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Review Article

Antibodies Against Melanoma Antigens - Clinical and Therapeutical Markers

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Abstract

Melanoma-Associated Antigens (MAA) are correlated with tumor development, progression and metastatic dissemination. MAA can be targeted in immunotherapy by specific antibodies or by cytotoxic T-cells. MAA are actually self-antigens and, thus, are weak immunogens because they induce various degrees of immune tolerance. Four families of MAA are involved in clinical monitoring and therapy efficacy, such as: melanocyte lineage/differentiation antigens, oncofetal/cancer-testis antigens, GAGE antigens and the extended family of cell-adhesion receptors. Antibodies against MAA are important players in the immune response generated in melanoma patients. These antibodies are found increased in melanoma patients and are proposed as biomarkers for diagnosis, prognosis and therapy monitoring, especially in the immune therapy domain. The anti-tumoral function of antibodies is determined by its isotype and subclass, hence IgG4 has an immune-suppressive action and its level is correlated with a poor prognosis in melanoma while IgG2 has anti-tumoral properties. There are still debates regarding the role of auto-antibodies in immune therapy, if their presence is a sign of therapy toxicity or a sign therapy efficacy. New therapies, like CAR T-cells, relying on melanoma antigens are described. In immune-therapy, autoantibodies associating severe immune related adverse effects were identified in melanoma patients, but their presence was connected with a good treatment response. In the immune-therapy domain, T-lymphocytes are the main focus, but another importanT-cell, slightly neglected in melanoma, B-cell and its antitumor functions can be important in developing the next generation of immuno-oncology therapies. Evaluating B-cells as both generators of antibodies and antigen presenting cells can widen the immune-based therapies in melanoma.

Keywords: Melanoma-Associated Antigens; Antibodies; B Lymphocyte; Biomarkers; Immune Therapy

Introduction

During oncogenesis, autologous normal cells suffer an array of events that drives them to cancer cells. These cells are characterized by different gene transcription and various deregulated protein expression profiles. Within tumorigenesis complex process genes suffer mutations, transcribe aberrant proteins with deregulated misfolding, have abnormal post-translational modifications or/and are overexpressed. All these changes and others alter the cell's protein abundance; can change their normal location, so that these proteins can become tumour-associated antigens inducing the immunogenic process. In the Tumour Microenvironment (TME), in correlation with the tumorigenesis process, several molecular deregulations can also lead to the initiation of immune responses henceforward to the production of tumour-associated autoantibodies. Thus, autoantibodies that appear associated to solid tumor development can be good immunological biomarkers of neoplasia mirroring the abnormal cellular mechanisms triggered by tumorigenesis. Autoantibodies that are generated within tumorigenesis can generate a specific signature of the immune response and within the clinical settings can become a good marker of early detection of cancer or its

recurrence. Therefore, in the last years, autoantibodies as diagnostic and prognostic biomarkers evaluated at the circulatory level have gained increased importance [1].

B-lymphocytes, the immune cells that are generating antibodies, have an important role in cutaneous melanoma and in the generated immune response. Recently reviewed, it was shown that tumour-infiltrating B-cells in melanoma have contradictory functions. There are studies that show that their presence is associated with the clinical outcome of the patients, while there are studies that do not find any association. These contradictory results reside in the different B-cell subsets that were studied and/or in the use of a single marker that is not differentiating multiple subsets. Within tumours, immune structures can be developed such as the Tertiary Lymphoid Structures (TLS) comprising organized T-cells and B-cells that are actively generating an onsite anti-tumour response. TLS presence was reported to be associated with improved response to immune checkpoint blockade and hence with melanoma patients' survival.

Antibodies that were investigated in melanoma patients can be directed against melanoma antigens and/or can be directed to other elements appending to TME. However, autoantibody production was found increased in melanoma patients and their involvement as biomarkers for diagnosis, prognosis and treatment/toxicity response was thoroughly studied in the recent years. The actual action of antibodies in the anti-tumoral response is a subject of intense studies. It was demonstrated in melanoma patients that the isotype and the subclass of antibodies govern their function. Thus, in melanoma while IgG4 has an immune-suppressive role, associating poor patient survival, high IgG2 levels correlate with good response to Immune Checkpoint Inhibitor therapy (ICI) [2].

