ NL6828) under a broader protocol to investigate tissue and clinical predictors of outcome in patients receiving cancer immunotherapy.

Statistical analysis

Clinicopathological characteristics were presented using count and percentage for categorical variables, median and range for continuous variables. Pearson chi-square or Fisher's exact tests were used for analysis of proportions across groups. Survival curves were plotted using the Kaplan-Meier method and differences in probability of surviving between the strata were evaluated by log-rank (Mantel-Cox) test. Bonferroni's correction was used for comparison of patients according to statin intensity (high-intensity statins versus low/moderate-statins versus no statins). The hazard ratios (HR) of progression and death were calculated using univariable/multivariable logistic regression and univariable/multivariable Cox proportional hazard model. Fixed multivariable analysis was performed separately in MPM and aNSCLC patients to take into account the different variables (e.g. histological subtype) that could affect patient outcome in each tumor type. The key covariates were: age (< 70 versus ≥ 70 years old), gender (female versus male), smoking status (never smokers versus current/former smokers), Eastern Cooperative Oncology Group Performance Status (ECOG PS) (0 versus ≥ 1), , histological subtype (epithelioid versus non-epithelioid for MPM; adenocarcinoma versus non-adenocarcinoma for aNSCLC). R 3.6.0 (R Foundation for Statistical Computing) was used for statistical analysis, with all estimates being reported with corresponding 95% confidence intervals and a two-tailed level of significance of $P < .05$.

Results

Patient characteristics

Data from 261 patients (82 MPM and 179 aNSCLC) treated with PD-1 inhibitors alone as second or further-line were analysed, whose 253 had available data on statins at baseline (80 MPM and 173 aNSCLC). Overall survival data were available for 166 aNSCLC and 80 MPM patients, respectively. Two hundred and sixteen (95%) of patients had an ECOG PS of 0 or 1 at start of treatment. Two hundred and six (79%) were treated in second-line. The frequency of statin use at start of anti-PD1 treatment was relatively similar in MPM (33%) and aNSCLC (22%) patients (**Table 1 and Supplementary Table 2**).

Table 1. Patient characteristics (whole cohort).

Characteristic	No. (%)
Total	261
Median age (range)	67 (26-89)
Sex	
Male	189 (72)
Female	72 (28)
ECOG PS	
0	66 (25)
1	150 (57)
≥2	10 (4)
unknown	35 (14)
Smoking status	
Never smoker	34 (13)
Current/former smoker	193 (74)
Unknown	34 (13)
Tumor type[a]	
aNSCLC	179 (69)
Adenocarcinoma	109 (61)
Non-adenocarcinoma	66 (37)
Unknown	4 (2)
MPM	82 (31)
Epithelioid	54 (66)
Sarcomatoid/biphasic	21 (26)
Unknown	7 (8)
Prior treatment lines	
1	206 (79)
2	43 (16)
>2	12 (5)
Tumor response	
CR	3 (1)
PR	49 (19)
SD	75 (29)
PD	119 (46)
Unknown	15 (5)
Statin use	

Table 1. Continued.

Characteristic	No. (%)
Yes	67 (26)
No	186 (71)
Unknown	8 (3)
Statin intensity[b]	
Low	2 (3)
Moderate	42 (63)
High	14 (21)
Unknown	9 (13)
Statin lipophilicity[b]	
Lipophilic	51 (76)
Hydrophilic	12 (18)
Unknown	4 (6)
Statin type[b]	
Atorvastatin	21 (30)
Rosuvastatin	9 (15)
Simvastatin	29 (43)
Others	8 (12)
Median OS (months)	9.4 (95% CI 8.3-12.0)
Median PFS (months)	3.9 (95% CI 2.8-5.2)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; aNSCLC, advanced non-small cell lung cancer; MPM, malignant pleural mesothelioma; CR, complete response; PR, partial response, SD, stable disease; PD, progressive disease; OS, overall survival; CI, confidence interval; PFS, progression-free survival.
[a] Percentages of histological subtypes refer to each tumor type taken alone.
[b] Percentages refer to only patients taking statins at baseline.

Clinicopathological characteristics were typical of advanced MPM and aNSCLC patients and were balanced between those who were taking statins or not, with the exception of age (older patients in the statin group) and gender (statin use more common among male patients) (**Table 2**).

Table 2. Association between baseline statin use and key clinicopathologic features of MPM and aNSCLC patients.

Characteristic	No statins (%)	Statins (%)	*P value*[a]
Total	186 (73)	67 (27)	
Age			
<65	75 (40)	17 (25)	.04[b]
≥65	111 (60)	50 (75)	
Sex			
Male	129 (69)	55 (82)	.06
Female	57 (31)	12 (18)	
ECOG PS			
0	45 (24)	21 (31)	.32
≥1	115 (62)	37 (55)	
Unknown	26 (14)	9 (14)	
Smoking status			
Never-smoker	26 (14)	8 (12)	.74
Smoker	134 (72)	52 (78)	
Unknown	26 (14)	7 (10)	
Prior treatment lines			
1	149 (80)	52 (78)	.79
2	26 (14)	15 (22)	
>2	11 (6%)	0	
Tumor type			
aNSCLC	133 (71)	40 (60)	
Adenocarcinoma	82 (62)	23 (58)	.78
Non-adenocarcinoma	48 (36)	16 (40)	
Unknown	3 (2)	1 (2)	
MPM	53 (29)	27 (40)	
Epithelioid	36 (68)	16 (59)	1.00
Sarcomatoid/biphasic	14 (26)	7 (26)	
Unknown	3 (6)	4 (15)	
Tumor response			
aNSCLC			
CR/PR	28 (21)	14 (35)	.04[b]
SD/PD	95 (71)	21 (52)	
Unknown	10 (7)	5 (12)	
MPM			
CR/PR	3 (6)	6 (22)	.03[b]
SD/PD	50 (94)	21 (78)	
Unknown	0	0	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; aNSCLC, advanced non-small cell lung cancer; MPM, malignant pleural mesothelioma; CR, complete response; PR, partial response, SD, stable disease; PD, progressive disease.
[a]Chi-square test comparing proportions between statin users and no users. *P* values were calculated excluding unknown values.
[b]Statistically significant ($P < .05$).

Association between statin use and clinical activity of PD-1 inhibitors.
In the whole study cohort, at a median follow up of 17.0 months (95% confidence interval [CI] 15.8-21.9), statin use was associated with significantly improved ORR (32% versus 18%, *P* = .02), significantly better PFS (median 6.7 versus 2.9 months, hazard ratio [HR] 0.57, 95% CI 0.39–0.83, *P* < .01), and better OS (median 13.1 versus 8.7 months, HR 0.67, 95% CI 0.45–1.00, *P* = .05) (**Figure 1**).

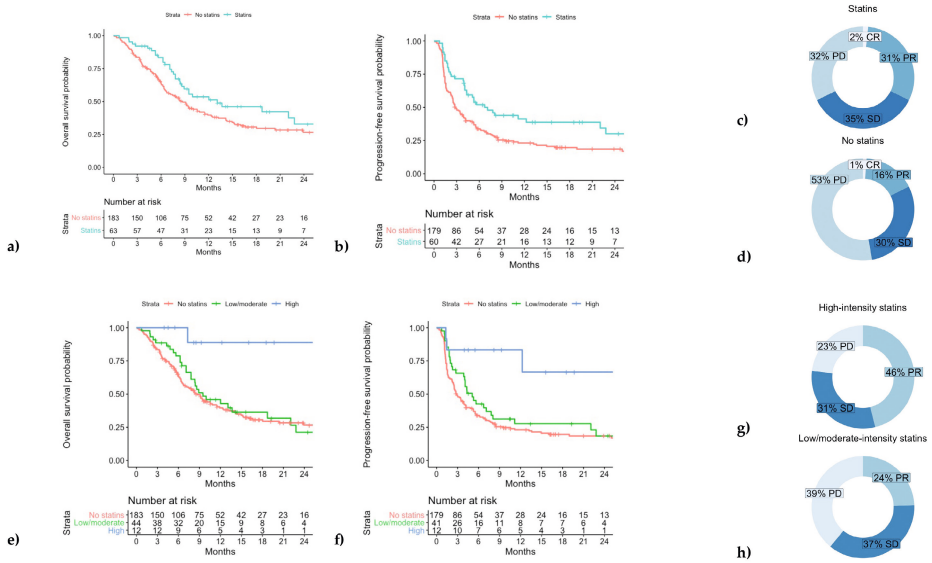

Figure 1. Overall survival (OS) (a), progression-free survival (PFS) (b), and tumor response (c and d) in thoracic cancer patients treated with PD-1 inhibitors, stratified by use of statins at baseline. OS (e), PFS (f), and tumor response (g and h) in thoracic cancer patients treated with PD-1 inhibitors, stratified by statin intensity at baseline. *P*-values calculated with log-rank tests. Numbers at risk between strata are shown directly below the Kaplan-Meier graph. Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

In MPM patients, statin use was associated with significantly increased ORR (22% versus 6%, *P* = .03). PFS and OS were also significantly longer in those patients with statins than in those with no statins (median PFS, 6.7 versus 2.3 months, HR 0.42, 95% CI 0.23–0.77, *P* < .01; median OS, not reached versus 6.0 months, HR 0.43, 95% CI 0.21–0.85, *P* = .01) (**Figure 2**).

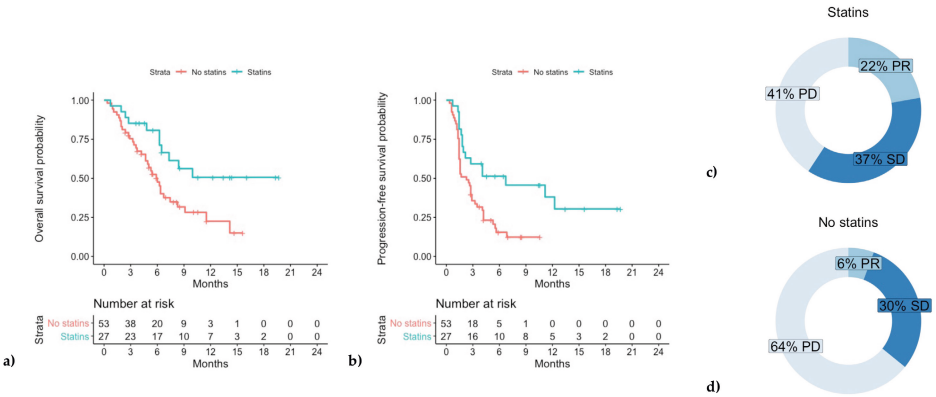

Figure 2. Overall survival (OS) (a), progression-free survival (PFS) (b), and tumor response (c and d) in MPM patients treated with PD-1 inhibitors with available survival data (n=80), stratified by use of statins at baseline.

In aNSCLC patients, statin use was associated with significantly increased ORR (40% versus 22%, *P* = .04). PFS was also significantly longer in those patients with statins than in those not taking statins (median 7.8 versus 3.6 months, HR 0.59, 95% CI 0.37–0.97, *P* = .03) while no difference in OS was observed between the two groups (median 13.1 versus 10.1 months, HR 0.79, 95% CI 0.49–1.28, *P* = .35) (**Figure 3**).

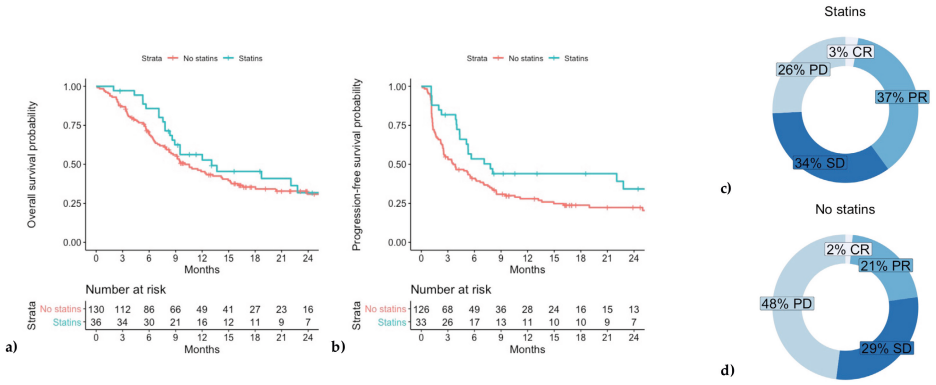

Figure 3. Overall survival (OS) (a), progression-free survival (PFS) (b), and tumor response (c and d) in aNSCLC patients treated with PD-1 inhibitors (n=166 for OS, n=159 for PFS), stratified by use of statins at baseline.

We next carried out a multivariable analysis of the effect of baseline statin use, adjusting for classical prognostic factors (age, gender, smoking status, ECOG PS, histological subtype) relevant to MPM and aNSCLC, respectively. In MPM, the impact

of statins remained significant for PFS (HR 0.36, 95% CI 0.16–0.81, *P* = .01) and OS (HR 0.27, 95% CI 0.10–0.74, *P* = .01) (**Table 3**). In aNSCLC, statins were not significantly associated with OS (HR 0.68, 95% CI 0.38–1.22, *P* = .20) but remained significantly associated with PFS (HR 0.52, 95% CI 0.29–0.93, *P* = .03) (**Table 3**).

Table 3. Multivariable analyses for PFS and OS in MPM and aNSCLC cohorts.

