Chapter

The Immune System of Mesothelioma Patients: A Window of Opportunity for Novel Immunotherapies

Fabio Nicolini and Massimiliano Mazza

Abstract

The interplay between the immune system and the pleural mesothelium is crucial both for the development of malignant pleural mesothelioma (MPM) and for the response of MPM patients to therapy. MPM is heavily infiltrated by several immune cell types which affect the progression of the disease. The presence of organized tertiary lymphoid structures (TLSs) witness the attempt to fight the disease *in situ* by adaptive immunity which is often suppressed by tumor expressed factors. In rare patients physiological, pharmacological or vaccine-induced immune response is efficient, rendering their plasma a valuable resource of anti-tumor immune cells and molecules. Of particular interest are human antibodies targeting antigens at the tumor cell surface. Here we review current knowledge regarding MPM immune infiltration, MPM immunotherapy and the harnessing of this response to identify novel biologics as biomarkers and therapeutics through innovative screening strategies.

Keywords: Malignant pleural mesothelioma (MPM), Immunotherapy, Fully human antibody, Tertiary lymphoid structure (TLS), BCR repertoire

1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive neoplasm principally due to asbestos exposure with a poor prognosis and a median overall survival (OS) of only 14 months [1]. Heavy asbestos utilization during earlier decades in Europe is the cause of actual disease incidence [2] and, despite many countries have banned asbestos use in recent years, a peak of MPM incidence is expected for 2020s due to a long latency and delayed disease onset [1, 3–5]. On the contrary, other countries that still make use asbestos are very likely to observe a substantial increase of asbestos-related disease and MPM in the future. BRCA1 associated protein-1 (*BAP1*) protein is an important player in DNA repair mechanisms, cell cycle control, carcinogenesis and apoptosis and almost 60% of MPM patients have BAP1 mutation [6–13]. BAP1 mutational status determines the insurgence of MPM [9, 10, 12–15], and influences the response to chemotherapy [16] and patient' s clinical outcome [17]. When other gene alterations are coupled to BAP1 mutation, synthetic lethality approaches could be evaluated as therapeutic options [18, 19]. Other frequent mutations are in the

genes NF2, LATS2, TP53, SETD2 and TERT promoter as recently reported and are associated with different histotypes of MPM with epithelioid, biphasic and sarcomatoid features [20]. MPM is characterized by a lack of early and specific symptomatology and few reliable biomarkers and screening tools are available causing a late prognosis. As we recently reviewed [21], current therapies in clinical practice consist of surgery, radiotherapy and chemotherapy and innovative therapeutic approaches are being explored. From this survey emerged that new therapeutic modalities and prognostic biomarkers are urgently needed in order to grant a fair chance of survival to all MPM patients. Here we describe the interplay of the immune system and MPM at the tumor tissue level and envision strategies to take advantage of it and derive novel fully human MPM-targeting antibodies to be used as biomarkers and for the design of novel immunotherapies.

2. Inflammatory response and carcinogenesis in MPM

MPM's development is intertwined with the inflammatory response provoked by asbestos exposure. Asbestos fibers and fluid enter the pleural space where they reach the outer pulmonary parenchyma inducing an inflammatory response [22]. Later steps see macrophage infiltration guided by the presence of the chemokine CCL2 generated by mesothelial cells in response to asbestos fibers contact. Reactive oxygen species (ROS) and nitrogen species are produced by macrophages that, together with already present nitrogen and oxygen species generated from iron particles associated with the fibers, create reactive and dangerous free radicals responsible for mutagenic events and genomic instability [23–25].

Normally, cells which suffer genotoxic DNA damage undergo PARP-dependent apoptosis. Despite that, an *in vitro* study [26] demonstrated that damaged human mesothelial cells could be rescued and skip apoptosis by TNF-alfa produced by macrophages and by other intracellular pathways activated in mesothelial cells, such as NFkB [26–28]. Conversely, TNF-alfa receptor knock-out mice are protected from fibroproliferative lesions when exposed to asbestos fibers [29]. In summary, among innate immune system players, macrophages contribute to genomic alterations as well as survival of mesothelial cells in a context of inflammatory response to asbestos fibers.

3. Immune cell infiltrate in MPM

3.1 Tumor-Associated Macrophages

Tumor-Associated Macrophages (TAMs) are the most abundant cells infiltrating the pleural effusions [30–33] and are associated with poor prognosis [32, 34, 35]. *In vitro* and *in vivo* experiments support TAMs as potential targets for MPM treatment. Chemokines released by mesothelioma cells such as CCL4, CCL5, CXCL12 and, in particular, CCL2, are chemoattractants for monocytes [36–38]. CCL2 concentration is particularly high in malignant pleural effusions with respect to benign lesions or lung adenocarcinoma pleural effusions [39, 40] and affects CCR2-expressing monocyte trafficking in MPM [41]. When recruited to MPM lesions, monocytes and macrophages switch to immunosuppressive cells under the influence of growth factors such as M-CSF, IL-34, MCSF [41, 42] and cytokines such as IL-10 and TGF-β released by MPM cells. Those cytokines act both on monocyte and macrophage development and activation but also exert autocrine feedback loop functions on MPM cells [42, 43]. Also, the macrophage checkpoint marker and "do not eat

me" signal CD47 is found to be highly expressed in the majority of patients with epithelioid mesothelioma [44]. In mesothelioma, TAMs show an immunosuppressive phenotype, characterized by $CD14^{mid}CD16^{hi}$ expression, reduced phagocytic activity and increased IL-10 production [45]. In addition, in vitro co-culture of TAMs with MPM cells boosts tumor proliferation and concomitantly reduces sensitivity to chemotherapy treatment [41]. Pro-tumoral activity of TAMs is also evident in mesothelioma mouse models where the removal of macrophages reduces the number of tumor nodules, metastases and tissue invasiveness [46].

3.2 Myeloid-Derived Suppressor Cells

Granulocytes and neutrophils are also present in MPM microenvironment and recruited by CXCR2 or CXCL5 and CXCL1 chemokines, respectively [36, 47]. Also, polarization and phenotype of granulocytes are affected by growth factors from the mesothelioma secretome which increases their expression of CD11b, CD15 and CD66b markers. These cells function as Myeloid-Derived Suppressor Cells (MDSC) and negatively affect T-cell proliferation via the production and release of ROS [48]. Also, the presence of consistent neutrophilic infiltrate as well as high numbers of neutrophils in the peripheral blood is associated with poor prognosis in epithelioid mesothelioma [49, 50]. However, MDSC targeting in MPM is still debated and controversial and requires further investigations.

3.3 T-lymphocytes

$CD4^+$ and $CD8^+$ T-lymphocytes are present in MPM microenvironment but in lower numbers compared to macrophages [32, 51–53]. T-regulatory cells (Tregs) are also present in MPM tissue but are less abundant compared to other solid tumors [54]. Principal chemokines present in mesothelioma secretome involved in T-cell trafficking are CXCL12, CXCL10 and CCL5. CXCR3, the receptor of CXCL10 chemokine, is upregulated in mouse models of MPM [47]. CCL5 concentration is high in MPM patients' peripheral blood with respect to asbestos workers and healthy individuals [55] while its receptor CCR5 is expressed on T-cell infiltrating pleural effusions [56]. As discussed in the following chapters, T-cells activation and programming is determined by the presence of neo antigenic stimuli [57, 58] and immune checkpoint expression [59, 60] in specialized immune structures organized *in situ*.

4. Tertiary lymphoid structures in solid tumors and MPM: where the anti-tumor response begins

Secondary lymphoid organs (SLOs) are lymphoid regions wherein dendritic cells (DCs) present antigens to T-cells in a major histocompatibility complex (MHC)-dependent way acting an efficient adaptive response against cancer, requiring the migration of DCs from the tumor site to the SLOs [61]. Consequently, B-cells are also activated in the SLOs by CD4+ T-cells, begin to proliferate and form a secondary follicle that will be converted to a germinal center (GC). This process induces T and B-lymphocyte proliferation and differentiation into effector T-cells and memory B-cells (MBCs), respectively, that migrate into the tumor contributing to cancer cells elimination, unless unfavorable/antagonizing events or exhaustive signals are in place. However, studies on the role of the immune system in tumors revealed that anti-tumor mechanisms can take place also at the tumor site within organized lymphoid aggregates similar to SLOs [62] called tertiary lymphoid structures (TLSs) [63].

TLSs are also present in the stroma, at the invasive margin and/or in the core of different tumor types [63, 64]. TLSs are composed of a T-cell-rich zone together with mature DCs but also by B-cell rich-GC surrounded by plasma cells (PCs). Inside TLSs tumor antigens are presented to T-cells by DCs. and both T- and B- cells are activated, begin to proliferate and to differentiate to effector memory T helper (TH) cells, effector memory cytotoxic T-cells, MBCs or antibody-producing PCs [53, 65–69]. High numbers of CD8+ and CD4+ T-cells in tumors determine TLS density [70] and evidence indicates a positive correlation of TLS density on OS and disease-free survival in lung cancer [66, 70–72], colorectal cancer [73, 74], pancreatic cancer [75, 76], oral squamous cell carcinoma [77] and invasive breast cancer [65, 78–80].

Importantly, its prognostic value is independent of tumor–node–metastasis (TNM) staging in most malignancies suggesting TLS can induce a systemic long-lasting anti-tumor response. High endothelial venules (HEVs) similar to those that allow entry of lymphocytes into SLOs could be detected near TLSs [65]. In this context HEVs allow lymphocytes to enter into tumors. Therefore, therapeutic approaches that enhance HEV formation would be beneficial to improve anti-tumor immune responses. Tregs negatively regulate HEV formation and their absence in cancer murine models promotes T-cell activation and tumor infiltration, favoring the eradication of the lesions [81, 82]. Also other immunosuppressive cell types, such as MDSCs, regulatory B-cell (Bregs) and cytokines, like TGFβ and IL-10, play a part in the development of an immunosuppressive tumor microenvironment (TME).