Our review retrieved the last 10 years publications from PubMed and Scopus data bases, it included reviews and original papers, excluding the following: study cases, communication abstracts at conferences, non-English language published papers and papers focusing on veterinary melanomas. If in some paragraph we have included older papers is just to proove that the application of the mentioned study did not match a real clinical translations as in the case of ganglioside vaccination in melanomas. The key words that were used for selection where "melanoma antigens", "antibody", "melanoma peptides". Using the key words and available data bases between 1964 and 2025 over 5,100 papers could be retrieved, out of which just over 1,600 had all three key words associated. For the last 10 years publications we have obtained 345 papers focusing on the subject and after excluding as mentioned study cases, abstracts at conferences, non-English language papers and studies on veterinary melanomas we remained with the 126 core papers that were cited within our review.

In this review, melanoma-associated antigens, antibodies against them and antibodies against other additional elements from TME will be discussed. Recent data regarding the antibodies involvement in cutaneous melanoma will be discussed regarding their diagnostic, prognostic and therapy value in this highly immunogenic solid tumor.

Melanoma Antigens

Melanoma-Associated Antigens (MAA) are still subject of intense study as their identification and characterization can shed new light on several fundamental aspects such as tumor development, progression and metastatic dissemination, but also in clinical practice these antigens can aid diagnostic, prognostic and therapy monitoring. Moreover, MAA can be targets for immunotherapy through specific antibodies or cytotoxic T-cells. One very interesting point regarding MAA is that most tumor antigens are actually self-antigens and, thus, are weak immunogens because they induce various degrees of immune tolerance [3]. Another point is that, MAA are differently expressed on tumour cells hence their expression and further presentation of antigens is heterogeneous, leading to an unstable antigenic profile during tumor proliferation and dissemination [4].

Tumor MAA are very diverse, they have different biochemical composition, such as differences in proteins and/or in carbohydrates. Tumor MAA have differenT-cellular localization, they can be intracellular or localized on the tumor's cell membrane. Last, but not least they will differ in concordance with the tumor stage, primary or metastatic. MAA can have various roles; from diagnostic tools to immunotherapy efficacy markers [5], an outline of the main MAA along with some of their characteristics are presented in Table 1. The presented classification and the detailed description of MAA was comprehensively published by Pitcovski, et al. [3].

Antigen Class	Antigen Type	Cellular Function	Comments	Ref.
Differentiation antigens	gp100, Melan-A, tyrosinase	Involved in melanocyte differentiation and melanin production	Strongly expressed in both primary and metastatic melanomas; expressed consistent across melanoma stages	[5]
Cancer-Testis Antigens	MAGE-A1, MAGE A4, NY-ESO-1	Proteins are restricted to male germ cells, they become again expressed in tumor settings	High expression in metastatic melanoma compared to primary lesions; MAGE-A1 expression in ulcerated primary melanomas is an adverse prognostic factor	[5]
	BAGE, GAGE -1, -2,-3, -6	Expressed in the normal trophoblast of the ovary and occasionally in placenta, they become again expressed in tumor settings		
Tumor-specific antigens resulting from mutations	CDK4	Cell division cycle has various regulators, CDK (cyclin-dependent kinase) being one of these	Subtle mutations of normal cellular proteins, induce proteins expression in tumors and CDK4 pathway is a frequently altered signaling pathway in melanomas	[6]
	Beta-catenin	WNT/β-catenin pathway is crucial in embryonic development and adult tissue homeostasis, including cell migration, hematopoiesis and wound repair	β-catenin signaling has been associated to both tumour formation and progression in melanoma; an inverse relationship of active β-catenin signaling and T-cell infiltration was been reported	[7,8]
Serological analysis of tumor antigens by recombinant cDNA expression cloning (SEREX)	D-1	D-1 (37 kDa) protein identified in melanoma.	It was initially discovered using serologic-based cloning strategies that screened melanoma cDNA expression libraries	[9]
	SSX-2	SSX-2 is an immunodominant epitope recognized by CD4+ T-cells in melanoma.	Silencing SSX-2 in melanoma cells impairs tumor growth, migration and invasion, as well as inhibits the formation of distant metastases	[10]

Table 1: Tumor melanoma antigens classification.