Test variables	PFS HR (95% CI)	*P* value	OS HR (95% CI)	*P* value
MPM				
No statins (ref.)/Statins	0.36 (0.16-0.81)	.01[a]	0.27 (0.10-0.74)	.01[a]
Age <70 (ref.)/≥70	0.97 (0.46-2.03)	.94	0.78 (0.34-1.78)	.55
Female (ref.)/Male	0.83 (0.31-2.19)	.71	1.32 (0.39-4.43)	.65
Never smokers (ref.)/Current-Former smokers	1.27 (0.60-2.66)	.52	1.01 (0.44-2.33)	.96
ECOG PS	1.62 (0.71-3.68)	.24	1.82 (0.68-4.97)	.22
0 (ref.)/≥1				
Histological subtype	1.34 (0.58-3.11)	.48	1.70 (0.66-4.38)	.26
Epithelioid (ref.)/Non epithelioid				
aNSCLC				
No statins (ref.)/Statins	0.52 (0.29-0.93)	.03[a]	0.68 (0.38-1.22)	.20
Age <70 (ref.)/≥70	1.49 (0.94-2.35)	.08	1.84 (1.12-3.03)	.02[a]
Female (ref.)/Male	0.80 (0.49-1.32)	.39	0.61 (0.35-1.04)	.07
Never smokers (ref.)/Current-Former smokers	0.84 (0.39-1.78)	.65	0.83 (0.37-1.84)	.64
ECOG PS	1.19 (0.75-1.89)	.44	1.60 (0.98-2.64)	.06
0 (ref.)/≥1				
Histological subtype	1.00 (0.62-1.59)	.99	1.02 (0.61-1.68)	.93
Adenocarcinoma (ref.)/Non adenocarcinoma				

Abbreviations: CI, confidence interval; PFS, progression-free survival, HR, hazard ratio; OS, overall survival; MPM, malignant pleural mesothelioma; ECOG PS, Eastern Cooperative Oncology Group performance status; aNSCLC, advanced non-small cell lung cancer.
[a] Statistically significant (*P* < .05).

To further investigate the role of statins as predictor of clinical activity in PD-1 inhibitors treated patients, we assessed survival of 77 MPM patients treated with standard chemotherapy (of which 13 (17%) were taking statins at baseline) (**Supplementary Table 3**), and we found no association between statin use, ORR (18% versus 17%, *P* = 1) and survival outcomes (median PFS, 6.0 months versus 6.3 months, *P* = .60; median OS, 11.3 months versus 16.7 months, *P* = .20) (**Supplementary Figure 1**).

Impact of statin intensity on clinical activity of PD-1 inhibitors.
We also examined the effect of baseline statins according to their intensity. In the whole cohort, patients receiving high-intensity statins were compared with those not receiving statins. Use of high-intensity statins was associated with increased ORR (46% versus 18%, $P = .03$), better PFS (median not reached versus 2.9 months, HR 0.20, 95% CI 0.06–0.65, $P < .01$) and better OS (median not reached versus 8.7 months, HR 0.10, 95% CI 0.01–0.76, $P = .02$) (**Figure 1**).

By looking separately at patients taking low/moderate-intensity statins and comparing them with no users, we found no differences in terms of ORR (24% versus 18%, $P = .43$), PFS (median 5.1 versus 2.8 months, HR 0.76, 95% CI 0.51–1.13, $P = .17$) and OS (median 9.5 versus 8.7 months, HR 0.89, 95% CI 0.59–1.35, $P = .59$) (**Figure 1**).

Finally, no difference in terms of PFS and OS was found according to the type and lipophilicity of statins (**Supplementary Figure 2 and Supplementary Figure 3**).

Discussion

This study reports that baseline statin use was associated with improved clinical activity of PD-1 inhibitors in MPM and aNSCLC patients. This association resulted to be intensity-dependent, as use of high-intensity but not of low/moderate-statins led to better outcomes.

The mechanism by which statins might boost clinical activity of PD-1 inhibitors in cancer is a matter of study [11]. Preliminary evidences suggested that blocking the MVA pathway might have a direct antitumor effect by interacting with key oncogenic molecules such as p53, MYC and PI3K [12]. According to Xia et al. [9], the antitumor effect of statins might be attributed to an indirect immunotherapeutic effect. More precisely, lipophilic statins were demonstrated to increase antigen occupation on dendritic cells thus boosting $CD4^+$ and $CD8^+$ responses in murine models. In the same model, lipophilic statins were also found to strongly synergize with PD-1 inhibitors, representing the biological rationale underlying our study.

Previous analyses in cancer patients were not always consistent in reporting an association between statin use and improved outcomes [13–15]. This is further supported by our investigation of MPM patients treated with standard chemotherapy. In this case, no association between statin use and outcomes was found, thus confirming that the potential of statins in cancer treatment might differ according to the characteristics of the disease and the combined drug.

Interestingly, our study suggests that statin intensity (meaning the LDL-C lowering that should occur with the specific statin and dosage) is essential in establishing their

association with response to immunotherapy. In particular, only high-intensity statins showed the ability to boost the activity of PD-1 inhibitors. If statins work as vaccine adjuvants, their temporal accumulation in combination with antigens is probably essential to induce adaptive immune responses [16]. To note, contrary to what showed in pre-clinical models [9], the lipophilicity of statins was not associated with clinical activity of PD-1 inhibitors in our series.

Our study has some limitations. First, it might be argued that patient characteristics other than statin use could have influenced the different prognosis. However, none of the factors known to normally affect clinical activity of PD-1 inhibitors (such as ECOG PS and smoking status) differ by statin use in our cohort. We also conducted multivariable analysis to adjust for significant prognostic factors in both MPM and aNSCLC, which still showed a positive impact of statin use. In addition, we found statins to be not only associated with PFS and OS but also with ORR to PD-1 inhibitors. Radiological response is much less likely to be affected by comorbidities/other patient conditions, thus further strengthening the observed link. Secondly, we did not adjust our estimates for tumor-intrinsic factors such as PD-L1 status and tumor mutational burden, which may differ in patients exposed to statins before anti-PD1 treatment. Finally, it was not possible to ascertain for how long patients were taking statins before anti-PD1 treatment. Therefore, these correlative analyses warrant further testing in prospective studies.

Conclusions

Altogether, our results showed that statin use is associated with improved clinical activity in thoracic cancer patients receiving PD-1 inhibitors in an intensity-dependent manner. Whether statin use reflects a general prognostic association or is causatively linked with improved clinical activity of PD-1 inhibitors still need to be assessed within randomized trials. If our results were prospectively confirmed, statins could represent an optimal strategy of drug repurposing in combination with anti-PD-1 therapy to improve outcome in patients with thoracic malignancies.

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Supplementary Material

Supplementary Table 1. Definition of statin intensity according to the 2018 American College of Cardiology/American Heart Association Guideline on the Management of Blood Cholesterol.

	High-Intensity	Moderate-intensity	Low-intensity
LDL-C Lowering*	≥50%	30% to 49%	<30%
Statins	Atorvastatin (40mg) 80mg Rosuvastatin 20 (40 mg)	Atorvastatin 10mg (20mg) Rosuvastatin 5 mg (10 mg) Simvastatin 20-40 mg	Simvastatin 10 mg
	-	Pravastatin 40mg (80mg) Lovastatin 40 mg (80 mg) Fluvastatin XL 80 mg Fluvastatin 40 mg BID Pitavastatin 1-4 mg	Pravastatin 10-20mg Lovastatin 20 mg Fluvastatin 20-40 mg

BID indicates twice daily; FDA, U.S. Food and Drug Administration; LDL-C, low-density lipoprotein cholesterol; RCT, randomized controlled trial.
Percent LDL-C reductions with the primary statin medications used in clinical practice (atorvastatin, rosuvastatin, simvastatin) were estimated using the median reduction in LDL-C from the VOYAGER database. Reductions in LDL-C for other statin medications (fluvastatin, lovastatin, pitavastatin, pravastatin) were identified according to FDA-approved product labeling in adults with hyperlipidemia, primary hypercholesterolemia, and mixed dyslipidemia.
*LDL-C lowering that should occur with the dosage listed below each intensity.

Supplementary Table 2. Statin characteristics in MPM and aNSCLC patients.

Characteristic	No. (%)
Statin use	
aNSCLC	
Yes	40 (22)
No	133 (74)
Unknown	6 (4)
MPM	
Yes	27 (33)
No	53 (65)
Unknown	2 (2)
Statin dose	
aNSCLC	
Low	0
Medium	25 (63)
High	6 (15)
unknown	9 (22)
MPM	
Low	2 (7)
Medium	17 (63)
High	8 (30)
Unknown	0
Statin lipophilicity	
aNSCLC	

Supplementary Table 2. Continued.

Characteristic	No. (%)
Lipophilic	30 (75)
Hydrophilic	6 (15)
Unknown	4 (10)
MPM	
Lipophilic	21 (78)
Hydrophilic	6 (22)
Unknown	0
Statin type	
aNSCLC	
Atorvastatin	11 (27)
Rosuvastatin	4 (10)
Simvastatin	18 (46)
Others	7 (17)
MPM	
Atorvastatin	10 (38)
Rosuvastatin	5 (18)
Simvastatin	11 (40)
Others	1 (4)

Abbreviations: aNSCLC, advanced non-small cell lung cancer; MPM, malignant pleural mesothelioma. Percentages refer to only patients taking statins at baseline and to each tumor type taken alone.

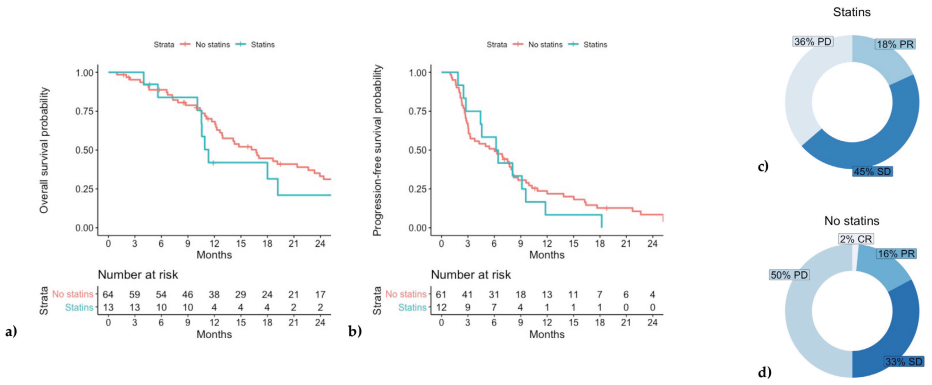
Supplementary Table 3. Clinical characteristics of MPM patients receiving standard first-line chemotherapy. Information about statin use and survival was available for 77 MPM patients.

Characteristic	No. (%)
Total	103
Median age	72 (48-83)
Sex	
Male	85 (82)
Female	18 (18)
ECOG	
0	58 (56)
1	31 (30)
≥2	6 (6)
Unknown	8 (8)
Smoking status	
Never smoker	37 (36)
Current/former smoker	57 (55)
Unknown	9 (9)
Histological subtype	
Epithelioid	65 (63)
Sarcomatoid/biphasic	32 (31)
Unknown	6 (6)
First-line chemotherapy	

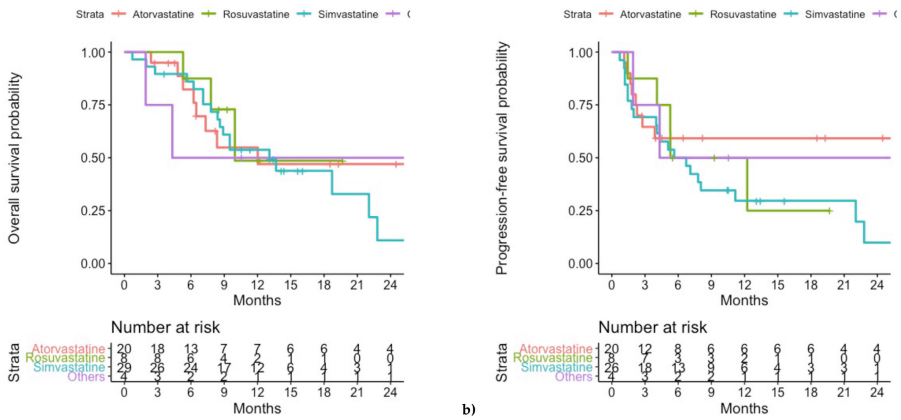
Supplementary Table 3. Continued.

Characteristic	No. (%)
Cisplatin plus Pemetrexed	25 (24)
Carboplatin plus Pemetrexed	40 (39)
Pemetrexed alone	16 (16)
Platinum based monotherapy	20 (19)
Unknown	2 (2)
Maintenance chemotherapy	
Yes	13 (13)
No	83 (80)
Unknown	7 (7)
Tumor response	
CR	1 (1)
PR	13 (12)
SD	33 (32)
PD	50 (49)
Unknown	6 (6)
Statin use	
Yes	13 (13)
No	66 (64)
Unknown	24 (23)
Statin intensity[a]	
Low	0
Moderate	10 (77)
High	2 (15)
Unknown	1 (8)
Statin characteristic[a]	
Lipophilic	10 (77)
Hydrophilic	3 (23)
Unknown	0

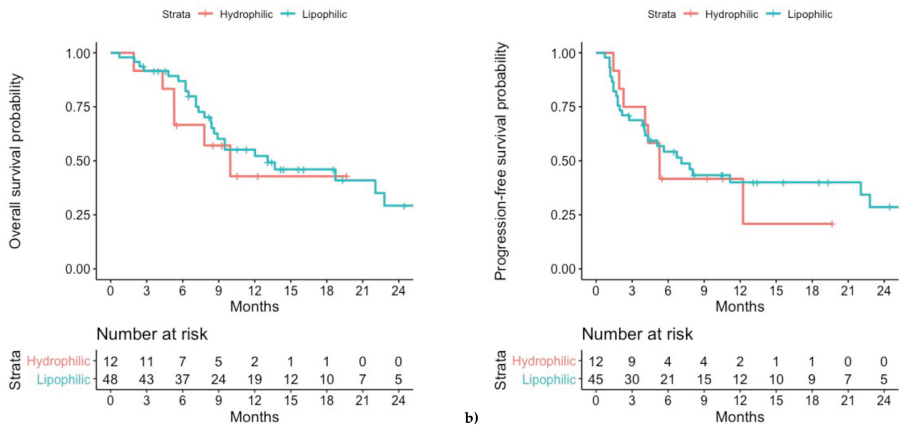
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; CR, complete response; PR, partial response, SD, stable disease; PD, progressive disease.
[a] Percentages refer to only patients taking statins at baseline.