Tumor-resident Tregs co-express high levels of CTLA-4, OX-40 and GITR compared to effector T-cells and In murine models of MPM, the combination of anti-OX-40 and anti-CTLA-4 antibodies has synergistic effect on CTLA-4$^+$, OX-40$^+$ tumor resident T-regs and contributing to a clear tumor regression when compared to single-antibody treatment [83]. Coherently with this point, combined anti-angiogenic and anti-PD-L1 therapies favor HEV and TLS formation in murine models of breast cancer and neuroendocrine pancreatic tumors [84] suggesting that a powerful anti-tumor systemic response by ICIs is sustained, if not triggered, by the presence of TLSs *in situ*. TLS heterogeneity among human cancers has been analyzed via a pan-cancer gene expression analysis of TME cellular composition on The Cancer Genome Atlas (TCGA) data and MPM, as well as lung adenocarcinoma and lung squamous cell carcinoma, display high expression of a 12-chemokine gene signature associated with TLS presence [85] suggesting TLSs are frequent, but also heterogeneous [86].

Seventy percent of MPM cases contain lymphoid aggregates and about 30% of them contain GCs [31]. These aggregates are functionally similar to TLSs, in which T- and B- lymphocytes are apart in two adjacent regions surrounded by HEV, as already shown for ovarian and prostate cancer [87, 88]. Intratumoral CD8+ T-lymphocytes in high numbers are an independent good prognostic marker for MPM patients [68]. Additionally, structural inter- or intra-chromosomal rearrangements and single nucleotide variants have been recently reported from mate-pair and RNA sequencing-based analyses on mesothelioma specimens predicting the expression of potentially-targetable neoantigens [58]. Moreover, some of these neoantigens bind patient-specific MHC and specific tumor-infiltrating T-cell clones are expanded as observed through TCR repertoire analysis [58]. Indeed, TCR diversity and mutation/neoantigen load are inversely correlated, but both active and suppressive TME immune components, such as Treg and CD8+ T-cells, were present and equally balanced suggesting a scenario where activated anti-tumor CD8+ T-cells are counteracted by pro-tumoral immune suppressive molecules and Treg cells [57] or activated CD8+ T and CD4+ T-helper cells displaying phenotypic markers of exhaustion like PD-1, TIM-3 and LAG3 [59].

5. The importance of B-cell infiltration in solid tumors and MPM

B-cell follicles in TLS from non-small cell lung cancer and ovarian cancers contain bona fide Ki67+ GC B-cells expressing the activation-induced deaminase (AID) gene, that codes a critical enzyme in somatic hypermutation and class switch recombination processes typical of immunoreceptor genes, as well as, of BCL-6, the transcription factor involved in the late stage of B-cell maturation [66, 89]. Additionally, the presence of CD38+ CD138+ PCs around the follicle is highly suggestive of antibody production *in situ* [90]. Indeed, micro-dissected follicles subjected to BCR repertoire analysis revealed clonal amplification compared to peripheral B-cells, supporting the idea that locally presented antigens can elicit specific B-cell responses in several malignancies [87, 89, 91–94].

Additionally, PCs isolated from dense aggregates in tumor stroma [90], produce anti-tumor antibodies of the immunoglobulin G (IgG) isotype *in vivo* whose mechanism of action has not been yet determined. One possibility is that anti-tumor IgGs produced locally increase antigen presentation by DCs and/or directly promote the activity of specific subsets of CD4+ T-cells endowed with Fcγ receptors (FcγRs) [95]. The presence of IgG deposits in TLS, the spatial organization of TLSs that may favor DC priming by locally produced IgGs and the observation that tumor-derived immune complexes increase the expression of the co-stimulatory molecule CD86 on DCs *in vivo* [87] suggest that these mechanisms take place. In favor of the latter are the results of a meta-analysis in a large set of human cancers showing that the prognostic effect of T-cells is generally stronger when tumor-infiltrating B-cells or PCs are present, supporting the hypothesis that a coordination between cellular and humoral adaptive immune responses is crucial for effective anti-tumor adaptive responses [96].

The role of B-cells and the association of B-cell rich TLSs with survival and anti-PD-1 immunotherapy response in sarcoma and melanoma have been recently established [97, 98]. Interestingly B-cells are the strongest prognostic factor even in the context of low CD8+ T-cells [97] in sarcoma and class-switched MBCs are specifically enriched in melanoma ICI-treated responders [99]. In murine models of MPM treated with immunotherapy, the presence of B-cells is essential for good prognosis, indicating that antibodies are generated and contribute significantly and essentially to the therapeutic effect [100]. Consistently, B-lymphocyte infiltration in MPM tissue positively correlates with prognosis [38] although variable in its extent [101]. Moreover clinical [52] and preclinical data on B-lymphocytes contribution to MPM prognosis suggest that they elicit an adaptive cytotoxic immune response rather than acting directly as antigen presenting cells (APCs) [100, 102]. In this respect MPM and other solid tumors share many similarities and provide a solid opportunity to develop novel immunotherapies via the identification of MPM targeting molecules in patients.

6. Immunotherapy in MPM

Immune checkpoint (IC) proteins, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) and PD-L1, are regulators of the immune system that preserve homeostasis and hinder autoimmunity in physiological conditions [103]. ICs overexpression in MPM keeps anti-tumor immune response in check contributing to the creation of a local immunosuppressive TME [31, 104]. IC inhibitors (ICIs), i.e. antibodies targeting ICs, are used as immunomodulatory agents to interfere with the CTLA-4/B7.1/2 or PD-1/PD-L1 axes thereby helping to overcome tumor-immune escape [95, 105, 106].

Recently, PD-L1 expression in MPM has been assessed on tissue microarrays using two different FDA-approved antibodies and 22–27% of cases were positive for PD-L1 (1% cut off) [107]. PD-L1 is expressed in a high proportion of biphasic and sarcomatoid MPM cases and its positivity >1% is associated with a significant 10-months reduction in median OS compared to PD-L1 negative tumors [108, 109]. Similarly, high PD-L1 expression (>50%) in epithelioid MPM patients correlates with shorter PFS (6.7 vs. 9.9 months) [108]. Despite its prognostic value [59, 60, 110], PD-L1 expression is not a valid predictive marker of response to anti-PD-L1 therapies for several tumor types [111, 112], including MPM [113]. Anti-PD-1/PD-L1 therapies were tested in different trials in MPM patients [114–121]. Combination of pembrolizumab with PPC in first-line treatment compared to pembrolizumab or PPC alone, is currently being evaluated in the phase III trial NCT0278417, while nivolumab is being investigated in the randomized phase III trial CONFIRM (NCT03063450) in comparison with placebo [119]. Durvalumab activity, a PD-L1 inhibitor, in combination with first-line CCP was tested in the DREAM study (ANZ clinical trial registry number: ACTRN12616001170415). This combination resulted in an ORR of 61% using mRECIST and 53% using iRECIST criteria and in a 6 months PFS of 71% (mRECIST). On the basis of these observations a randomized phase 3 trial will be started [122].

ICs expression is controlled at different stages of T-lymphocyte activation and variable in tumor cells. For these reasons, a combination strategy employing two different ICIs in addition to chemotherapy has been proposed to achieve a synergistic effect by overcoming immune-resistance observed in some MPM patients. Encouraging results observed for different ICIs in combination [113, 123, 124] prompted the investigation of the nivolumab plus ipilimumab combination in comparison to standard PPC alone as first-line option in the phase III clinical trial Checkmate-743 (NCT02899299). Checkmate-743 has clearly demonstrated the benefit of nivolumab in combination with ipilimumab in first line mesothelioma treatment and based on those results obtained approval from FDA from October 2020. On 22 April 2021, the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion recommending a change to the terms of the marketing authorization for the medicinal product Opdivo (nivolumab) in combination with ipilimumab for the first line treatment of adult patients with unresectable malignant pleural mesothelioma in Europe as well.

At present, efficacy and safety of adoptive T-cell therapies, in particular chimeric antigen receptor-transduced T-cells (CAR-T), in MPM and other solid tumors are under investigation [125, 126]. CAR-T-cells directed against mesothelin (MSLN), a glycoprotein expressed on MPM and other solid tumor cells, with a limited presence on normal tissues [127], represent a promising therapeutic option [128, 129]. Recently, Adusumilli and colleagues reported the outcome of a phase I clinical trial, NCT02414269, [130, 131] on MPM patients with pleural metastases from lung or breast cancer treated with anti-MSLN CAR-T-cells. Of note, the inclusion of anti-PD-1 therapy was crucial to elicit clinical efficacy and avoid T-cell exhaustion since no patient had an objective response before pembrolizumab addition showing the importance of conditioning the immune suppressive features of the TME also in this therapeutic setting.

Pembrolizumab plus anti-MSLN CAR-T-cell combination showed the best clinical outcome with an ORR of 63% (10/16) and a DCR of 75% (12/16). No evidence of on-target, off-tumor or therapy related toxicities higher than grade 1 was observed. Although applied to a limited number of patients so far, CAR-T therapies against MPM have shown really impressive results highlighting the different efficacy for advanced cell therapies compared to small molecule drugs or antibodies. Recently,

a comprehensive review about immunotherapy in MPM has been published [132]. However, the limited availability of therapeutic targetable antigens hinders the efficacy of CAR based strategies for MPM patients. More targets are needed for MPM treatment in the future.

7. Making a hot tumor microenvironment

ICIs effectiveness in MPM treated patients highlight the presence of potentially active immune cells *in situ that* if properly unleashed can elicit anti-tumor responses. However, to achieve this goal, TME must be modified in order to abolish/interfere with specific immune suppressive cues. Interestingly, Barsky and colleagues recently reported a case of a man with MPM treated with a combination of palliative radiation and immune-gene therapy (GM-CSF) [133]. The outcome of this treatment combination was outstanding, resulting in a so-called "abscopal effect".