Tumour specific antibodies from both patient serum and tumour microenvironment, can recognize a wide range of antigens, including self-antigens, abnormally modified proteins and normal molecules. Antibody responses directed against MAA were found in less than 2% melanoma patients and they are mainly directed to MAGE-A1, -A3 or SSX-2. Autoantibodies against NY-ESO-1, can be detected in 9% of the patients, while other groups reported that antibodies against GAGE family are found in 6% of melanoma patients [11,12].

Immunotherapy has to overcome the immune suppression of TME generated by a complex array of immune-suppressive immune cells and a myriad of immune-suppressive molecules like cytokines, chemokines, growth factors that sustain this pro-tumoral environment [13]. This immune suppressive milieu down-regulates the complex phenomenon of processing and presentation of peptides in association with the Major Histocompatibility Complex (MHC) [14].

There are several tactics to surpass the immune-suppression within TME in advanced melanoma. One approach is to use monoclonal antibodies against the negative regulators of T-cell activation, increasing the anti-tumor immunity. These antibodies can enhance the immune response against MAA [15]. Therefore, two of these Immune Checkpoint Inhibitors (ICI), anti-cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) (Ipilimumab) and anti-Programmed Death receptor-1 (PD-1) (pembrolizumab) were the first antibodies that were approved in the last decade [5]. Another approach to overcome immune-suppression is to use *ex-vivo* expanded tumor-infiltrating lymphocytes toward specific MAA as adoptive cell therapy [16]. Because preclinical and clinical studies were positive for this type of approach, FDA approved in 2024, Amtagvi, this adoptive therapy being actually the first one approved for solid tumors [17].

Another interesting approach that is based on MAA is the development of cancer vaccines including the mRNA-based vaccines. TCGA (The Cancer Genome Atlas) and GTEx (Genotype-Tissue Expression) data bases were used to retrieve gene expression information on several hundreds of clinical samples. Correlating over-expressed genes, mutated genes and Immune-Related Differentially Expressed Genes (IRDEGs) new tumor antigens were searched. This recent study identified five new tumor antigens: Protein tyrosine phosphatase, receptor type, C (PTPRC), Sialic Acid-Binding Immunoglobulin-Type Lectins (SIGLEC10), Caspase recruitment domain-containing protein 11 (CARD11), Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1), ADAM Like Decysin 1 (ADAMDEC1). All these identified tumor antigens were associated with good overall survival and disease-free survival of melanoma patients. In the framework of these antigens, Ping et al identified two immune subtypes of melanoma, one that is immunophenotypically “cold”, while the other was characterized as “hot". Cold tumors are characterized by a specific pattern of infiltrating immune cells: low B-cells memory, low CD8+ T-cells, natural killer cells (NK) in their resting stage, low CD4+T memory cells, all these cellular characteristics representing a low inflammatory microenvironment. Hot tumors have a high infiltration of immune cells harboring an intense inflammatory milieu [18]. Prior to Ping’s et al study it was shown that CARD11-BCL10- MALT1 signaling converts resting Tregs to effector Tregs and that inhibitory receptor LILRB1 could be another immune target as it is involved in the control of MHC class I - mediated phagocytosis of macrophages [19,20]. Antigen Presenting Cells (APCs) in general and macrophages in particular, have a seminal role in the antigen-specific immune response because naive T-cells interact with APCs for the T-cell activation process to be developed [21]. Therefore, there are also new melanoma antigens that can be of interest in the domain as cold tumors patients can beneficiate on specific vaccination [18].