Supplementary Figure 1. Overall survival (OS)(a), progression-free survival (PFS) (b) and tumor response (c and d) in MPM patients (n=77) treated with standard first-line chemotherapy, stratified by use of statins at baseline.



Supplementary Figure 2. Overall survival (OS) (a) and progression-free survival (PFS) (b) in the whole patient cohort, stratified by statin type at baseline.



Supplementary Figure 3. Overall survival (OS) (a) and progression-free survival (PFS) (b) in the whole patient cohort, stratified by statin lipophilicity at baseline.

CHAPTER 7

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

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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 19th, 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 29th, 2019 to December 22th, 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)
≥2	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)
≥3	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) (**Fig. 1A**), and median OS (mOS) was 7.0 months (95% CI: 4.7–not reached) (**Fig. 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%.

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) (**Fig. 1C**), and mOS was 7.2 months (95% CI: 5.9–not reached) (**Fig. 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**).

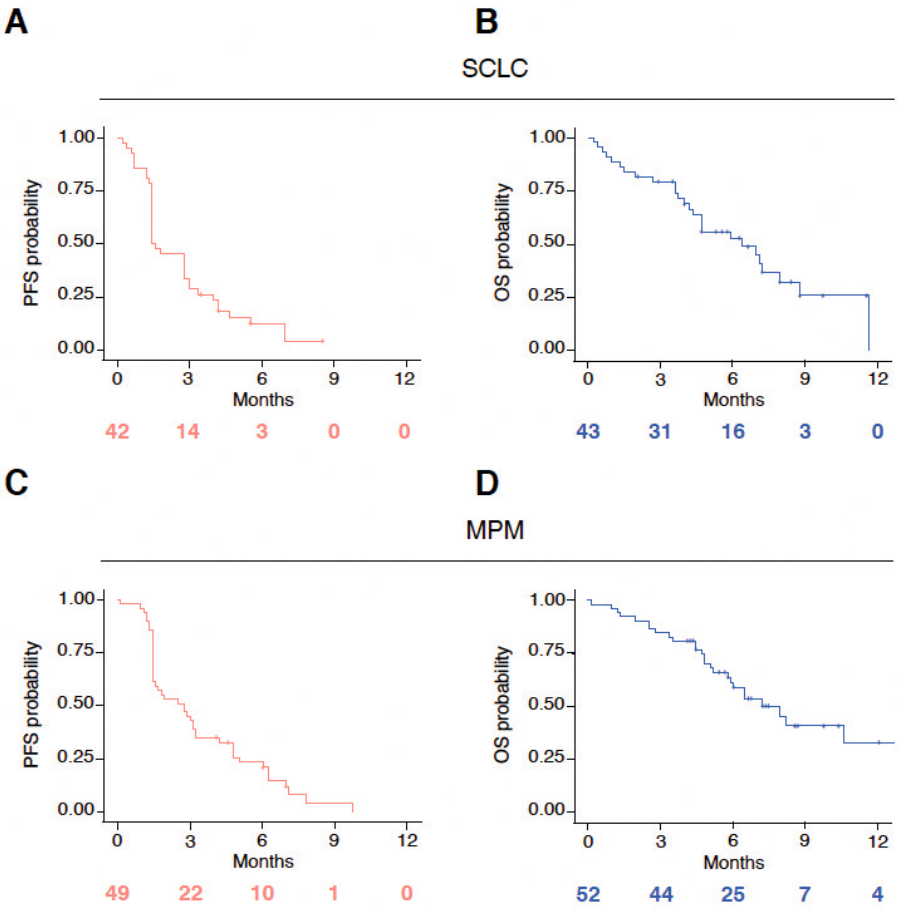

Figure 1. Kaplan Meier analyses in patients with small cell lung cancer (SCLC) and malignant pleural mesothelioma (MPM). **A**: Progression-free survival of SCLC patients (entire cohort). **B**: Overall survival of SCLC patients (entire cohort). **C**: Progression-free survival of MPM patients (entire cohort). **D**: Overall survival of MPM patients (entire cohort).

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).

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

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**).

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 Fig. 2**).

Although the relative proportion of the total monocyte population did not change significantly during therapy (**Fig. 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 (**Fig. 2B and 2C;** see for gating: **Suppl. Fig. 2**). This decrease of classical monocyte frequencies was paralleled by a significant relative increase of intermediate monocytes in both SCLC (**Fig. 2B**) and MPM (**Fig. 2C**). Interestingly, we found that SCLC patients with lower frequencies

of classical monocytes before treatment with lurbinectedin, had a longer PFS (**Data Supplement Fig. 3**).

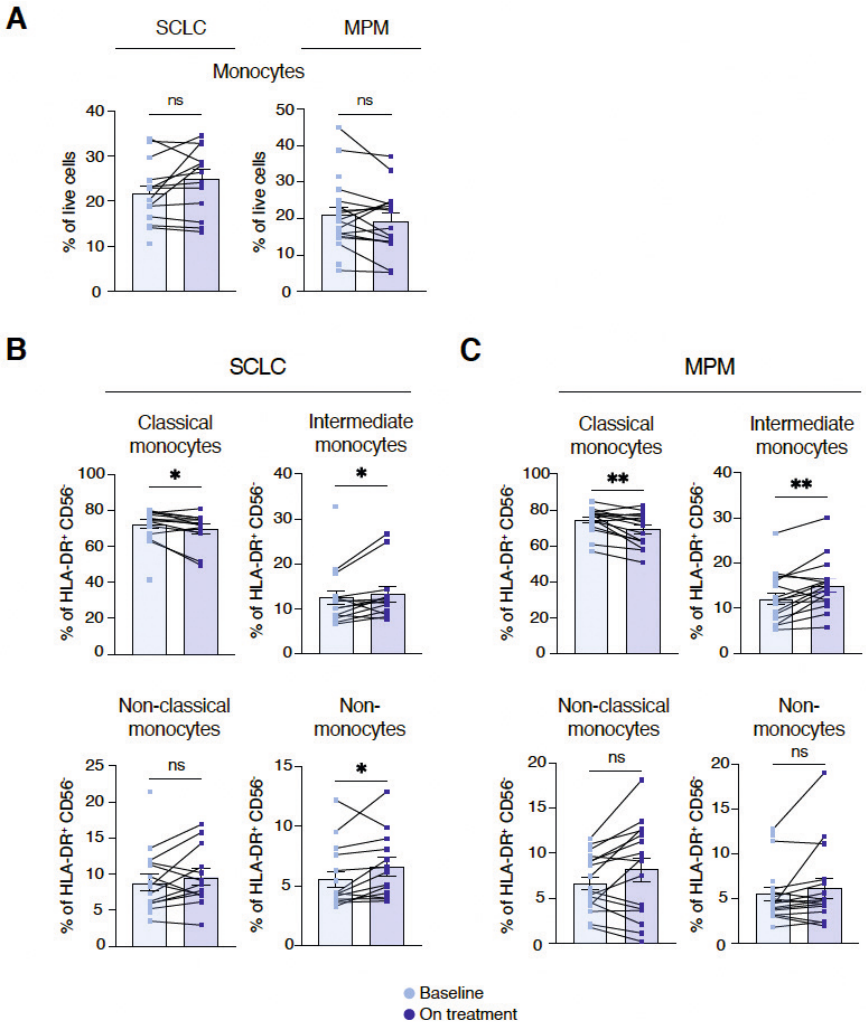

Figure 2. Lurbinectedin treatment is associated with depletion of the classical monocyte subset. **A**: Percentage of monocytes ($CD14^+$ $CD16^{+/-}$ and $CD14^-$ $CD16^+$) at screening an on-treatment time points in small cell lung cancer (SCLC) patients (left) and malignant pleural mesothelioma (MPM) 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 (**Fig. 3A**), and of NK and NKT cells in both SCLC and MPM (**Fig. 3B**). This increase in proliferation was independent of clinical response (**Data Supplement Fig. 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 Fig. 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 Fig. 6**).

In addition to T cell proliferation, we assessed the expression of a variety of co-stimulatory and –inhibitory receptors on circulating T cells (**Fig. 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 (**Fig. 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.

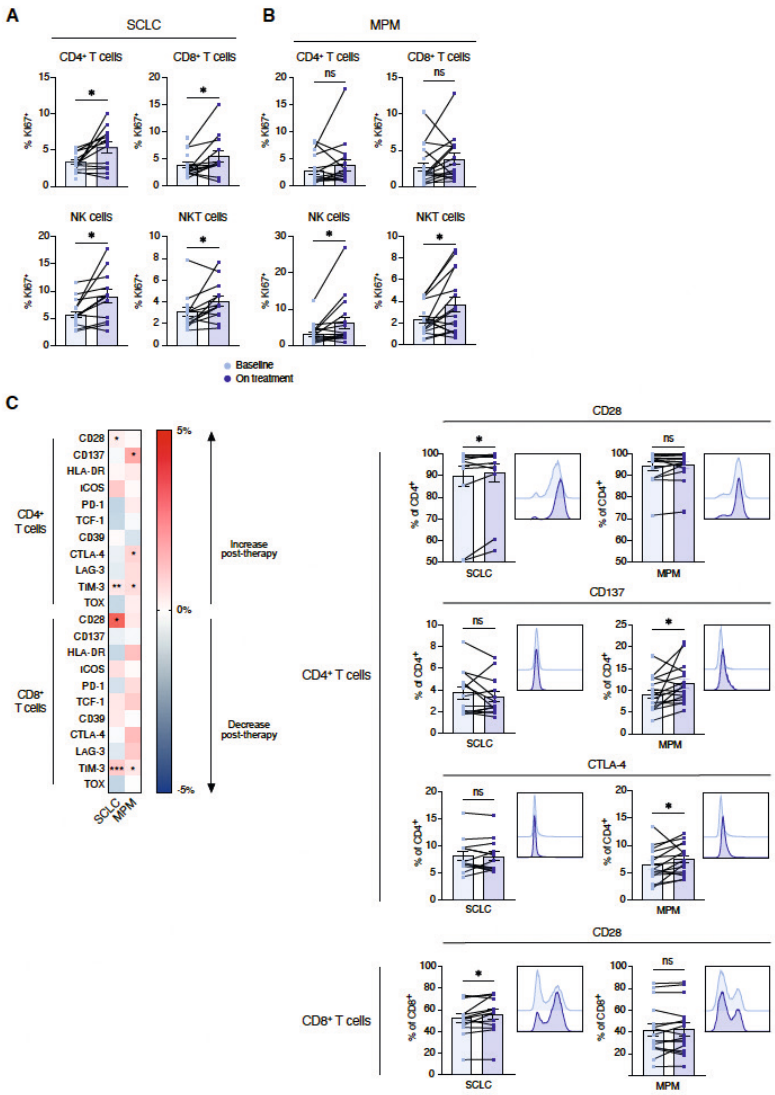

Figure 3. Lurbinectedin modulates proliferation and alters phenotype of circulating lymphocyte subsets. **A**: Percentage of Ki67$^+$ CD4$^+$ T cells, CD8$^+$ T cells, NK and NKT cells, at screening and on-treatment time points in small cell lung cancer (SCLC) patients. **B**: Percentage of Ki67$^+$ 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/m² every 3 weeks until progression or unacceptable toxicity. Dose reductions were performed in steps of 0·6 mg/m², with a minimal dose of 2·0 mg/m². 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
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.

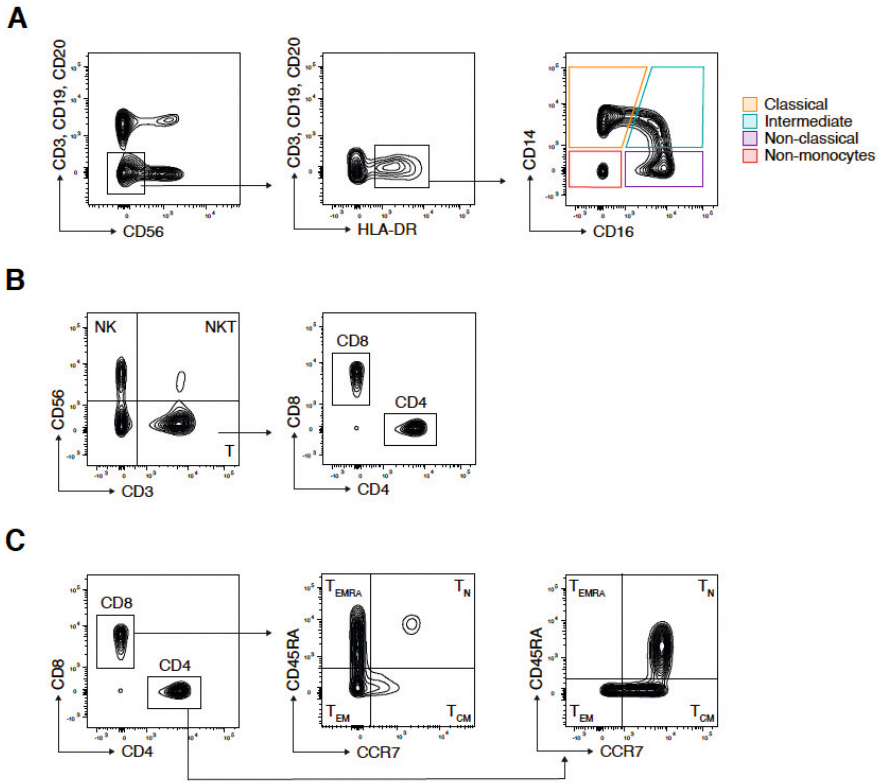
Characteristic	SCLC (n=20)	MPM (n=19)
Median age, years (range)	65 (56-77)	73 (58-79)
Gender, male, No. (%)	9 (45)	17 (89)
Median time from diagnosis to start of lurbinectedin, months (IQR)	14.0 (11.0-22.8)	13.0 (10.4-26.3)
Smoking status, No. (%)		
Former/current	12 (60)	11 (58)
Never	1 (5)	3 (16)
Unknown	7 (35)	5 (26)
ECOG PS at start of lurbinectedin, No. (%)		
0	2 (10)	3 (16)
1	17 (85)	12 (63)
≥2	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)
≥3	8 (40)	4 (21)
Prior immunotherapy, No. (%)	2 (10)	43 (83)