The abscopal effect is observed when a localized radiation induces an anti-tumor response at distant sites. RT can trigger an immunogenic cell death (ICD) [134, 135] and can stimulate antigen-specific, adaptive immunity [136]. ICD sets the stage for anti-tumor immune responses which include the release of tumor antigens by irradiated tumor cells, the cross-presentation of tumor-derived antigens to T-cells by antigen-presenting cells (APCs), and the migration of effector T-cells from the lymph nodes to distant tumor sites, suggesting that irradiated tumors can act as an *in situ* vaccine if appropriate conditions are in place [137–139]. The overall incidence of the abscopal effect of RT alone is low with 46 clinical cases reported from 1969 [139]. Those poor numbers witness the insufficiency of RT alone to overcome the immune resistance of malignant tumors. Immunotherapy can lower host immune tolerance towards tumors, therefore the combination of RT and immunotherapy can amplify the anti-tumor immune response, a hypothesis currently under investigation in the trial NCT02959463 where adjuvant pembrolizumab after RT in lung-intact MPM patients is tested. In a murine model of MPM, the abscopal effect can be induced by local RT and enhanced by immune suppressive CTLA-4 blockade as infiltrated T-cells, both in primary and secondary tumor sites, are predominantly cytotoxic CD8+ T-cells while Tregs are reduced [140]. Those observations corroborate the idea that a systemic tumor response can be unleashed by a local treatment thereby modifying the features of the TME.

8. The quest for specificity in malignant mesothelioma: how can we fill this gap?

Adoptive cell therapies in combination with ICIs are showing promising results for MPM patients. Their specificity or preference of targeting is granted almost exclusively by the use of antibodies or their derived fragments that are directed to tumor specific/associated antigens. First attempts of therapy using murine monoclonal antibodies (mAbs) in cancer patients failed due to neutralizing antibodies generation and to mismatch with components of the human immune system. These results highlighted the importance of using human or human compatible/tolerable biomolecules and prompted the design of novel screening platforms to find them. Antigen unbiased screening methods (**Figure 1**) can be used to this end to test *a priori* the targeting ability of antibodies to cells postponing the identification of antigens to lead candidates only.

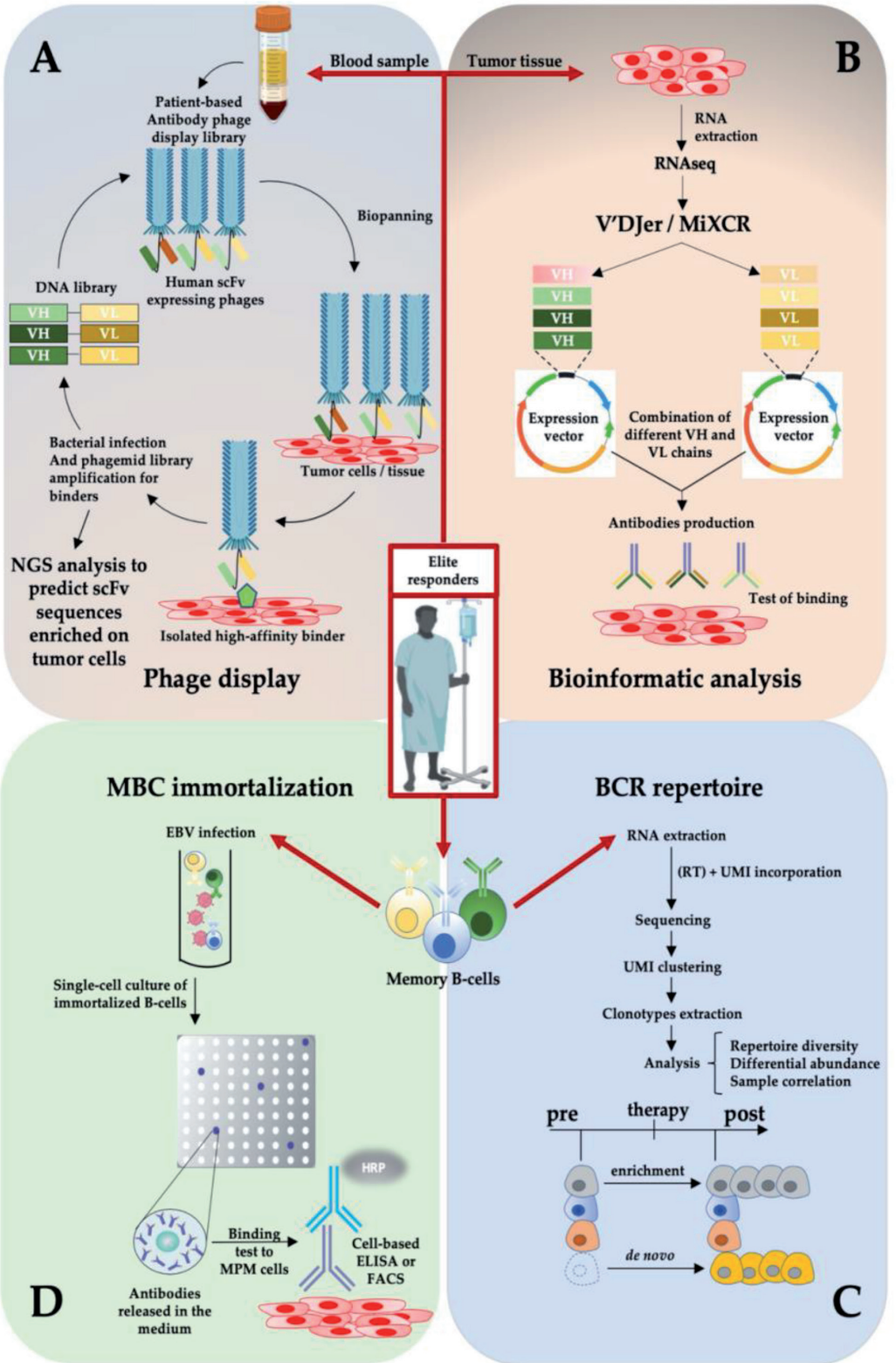

Figure 1.
Schematic representation of 4 antigen-unbiased screening strategies to obtain fully human tumor targeting antibodies. Panel ***A****: Patient derived scFv phage display libraries can be generated from MPM patient peripheral blood B cells. Those libraries are used to screen for novel specificities. Phage displayed scFvs undergo selection through consecutive rounds of panning on tumor cells to enrich for specific binders. NGS analysis allows the prediction of scFv sequences enriched on tumor cells. Panel* ***B:*** de novo *formed sequences, like those codifying the BCRs of infiltrated immune cells in tumor tissue can be retrieved using specific bioinformatic tools. Combinations of different heavy (VH) and light (VL) variable chains are used to generate candidate antibodies to be screened on cell or tumor tissues. Panel* ***C:*** *Analysis of BCR repertoire could be performed from memory B-cells from MPM patients. Enriched or de novo formed sequences could be monitored before and after a specific treatment in order to identify specific clones. Panel* ***D:*** *Memory B-cells from MPM patients can be immortalized through EBV infection and the immunoglobulins released in the medium of clonal cell cultures are tested on tumor cells by FACS or ELISA assays.*

9. From today's patients the future cures for MPM

As explained above, patients develop an immune response against MPM that, if unleashed, can be very effective. The presence of TLSs and the development of oligoclonal families of B-cells inside or at the border of MPM tissue are positive prognostic features and constitute a window of opportunity to capture human therapeutic antibodies. Now the next question is: how can we exploit this powerful reservoir of biologics to isolate or design targeting drugs? In other words: what technologies are available to take up this challenge?

10. BCR repertoire from sequencing data

Bulk RNA-Seq data from tumor tissue contain a hitherto overlooked picture of tumor and its ecosystem. Typically, data are analyzed to assess the expression of known transcripts, while *de novo* formed sequences, like those generated by T- and B-cells in the assembly and generation of their specific receptors, are usually disregarded since they fall off from the comparison with the reference transcriptome. However, these sequences can be retrieved from raw data and employed to extract the sequence of TCRs and BCRs from tumor tissue infiltrated immune cells using specific bioinformatic tools. One of them is MiXCR [141], a universal tool which takes raw sequencing data, including RNA-seq, as input and profiles TCR and BCR repertoires. As a reference, it uses a built-in library of V, D, J and C gene sequences from the human or mouse genome. MixCR output provides a list of clonotypes derived by assembling identical and homologous reads, corrected for sequencing errors.

V'DJer is another software that can process RNA-seq data for this purpose [142]. It can be run on BCR light and heavy chain data and employs unmapped paired end short reads aligning them against a reference transcriptome. Then, V'DJer detects VDJ rearrangements, generates contigs and quantifies the ones that represent the most abundant portions of the BCR repertoire. When the expression levels of BCR are low, there is an option to increase sensitivity of the algorithm at the cost of increasing the demand for computational resources. V'DJer has been used, for example, to retrieve antibodies from RNA sequencing data of melanoma patients from TCGA repository [142, 143]. At present, TCGA contains expression analyses of 87 MPM patients (TCGA-MESO) that could be used for this purpose. In addition, RNA can be obtained from FFPE samples containing TLSs in prospective and retrospective patients' cohorts.

It is possible to infer the sequence of resident B-cell clones by applying bioinformatic tools to RNA-Seq or by sequencing amplicons for immunoglobulin chains using specific sets of degenerate universal primers from whole tissue DNA or RNA/cDNA. The latter approach is implemented by the immunoSEQ platform (Adaptive Biotechnologies, Seattle, WA). In contrast to profiling using bulk RNA-Seq data, it is more precise since the experimental design is optimized to identify the BCR repertoire through the ImmunoSeq Analyzer software which is specific for this purpose. Its starting material can be both genomic DNA (gDNA) and cDNA: in order to assess clonal expansion of B-cells in tissues, gDNA is the best solution since each cell contains the same copy number, while mRNA transcripts can be very different among cells, depending on cellular activation and even the retrotranscription procedure can add other confounding factors. However, cDNA is a better choice if the goal is to find the most abundantly produced antibodies in situ, since there is a difference in the mRNA expression between activated and naive B-cells. Finally, independently of the method employed for their derivation,

identified immunoglobulin heavy and light chain sequences can be assembled to produce candidate antibodies and test them for MPM target cells binding.