In 2017 Sahin, et al., have defined the concept of individualized mutanome vaccines to be used in skin melanomas [22]. The team has shown that personalized mRNA vaccines can be developed, based on mutanome identification, new epitopes prediction and further selection. There are several on-going clinical trials focusing on mRNA vaccination in melanoma. mRNA cancer vaccine against twenty MAAs combined with Pembrolizumab were tested in patients with complete resection of the tumor, but with a high risk of recurrence [23]. Ipilimumab combined with mRNA encoding MAAs, cluster of differentiation 70, ligand of cluster of differentiation 40 and TLR4 (Toll-like receptor) were tested in two phase II clinical studies [24,25]. There are now phase 1 and 2 clinical trials on mRNA vaccination combined with already classical drugs in melanoma. Thus, mRNA encoding MAAs (NY-ESO-1, MAGE-A3, Tyrosinase and TPTE) is tested in combination with Celiplimab (PD-1 blocker) or other PD-1 inhibitors [26-28]. Additionally, the group of Sahin showed the response of inoperable melanoma patients treated with RNA-LPX encoding four MAAs in combination or not with PD1 blockers. The patients’ durable clinical responses were correlated with strong CD4+ and CD8+ T-cell response against specific MAAs and it was shown that the vaccine synergizes with anti-PD1 therapy in patients resistant to ICI monotherapy [29,30].

The main limitations of the clinical translation of mRNA cancer vaccines is the inefficient delivery of mRNA and the complex immune response that it can generate. Decreased bioavailability leads to minimal protein production and low therapeutic efficacy. To overcome the innefficient delivery of the mRNA several molecular designes were implied and a comprehensive descrisption of them was published by Neill, et al., in 2024 [31]. Exogenous mRNA is immunogenic and although it activates the immune system it also increases mRNA degradation and antigen expression [32]. Moreover, exogenous mRNA can generate

within the innate immune system, secretion of inflammatory cytokines that can lead to unwanted effects and it can decrease vaccination effect [33]. There are numerous clinical trials completed or on-going regarding testing mRNA vaccination in melanoma patients in combination or not with other immune therapies and additional limitations can be found in the paper of Van Hoecke, et al. [34].

There are 4 extended families of MAA (Table 1) that got attention in the last decade in terms of clinical monitoring and therapy efficacy, because their increased expression was significantly associated with an increased tumor infiltration of several immune cells like macrophages, DCs and B-lymphocytes. This increased infiltration signifies that antigens are presented to T-cells to initiate specific immune responses [35]. Cold and hot tumors, as described above, have different immune mechanisms and hence the therapeutic strategies should be different. In cold tumors, an activation of the immune elements should be done, while in hot tumors the inflammatory exacerbated process should be also triggered. Tumor cells in melanoma express by themselves an array of cytokines (e.g. IL-1, IL-6, IL-8, TNF-α, TGF-β) that are involved in tumor progression, therefore inflammatory elements within hot and cold tumors are to be considered [36].

Within TME, not only T-cells that comprise the majority of Tumor Infiltrating Lymphocyte (TIL) are important. As shown by different groups, including ours, actively contributing to TME and sustaining an intense crosstalk with tumor cells are several other immune cells like Langerhans Cells (LCs), Dendritic Cells (DCs), macrophages, masT-cells and NK cells [4,37,38]. Melanomas have a high diversity in LCs content, they can have high amounts of LCs or these cells can be absent. It is most probable that subtle molecular characteristics of LCs govern the cells' ability to populate the tumor. Recent studies proved that LCs capacity to infiltrate the tumor resides also in the expression of different TLRs [4,39]. LCs from melanoma patients' Sentinel Lymph Nodes (SLNs) have molecular characteristics that favor immune tolerance, like Indoleamine 2,3-Dioxygenase (IDO1) expression and altered antigen presentation machinery [40,41].