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

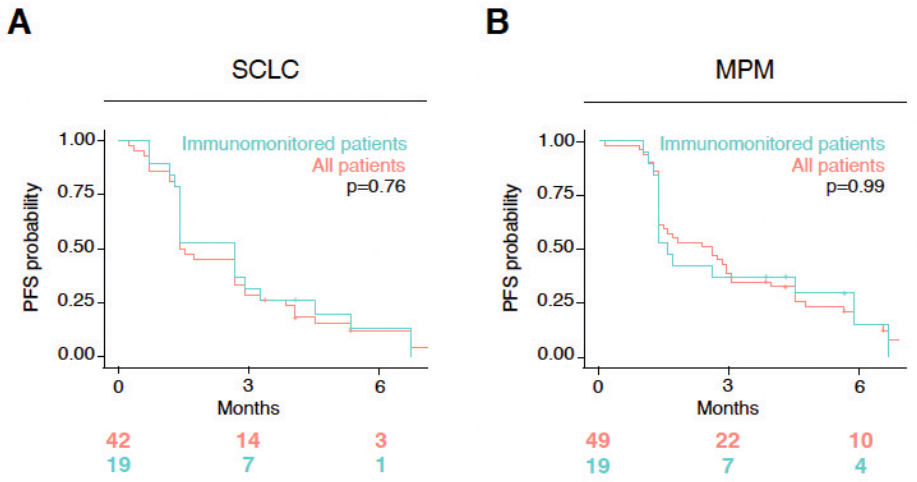
Characteristic	SCLC (n=20)	MPM (n=19)
Time since last cycle of systemic treatment, months (range)	1.9 (0.8-7.4)	1·6 (0·8-21·2)
<90 days	18 (90)	13 (68)
≥90 days	2 (10)	6 (32)
Unknown	0 (0)	0 (0)
Response to lurbinectedin, No. (%)		
PD	13 (65)	12 (63)
SD	3 (15)	7 (37)
PR	3 (15)	0 (0)
Unknown	1 (5)	0 (0)

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.

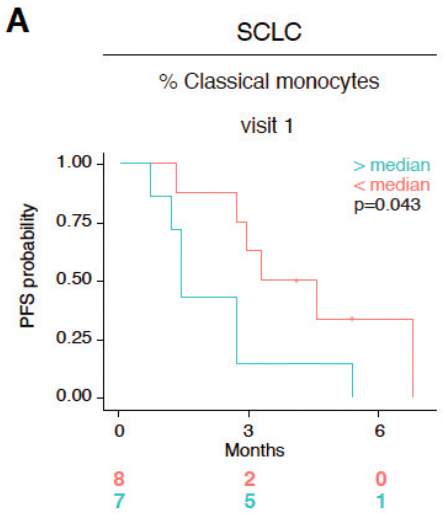
Supplemental Figures



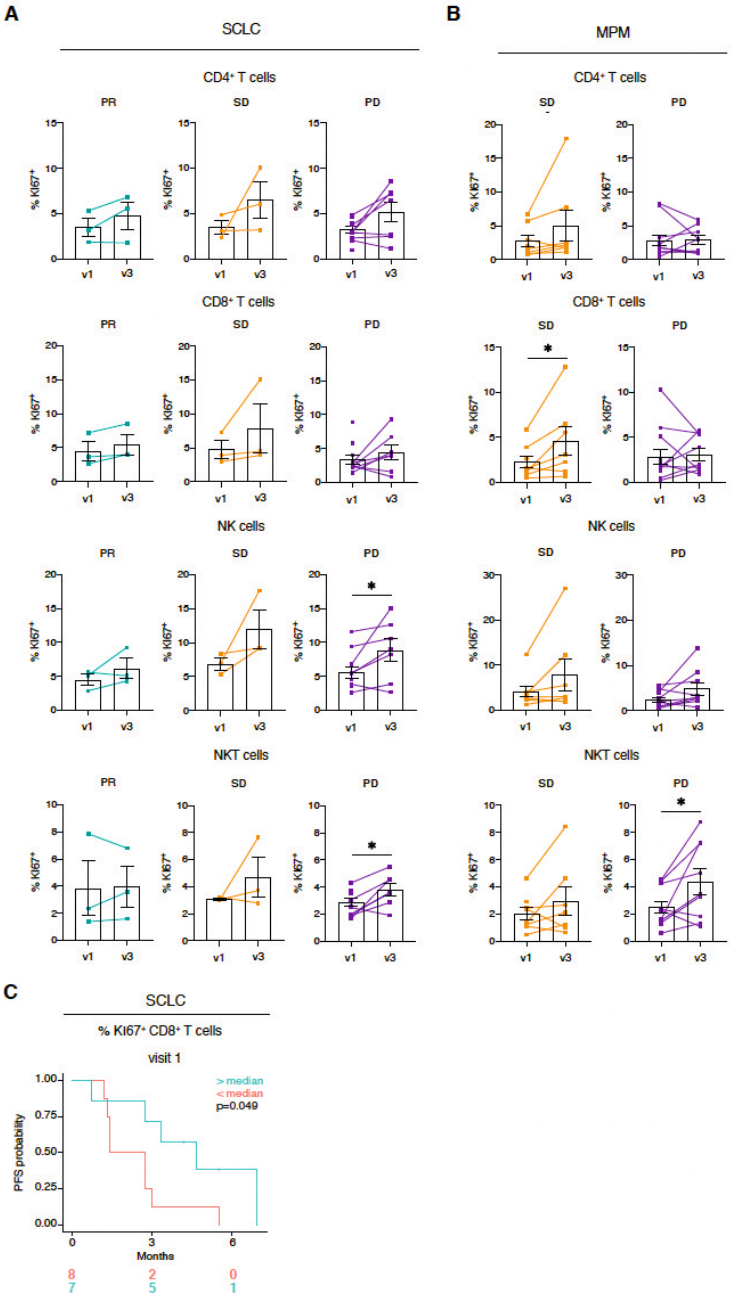
Supplemental Figure 1. Gating strategies. **A:** Gating strategy for the circulating myeloid subsets. **B.** Gating strategy for NK cells, NKT cells and T cells. **C**: Gating strategy for $CD4^+$ T cell and $CD8^+$ T cell subsets.



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.

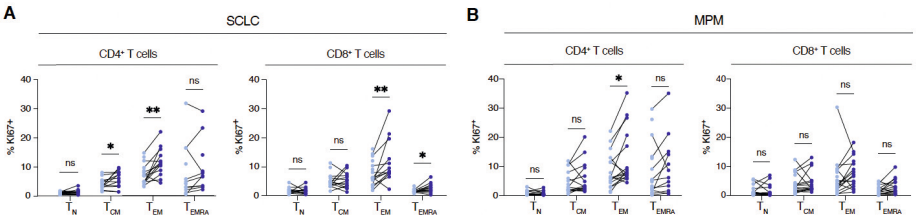


Supplemental Figure 3. Kaplan–Meier analysis showing differences in progression-free survival between SCLC patients exhibiting a lower (red) or higher (blue) proportion of classical monocytes prior to treatment.

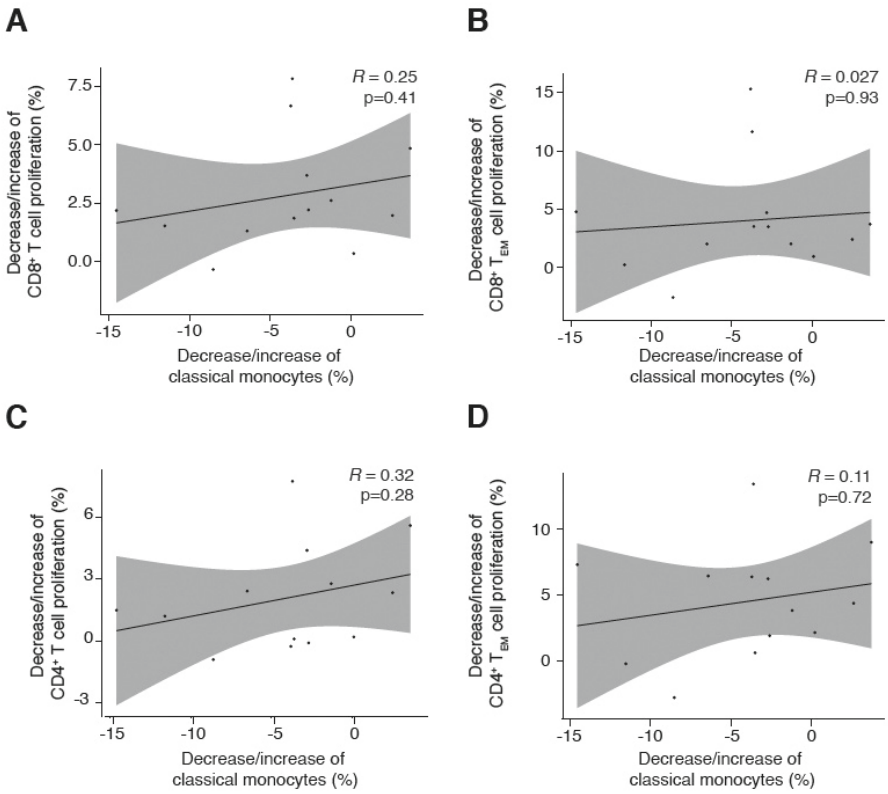


Supplemental Figure 4. Lurbinectedin effect on proliferation of circulating lymphocytes is independent of clinical response. **A**: Comparison between small cell lung cancer (SCLC) patients with partial response (PR), stable disease (SD) and progressive disease (PD) for the percentage of Ki67$^+$ CD4$^+$ 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. 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 8

Dendritic Cell therapy (MesoPher) In Combination With Extended-Pleurectomy/Decortication After Chemotherapy in Subjects With Resectable Mesothelioma: protocol description of the ENSURE feasibility study and results in the first patient

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Submitted

Introduction

Malignant pleural mesothelioma (MPM) is an uncommon but aggressive neoplasm with low survival rates. For patients with early stage - resectable MPM the role of radical surgery remains controversial and multimodal treatment might improve patients' prognosis. Dendritic cell therapy (DCT) with Mesopher proved to be safe and yielded promising results in patients with MPM, with single agent radiological activity[1–3], representing the rationale for a combined (neo)adjuvant approach with extended pleurectomy/decortication (eP/D) surgery.

Trial design

This open label, single center, phase I study will evaluate the feasibility of DCT with Mesopher performed before and after eP/D in patients with resectable epithelioid MPM. Safety and immunological effects of (neo)adjuvant DCT will be determined. Sixteen adult patients diagnosed with resectable epithelioid MPM will be enrolled following first-line chemotherapy. Before standard-of-care chemotherapy, a leukapheresis will be performed from which monocytes will be isolated and used for further differentiation into DCs. Hereafter, the DC will be loaded with allogeneic MPM tumor cell line lysate (Pheralys) and maturated using the Jonuleit cytokine cocktail (**Figure 1**).

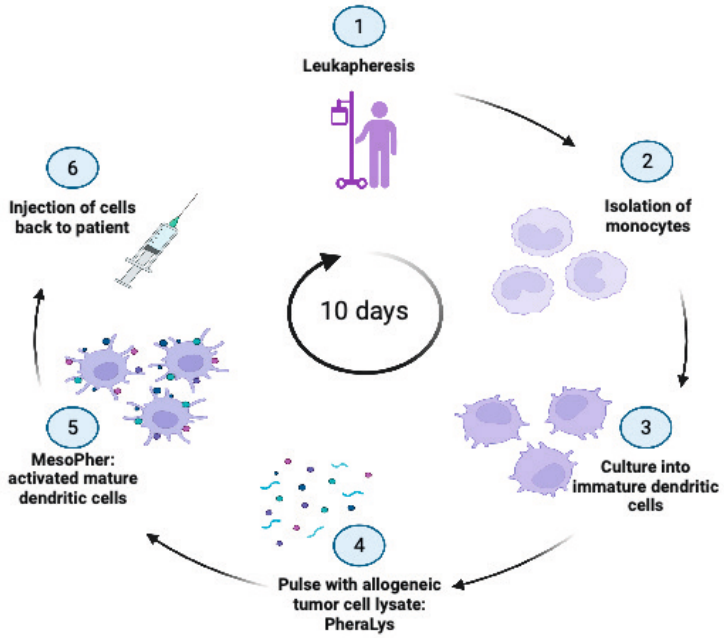

Figure 1. Mesopher production process.

The subsequently formulated drug product (Mesopher) will be re-injected 4 weeks after completing chemotherapy, 2 times every other week. Four weeks after the first injection with DCT, patients will undergo eP/D surgery followed by three bi-weekly injections with DCT, starting 4 weeks after surgery. In total, five DC vaccinations will be administered (**Figure 2**).

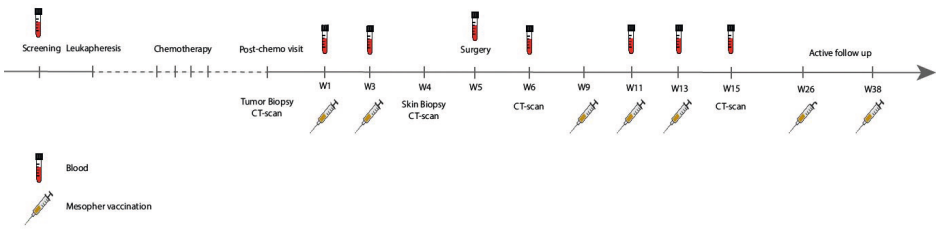

Figure 2. Study procedures and timeline.