11. Memory B-cell receptor repertoire in MPM patients

A second powerful approach to obtain human antibodies targeting MPM cancer cells exploits directly the immune system of patients. Individuals exposed to viral agents, parasites and tumors develop an adaptive response against non-self and neoantigens. Anti-cancer treatments such as vaccines and ICIs elicit impressive clinical responses (reviewed in [95]) and an immunological memory in subgroups of cancer patients ("elite responders") has been reported. MPM is not characterized by high mutational burden [15] an important determinant of the response to checkpoint blockade.

The efficacy of the anti-PD-1 pembrolizumab was shown by Alley and colleagues in KEYNOTE-028 [116]. In addition, ipilimumab in combination with anti-TGFβ and anti-CD25 antibodies of syngeneic MPM in BALB/c animals resulted in: i) disease eradication in treated mice; ii) elevated levels of tumor-specific IgG antibodies in healed animals; iii) failure to regrow tumors in cured mice when re-challenged with the same tumor; iv) importantly, no response in the absence of B-cells, suggesting that antibodies generated upon treatment contribute significantly to the curative effect [100]. Besides that, CD20+ B-cells infiltration in MPM tumor tissue is a positive prognostic factor as previously discussed [38].

Therefore, the immune system of elite responders can be mined to isolate MBCs producing targeting antibodies. MBCs derive mostly from affinity matured and somatically hypermutated B-cells arising in the germinal centers [144] and constitute a reservoir of high-affinity antibody producers. These features make the MBC pool very attractive so biotech and pharma companies invest in the design of screening platforms to exploit it. For example, Oncoresponse, a company that developed a proprietary, clinically validated human-antibody discovery platform in partnership with MD Anderson Cancer Center follows this paradigm and identifies therapeutically relevant antibodies from patients showing elite response against cancer after immunotherapy. MBCs are easily accessible from the peripheral blood of donors and are suitable for viral immortalization to generate lymphoblastoid cultures for high throughput screens. MBC immortalization is usually performed by infection of peripheral MBCs by Epstein Barr Virus (EBV) [145] or by BCL-6/BCL-XL expressing vectors [146]. Those procedures generate cells that express BCR on the membrane and release their antibody into culture medium at the same time. BCR presence is exploited to isolate cells binding to labeled soluble antigens by cell sorting [146] so that subsequently immunoglobulin sequences from isolated cells can be cloned into expression vectors for large-scale antibody production. Companies like Humabs and AIMM therapeutics exploit those strategies to raise antibodies against specific targets. However, the same technology can be used to isolate targeting antibodies in an antigen unbiased manner as shown for melanoma via cell-based screenings of EBV immortalized B-cells [147]. In addition, human plasmablasts and MBCs can be cultured for a limited time using specific cytokines [147–152].

Importantly, these approaches to retrieve targeting antibodies do not rely on a prior knowledge of the target. Target identification in this case is postponed, initially drawing on the demonstration of efficacy and specificity towards MPM cancer cells. MBCs receptor repertoire can be obtained also from peripheral blood or draining lymph node purified MBCs by RNA-Seq mining for *de novo* formed or highly enriched variants after treatment in elite responders [142]. Advantages and

Approach	Antigen display	Advantages	Disadvantages
Phage-display technology with patient derived scFv antibody libraries	Antigen on cell surface	• Cheap instrumentation • Used with any cell type • Established technology • Fastest strategy to lead candidates • NGS driven selection of candidates	• Affinity maturation step is often needed • Reformatting in IgG format, if needed • Binding to normal human tissues to establish specificity *a posteriori*
BCR repertoire from the peripheral blood of elite responders pre and post therapy	Antigen on cell surface	• Availability of blood samples from elite responders • Antibodies are derived from affinity matured human immunoglobulins	• Possible downsampling • Cloning and production of candidate antibodies is required • VH and VL pairs are not known (unless single cell sequencing is used) • Requires a test of binding specificity to normal human tissues *a posteriori*
Bioinformatic analysis of BCR repertoire in tumor tissue	Antigen on cell surface	• Availability of large number of FFPE samples • Applicable to retrospective case series • Applicable to any RNA-Seq dataset	• Requires cloning and production of the antibodies • Possible downsampling due to low quality or limited sample material • VH and VL pairs cannot be known • Requires a test of binding specificity to normal human tissues *a posteriori*
MBC immortalization	Antigen on cell surface	• Easy availability of elite responder samples (blood/PBMCs) • Established protocols • Isolation of *in vivo* high-affinity matured and human compatible immunoglobulins • Basic technical expertise on viral manipulation	• Requires a BSL2 area • Identification of the antigens can be technically challenging • Requires a test of binding specificity to normal human tissues *a posteriori*

Table 1.
Advantages and drawbacks of antigen-unbiased screens to obtain fully human antibodies.

drawbacks of the different screening strategies for fully human antibody selection are summarized in **Table 1**.

12. Phage display screening using patient-derived scFv antibody libraries

A useful strategy to select human antibody fragments (Fabs and scFvs) against specific antigens or cells is phage display (reviewed in [153]). The importance of phage display has been restated in 2018 by the award of Nobel Prize in Chemistry

to George P. Smith and Sir Gregory P. Winter"for the phage display of peptides and antibodies". Phage display has allowed the production of clinically relevant antibodies (reviewed in [154]). The presence of BCRs in TME, SLOs and in the peripheral blood of MPM and other tumor patients allows for the generation of patient derived scFv phage display libraries [155] that can be used to screen for novel specificities. Phage displayed scFvs undergo selection through consecutive rounds of panning on tumor cells to enrich for specific binders (**Figure 1**). Identified antibodies can be reformatted to fully human antibodies or used as fragments or building blocks for CAR constructs.

Importantly those antibodies will derive from the permutation of original VH and VL sequences of the B-cell repertoire during library preparation while for EBV immortalized cells VH and VL pairs will be the original ones as in the patient. Classically single bacterial clones were selected and grown to produce antibodies or phages displaying specific antibodies in order to test individually their targeting of a cell of interest. Nowadays, next generation sequencing provides an efficient, quantitative and quick analytical tool to assess the evolution of complexity of phage antibody-display libraries during consecutive biopanning enrichment stages. Phage clonal evolution during screening can be studied and used to identify putative candidate antibodies and promote their cloning and production for further testing their binding to cells [156].

An unbiased phage display approach has been used to identify tumor-targeting scFvs for both sarcomatoid and epithelioid MPM. In this study, 95 mesothelioma-targeting scFvs were identified and 21 candidates were characterized for binding by FACS and IHC and for their *in vitro* internalization capacity by MPM cells with the goal to deliver conjugated anti-tumor drugs directly inside tumor cells [157]. Further analyses identified MCAM/CD146 as one of the antigens. CD146 had been previously described as a marker in advanced melanoma [158] and other tumors [159, 160], it is expressed in all MPM histotypes and by a limited spectrum of normal human adult tissues [161]. The clinical utility of MCAM/CD146 detection in pleural effusion fluid and peripheral blood samples as a diagnostic and prognostic marker for MPM [162] is under evaluation. The generation of a phage antibody-display library from the entire antibody genes repertoire of a cancer patient has been also attempted. Rare cancer targeting antibodies have been identified by this strategy [163]. However, the immunodominance phenomenon typical of certain cancers [153, 164, 165] has hindered a wider use of this strategy in early attempts.

13. Conclusions

Despite amazing efforts made by the scientific community and the therapeutic options developed over the last decades, the discovery of a curative treatment for MPM is still elusive and constitutes an unmet clinical need. To-date, the most promising therapeutic approaches comprise immunotherapies and CAR-based therapies that have shown impressive although preliminary clinical results. The field needs to bet on and implement these novel approaches towards novel targets and antigens to cope with tumor heterogeneity and to provide effective treatments to be used in combination. The most innovative screening technologies for the generation of fully human antibodies are in place and combine elements from fields of science that started far apart and came together to serve the purpose. These include protein engineering, next-generation sequencing (NGS), virology and cell biology providing an opportunity to find novel and unknown therapeutic targets for MPM and cancer in general. Based on these premises, we believe that a future breakthrough in MPM management will come from the design of novel

ATMPs engineered to target antigens that are still unknown but that can be identified via unbiased screening strategies.

Acknowledgements

We thank Alicja M. Gruszka for her help in proofreading and editing this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Author details

Fabio Nicolini* and Massimiliano Mazza*
Immunotherapy, Cell Therapy and Biobank (ITCB), IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy

*Address all correspondence to: fabio.nicolini@irst.emr.it and massimiliano.mazza@irst.emr.it

References

[1] Carbone M, Adusumilli PS, Alexander HR Jr, Baas P, Bardelli F, Bononi A, et al. Mesothelioma: Scientific clues for prevention, diagnosis, and therapy. CA A Cancer J Clin. 2019 Jul 30;69(5):402-29.

[2] Kameda T, Takahashi K, Kim R, Jiang Y, Movahed M, Park E-K, et al. Asbestos: use, bans and disease burden in Europe. Bull World Health Organ. 2014 Nov 1;92(11):790-7.

[3] Peto J, Decarli A, La Vecchia C, Levi F, Negri E. The European mesothelioma epidemic. Br J Cancer. 1999 Feb;79(3-4):666-72.

[4] Henley SJ, Larson TC, Wu M, Antao VCS, Lewis M, Pinheiro GA, et al. Mesothelioma incidence in 50 states and the District of Columbia, United States, 2003-2008. Int J Occup Environ Health. 2013 Jan;19(1):1-10.

[5] Beckett P, Edwards J, Fennell D, Hubbard R, Woolhouse I, Peake MD. Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. Lung Cancer. 2015 Jun;88(3):344-8.

[6] Cheung M, Testa JR. BAP1, a tumor suppressor gene driving malignant mesothelioma. Transl Lung Cancer Res. 2017 Jun;6(3):270-8.