Antigens and Epitopes Recognized by Antibodies in Melanoma

Around 30% of B-cells harvested from melanoma patients can produce antibodies that recognize MAA [42]. Epitopes that are recognized by B-cells are in conformational sequences, rarely linear peptides, in contrast to the ones that T-cell recognize that are frequently linear peptides [43,44]. Protein arrays assays can screen the protein antigens that are recognizable by antibodies, but the technology cannot offer information regarding the epitope sequences [45]. Protein arrays can identify autoantibodies against MAA and/or against Post-Translationally Modified (PTM) proteins. In melanoma, the classic T-cell epitope (YMDGTMSQV) is a PTM structure that has an alteration in the glycosylation sequence induced by tyrosinase. These PTMs increase the number of targets for specific antibodies [46,47]. Epitope spreading is an important process in tumorigenesis because as the initial epitope is recognized by the adaptive immune cells, when the tumor evolves, multiple epitopes appear and this recognition can be expanded to other new epitopes [48]. T-cell epitope spreading in melanoma patients subjected to immunotherapy was shown several years ago [49]. In B-cell however, the event was only recently described in melanoma [50]. The APC role of B-cells infiltrating the tumor is extremely important in the epitope spreading event as during therapy tumor cells can display mutant or wild type antigens in various tumor development phases [50]. B-cell epitope spreading probably is a phenomenon that appears simultaneously with their APC function. B-cell Receptor (BCR) binds antigen from a dying tumor cell, processes it and presents its novel epitopes to T-cells, activating thus the adaptive immune response [51].

In tumor immunology, MAA have an important role of generating and sustaining the immune response. As described more than 10 years ago and recently studied tumors initiate a cyclic immunity process that, if immunity is strong enough, would amplify and broaden anti-tumoral T-Cell responses. Regulatory loops are developed in this cycle where inhibitory factors can halt or limit the development of immunity processes. This cycle is initiated by tumor antigens and is fulfilling several steps that take place in the lymphatic circulation, in blood and in the tissue that has generated the tumor. Fig. 1 describes the immunity cycle of a melanoma tumor. In patients there is a critical balance between T effector cells and the immune-suppressive T regulatory cells, this balance being of outmost importance for the clinical outcome of the patient. After being primed in the lymph nodes, the activated effector T-cells infiltrate the tumor and their T-Cell Receptor (TCR) interacts with MAA in MHC restriction, killing the tumor cell. There are several immune glitches in this cycle in a developing tumor, such as the stealthiness of tumor antigens, DCs and T-cells can be a-reactive and are unable to recognize tumor antigens as foreign, creating hence immune-suppressive T regulatory cells. Additionally, there is a lack of immunogenic co-stimulatory signals from pro-inflammatory cytokines and factors released by the dying tumor cells or by the gut microbiota. Moreover, T-cells' trafficking is hindered and the immune

cells do not infiltrate in effective numbers the tumor, and/or even if they are in high numbers they are switched off by the immune-suppressive events [52].