Tumor material will be collected before starting neo-adjuvant DCT and at time of surgery. Tumor-specific immune activation will be investigated on both tumor material and peripheral blood samples prior and post DCT by using flow cytometry, imaging mass cytometry, and T-cell receptor (TCR) repertoire analysis. The trial is active at the Erasmus MC, Rotterdam (NL), enrolling patients since January 2022.

Study objectives

Primary objective: To assess whether (neo)-adjuvant DCT with Mesopher is feasible in resectable epithelioid MPM patients after first-line chemotherapy.

Secondary objectives: Safety, efficacy (as measured by progression free and overall survival).

Exploratory objective: Determine the anti-tumor immune response induced by (neo)adjuvant DCT. Tumor-specific immune activation will be investigated on both tumor material and peripheral blood samples prior and post DCT by flow cytometry, multiplex immunofluorescence, and T-cell receptor (TCR) repertoire analysis.

Study population

Inclusion criteria

✓ Histologically confirmed diagnosis of epithelioid MPM.
✓ Resectable disease defined by stage cT1-3, N0-1, M0 (I to IIIA)
✓ Eligibility for 2 to 4 cycles of platinum-based chemotherapy.
✓ Fit to undergo an eP/D with optional removal of hemidiaphragm and pericardium.
✓ Tumor tissue available after completing chemotherapy and before starting treatment with DCT.
✓ Adequate bone-marrow, renal, and liver function.
✓ ECOG performance status of 0 or 1.

Exclusion criteria

✓ Clinical or radiological invasion of mediastinal structures and widespread chest wall invasion (stage T4). Involvement of N2 nodes.
✓ Stage IV (metastatic disease).
✓ Any different histology from the epithelioid MPM.
✓ Unavailability of tumor tissue after completing chemotherapy.
✓ Use of >10 mg of prednisolone or equivalent/day (or other immunosuppressive agents)
✓ Prior treatment of any kind for MPM.
✓ Any previous malignancy.
✓ Major surgical procedure in the last month.

Sample size calculation
The primary objective of the study is to determine the feasibility of applying MesoPher as neo-adjuvant and adjuvant therapy in combination with eP/D in MPM patients.

Providing a specific statistic calculation assessing the number of patients to prove feasibility is hard to achieve. We will start enrolling 16 MPM patients. By doing this, we will be able to estimate a compliance rate of 43% to within a 95% confidence interval of +/-24%.

Trial identification: nct05304208

Case report

The first patient treated with (neo)adjuvant DCT (Mesopher) and eP/D is a 52-years old female patient. Her history started in October 2021, when she presented to our attention with dyspnea, reporting idiopathic recurrent pneumonia since a few months. The patient had no relevant medical history except for a hysterosalpingo-oophorectomy in 2000 and no known asbestos exposure. Based on CT findings, she underwent a video-assisted thoracoscopic surgery (VATS) biopsy on the right pleura and was diagnosed with epithelioid mesothelioma. The PET scan confirmed moderate (18)F-fluorodeoxyglucose (FDG) accumulation on the right pleura with pleural effusion.

Since surgeons deemed the tumor resectable and the patient fit to receive an extended operation, she was eligible for the ENSURE trial and was included in December 2021.

The patient underwent leukapheresis without any complications. According to protocol, she received 4 courses of cisplatin-pemetrexed chemotherapy as per standard of care. Because of worsening of renal function cisplatin was shifted to carboplatin on the fourth course. The CT scan performed at the end of chemotherapy revealed a stable disease. To gain pre-DC treatment tumor material, a new VATS biopsy was performed as per study design. Renal function gradually improved after interruption of chemotherapy and the two neoadjuvant DC vaccinations were administered one week and three weeks after the VATS procedure. This was followed by a delayed skin testing (as per protocol) for translational purposes. The patient was then referred to surgery within the predefined timeline. eP/D was performed without complications and the patient was discharged after 6 days. The pathologic examination revealed epithelioid mesothelioma on both visceral and parietal pleura and showed limited therapy effect. Translational research on the pre- and post-DCT tumor samples is ongoing. After surgery, the patient received adjuvant treatment with three DC vaccinations without treatment-related delay. No severe toxicity developed

during DC vaccination. The only DC-related adverse events consisted of fever, cold chills, malaise, and injection site reactions (i.e. erythema, induration, and itching), all limited to CTCAE grade 1 toxicity. At the post-surgical radiological evaluations, no signs of disease recurrence were observed. After more than 12 months since trial inclusion, the patient is still in good medical conditions (ECOG PS 1) and no signs of relapse have emerged. DCT with Mesopher resulted feasible and safe in this patient as she completed neo-adjuvant plus adjuvant DCT (5 administrations in total) and surgery within the predefined timeline and without extended treatment-related delay. As per study protocol, a 6th and a 7th optional DC vaccination will be planned in the next months according to patient conditions.

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CHAPTER 9

General Discussion

Since the discovery of oncogenic driver mutations which are implicated in the development and propagation of cancer, drug development in oncology has shifted towards a "personalized medicine" approach. Differently from traditional chemotherapy, new targeted agents have been specifically investigated and approved for patients whose tumors harbors specific genomic alterations. One example is represented by targeted therapies in non-small cell lung cancer (NSCLC) patients with activating mutations in EGFR, BRAF, and rearrangements in ALK and ROS1[1]. In the last decade, the advent of immune check-point inhibitors (ICIs) has deeply reshaped the treatment paradigm of lung cancer and mesothelioma, leading to a Renaissance of immunotherapy with a consistent survival benefit across different indications. However, the number of clinical trials in this new field has often outpaced researchers' ability of tailoring the right immunotherapy for the right patient. So far, PD-L1 expression represents the only biomarker which help patient selection for ICI treatment in clinical practice[2], yet its adoption comes along with many pitfalls related to test and tumor heterogeneity and could not be extended besides NSCLC to small cell lung cancer (SCLC) or malignant pleural mesothelioma (MPM). The development of smart immunotherapy combinations has also been hampered by the design of clinical trials with little scientific rationale.

The main goal of this thesis was to assess the current impact of immunotherapy in terms of safety and effectiveness for lung cancer and MPM patients treated in the real-world. In addition, by investigating a more holistic approach which takes into account not only tumor but also patient-related factors as determinants of response/toxicity we sought to inform a rational development of new immunotherapy combinations and finally improve the observed outcomes.

In **Chapter 2** we discussed how the conduction of well-designed, biomarker-driven clinical trials, represents a major challenge in cancer of rare incidence such as MPM. Too often, the scientific evidence supporting the implementation of new agents in this neglected disease was incomplete, mainly based on nonrandomized studies with surrogate endpoints not being replicated in the real-life context. Only by acknowledging MPM the inter- and intra-patient heterogeneity of this disease, which became clearer with a better appreciation of its pathobiology, we will be able to move beyond single immune check point inhibition in MPM (**Chapter 3**).

Although prone to selection bias in general, well designed cohort studies in real-world population provide researchers with more insights into the number of (treatment-related) adverse events and actual survival times. An example of data which can be abstracted from real-world analyses is illustrated in **Chapter 4**, where we confirmed that adopting an extended interval dosing (ED) of ICIs represents a safe and feasible approach across multiple cancer types. In **Chapter 5,** we looked at real-world clinical outcomes of nivolumab treatment in pre-treated MPM patients,

showing that objective response rate (ORR) and median overall survival (mOS) were lower compared to those reported in phase II trials.

In line with a number of studies which have reported a link between concomitant baseline medications and response to immunotherapy, in **Chapter 6** we showed that statins are associated with better clinical outcome in MPM and advanced NSCLC patients treated with PD-1 inhibitors in an intensity-dependent manner.

Starting from a manageable safety profile and clinical activity of lurbinectedin in pre-treated patients with SCLC and MPM, in **Chapter 7** we reported on the immune-modulatory effect of this chemotherapy, as determined by the circulating immune profile of patients. The observed results make lurbinectedin a potential backbone for future immunotherapy combinations in SCLC and MPM. Finally, in **Chapter 8**, we proposed an alternative immunotherapy approach by using (neo)adjuvant DC vaccination (Mesopher) in resectable MPM patients.

Taken together, findings presented in this thesis reveal the complexity of the interaction between lung cancer, MPM and the immune system. Only interrogating cancer immunity through different available approaches (e.g. real-world data) and from a broader perspective (e.g. investigating patient-related factors) will likely increase physicians' ability to select appropriate immunotherapy in the coming few years, moving further from the "one size fits all" approach. Below, we discuss the strengths and limitations of our research in the context of available literature to dissect the underpinnings of how immunotherapy constitute its effect and ultimately improve drug delivery for lung cancer and mesothelioma patients.

Tailoring immunotherapy for lung cancer and mesothelioma

Safety of new extending interval dosing of immune check point inhibitors

In the last couple of years, the number of indications for which ICIs have become available considerably extended. Expectations are high that ICIs will remain the cornerstone of many cancer treatments patients in the future. ICIs act by modulating endogenous regulatory immune mechanisms to enhance immune system activation against the tumor[3,4]. In some treated patients, this activation can occur broadly and relatively non-specific, leading to the onset of immune-related adverse events (irAEs). As irAEs can be life-threatening or even lead to death, a careful and tailored usage of ICIs is recommended to early identify patients at high-risk of toxicity as well as reduce or avoid severe irAEs. During clinical development of most of the approved PD-1 and PD-L1 inhibitors, the exposure–efficacy as well as the exposure–safety relationship was flat or nonsignificant over the dose ranges tested[5]. This made the pair with PK data reporting a low distribution outside the plasma compartment[6,7], highlighting a comparable risk-benefit profile with ICIs between weight-based

and flat dosing and leading to FDA and EMA approvals of the latter. Flat doses are expected to shorten dosage preparation time, improve ease of administration, thus shortening patient waiting time. Recently, long life expectancy of patients treated with ICIs, high healthcare costs, together with the urge of reducing avoidable hospital admissions during COVID-19 crises[8], led to an increasing interest in longer interval dosing ICI schedules. Based on additional PK exposure simulation and clinical trial data, adoption of flat ED ICIs, pembrolizumab 400 mg Q6W and nivolumab 480 mg Q4W, seemed to offer similar outcomes and safety compared with canonical interval dosing (CD) schedules (200 mg Q3W and 240 mg Q2W, respectively)[9–11]. For this reason, in the real-life setting an increasing percentage of patients have been shifted to (or treated upfront with) ED ICIs. A recently published real-world study, although performed in a limited cohort of 45 NSCLC patients[12], reported the arising of new or worsening irAEs due to switching of pembrolizumab interval dosing from 200 mg Q3W to 400 mg Q6W. Contrary, in **Chapter 4** we investigated a larger pan-cancer cohort of 835 patients and we confirmed that switching ICI monotherapy from CD to ED did not affect the safety profile, thus representing a safe and feasible option also outside of clinical trials. In the multicentre EDICI ("Extended interval Dosing in patients receiving Immune Checkpoint Inhibitors") study, dermatitis, asthenia, and diarrhea/colitis were the most common irAEs during ED, and only 6% of patients returned to CD after switching to ED. Showing a progressive reduction of toxicities after switching to ED, our study somehow corroborates previous observations indicating that prolonged ICI treatment does not lead to an increased cumulative toxicity[11,13,14]. Noteworthy, some irAEs during ED represented de novo toxicity, meaning that patients had not experienced any irAEs during CD. This fact reveals that the pathobiology of immune-related toxicity might differ between the two schedules. In particular, 43% of any grade and 30% of G3/G4 irAEs occurred after only one ED administration, suggesting a special warning during the early cycles after switching probably due to a rapid change in peak concentrations (C_{max}). Being aware of the occurrence of de novo toxicity and of the intrinsic limitations of a retrospectively collected dataset, our data will hopefully reassure physicians that ED schedules represent an important alternative for ICI treatment of cancer patients (including lung cancer and MPM where ICI is used up to 2 years). Beside representing a reasonable option during pandemic times when patients' access to oncology departments were remodulated, the ED schedule will help reducing the so-called "time-toxicity"[15], a concept introduced by Gupta et al. as the "time spent in coordinating care and in frequent visits to a health care facility (including travel and wait times)"[16]. So far, ED ICI adoption has been lower than expected[17], probably because of a general inertia of prescribers and because of doubts regarding ED efficacy, given the criteria on which they have been approved. To further support ED adoption, investigations looking at the safety of this approach when used upfront (and compare it with upfront CD) as well as investigating ED effectiveness data outside of clinical trials are awaited. Strohbehn et al.[17] looked at data from the Veterans Health Administration and, based on an intention-to-treat analysis,

they found no differences in time-to-treatment discontinuation (TTD) between CD and ED pembrolizumab. Although frequently used, the correlation between TTD and overall survival (OS) has not been confirmed for every cancer type[18] and data from Strohbehn et al. need longer follow up and external validation. Nevertheless, these data are consistent with pharmacokinetic, pharmacodynamic, and single-arm clinical trial findings and complement our safety data, suggesting the near-equivalence of CD vs ED ICIs. As the two dosing regimens are equally effective, physicians should encourage a tailored and patient-centred approach[19]. On a broader perspective, this should take into account not only the impact on individual patients but also on the global health system. Using an ICI flat dosing might not always represent the most sustainable choice, as it results in an excess of 25% to 40% in drug dose and of 25% increase in drug costs compared to body weight–based dosing[20]. The best strategy would imply to improve the cost-effectiveness of ICI therapy while preserving efficacy, safety, and quality of life for the patients. To this extent, our group at the Erasmus MC Cancer Institute has proposed a hybrid dosing, which combines weight-based dosing with the registered fixed-dose as a maximum dose (dose cap). This approach leads to a sharp cost reduction (**Table 1**) and can be even improved when vial sharing and (electronic) rounding on whole ampoules are adopted. When implemented globally, through the mean of well-designed pharmacoeconomic studies, this novel strategy could potentially save billions of dollars to our health systems[21].