[7] Yu H, Pak H, Hammond-Martel I, Ghram M, Rodrigue A, Daou S, et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. Proc Natl Acad Sci USA. 2014 Jan 7;111(1):285-90.

[8] Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. Nature Publishing Group. 2013 Mar;13(3):153-9.

[9] Affar EB, Carbone M. BAP1 regulates different mechanisms of cell death. Cell Death Dis. Nature Publishing Group; 2018 Nov 19;9(12):1151-3.

[10] Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P, et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. Nat Cell Biol. Nature Publishing Group; 2018 Oct;20(10):1181-92.

[11] Dey A, Seshasayee D, Noubade R, French DM, Liu J, Chaurushiya MS, et al. Loss of the tumor suppressor BAP1 causes myeloid transformation. Science. 2012 Sep 21;337(6101):1541-6.

[12] Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M, et al. BAP1 regulates IP3R3-mediated Ca2+ flux to mitochondria suppressing cell transformation. Nature. Nature Publishing Group; 2017 Jun 22;546(7659):549-53.

[13] Bononi A, Yang H, Giorgi C, Patergnani S, Pellegrini L, Su M, et al. Germline BAP1 mutations induce a Warburg effect. Cell Death Differ. 2017 Oct;24(10):1694-704.

[14] Betti M, Casalone E, Ferrante D, Aspesi A, Morleo G, Biasi A, et al. Germline mutations in DNA repair genes predispose asbestos-exposed patients to malignant pleural mesothelioma. Cancer Letters. 2017 Oct 1;405:38-45.

[15] Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet. Nature Publishing Group; 2016 Apr;48(4):407-16.

[16] Hassan R, Morrow B, Thomas A, Walsh T, Lee MK, Gulsuner S, et al.

Inherited predisposition to malignant mesothelioma and overall survival following platinum chemotherapy. Proc Natl Acad Sci USA. National Academy of Sciences; 2019 Apr 30;116(18): 9008-13.

[17] Farzin M, Toon CW, Clarkson A, Sioson L, Watson N, Andrici J, et al. Loss of expression of BAP1 predicts longer survival in mesothelioma. Pathology. 2015 Jun;47(4):302-7.

[18] Srinivasan G, Sidhu GS, Williamson EA, Jaiswal AS, Najmunnisa N, Wilcoxen K, et al. Synthetic lethality in malignant pleural mesothelioma with PARP1 inhibition. Cancer Chemother Pharmacol. Springer Berlin Heidelberg; 2017 Oct;80(4): 861-7.

[19] Zauderer MG, Szlosarek P, Le Moulec S, Popat S, Taylor P, Planchard D, et al. Phase 2, multicenter study of the EZH2 inhibitor tazemetostat as monotherapy in adults with relapsed or refractory (R/R) malignant mesothelioma (MM) with BAP1 inactivation. JCO. 2018;36 (15_suppl):8515-5.

[20] Quetel L, Meiller C, Assié J-B, Blum Y, Imbeaud S, Montagne F, et al. Genetic alterations of malignant pleural mesothelioma: association with tumor heterogeneity and overall survival. Mol Oncol. John Wiley & Sons, Ltd; 2020 Jun;14(6):1207-23.

[21] Nicolini F, Bocchini M, Bronte G, Delmonte A, Guidoboni M, Crinò L, et al. Malignant Pleural Mesothelioma: State-of-the-Art on Current Therapies and Promises for the Future. Front Oncol. 2019;9:1519.

[22] Donaldson K, Murphy FA, Duffin R, Poland CA. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol. BioMed Central; 2010 Mar 22;7(1):5-17.

[23] Park SH, Aust AE. Participation of iron and nitric oxide in the mutagenicity of asbestos in hgprt-, gpt+ Chinese hamster V79 cells. Cancer Res. Cancer Res; 1998 Mar 15;58(6):1144-8.

[24] Kamp DW, Israbian VA, Preusen SE, Zhang CX, Weitzman SA. Asbestos causes DNA strand breaks in cultured pulmonary epithelial cells: role of iron-catalyzed free radicals. Am J Physiol. American Physiological Society Bethesda, MD; 1995 Mar;268 (3 Pt 1):L471-80.

[25] Hei TK, Piao CQ, He ZY, Vannais D, Waldren CA. Chrysotile fiber is a strong mutagen in mammalian cells. Cancer Res. Cancer Res; 1992 Nov 15;52(22):6305-9.

[26] Yang H, Bocchetta M, Kroczynska B, Elmishad AG, Chen Y, Liu Z, et al. TNF-alpha inhibits asbestos-induced cytotoxicity via a NF-kappaB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. Proc Natl Acad Sci USA. National Academy of Sciences; 2006 Jul 5;103(27):10397-402.

[27] Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C, et al. Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. Proc Natl Acad Sci USA. National Academy of Sciences; 2010 Jul 13;107(28):12611-6.

[28] Padmore T, Stark C, Turkevich LA, Champion JA. Quantitative analysis of the role of fiber length on phagocytosis and inflammatory response by alveolar macrophages. Biochim Biophys Acta Gen Subj. 2017 Feb;1861(2):58-67.

[29] Liu JY, Brass DM, Hoyle GW, Brody AR. TNF-alpha receptor

knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. Am J Pathol. 1998 Dec;153(6):1839-47.

[30] Lievense LA, Cornelissen R, Bezemer K, Kaijen-Lambers MEH, Hegmans JPJJ, Aerts JGJV. Pleural Effusion of Patients with Malignant Mesothelioma Induces Macrophage-Mediated T Cell Suppression. J Thorac Oncol. 2016 Oct;11(10):1755-64.

[31] Marcq E, Siozopoulou V, De Waele J, van Audenaerde J, Zwaenepoel K, Santermans E, et al. Prognostic and predictive aspects of the tumor immune microenvironment and immune checkpoints in malignant pleural mesothelioma. Oncoimmunology. Taylor & Francis; 2017 Jan 20;6(1):1-10.

[32] Burt BM, Rodig SJ, Tilleman TR, Elbardissi AW, Bueno R, Sugarbaker DJ. Circulating and tumor-infiltrating myeloid cells predict survival in human pleural mesothelioma. Cancer. John Wiley & Sons, Ltd; 2011 Nov 15;117(22): 5234-44.

[33] Awad MM, Jones RE, Liu H, Lizotte PH, Ivanova EV, Kulkarni M, et al. Cytotoxic T Cells in PD-L1-Positive Malignant Pleural Mesotheliomas Are Counterbalanced by Distinct Immunosuppressive Factors. Cancer Immunology Research. American Association for Cancer Research; 2016 Dec;4(12):1038-48.

[34] Cornelissen R, Hegmans JPJJ, Maat APWM, Kaijen-Lambers MEH, Bezemer K, Hendriks RW, et al. Extended Tumor Control after Dendritic Cell Vaccination with Low-Dose Cyclophosphamide as Adjuvant Treatment in Patients with Malignant Pleural Mesothelioma. Am J Respir Crit Care Med. 2016 May;193(9):1023-31.

[35] Tanrikulu AC, Abakay A, Komek H, Abakay O. Prognostic value of the lymphocyte-to-monocyte ratio and other inflammatory markers in malignant pleural mesothelioma. Environ Health Prev Med. BioMed Central; 2016 Sep;21(5):304-11.

[36] Hegmans JPJJ, Hemmes A, Hammad H, Boon L, Hoogsteden HC, Lambrecht BN. Mesothelioma environment comprises cytokines and T-regulatory cells that suppress immune responses. Eur Respir J. European Respiratory Society; 2006 Jun;27(6): 1086-95.

[37] Li T, Li H, Wang Y, Harvard C, Tan J-L, Au A, et al. The expression of CXCR4, CXCL12 and CXCR7 in malignant pleural mesothelioma. J Pathol. John Wiley & Sons, Ltd; 2011 Mar;223(4):519-30.

[38] Ujiie H, Kadota K, Nitadori J-I, Aerts JG, Woo KM, Sima CS, et al. The tumoral and stromal immune microenvironment in malignant pleural mesothelioma: A comprehensive analysis reveals prognostic immune markers. Oncoimmunology. Taylor & Francis; 2015 Jun;4(6):e1009285.

[39] Blanquart C, Gueugnon F, Nguyen J-M, Roulois D, Cellerin L, Sagan C, et al. CCL2, galectin-3, and SMRP combination improves the diagnosis of mesothelioma in pleural effusions. J Thorac Oncol. 2012 May;7(5):883-9.

[40] Gueugnon F, Leclercq S, Blanquart C, Sagan C, Cellerin L, Padieu M, et al. Identification of novel markers for the diagnosis of malignant pleural mesothelioma. Am J Pathol. 2011 Mar;178(3):1033-42.

[41] Chéné A-L, d'Almeida S, Blondy T, Tabiasco J, Deshayes S, Fonteneau J-F, et al. Pleural Effusions from Patients with Mesothelioma Induce Recruitment of Monocytes and Their Differentiation into M2 Macrophages. J Thorac Oncol. 2016 Oct;11(10):1765-73.

[42] Cioce M, Canino C, Goparaju C, Yang H, Carbone M, Pass HI. Autocrine

CSF-1R signaling drives mesothelioma chemoresistance via AKT activation. Cell Death Dis. Nature Publishing Group; 2014 Apr 10;5(4):e1167-7.

[43] Fujii M, Toyoda T, Nakanishi H, Yatabe Y, Sato A, Matsudaira Y, et al. TGF-β synergizes with defects in the Hippo pathway to stimulate human malignant mesothelioma growth. J Exp Med. 2012 Mar 12;209(3):479-94.

[44] Schürch CM, Forster S, Brühl F, Yang SH, Felley Bosco E, Hewer E. The "don't eat me" signal CD47 is a novel diagnostic biomarker and potential therapeutic target for diffuse malignant mesothelioma. Oncoimmunology. Taylor & Francis; 2017;7(1):e1373235.