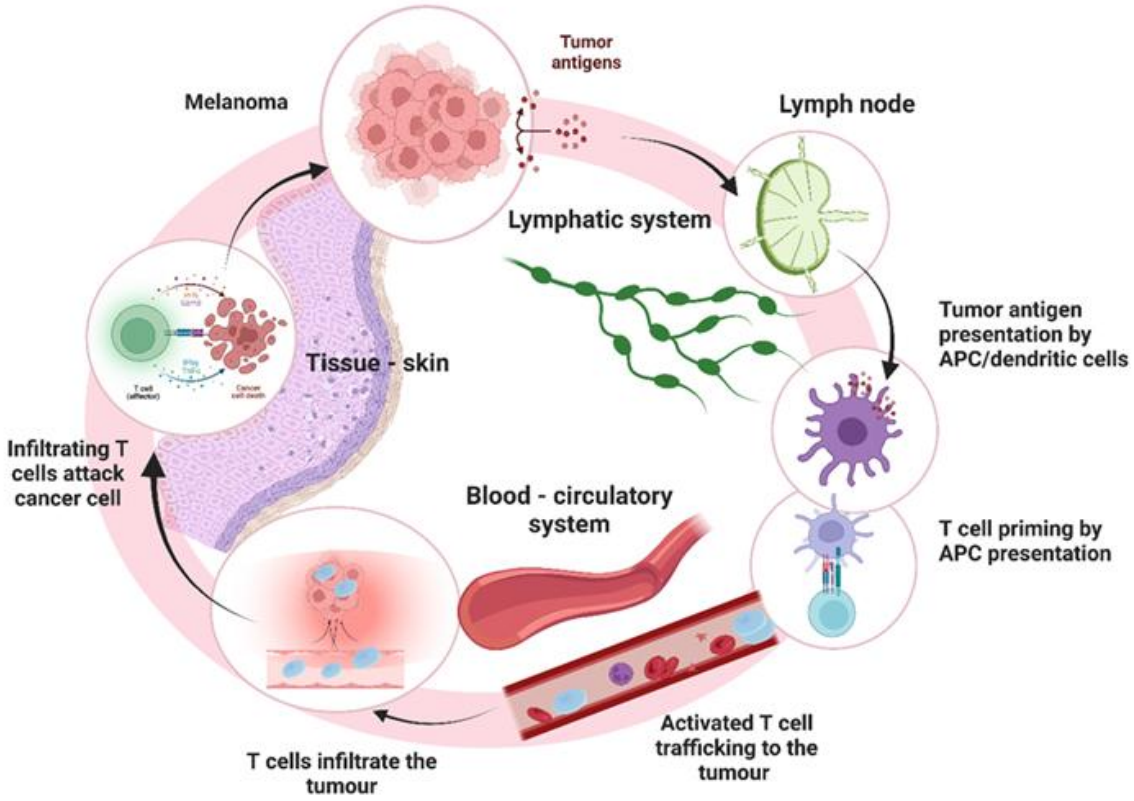

Figure 1: Tumor antigens cycle developing specific anti-tumor immunity. Tumor antigens are released early in the development of the complex process of tumorigenesis. Entering the lymph nodes tumor antigens are captured by dendritic cells where they are processed and presented to T-cells that are primed for these specific antigens. Primed T-cells circulate within the blood and extravasate in the tumor tissue becoming T-cell infiltrating cells. In the tissue, effector T-cell will recognize tumor cells and will kill them. Killing tumor cells will release tumor-associated antigens that will fuel the cycle again so that the immune response intensifies.

Mechanisms of Antigen Presenting Melanoma Antigens and B-lymphocytes Roles

Antigen presenting process is a seminal event in developing a proper immune response and pivotal in immune-oncology. APC will process and present to T-lymphocytes the peptides generating the activation of T-cells. Due to this first immune-generating response cellular contact (denominated as immune synapse). T-cells were the focus of immune therapies in melanoma [53,54]. In recent years B-lymphocytes, generators of antibodies, caught the researchers' interest regarding their involvement in the anti-tumoral events, their antigenic reactivities, the expression of certain isotypes and their overall role in tumor immune surveillance. As melanoma is an immunogenic tumor, these last years have shown that humoral immunity has a substantial role in the anti-tumoral complex processes.

B-lymphocytes in melanoma context can have two opposite actions, these cells can suppress tumor growth or they can promote it depending on TME characteristics, on the particular phenotypes and their antibody repertoires [55]. Studies have shown that B-lymphocytes immune responses are highly involved in the disease outcome [56] and the clinical response to immunotherapy [57].