Table 1. Overview of ICI hybrid dosing regimens and potential savings.

Drug	FDA.EMA Approved dose	Hybrid dosing	% Savings
Pembrolizumab	Q3W: 2 mg/kg 200 mg	Q3W: <65 kg 100 mg >65 kg 150 mg*	~22%
	Q6W: 400 mg	Q6W: <65 kg 200 mg 35-90 kg 300 mg >90 kg 400 mg	
Nivolumab	Q2W: 3 mg/kg 240 mg	Q2W: 3 mg/kg max. 240 mg**	~10%
	Q3W: 360 mg	Q3W: 4.5 mg/kg max. 360 mg**	
	Q4W: 480 mg	Q4W: 6 mg/kg max. 480 mg**	

* With vial sharing
** With rounding of <10% of calculated dose on whole ampules
N.a.= no available

Real world effectiveness of immune check point inhibitors

Unlike other solid tumors (including NSCLC), a promising actionable oncogenic driver with therapeutic impact has never been identified in MPM (**Chapter 2**). For this reason, treatment of this rare neoplasm has never shifted towards a "personalized medicine" perspective[22]. After the introduction of single immune check point inhibition in the treatment armamentarium of lung cancer, researchers have attempted to replicate the same results in the setting of MPM, yet findings have been conflicting and inconsistent. As discussed in **Chapter 3**, tumor responses have been variable and most importantly unpredictable. In particular, initial results from phase II studies with single ICI in pre-treated MPM patients have not been confirmed in larger, randomized, phase III trials and in real-world analyses. Although the CONFIRM trial[23] showed the superiority of nivolumab over supportive care, no survival differences were observed in the only randomized trial with active control[24]. In the phase III, open-label, PROMISE-meso trial, MPM patients pre-treated with platinum-based chemotherapy were randomized to receive either pembrolizumab 200 mg Q3W or institutional choice single-agent chemotherapy (gemcitabine or vinorelbine)[24]. At data cut-off for the primary end point analysis, no difference in progression-free survival (PFS) was identified. Median PFS was 2.5 months (95%CI: 2.1-4.2) for pembrolizumab versus 3.4 months (95%CI: 2.2-4.3) for chemotherapy (p = 0.76). To note, nearly four times more patients responded to immunotherapy (objective response rates (ORRs) were 22% with pembrolizumab vs 6% with chemotherapy, p = 0.004), but these responses were not translated into delayed progression or improved survival.

In **Chapter 5**, we investigated the real-world clinical outcomes of nivolumab treatment in 107 pre-treated MPM patients included in the Dutch expanded access program. We observed an ORR of 10%, a mPFS of 2.3 months and a mOS of 6.7 months. Compared to clinical trials data[24–27], our ORR and mOS were inferior, a fact that might be attributed to the lack of strict inclusion criteria in our analysis, which led to a less selected patient population. A recent metanalysis included our study among other 13 aiming at identifying the efficacy of PD-1/PD-L1 monoclonal antibodies (mAbs) in chemotherapy pre-treated MPM patients. In a total of 888 patients, ORR and disease control rate (DCR) were 18.1% and 55.4%, while mPFS and mOS ranged from 2.1 to 5.9 and from 6.7 to 20.9 months, respectively[28]. Although disappointing, these results should be put in the specific context of an orphan disease such as MPM. Following progression to first-line chemotherapy, standard treatments have repeatedly shown only modest efficacy, with ORR of 8.6%, DCR of 54.8%, mPFS and mOS of 3.4 and 7.8 months, respectively[29]. As shown in **Chapter 7**, no tumor responses were registered in a real-world cohort of MPM pre-treated patients who received lurbinectedin, again confirming the poor performance of any therapy in this setting.

In MPM patients treated with nivolumab, we also observed a correlation between ORR and OS, with patients achieving partial response (PR) experiencing long-term survival. Although ORR has never been demonstrated to be a surrogate marker of survival in the context of MPM chemotherapy[28] and different criteria for response evaluation such as mRECIST and iRECIST have been used across studies, duration of response has repeatedly resulted longer with ICIs (ranging from 11.1 to 15.2 months with single PD-1/PD-L1 inhibition in phase I-II trials)[30]. Therefore, careful statistical considerations should be adopted when evaluating immunotherapy before denying the validity of this approach. We hypothesize that the lack of significant survival benefit observed in the PROMISE-meso trial in terms of mPFS and mOS, despite a higher ORR, might be due to the limited number of patients achieving response combined with most patients experiencing early progression. In fact, if only a minority of patients (10–20%) respond to therapy, mPFS and mOS will not be influenced, as more than 50% of the patients will progress or die earlier according to the aggressive course of disease. Different endpoints able to capture benefit across the entire population (e.g., hazard ratios [HRs] over time and milestone survival), should be considered when designing clinical trials for single immune check point inhibition in MPM[31,32].

The presence of a minority of long-term responders to ICIs in our study and in the PROMISE-meso underlines the importance to identify reliable predictive biomarkers in this setting to improve tailoring of the treatment. Besides that, two other options may be pursued to optimize the use of ICIs in MPM patients. The first one is to move ICIs toward the first-line setting, where the reinvigoration of the immune

system may be stronger and more efficient, as proved by the Checkmate 743 trial which showed a significant improvement in OS of nivolumab plus ipilimumab (anti-PD1 and anti-CTLA4, respectively) versus platinum-based chemotherapy[33]. Moving immunotherapy to the (neo)adjuvant setting is even more promising, as surgically shrinking tumor size may potentially alleviate immune inhibition and T-cell exhaustion[34]. However, the OS benefit observed in the Checkmate-743 by using double check-point inhibition was not consistent in every subgroup, resulting lower in patients with epithelioid histology, and in those with tumors not expressing PD-L1. Therefore, second option may be to combine ICIs with agents other than immunotherapy. As epithelioid MPM is more chemo sensitive while ICIs conferred a striking survival advantage especially in non-epithelioid MPM, it is possible that chemo-immunotherapy confers a particular advantage for patients with epithelioid MPM. Three recent phase II trials have tested the combination of standard chemotherapy with ICIs as first-line treatment in MPM patients, showing response rates ranging from 48% and 78% with 65% to 92% of patients still alive at 1 year[35–37] and leading to initiation of the phase 3 DREAM3R trial, which will determine the efficacy of adding durvalumab to cisplatin/carboplatin and pemetrexed[38]. These promising data, when compared to our study with single agent nivolumab, underline the importance of moving beyond single check point inhibition to prevent primary ICI resistance in a significant subset of MPM patients.

Moving beyond single immune check point inhibition

Revisiting the concept of tumor microenvironment: the role of drug repurposing
After an initial Renaissance, the new era of immunotherapy for lung cancer and MPM will necessarily rely on a tailored approach which builds upon proper biomarker selection and a more rational design of drug combinations. Thinking in terms of TME has helped to acknowledge the role of nongenetic and noncell-intrinsic factors in cancer development. However, the concept of a tumor macroenvironment or 'tumor organismal environment' (TOE)[39] might better delineate the critical interactions that occur between the very local environment and the entire organism during cancer progression and that are needed for an effective anti-tumor immunity. Some of the shortcomings faced by immunotherapy in lung cancer and MPM may trace back to the fact that distant factors commonly excluded from the TME (extrinsic mechanisms of response/resistance) were not considered[40]. To this extent, the impact of host-related factors on immunotherapy has begun to be explored, especially in the field of ICIs[41–45].

Because of comorbidities, thoracic cancer patients are often already receiving different non-cancer medications at time of starting cancer therapy[46]. Besides pharmacodynamic and pharmacokinetic interactions, some concomitant medications could exert immunomodulatory effects both systemically and within the TME,

thus affecting the clinical outcomes of lung cancer and MPM treated with ICIs[47,48]. In **Chapter 6**, we found an association between baseline statin use and improved clinical outcomes in MPM and advanced NSCLC patients treated with PD-1 inhibitors. Interestingly, in our multicenter study this association was intensity-dependent, as use of high-intensity but not of low/moderate-statins led to better outcomes. Contrarily, no association between statins and outcome was found in the control MPM chemotherapy cohort. The association between statin use and improved outcomes has not always been consistent across cancer patients[49–51]. In a large cohort of patients with metastatic NSCLC and PD-L1 tumor expression ≥50% receiving first-line pembrolizumab monotherapy, Cortellini et al. did not find any association between baseline statins and improved outcomes[52]. However, this study did not report data on statin intensity (meaning the LDL-C lowering that should occur with the specific statin and dosage), which in our cohort proved to be essential in establishing their association with response to immunotherapy. Another study assessing outcomes of patients with advanced NSCLC, melanoma or renal cell carcinoma receiving PD(L)1 inhibitors showed that baseline statin use was an independent predictor of improved ORR, but not with PFS and OS[53]. In our study, the association was more evident for MPM, where statin use independently correlated with better ORR, PFS, and OS than for NSCLC, where no significant difference in OS was found. Recently, Santoni et al. replicated our findings in a cohort of metastatic renal cell carcinoma patients treated with nivolumab. They found statin exposure to be associated with longer median PFS and OS in both patients aged ≥70 and <70 years old[54].

Many pre-clinical studies support a potential role of statins in boosting the clinical activity of PD-1 inhibitors. Statins inhibit the production of cholesterol by interfering with the mevalonate pathway (MVP). MVP gene expression is in turn controlled by Sterol Response Element Binding Proteins (SREBPs), a family of transcription factors that are key regulators of cholesterol homeostasis[55]. In addition to a direct antiproliferative effect due to the interaction with key oncogenic molecules such as p53, MYC, and PI3K[55], inhibiting the MVP also hinders prenylation, a posttranslational modification which leads to the addition of hydrophobic molecules, such as geranylgeranyl diphosphate (GGPP), to proteins[56,57]. In KRAS-mutant cancers, statin-mediated inhibition of KRAS prenylation induced severe endoplasmic reticulum (ER) stress, enhancing the expression of 'eat me' signals and promoting immunogenic cell death (ICD) of KRAS-mutant cancer cells. By this mechanism, statins also enhanced the cross-priming ability of dendritic cells (DCs), thereby boosting $CD8^+$ T-cell immune responses in mice with KRAS-mutant tumors[58]. According to Xia et al., inhibition of small GTPases proteins by statins would also lead to an alteration of their internal cell membrane anchorage, prolonging antigen retention on cell membrane and again strengthening antigen presentation to T cells[57]. Finally, by blocking GGPP production, statins might downregulate Rac1 and Rac2 signaling, thereby suppressing PD-1 expression on T cell receptor (TCR)-stimulated Treg cells (**Figure**

1)[59]. Despite accumulating pre-clinical evidence supporting the immunomodulatory effects of statins in the context of cancer, findings from cancer patients mainly derive from retrospective datasets and potential selection biases cannot be excluded. Unfortunately, in our study we lacked information regarding statin time-exposure, as patients were included in the study only since the start of anti-PD1 treatment[60,61]. Moreover, although a multivariable analysis was conducted to adjust for significant prognostic factors in both MPM and NSCLC, other tumor-related (such as PD-L1 status and tumor mutational burden) and patient-related (such as circulating lipid levels) factors could have influenced the different prognosis.

In a separate cohort (data not published yet) we assessed the role of circulating lipid profile components (total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins, triglycerides) in predicting prognosis of advanced cancer patients treated with ICIs. We found that patients with hypercholesterolemia had longer OS compared to those with normal cholesterol levels. More precisely, patients with higher HDL presented improved OS compared to those with lower plasmatic concentration. Conversely, patients with hypertriglyceridemia detained significant shorter PFS and OS. As statins are drugs commonly used to lower plasmatic cholesterol levels, the better prognosis observed in hypercholesterolemic patients suggests that statins might exert their anti-cancer/immune-modulatory effect through mechanisms other than the lipid-lowering effect, such as the ones previously cited[57,62].

In conclusion, prospective and well-controlled clinical trials are needed to understand whether statin use reflects a general prognostic association or a causal link with improved clinical activity of PD-1 inhibitors exists. Validating these findings back to appropriate pre-clinical models would also help estimating the contribution of baseline lipid profile and statin time-exposure in affecting baseline tumor characteristics as well as anti-tumor activity of these agents, to ultimately test statins as drug repurposing strategy in combination with anti-PD1 therapy for lung cancer and MPM.