[45] Izzi V, Chiurchiù V, D'Aquilio F, Palumbo C, Tresoldi I, Modesti A, et al. Differential effects of malignant mesothelioma cells on THP-1 monocytes and macrophages. Int J Oncol. Int J Oncol; 2009 Feb;34(2):543-50.

[46] Miselis NR, Wu ZJ, Van Rooijen N, Kane AB. Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma. Molecular Cancer Therapeutics. American Association for Cancer Research; 2008 Apr;7(4):788-99.

[47] Rehrauer H, Wu L, Blum W, Pecze L, Henzi T, Serre-Beinier V, et al. How asbestos drives the tissue towards tumors: YAP activation, macrophage and mesothelial precursor recruitment, RNA editing, and somatic mutations. Oncogene. Nature Publishing Group; 2018 May;37(20):2645-59.

[48] Khanna S, Graef S, Mussai F, Thomas A, Wali N, Yenidunya BG, et al. Tumor-Derived GM-CSF Promotes Granulocyte Immunosuppression in Mesothelioma Patients. Clin Cancer Res. American Association for Cancer Research; 2018 Jun 15;24(12):2859-72.

[49] Kao SCH, Pavlakis N, Harvie R, Vardy JL, Boyer MJ, van Zandwijk N, et al. High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. Clin Cancer Res. American Association for Cancer Research; 2010 Dec 1;16(23):5805-13.

[50] Chee SJ, Lopez M, Mellows T, Gankande S, Moutasim KA, Harris S, et al. Evaluating the effect of immune cells on the outcome of patients with mesothelioma. Br J Cancer. Nature Publishing Group; 2017 Oct 24;117(9):1341-8.

[51] Marcq E, Siozopoulou V, De Waele J, van Audenaerde J, Zwaenepoel K, Santermans E, et al. Prognostic and predictive aspects of the tumor immune microenvironment and immune checkpoints in malignant pleural mesothelioma. Oncoimmunology. Taylor & Francis; 2017 Jan 20;6(1):1-10.

[52] Chee SJ, Lopez M, Mellows T, Gankande S, Moutasim KA, Harris S, et al. Evaluating the effect of immune cells on the outcome of patients with mesothelioma. Nature Publishing Group; 2017 Aug 17;117(9):1341-8.

[53] Anraku M, Cunningham KS, Yun Z, Tsao M-S, Zhang L, Keshavjee S, et al. Impact of tumor-infiltrating T cells on survival in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg. 2008 Apr;135(4):823-9.

[54] DeLong P, Carroll RG, Henry AC, Tanaka T, Ahmad S, Leibowitz MS, et al. Regulatory T cells and cytokines in malignant pleural effusions secondary to mesothelioma and carcinoma. Cancer Biol Ther. Taylor & Francis; 2005 Mar;4(3):342-6.

[55] Comar M, Zanotta N, Bonotti A, Tognon M, Negro C, Cristaudo A, et al. Increased levels of C-C chemokine RANTES in asbestos exposed workers and in malignant mesothelioma patients

from an hyperendemic area. PLOS ONE. Public Library of Science; 2014;9(8): e104848.

[56] Davidson B, Dong HP, Holth A, Berner A, Risberg B. Chemokine receptors are infrequently expressed in malignant and benign mesothelial cells. Am J Clin Pathol. 2007 May;127(5): 752-9.

[57] Kiyotani K, Park J-H, Inoue H, Husain A, Olugbile S, Zewde M, et al. Integrated analysis of somatic mutations and immune microenvironment in malignant pleural mesothelioma. Oncoimmunology. Taylor & Francis; 2017;6(2):e1278330.

[58] Mansfield AS, Peikert T, Smadbeck JB, Udell JBM, Garcia-Rivera E, Elsbernd L, et al. Neoantigenic Potential of Complex Chromosomal Rearrangements in Mesothelioma. J Thorac Oncol. 2019 Feb;14(2): 276-87.

[59] Marcq E, Waele JD, Audenaerde JV, Lion E, Santermans E, Hens N, et al. Abundant expression of TIM-3, LAG-3, PD-1 and PD-L1 as immunotherapy checkpoint targets in effusions of mesothelioma patients. Oncotarget. 2017 Oct 27;8(52):89722-35.

[60] Cedrés S, Ponce-Aix S, Zugazagoitia J, Sansano I, Enguita A, Navarro-Mendivil A, et al. Analysis of expression of programmed cell death 1 ligand 1 (PD-L1) in malignant pleural mesothelioma (MPM). Gangopadhyay N, editor. PLOS ONE. 2015;10(3):e0121071.

[61] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480(7378):480-9.

[62] Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol. 2006;7(4):344-53.

[63] Dieu-Nosjean M-C, Giraldo NA, Kaplon H, Germain C, Fridman WH, Sautès-Fridman C. Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. Immunol Rev. John Wiley & Sons, Ltd (10.1111); 2016 May;271(1):260-75.

[64] Sautès-Fridman C, Lawand M, Giraldo NA, Kaplon H, Germain C, Fridman WH, et al. Tertiary Lymphoid Structures in Cancers: Prognostic Value, Regulation, and Manipulation for Therapeutic Intervention. Front Immunol. 2016 Oct 3;7(27):4410-1.

[65] Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, et al. Human Solid Tumors Contain High Endothelial Venules: Association with T- and B-Lymphocyte Infiltration and Favorable Prognosis in Breast Cancer. Cancer Res. 2011 Aug 30;71(17):5678-87.

[66] Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B Cells in Tertiary Lymphoid Structures Is Associated with a Protective Immunity in Patients with Lung Cancer. Am J Respir Crit Care Med. 2014 Apr;189(7):832-44.

[67] Ettinger DS, Wood DE, Akerley W, Bazhenova LA, Borghaei H, Camidge DR, et al. NCCN Guidelines Insights: Malignant Pleural Mesothelioma, Version 3.2016. Vol. 14, Journal of the National Comprehensive Cancer Network : JNCCN. 2016. pp. 825-36.

[68] Yamada N, Oizumi S, Kikuchi E, Shinagawa N, Konishi-Sakakibara J, Ishimine A, et al. CD8+ tumor-infiltrating lymphocytes predict favorable prognosis in malignant pleural mesothelioma after resection. Cancer Immunol Immunother. 2010 Oct;59(10):1543-9.

[69] Suzuki K, Kadota K, Sima CS, Sadelain M, Rusch VW, Travis WD, et al. Chronic inflammation in tumor

stroma is an independent predictor of prolonged survival in epithelioid malignant pleural mesothelioma patients. Cancer Immunol Immunother. 3rd ed. 2011 Dec;60(12):1721-8.

[70] Goc J, Germain C, Vo-Bourgais TKD, Lupo A, Klein C, Knockaert S, et al. Dendritic Cells in Tumor-Associated Tertiary Lymphoid Structures Signal a Th1 Cytotoxic Immune Contexture and License the Positive Prognostic Value of Infiltrating CD8+ T Cells. Cancer Res. 2014 Feb 2;74(3):705-15.

[71] Dieu-Nosjean M-C, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-Term Survival for Patients With Non–Small-Cell Lung Cancer With Intratumoral Lymphoid Structures. JCO. 2008 Sep 20;26(27): 4410-7.

[72] Silina K, Soltermann A, Attar FM, Casanova R, Uckeley ZM, Thut H, et al. Germinal Centers Determine the Prognostic Relevance of Tertiary Lymphoid Structures and Are Impaired by Corticosteroids in Lung Squamous Cell Carcinoma. Cancer Res. American Association for Cancer Research; 2018 Mar 1;78(5):1308-20.

[73] Di Caro G, Bergomas F, Grizzi F, Doni A, Bianchi P, Malesci A, et al. Occurrence of Tertiary Lymphoid Tissue Is Associated with T-Cell Infiltration and Predicts Better Prognosis in Early-Stage Colorectal Cancers. Clin Cancer Res. 2014 Apr 15;20(8):2147-58.

[74] Posch F, Silina K, Leibl S, Mündlein A, Moch H, Siebenhüner A, et al. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. Oncoimmunology. Taylor & Francis; 2017 Dec 20;7(2):1-13.

[75] Hiraoka N, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. Br J Cancer. Nature Publishing Group; 2015 May 5;112(11):1782-90.

[76] Castino GF, Cortese N, Capretti G, Serio S, Di Caro G, Mineri R, et al. Spatial distribution of B cells predicts prognosis in human pancreatic adenocarcinoma. Oncoimmunology. Taylor & Francis; 2016 Apr 5;5(4):1-14.

[77] Wirsing AM, Ervik IK, Seppola M, Uhlin-Hansen L, Steigen SE, Hadler-Olsen E. Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. Modern Pathology. Springer US; 2018 Jun 7;:1-13.

[78] Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, et al. CD4+ follicular helper T cell infiltration predicts breast cancer survival. Journal of Clinical Investigation. 2013 Jun 17;123(7):2873-92.

[79] Lee HJ, Kim JY, Park IA, Song IH, Yu JH, Ahn J-H, et al. Prognostic Significance of Tumor-Infiltrating Lymphocytes and the Tertiary Lymphoid Structures in HER2-Positive Breast Cancer Treated With Adjuvant Trastuzumab. Am J Clin Pathol. 2015 Aug 1;144(2):278-88.

[80] Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol. Nature Publishing Group; 2016 Apr;13(4):228-41.

[81] Colbeck EJ, Jones E, Hindley JP, Smart K, Schulz R, Browne M, et al. Treg Depletion Licenses T Cell-Driven HEV Neogenesis and Promotes Tumor Destruction. Cancer Immunology Research. 2017 Nov;5(11):1005-15.

[82] Joshi NS, Akama-Garren EH, Lu Y, Lee D-Y, Chang GP, Li A, et al. Regulatory T Cells in Tumor-Associated Tertiary Lymphoid Structures Suppress

Anti-tumor T Cell Responses. Immunity. 2015 Sep 15;43(3):579-90.

[83] Fear VS, Tilsed C, Chee J, Forbes CA, Casey T, Solin JN, et al. Combination immune checkpoint blockade as an effective therapy for mesothelioma. Oncoimmunology. Taylor & Francis; 2018 Sep 12;7(10):1-14.

[84] Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, et al. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HEV formation. Sci Transl Med. 2017 Apr 12;9(385): eaak9679.

[85] Coppola D, Nebozhyn M, Khalil F, Dai H, Yeatman T, Loboda A, et al. Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. Am J Pathol. 2011 Jul;179(1):37-45.

[86] Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nature Publishing Group. Nature Publishing Group; 2019 Jun;19(6):307-25.

[87] Montfort A, Pearce O, Maniati E, Vincent BG, Bixby L, Böhm S, et al. A Strong B-cell Response Is Part of the Immune Landscape in Human High-Grade Serous Ovarian Metastases. Clin Cancer Res. 2017 Jan 2;23(1): 250-62.

[88] García-Hernández M de LL, Uribe-Uribe NO, Espinosa-González R, Kast WM, Khader SA, Rangel-Moreno J. A Unique Cellular and Molecular Microenvironment Is Present in Tertiary Lymphoid Organs of Patients with Spontaneous Prostate Cancer Regression. Front Immunol. 2017 May 17;8:87-21.

[89] Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, et al. CD20+ Tumor-Infiltrating Lymphocytes Have an Atypical CD27- Memory Phenotype and Together with CD8+ T Cells Promote Favorable Prognosis in Ovarian Cancer. Clin Cancer Res. 2012 Jun 14;18(12):3281-92.

[90] Kroeger DR, Milne K, Nelson BH. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. Clin Cancer Res. 2016 Jun 14;22(12):3005-15.

[91] Coronella JA, Spier C, Welch M, Trevor KT, Stopeck AT, Villar H, et al. Antigen-Driven Oligoclonal Expansion of Tumor-Infiltrating B Cells in Infiltrating Ductal Carcinoma of the Breast. JI. 2002 Aug 15;169(4):1829-36.

[92] Schlößer HA, Thelen M, Lechner A, Wennhold K, Garcia-Marquez MA, Rothschild SI, et al. B cells in esophago-gastric adenocarcinoma are highly differentiated, organize in tertiary lymphoid structures and produce tumor-specific antibodies. Oncoimmunology. Taylor & Francis; 2018 Oct 29;8(1):e1512458.

[93] Nzula S, Going JJ, Stott DI. Antigen-driven clonal proliferation, somatic hypermutation, and selection of B lymphocytes infiltrating human ductal breast carcinomas. Cancer Res. 2003 Jun 15;63(12):3275-80.

[94] Cipponi A, Mercier M, Seremet T, Baurain J-F, Théate I, van den Oord J, et al. Neogenesis of Lymphoid Structures and Antibody Responses Occur in Human Melanoma Metastases. Cancer Res. 2012 Aug 14;72(16):3997-4007.

[95] Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. Nature

Publishing Group; 2018 Dec 13;50(12):165-11.

[96] Wouters MCA, Nelson BH. Prognostic Significance of Tumor-Infiltrating B Cells and Plasma Cells in Human Cancer. Clin Cancer Res. 2018 Dec 15;24(24):6125-35.

[97] Petitprez F, de Reyniès A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature. Nature Publishing Group; 2020 Jan;577(7791):556-60.

[98] Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. Nature Publishing Group; 2020 Jan;577(7791):561-5.

[99] Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. Nature Publishing Group; 2020 Jan;577(7791):549-55.

[100] Krishnan S, Bakker E, Lee C, Kissick HT, Ireland DJ, Beilharz MW. Successful combined intratumoral immunotherapy of established murine mesotheliomas requires B-cell involvement. J Interferon Cytokine Res. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA; 2015 Feb;35(2):100-7.

[101] Minnema-Luiting J, Vroman H, Aerts J, Cornelissen R. Heterogeneity in Immune Cell Content in Malignant Pleural Mesothelioma. Int J Mol Sci. Multidisciplinary Digital Publishing Institute; 2018 Apr;19(4):1041-12.

[102] Jackaman C, Cornwall S, Graham PT, Nelson DJ. CD40-activated B cells contribute to mesothelioma tumor regression. Immunol Cell Biol. 2011 Feb;89(2):255-67.

[103] Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol. 2016 Feb;39(1):98-106.

[104] Pasello G, Zago G, Lunardi F, Urso L, Kern I, Vlacic G, et al. Malignant pleural mesothelioma immune microenvironment and checkpoint expression: correlation with clinical-pathological features and intratumor heterogeneity over time. Ann Oncol. 2018 May 1;29(5):1258-65.

[105] Nowak AK, Forde PM. Immunotherapy trials in mesothelioma - promising results, but don't stop here. Nat Rev Clin Oncol. 2019 Dec;16(12):726-8.

[106] Wei SC, Duffy CR, Allison JP. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discov. American Association for Cancer Research; 2018 Sep;8(9): 1069-86.

[107] Chapel DB, Stewart R, Furtado LV, Husain AN, Krausz T, Deftereos G. Tumor PD-L1 expression in malignant pleural and peritoneal mesothelioma by Dako PD-L1 22C3 pharmDx and Dako PD-L1 28-8 pharmDx assays. Human Pathology. 2019 May;87:11-7.

[108] Brosseau S, Danel C, Scherpereel A, Mazieres J, Lantuejoul S, Margery J, et al. Shorter Survival in Malignant Pleural Mesothelioma Patients With High PD-L1 Expression Associated With Sarcomatoid or Biphasic Histology Subtype: A Series of 214 Cases From the Bio-MAPS Cohort. Clin Lung Cancer. 2019 Sep;20(5):e564-75.

[109] Nguyen BH, Montgomery R, Fadia M, Wang J, Ali S. PD-L1 expression associated with worse survival outcome in malignant pleural mesothelioma. Asia Pac J Clin Oncol.

John Wiley & Sons, Ltd (10.1111); 2018 Feb;14(1):69-73.

[110] Gennen K, Käsmann L, Taugner J, Eze C, Karin M, Roengvoraphoj O, et al. Prognostic value of PD-L1 expression on tumor cells combined with CD8+ TIL density in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy. Radiat Oncol. BioMed Central; 2020 Jan 2;15(1):5-12.

[111] Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. BMJ. 2018 Sep 10;362:k3529.

[112] Conroy JM, Pabla S, Nesline MK, Glenn ST, Papanicolau-Sengos A, Burgher B, et al. Next generation sequencing of PD-L1 for predicting response to immune checkpoint inhibitors. J Immunother Cancer. BMJ Specialist Journals; 2019 Jan 24;7(1):18.

[113] Scherpereel A, Mazieres J, Greillier L, Lantuejoul S, Dô P, Bylicki O, et al. Nivolumab or nivolumab plus ipilimumab in patients with relapsed malignant pleural mesothelioma (IFCT-1501 MAPS2): a multicentre, open-label, randomised, non-comparative, phase 2 trial. Lancet Oncol. 2019 Feb;20(2):239-53.

[114] Calabrò L, Morra A, Fonsatti E, Cutaia O, Amato G, Giannarelli D, et al. Tremelimumab for patients with chemotherapy-resistant advanced malignant mesothelioma: an open-label, single-arm, phase 2 trial. Lancet Oncol. 2013 Oct;14(11):1104-11.

[115] Maio M, Scherpereel A, Calabrò L, Aerts J, Cedres Perez S, Bearz A, et al. Tremelimumab as second-line or third-line treatment in relapsed malignant mesothelioma (DETERMINE): a multicentre, international, randomised, double-blind, placebo-controlled phase 2b trial. Lancet Oncol. 2017 Sep;18(9):1261-73.

[116] Alley EW, Lopez J, Santoro A, Morosky A, Saraf S, Piperdi B, et al. Clinical safety and activity of pembrolizumab in patients with malignant pleural mesothelioma (KEYNOTE-028): preliminary results from a non-randomised, open-label, phase 1b trial. Lancet Oncol. 2017 May;18(5):623-30.

[117] Desai A, Karrison T, Rose B, Tan Y, Hill B, Pemberton E, et al. OA08.03 Phase II Trial of Pembrolizumab (NCT02399371) In Previously-Treated Malignant Mesothelioma (MM): Final Analysis. Journal of Thoracic Oncology. Elsevier Inc; 2018 Oct 1;13(Supplement):S339.

[118] Popat S, Curioni-Fontecedro A, Polydoropoulou V, Shah R, O'Brien M, Pope A, et al. LBA91_PRA multicentre randomized phase III trial comparing pembrolizumab (P) vs single agent chemotherapy (CT) for advanced pre-treated malignant pleural mesothelioma (MPM): Results from the European Thoracic Oncology Platform (ETOP 9-15) PROMISE-meso trial. annonc. 2019 Oct 1;30(Supplement_5).

[119] Fennell DA, Kirkpatrick E, Cozens K, Nye M, Lester J, Hanna G, et al. CONFIRM: a double-blind, placebo-controlled phase III clinical trial investigating the effect of nivolumab in patients with relapsed mesothelioma: study protocol for a randomised controlled trial. Trials. BioMed Central; 2018 Apr 18;19(1):233-10.

[120] Quispel-Janssen J, Zago G, Schouten R, Buikhuisen W, Monkhorst K, Thunissen E, et al. OA13.01 A Phase II Study of Nivolumab in Malignant Pleural Mesothelioma (NivoMes): with Translational Research (TR) Biopies. Journal of Thoracic Oncology. Elsevier; 2017 Jan 1;12(Supplement):S292-3.

[121] Okada M, Kijima T, Aoe K, Kato T, Fujimoto N, Nakagawa K, et al. Clinical

Efficacy and Safety of Nivolumab: Results of a Multicenter, Open-label, Single-arm, Japanese Phase II study in Malignant Pleural Mesohelioma (MERIT). Clin Cancer Res. 2019 Sep 15;25(18):5485.