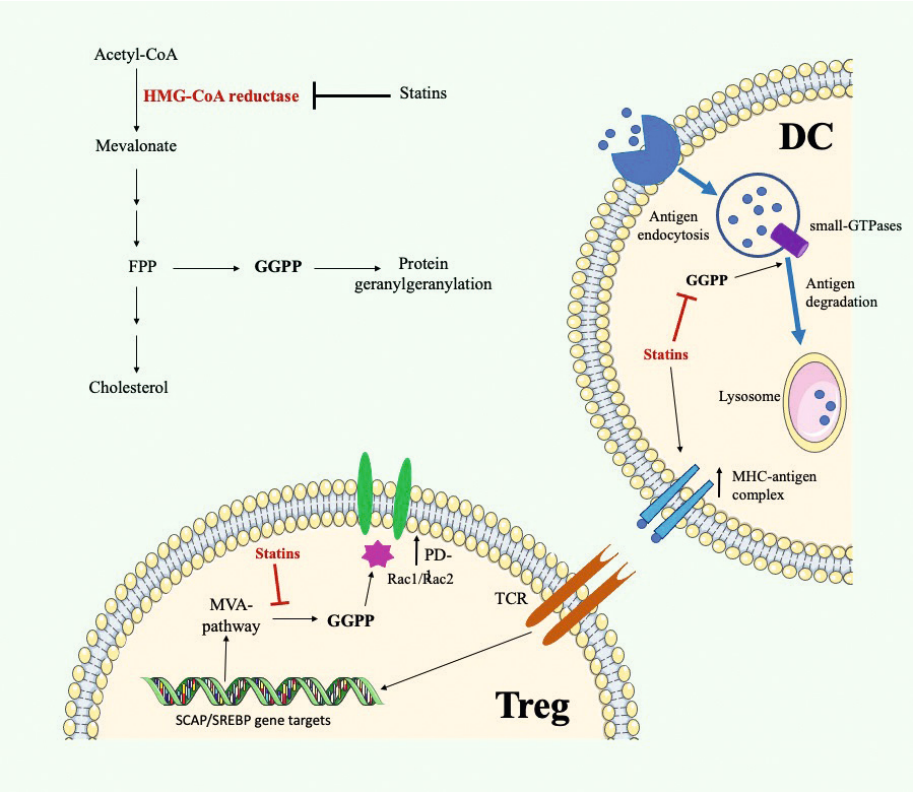

Figure 1. Off-target effects of statins modulate the tumor microenvironment (TME). The mevalonate (MVA) pathway is involved in several intracellular functions, including the synthesis of cholesterol and other isoprenoids, involved in protein post-translational prenylation. The latter leads to the addition of hydrophobic molecules, such as geranylgeranyl diphosphate (GGPP) to proteins, including to small GTPases proteins (e.g., Rab5 in DCs). Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme of the MVA pathway, blocking not only cholesterol but also GGPP production. In dendritic cells (DCs), the inhibition of geranylgeranylation of small GTPases, such as Rad5, by statins leads to the arrest of endosomal maturation, prolonged antigen retention, and enhanced antigen presentation by MHC-antigen complex. In T-regulatory (Treg) cells, T cell antigen receptor (TCR) stimulation by MHC/antigen complex leads to the expression of SREBP cleavage-activating protein (SCAP)/SREBP gene targets. Sterol regulatory element-binding protein (SREBP) stimulate MVA pathway and GGPP production, that, through protein geranylgeranylation promotes Rac1 and Rac2 signaling and, therefore, PD-1 upregulation on Treg surface. Statins, blocking the production of GGPP, might suppress PD-1 expression on TCR-stimulated Treg cells. Other abbreviations: farnesyl diphosphate (FPP). Figure derived from *Cantini et al., European Journal of Cancer 2021*.

The mechanisms of immune escape may differ across thoracic cancer types and diverse immunotherapeutic interventions are necessary to optimize clinical responses. Because of the extensive clinical experience and established efficacy, chemotherapy represents a particularly suited treatments for combination immunotherapy. In the past, chemotherapy was thought to be solely immunosuppressive[63]. More recently,

researchers have recognized the ability of certain chemotherapies to induce ICD through exposure or releasing of 'damage-associated molecular patterns' (DAMPs) molecules, such as calreticulin, ATP, and high mobility group protein box-1 (HMGB-1)[64]. By prompting the release of DAMPs and tumor antigens, chemotherapy promotes the infiltration of antigen-presenting cells (APCs) and the activity of cytotoxic T-lymphocytes (CTLs), potentially increasing tumor immunogenicity[65]. Meanwhile, chemotherapy is thought to promote anti-tumor immunity indirectly by reducing the activity of myeloid derived suppressor cells (MDSCs) in the TME[66,67].

In SCLC, because of the immunosuppressive TME and the rapidly progressive course, ICI monotherapy has showed only moderate benefit and using combinations with chemotherapy is even more necessary. Based on this assumption, atezolizumab or durvalumab (two PD-L1 inhibitors) were separately tested in combination with platinum-etoposide chemotherapy and became the new standard of care in the first-line setting of SCLC[68,69]. However, the magnitude of benefit in these trials was modest compared to NSCLC, suggesting that different immunosuppressive mechanisms might come into play in SCLC but also supporting the absence of a strong synergistic effect between standard-of-care chemotherapy and ICIs[70].

In addition to investigating new immunotherapies, changing chemotherapy backbone might then represent an appealing strategy. Compared to platinum-etoposide (the current chemotherapy backbone in ES-SCLC), anthracyclines for example proved to be more efficient ICD inducers because of their ability of enhancing calreticulin exposure on dying cancer cell[71].

In **Chapter 7**, we showed that lurbinectedin monotherapy was safe and effective in SCLC patients treated in the context of a named patient program at the Erasmus MC. Lurbinectedin is a new chemotherapy agent which had previously proved signs of efficacy in a phase II basket trial with 105 stage IV pre-treated SCLC patients[72,73]. In our real-world study, ORR and DCR after 12 weeks in pre-treated patients were 16% and 28%, respectively. These percentages were inferior when compared to the clinical trial[72] yet still very interesting as patients included in our cohort were more heavily pre-treated. In addition to clinical data, we sought to investigate potential off-targets effect of lurbinectedin including its immune-modulatory functions. We then performed a comprehensive immune monitoring on peripheral blood samples of treated patients, showing an association between lurbinectedin and depletion of circulating classical monocytes. Treatment was also associated with increased proliferation of $CD4^+$, $CD8^+$ T cells, and NK, NKT cells, as well as with alteration of co-stimulatory and co-inhibitory receptor expression on circulating lymphocytes. Unfortunately, the lack of tumor sample availability pre- and post- treatment precludes definitive conclusions on the immune-modulatory functions of lurbinectedin. Nevertheless, the alterations in circulating immune cell composition and phenotype make the pair

with previous data on pre-clinical models showing that lurbinectedin induces a dose- and time-dependent death in cultured monocytes and MDSCs[74] and with a possible synergy between lurbinectedin and ICIs due to ICD induction *in vitro*[75]. These findings render lurbinectedin a potential platform for future ICI combinations to be tested in the context of randomized clinical trials for SCLC and highlight the opportunity of conducting comprehensive immune monitoring on peripheral blood to inform rational drug development.

New pharmaceutic allies to strengthen the immune system: the rational development of immunotherapy combinations

In addition to repurposing drugs such as chemotherapy and statins, new therapeutic advances offer multiple possibility to tackle cancer-immunity cycle vulnerabilities[76]. Cancer types which display primary resistance to ICI monotherapy (such as MPM, SCLC, and to a minor extent NSCLC), often show an anergic and immunosuppressive TME, where both the effector arm (CTL response inside the tumor) and the inductive arm of the antitumoral immune response are suppressed. While the first may be restored using anti-PD(L)1 agents, cancer vaccines may be able to boost the second one leading to effective and durable tumor responses. Presentation of antigen in the context of appropriate presenting cells (APCs) is a requisite for initiating a CTL differentiation program starting from naive $CD8^+$ T cells. Moreover, generation of memory $CD8^+$ T cell relies on the quality of $CD4^+$ T cell help, which in turn is dependent on the IL-12 secreted by DCs[77]. As DCs are the most potent APCs[78] and the principal APCs with the ability to cross-prime (namely presenting exogenous antigens into the context on MHC class I molecules, which is necessary for the generation of CTLs)[79], developing DC-based vaccinations represents an appealing strategy for treatment of these cancer types. Our group at the Erasmus MC has developed a method to make DC therapy (DCT) more accessible by utilizing autologous DCs and exposing them to an allogeneic mesothelioma tumor lysate, thereby eliminating the need to obtain an autologous tumor lysate from every single patient. This approach proved to be safe, feasible and induced some radiological responses in the context of a phase I trial conducted in MPM patients[80]. This led to the conduction of a multicenter phase II/III study aimed to compare allogenic tumor lysate sensitized DCs (named as Mesopher) as maintenance treatment after first-line chemotherapy versus chemotherapy alone, which is currently ongoing[81]. In recent years, researchers started using cancer vaccines at an earlier stage of carcinogenesis, to prevent the establishment of tumor-mediated immunosuppressive environments and to anticipate the onset of redundant mutations that foster treatment resistance and tumor proliferation[79]. Besides showing the ability to reduce tumor load in some patients, DCT with Mesopher demonstrated a better outcome when injected earlier in tumor development in preclinical models. As shown in **Chapter 8**, this offered the rationale for testing a combined (neo)adjuvant approach with Mesopher and extended pleurectomy/decortication (eP/D) surgery in resectable MPM patients. In the open label, single center, phase 1 ENSURE trial, we aim to assess

whether (neo)-adjuvant DCT with Mesopher is feasible in early stage epithelioid MPM patients after first-line chemotherapy. Moreover, the neoadjuvant setting provides us with the unique possibility to conduct translational research, acquiring valuable information from blood and tissue collection before and after DCT. Many attempts have been made to show that DCT enhances intratumoral T-cell infiltration, yet many confounding factors (concomitant cancer treatments and availability of tumor tissue only at time of progression) has led to inconclusive results[80,82–84]. Through evaluation of immune cell infiltration in tumor tissue prior and post DCT and characterization of T-cell receptor (TCR) repertoire tumor-infiltrating T cells, the ENSURE trial will investigate whether Mesopher induces a (tumor-specific) immune response, offering novel insights into the mechanisms of action of DCT.

Concluding remarks and prospects for future investigation

Starting from a phase in which ''immune enhancement'' strategies (e.g. cytokines) were mainly investigated often resulting in poor objective responses and high toxicity rate, cancer immunotherapy now translated to "immune normalization" strategies, targeting specific tumor-induced immune deficiencies in the tumor micro-(or macro-)environment (e.g. PD-(L)1 inhibitors)[3]. Since their introduction, ICIs have been remarkable in achieving long-lasting "treatment-free survival" in a minority of patients, leading to a Renaissance of immunotherapy in thoracic oncology. However, development of these new treatment modalities was not always based on a "personalized medicine" approach, and still a wide percentage of lung cancer and MPM patients are resistant to single ICIs. In order to maximize the benefit of immunotherapy, a tailored approach based on biomarker selection and rational design of drug combinations should be pursued. To achieve that, as summarized in the first part of this thesis, clinical research needs to be coupled with translational investigations in the context of well-designed, biomarker-driven clinical trials, especially in the context of cancers with lower incidence such as MPM.

Evidence derived from real-world should be also encouraged, as they can reveal new ways to improve allocation of immunotherapy. Different from registries which often come with biases during data collection, thoroughly designed real-world cohort studies may provide useful and easy-to-access data to guide further research. In our pan-cancer cohort, adoption of ED ICIs did not affect the safety profile, representing a safe and feasible option also outside of clinical trials. Therefore, this treatment schedule represents an important alternative for treating physicians. Noteworthy, ICI flat-doses may result in an increase of drug costs putting a considerable strain on health-care budgets, and hybrid-dosing strategies should be rather adopted as they reduce the costs of these therapies while maintaining clinical efficacy and toxicity.

Effectiveness of ICI monotherapy in our MPM cohort did not recapitulate data observed in the context of clinical trials, probably reflecting a more heterogeneous

and less selected real-world population. However, we did identify some subgroups of MPM patients (those with a radiological response, higher PD-L1 tumor expression and higher albumin values) that derived a greater benefit from nivolumab, again showing the importance of better tailoring immunotherapy. Future randomized clinical trials on immunotherapy should always be coupled with extensive exploratory analyses looking at peripheral blood parameters and tumor samples.

The impact of concomitant medications as determinants of response and survival to immunotherapy in lung cancer and MPM also need to be considered. In particular, whether the impact of commonly used drugs such as statins on clinical activity of PD-(L)1 inhibitors reflects a mechanistic rather than an associative effect warrants further investigations, using appropriate pre-clinical models and dissecting TME characteristics according to patient lipid profile.

Comprehensive multi-tissue immune monitoring studies will be useful to unravel the mechanisms of anti-tumor immune responses directly in vivo, thereby designing new immunotherapy combinations with care. A recent review demonstrated no evidence of drug additivity or synergy in trials of combination therapies with ICIs, showing that clinical benefit from ICI combinations is mainly obtained by increasing the chances of response to the single agent[85]. One way to achieve drug additivity or synergy is via biomarker discovery and patient stratification. The other way is to tackle distinct vulnerabilities of the cancer immunity cycle while optimizing treatment sequencing. Verma et al. showed that using PD-1 blockade before cancer vaccines enhanced treatment resistance because of the suboptimally primed $CD8^+$ cell conditions imposed by the tumor, while optimal simultaneous treatment reversed resistance leading to sustained response[86]. Similarly, our group showed that DC therapy and sequential anti-PD1 treatment is safe and lead to promising survival outcomes in MPM patients[87]. Whether DCT is feasible also at an earlier stage of carcinogenesis, before immunosuppressive environments are established, is currently investigate in the context of the ENSURE trial. This (neo)adjuvant window-of-opportunity trial also represents an ideal platform to inform on how DCT elicits its anticancer effect not only systemically yet more specifically at the tumor site. Such studies are eagerly awaited, as they implement a model of iteration from clinical results to basic laboratory research and vice versa that will ultimately help us to move towards the next era of immunotherapy for lung cancer and MPM.

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SUMMARY

Lung cancer and malignant pleural mesothelioma (MPM) present a significant global health burden with the incidence and mortality of both increasing by year. Lung cancer, including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), has the highest mortality rate of all malignancies. MPM still represents an uncommon disease, yet its incidence and mortality are peaking worldwide. Survival rates for all these cancers are poor, as both lung cancer and MPM patients are often detected at a late stage. In the last decade, we witnessed an exponential growth in the appreciation of lung cancer and mesothelioma pathobiology, leading several new treatments to be investigated both in the early stage of the disease and in the advanced setting. The discovery of oncogenic driver mutations which are implicated in the development and propagation of lung cancer and mesothelioma have been essential to identify novel molecular therapeutic targets, representing the rationale for testing multiple targeted therapies. Moreover, a better understanding of the role that the immune system plays in controlling lung cancer and mesothelioma growth, along with the potential to effectively harness the antitumor immune response, have attracted a growing interest in cancer immunotherapy.