[122] Nowak AK, Lesterhuis WJ, Hughes BGM, Brown C, Kok PS, O'Byrne KJ, et al. DREAM: A phase II study of durvalumab with first line chemotherapy in mesothelioma—First results. JCO. American Society of Clinical Oncology; 2018 May 20;36(15_suppl):8503-3.

[123] Calabrò L, Morra A, Giannarelli D, Amato G, D'Incecco A, Covre A, et al. Tremelimumab combined with durvalumab in patients with mesothelioma (NIBIT-MESO-1): an open-label, non-randomised, phase 2 study. Lancet Respir Med. 2018 Jun;6(6):451-60.

[124] Disselhorst MJ, Quispel-Janssen J, Lalezari F, Monkhorst K, de Vries JF, van der Noort V, et al. Ipilimumab and nivolumab in the treatment of recurrent malignant pleural mesothelioma (INITIATE): results of a prospective, single-arm, phase 2 trial. Lancet Respir Med. 2019 Mar;7(3):260-70.

[125] Zeltsman M, Dozier J, McGee E, Ngai D, Adusumilli PS. CAR T-cell therapy for lung cancer and malignant pleural mesothelioma. Transl Res. 2017 Sep;187:1-10.

[126] Martinez M, Moon EK. CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol. Frontiers; 2019;10:128.

[127] Lv J, Li P. Mesothelin as a biomarker for targeted therapy. Biomark Res. BioMed Central; 2019;7(1):18-8.

[128] Zhao Y, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, et al. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. Cancer Res. 2010 Nov 15;70(22):9053-61.

[129] Zhang Z, Jiang D, Yang H, He Z, Liu X, Qin W, et al. Modified CAR T cells targeting membrane-proximal epitope of mesothelin enhances the antitumor function against large solid tumor. Cell Death Dis. Nature Publishing Group; 2019 Jun 17;10(7):476-12.

[130] Adusumilli PS, Zauderer MG, Rusch VW, O 039 Cearbhaill RE, Zhu A, Ngai DA, et al. Abstract CT036: A phase I clinical trial of malignant pleural disease treated with regionally delivered autologous mesothelin-targeted CAR T cells: Safety and efficacy. Cancer Res. 2019 Jul 1;79(13 Supplement):CT036.

[131] Adusumilli PS, Cherkassky L, Villena-Vargas J, Colovos C, Servais E, Plotkin J, et al. Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. Sci Transl Med. 2014 Nov 5;6(261):261ra151-1.

[132] de Gooijer CJ, Borm FJ, Scherpereel A, Baas P. Immunotherapy in Malignant Pleural Mesothelioma. Front Oncol. 2020;10:187.

[133] Barsky AR, Cengel KA, Katz SI, Sterman DH, Simone CB. First-ever Abscopal Effect after Palliative Radiotherapy and Immuno-gene Therapy for Malignant Pleural Mesothelioma. Cureus. 2019 Feb 20;11(2):e4102.

[134] Kepp O, Galluzzi L, Martins I, Schlemmer F, Adjemian S, Michaud M, et al. Molecular determinants of immunogenic cell death elicited by anticancer chemotherapy. Cancer Metastasis Rev. Springer US; 2011 Mar;30(1):61-9.

[135] Galluzzi L, Kepp O, Kroemer G. Immunogenic cell death in radiation therapy. Oncoimmunology. 2014 Oct 27;2(10):e26536-3.

[136] Zelenay S, Reis e Sousa C. Adaptive immunity after cell death. Trends in Immunology. 2013 Jul;34(7):329-35.

[137] Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991-8.

[138] Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. Science. American Association for the Advancement of Science; 2011 Mar 24;331(6024):1565-70.

[139] Abuodeh Y, Venkat P, Kim S. Systematic review of case reports on the abscopal effect. Current Problems in Cancer. Elsevier; 2016 Jan 1;40(1):25-37.

[140] Wu L, Wu MO, la Maza De L, Yun Z, Yu J, Zhao Y, et al. Targeting the inhibitory receptor CTLA-4 on T cells increased abscopal effects in murine mesothelioma model. Oncotarget. 2015 May 20;6(14):12468-80.

[141] Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nat Methods. Nature Publishing Group; 2015 May;12(5): 380-1.

[142] Mose LE, Selitsky SR, Bixby LM, Marron DL, Iglesia MD, Serody JS, et al. Assembly-based inference of B-cell receptor repertoires from short read RNA sequencing data with V'DJer. Bioinformatics. 2016 Dec 15;32(24):3729-34.

[143] Selitsky SR, Mose LE, Smith CC, Chai S, Hoadley KA, Dittmer DP, et al. Prognostic value of B cells in cutaneous melanoma. Genome Med. Genome Medicine; 2019 May 25;11(1):1-11.

[144] Victora GD, Nussenzweig MC. Germinal centers. Annu Rev Immunol. Annual Reviews; 2012;30(1):429-57.

[145] Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, et al. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Nat Med. 2004 Jul 11;10(8):871-5.

[146] Kwakkenbos MJ, van Helden PM, Beaumont T, Spits H. Stable long-term cultures of self-renewing B cells and their applications. Immunol Rev. 2016 Mar;270(1):65-77.

[147] Gilbert AE, Karagiannis P, Dodev T, Koers A, Lacy K, Josephs DH, et al. Monitoring the Systemic Human Memory B Cell Compartment of Melanoma Patients for Anti-Tumor IgG Antibodies. Lu S, editor. PLOS ONE [Internet]. Public Library of Science; 2011 Apr 29;6(4):e19330-15. Available from: http://dx.plos.org/10.1371/journal.pone.0019330

[148] Clargo AM, Hudson AR, Ndlovu W, Wootton RJ, Cremin LA, O'Dowd VL, et al. The rapid generation of recombinant functional monoclonal antibodies from individual, antigen-specific bone marrow-derived plasma cells isolated using a novel fluorescence-based method. MAbs. Taylor & Francis; 2014 Jan;6(1):143-59.

[149] Tickle S, Howells L, O'Dowd V, Starkie D, Whale K, Saunders M, et al. A fully automated primary screening system for the discovery of therapeutic antibodies directly from B cells. J Biomol Screen. SAGE PublicationsSage CA: Los Angeles, CA; 2015 Apr;20(4):492-7.

[150] Jin A, Ozawa T, Tajiri K, Obata T, Kondo S, Kinoshita K, et al. A rapid and

efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. Nat Med. Nature Publishing Group; 2009 Sep;15(9):1088-92.

[151] Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science. 2011 Aug 12;333(6044):850-6.

[152] Walker LM, Phogat SK, Chan-Hui P-Y, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009 Oct 9;326(5950):285-9.

[153] Dantas-Barbosa C, de Macedo Brigido M, Maranhao AQ. Antibody phage display libraries: contributions to oncology. Int J Mol Sci. Molecular Diversity Preservation International; 2012;13(5):5420-40.

[154] Alfaleh MA, Alsaab HO, Mahmoud AB, Alkayyal AA, Jones ML, Mahler SM, et al. Phage Display Derived Monoclonal Antibodies: From Bench to Bedside. Front Immunol. Frontiers; 2020;11:1986.

[155] Rothe A, Klimka A, Tur MK, Pfitzner T, Huhn M, Sasse S, et al. Construction of phage display libraries from reactive lymph nodes of breast carcinoma patients and selection for specifically binding human single chain Fv on cell lines. Int J Mol Med. Spandidos Publications; 2004 Oct;14(4):729-35.

[156] Rouet R, Jackson KJL, Langley DB, Christ D. Next-Generation Sequencing of Antibody Display Repertoires. Front Immunol. 2018 Feb 2;9:1315.

[157] An F, Drummond DC, Wilson S, Kirpotin DB, Nishimura SL, Broaddus VC, et al. Targeted drug delivery to mesothelioma cells using functionally selected internalizing human single-chain antibodies. Molecular Cancer Therapeutics. 2008 Mar 1;7(3):569-78.

[158] Lei X, Guan C-W, Song Y, Wang H. The multifaceted role of CD146/MCAM in the promotion of melanoma progression. Cancer Cell International. BioMed Central; 2015;15(1):3-11.

[159] de Kruijff I, Timmermans A, Bakker den M, Trapman-Jansen A, Foekens R, Meijer-Van Gelder M, et al. The Prevalence of CD146 Expression in Breast Cancer Subtypes and Its Relation to Outcome. Cancers. Multidisciplinary Digital Publishing Institute; 2018 May;10(5):134.

[160] Olajuyin AM, Olajuyin AK, Wang Z, Zhao X, Zhang X. CD146 T cells in lung cancer: its function, detection, and clinical implications as a biomarker and therapeutic target. Cancer Cell International. BioMed Central; 2019 Sep 25;:1-13.

[161] Bidlingmaier S, He J, Wang Y, An F, Feng J, Barbone D, et al. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. Cancer Res. American Association for Cancer Research; 2009 Feb 15;69(4):1570-7.

[162] Beije N, Kraan J, Bakker den MA, Maat APWM, van der Leest C, Cornelissen R, et al. Improved diagnosis and prognostication of patients with pleural malignant mesothelioma using biomarkers in pleural effusions and peripheral blood samples - a short report. Cell Oncol (Dordr). Springer Netherlands; 2017 Oct;40(5):511-9.

[163] Barbas CF, Kang AS, Lerner RA, Benkovic SJ. Assembly of combinatorial antibody libraries on phage surfaces: the

gene III site. Proc Natl Acad Sci USA. National Academy of Sciences; 1991 Sep 15;88(18):7978-82.

[164] Cai X, Garen A. Anti-melanoma antibodies from melanoma patients immunized with genetically modified autologous tumor cells: selection of specific antibodies from single-chain Fv fusion phage libraries. Proc Natl Acad Sci USA. 1995 Jul 3;92(14):6537-41.

[165] Wortzel RD, Urban JL, Philipps C, Fitch FW, Schreiber H. Independent immunodominant and immunorecessive tumor-specific antigens on a malignant tumor: antigenic dissection with cytolytic T cell clones. JI. 1983 May;130(5):2461-6.