Cancer immunotherapy is a type of medicine that treats cancer using the body's own immune system. On a healthy individual, the immune system can detect and destroy the abnormal cells preventing the development of cancers. However, cancer cells are sometimes able to restrain the immune system. This can be obtained for example by reducing the surface expression of molecules (tumor antigens) which are normally recognized by the immune system, by expressing (or inducing expression of) 'checkpoint' proteins that induce immune cell inactivation, or by attracting other immunosuppressive cells in the tumor environment. Compared to the past century, researchers' efforts recently shifted from ''immune enhancement'' strategies aimed at broadly amplifying immune activation to "immune normalization" strategies, targeting specific tumor-induced immune deficiencies in the tumor micro-(or macro-) environment. This "paradigm shift" brought to the introduction of therapeutic agents with a more beneficial tumor response-to-toxicity profile, leading to a Renaissance of immunotherapy in thoracic oncology. However, the rapidity of drug development in this new field often outpaced researchers' ability of tailoring the right immunotherapy for the right patient, and still a wide percentage of lung cancer and MPM patients do not respond to immunotherapy.

In this thesis, after reviewing the current role of immunotherapy for lung cancer and MPM patients treated in the real-world, we explored new avenues of this treatment modality by looking at a more tailored approach based on biomarker selection and rational design of new drug combinations.

In **Chapter 1**, the rationale for immunotherapy in lung cancer and mesothelioma is introduced. Of the many different immunotherapeutic approaches, immune-

checkpoint inhibitors (ICIs) are the most used by cancer physicians. ICIs are antagonistic antibodies directed towards known targets of exhaustion including PD-1 and CTLA-4 that enable immune cells to proliferate and respond more vigorously against the tumor. Starting from its approval in recurrent metastatic NSCLC in 2015, the use of ICIs has been recently expanded beyond NSCLC to include treatment of SCLC and MPM with a remarkable survival gain for some patients. However, primary and acquired resistance still occur in most of the cases, and tumor responses often come at the price of new forms of toxicities. This highlights the need for further fueling research in this setting. In NSCLC, differences in terms of immune composition and milieu have been reported among histology variants and among NSCLC harboring distinct mutational landscape and should be considered to fully unleash the potential of immunotherapy. In SCLC, chemotherapy-free ICI trials have not generated solid evidence and finding the perfect companion for ICIs (moving beyond the standard platinum-based chemotherapy) is crucial to improve patient outcomes.

As discussed in **Chapter 2**, mesothelioma treatment deserves separate considerations. The chronic inflammatory response to asbestos involved in the pathogenesis of MPM causes a unique tumor environment, with a high local immune suppression. In the last few years, mesothelioma genetics, epigenetics, and the tumor microenvironment (especially immune biology) have been studied more deeply and this knowledge has started to be properly applied to discover new therapies. However, multiple biases in the design of clinical trials and the peculiar biological features of MPM delayed the discovery of effective therapeutic agents. Most of the previous trials attempted to readapt drugs that succeeded in other cancer types to MPM, yet they were either too small or not stratified for predictive biomarkers, not taking into account the intrinsic heterogeneity of this disease. Results from phase II studies were often not replicated in larger, randomized, phase III trials, pointing out that the implementation of well-designed, biomarker-driven clinical trials with appropriate size and duration should be pursued.

In **Chapter 3**, we observed how results from first clinical trials using single immune check-point inhibition in MPM were conflicting. Recent data from the CheckMate 743 trial showed that the combination of nivolumab and ipilimumab (anti-PD-1 and anti-CTLA-4, respectively) prolonged overall survival (OS) compared to platinum-based chemotherapy (to a greater extent in non-epithelioid tumors), leading to a new standard of care in the first-line setting. These results highlighted the potential of combining ICIs with other immunotherapies, as well as targeted agents and old-school chemotherapy to improve prognosis in MPM. Since the "one size fits all" approach does not adapt to MPM, focus should lie on the heterogeneity of the genetic and epigenetic landscape and of the composition of tumor microenvironment to take a step further single immune check point inhibition and increase the number of patients who benefit from these approaches.

While randomized controlled trials remain the gold standard for evaluating the integration of immunotherapy in the clinical practice, well designed cohort studies in real-world populations also provide the opportunity to study the multifaceted aspects of cancer treatments, offering different insights into the number of (treatment-related) adverse events and actual survival times. Despite their favorable response-to-toxicity profile, in some cases ICIs can lead to a broad and non-tumor specific activation of the immune system, which becomes supra supraphysiologic and translates in the onset of immune-related adverse events (irAEs). Different ICI drugs and doses can lead to different toxicity features. Recently, long life expectancy of patients treated with ICIs, high healthcare costs, and the need to reduce avoidable hospital admissions during COVID-19 crises, led to an increasing interest in alternative longer dosing schedules. In **Chapter 4**, we investigated a pan-cancer cohort, showing that adoption of an extended interval dosing (ED) of ICIs did not affect the safety profile, representing a safe and feasible option for treating physicians. In addition, we corroborated previous evidence in the setting of canonical interval dosing (CD), finding that irAEs onset was associated with improved OS also in the context of ED treatment. In **Chapter 5**, we queried again the real-world evidence to investigate the effectiveness of nivolumab (an anti-PD1 ICI) in a population of pre-treated MPM patients. We observed that objective response rate (ORR) and median OS were lower compared to those reported in clinical trials, probably due to the lack of strict inclusion criteria in our analysis, which led to a less selected patient population. Interestingly, we reported the presence of a minority of long-term responders to ICIs in our study, a fact that underlines the importance to identify reliable predictive biomarkers in this setting.

After an initial Renaissance, the new era of immunotherapy for lung cancer and MPM will necessarily rely on a tailored approach, which besides proper biomarker selection builds upon a more rational design of drug combinations. Because of comorbidities, thoracic cancer patients often receive concomitant medications at time of starting cancer therapy. Apart from pharmacodynamic and pharmacokinetic interactions, some concomitant medications could exert immunomodulatory effects, thus affecting ICI efficacy. In **Chapter 6**, we found an association between baseline statin use and improved clinical outcomes in MPM and advanced NSCLC patients treated with PD-1 inhibitors. The association was intensity-dependent, as use of high-intensity but not of low/moderate-statins led to better outcomes. Our results are supported by pre-clinical studies which already shown how statins may exert an anti-cancer/immune-modulatory effect through mechanisms other than the lipid-lowering effect. Prospective and well-controlled studies will help understanding whether statin use reflects a general prognostic association or a causal link with improved clinical activity of PD-1 inhibitors can be established. Validating our findings back to appropriate pre-clinical models which consider baseline lipid profile and statin time-exposure would also shed light on the anti-tumor activity of these agents, to ultimately test

statins as drug repurposing strategy in combination with anti-PD1 therapy for lung cancer and MPM.

Chemotherapy represents another suited treatment for combination immunotherapy, because of the extensive clinical experience and established efficacy. In the past, chemotherapy was thought to be solely immunosuppressive. More recently, researchers have recognized chemotherapy ability of boosting anti-tumor immunity by either promoting an immunogenic cell death (ICD) or reducing the activity of immune-suppressive cells in the tumor microenvironment. In **Chapter 7**, we showed that lurbinectedin represents a safe and effective option in pre-treated SCLC patients. By assessing patient circulating immune profile, we also reported on the immune-modulatory effect of this chemotherapy. Lurbinectedin treatment was associated with depletion of circulating classical monocytes, as well as increased proliferation of $CD4^+$, $CD8^+$ T cells, and NK, NKT cells, and alteration of co-stimulatory and co-inhibitory receptor expression on circulating lymphocytes. The observed alterations in circulating immune cell composition and phenotype make the pair with pre-clinical data and render lurbinectedin a potential platform for future ICI combinations.

Multiple mechanisms of immune escape may come into play across thoracic cancer types and diverse immunotherapeutic interventions are necessary to optimize clinical responses. In addition to repurposing drugs such as chemotherapy and statins, new therapeutic advances such as cancer vaccination offer different possibilities to tackle cancer-immunity cycle vulnerabilities and induce immune responses. Cancer types which display primary resistance to ICI monotherapy (such as MPM, SCLC, and to a minor extent NSCLC), often show an anergic and immunosuppressive tumor microenvironment, where both the effector arm (T cell response inside the tumor) and the inductive arm of the antitumoral immune response are suppressed. While the first may be restored using ICIs, cancer vaccines may be able to boost the second one. In fact, presentation of antigen in the context of appropriate presenting cells (APCs) is a requisite for initiating a specific antitumor response. As dendritic cells (DCs) are the most potent APCs, developing DC-based vaccinations represents an appealing strategy for treatment of many cancer types. In **Chapter 8**, we presented the trial protocol of a phase I study with DC therapy (Mesopher) as (neo)adjuvant approach combined with extended pleurectomy/decortication (eP/D) surgery in patients with resectable epithelioid MPM. For this therapeutic approach, DCs are extracted from patient blood, loaded with allogeneic MPM tumor cell line lysate (Pheralys), maturated using an adjuvant and infused back to the patient. Using DC vaccination at an earlier stage of carcinogenesis, before immunosuppressive environments are established, we aim to anticipate the onset of redundant mutations and prevent treatment resistance. In addition, the availability of tumor tissue before and after DC therapy represents a unique opportunity to evaluate whether DC vaccination is capable to induce tumor-specific T cell response.

This thesis concludes with a Discussion in **Chapter 9**, where the findings are put into context. In the last few years, immunotherapy has deeply reshaped the treatment paradigm of lung cancer and mesothelioma, revamping the enthusiasm over this treatment modality. Many treatment combinations have been investigated in clinical trials, with often little scientific rationale. Only the adoption a more holistic approach, by interrogating cancer immunity through different available approaches (e.g. real-world data) and from a broader perspective (e.g. investigating patient-related factors), will likely increase physicians' ability to select appropriate immunotherapies in the coming few years, moving further from the "one size fits all" approach. To this regard, the advent of new technologies such as artificial intelligence may be crucial to translate multisource data into clinical decision aids, dissecting the underpinnings of how immunotherapy constitute its effect and ultimately improving drug delivery for lung cancer and mesothelioma patients.

ADDENDUM

Acknowledgments

PhD Portfolio

List of publications

Curriculum Vitae

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Luca Cantini,
Rotterdam, April 2023

PhD Portfolio

PhD training	Year	Workload (ECTS)
Courses		
Basic Course on 'R'	2019	1.80
Biomedical English Writing	2019	2.00
Basic and Translational Oncology	2019	1.80
Research Integrity Course	2022	1.00
Conferences & Presentations		
ESMO Translational Research Unit Visit, Brussels, BE	2017	0.60
International Congress on Targeted AnticancerTherapies (TAT) 2019, Paris, FR	2018	1.00
18th ESO-ESMO Masterclass in Clinical Oncology, Nauen OT Gross Berhnitz, DE	2019	1.50
Associazione Italiana di Oncologia Medica (AIOM)Conference 2019, Rome, IT	2019	0.75
8th ETOP Residential Workshop, Amsterdam, NL	2019	1.00
American Society of Clinical Oncology (ASCO)Conference 2020, Virtual Meeting	2020	1.00
European Society of Medical Oncology (ESMO)Conference 2020, Virtual Meeting	2020	1.00
9th ETOP Residential Workshop, Virtual	2020	0.30
Society for Immunotherapy of Cancer Winter School	2021	1.00
European Lung Cancer Congress (ELCC) 2021, VirtualMeeting	2021	1.00
World Conference on Lung Cancer (WCLC) 2021, Virtual Meeting	2021	2.00
European Society of Medical Oncology (ESMO)Conference 2021, Virtual Meeting	2021	1.50
European Lung Cancer Congress (ELCC) 2022, VirtualMeeting	2022	1.25
NVVI Annual meeting, Noordwijkerhout, NL	2022	0.75
American Society of Clinical Oncology (ASCO)Conference 2022, Chicago, USA	2022	1.50
AIOM Young Congress, Perugia, IT	2022	0.60
European Society of Medical Oncology (ESMO)Conference 2022, Paris, FR	2022	1.50
Associazione Italiana di Oncologia Medica (AIOM) Conference 2022, Rome, IT	2022	1.00

Teaching	Year	Workload (ECTS)
Reviewer MSc Thesis of Nura Tebayna	2021	1.00
Supervisor BSc Internship of Kick Slooff	2021	7.50
Total ECTS		**33.65**

Awards & Funding	Year	Amount
ESMO Translational Research Fellowship	2021	€ 80,000
IASLC Early Career Education Award	2021	NA
ELCC Travel Grant	2022	€ 600
NRS Travel Grant	2020	€ 1250

List of publications

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Curriculum Vitae

Luca Cantini was born on the 26th of June 1989 in Viareggio (LU), Italy. He attended high school at the Carducci Gymnasium in Viareggio (LU) and graduated in 2008. He then started medical school at University of Pisa and obtained his medical degree in 2015. After his studies, he started his Residency in Medical Oncology at the Polytechnic University of Marche (Ancona, Italy) under the supervision of Prof. Berardi. In 2019, he was visiting research fellow at the Pulmonary Department at the Erasmus MC (Rotterdam, the Netherlands). The following year he completed his medical specialty training in Italy becoming Medical Oncologist. In 2021, he was awarded with the ESMO Translational Research Fellowship, to carry on a project titled "ENSURE study - dENdritic cell therapy combined with SURgEry in mesothelioma", which allowed him to move back to the Erasmus MC under the supervision of Prof. Aerts. During the Fellowship, he decided to pursue a PhD trajectory at the same Institution. Besides the ESMO Fellowship, he has been awarded with the IASLC Early Career Education Award, the ESO Grant for participating to the "18th ESO/ESMO Masterclass in Clinical Oncology", the ESMO Grant for participating to the "Translational Research Unit Visit", the ESMO Travel Grant for participating to the "2022 European Lung Cancer Conference", and the NRS Travel Grant. He authored 53 manuscripts in peer-reviewed journals. Currently Luca is Medical Director at Labcorp Drug Development.