Several groups, including ours, have shown that B-lymphocytes have different pattern in melanoma tumors. B-cells can border the tumor margin, can infiltrate the tumor or even can develop small cellular aggregates like TLS. These specific topographies advocate for B-cells involvement in the anti-tumoral immune response. B-cells are not just witnesses of the T-cell-mediated immune response, but they are actively involved in both tumor progression and in treatment outcomes [58-62]. B-lymphocytes

have anti-tumor action through their specific tumor-clearing antibodies, mostly IgG1. These antibodies can sustain the Antibody DependenT-cell-Mediated Cytotoxicity (ADCC), Antibody DependenT-cell-Mediated Phagocytosis (ADCP) and complement activation. B-cells have also the function of APCs, activating CD4+ and CD8+ T-cells and inducing cytokine secretion (e.g. IFNγ and IL-2 by T-cells). Moreover, B-cells can aid other APCs including macrophages and DCs. In TLS, the APC function of B-cells and their activation is enhanced, leading to several important processes like somatic hypermutation (SHM), clonal amplification and isotype switching to more active antibodies' isotypes like IgG1.

B-lymphocytes can have direct cytotoxic actions on tumor cells through granzyme B secretion. Additionally, B-cells can express inflammatory antibodies like IgG4 and IL-10 produced by regulatory B-cell (Breg). Bregs negatively modulate the function of CD8+ T-cells, induce regulatory T-cells (Tregs) that have immune suppressive function and hinder the APC activity of DCs and macrophages, hence the APC process [42]. Moreover, it was shown in melanoma that Bregs can produce IgA at TME level dampening the anti-tumoral processes [59].

Reviewed comprehensively in 2022 by Rodgers, et al., it was shown that tumour-infiltrating B-cells associate in melanoma with contradictory prognostic data. These contradictory results can reflect the fact that various studies use different B-cell subset classification, different markers for phenotyping and usage of single versus multiple markers. As shown in the introductory part TLS encompass T-cells and B-cells within the tumours to engender a local anti-tumour immune response. TLS presence prognosticates an improved survival and a good response to immune checkpoint therapies [2]. In spite of the fact that in various studies B-cell infiltrate are reported differently, there is a consensus for the fact that low B-cell infiltrates are found in stage IV melanoma compared with earlier stages [62]. B-cells that infiltrate the advanced stages melanomas display a suppressive phenotype (high IL-10 and TGFB1 expression), with high expression of B-cell exhaustion markers (PDCD1, FCRL4, SIGLEC6 and CD22) [59].

B-cells in their APC capacity induce antigen-specific T lymphocyte priming, by complex molecular interactions such as the recognition of antigens through their Ig - BCR and assignation of CD40, a co-stimulatory molecule expressed by helper T-cells (Th). This process induces B-cell maturation, the internalization of antigen, its processing and further presentation of the relevant peptide within MHC-restriction [63]. During this process, B-cells will secrete an array of cytokines, (e.g. Tumor Necrosis Factor-a (TNFa), IL-10, Lymphotoxin (LT), IL-2, IL-6, IL-4 and Interferon-γ (IFNγ)). This array of cytokines will tailor both the TME and the systemic immune response [64] and are actively involved in tumor regression [60]. When B-cells were in vitro activated using CD40 ligand and afterward activated with MAA, they robustly acted as APCs, processed efficiently the antigens and presented relevant peptides in MHC class II restriction to specific CD4+ T-cell clones, inducing T-lymphocytes specific for MAA [65]. More than 20 years ago it was shown that circulating B-lymphocytes are efficient APCs in vitro and that these cells can induce the proliferation of tumor antigen-specific memory and naive CD4+ and CD8+ T-cells [66]. In in vitro studies it was shown that B-cells can also directly kill murine 4TI breast cancer cell lines using the Fas/FasL-dependent process without IL-10 [67] but with IL-2 being present [68]. Therefore, B-cells are involved in seminal processes in the anti-tumoral complex processes from antigen presentation to tumor regression.

Within melanoma there are multiple B-cell subpopulations suggesting there are multiple roles played by B-cells in the immune response. There are two populations of B-cells that produce specific antibodies: plasmablasts, rapidly produced, short-lived cells involved in the early antibody response and plasma cells, long-lived cells supporting long lasting humoral immunity. In melanoma the presence of both plasmablasts and plasma cells indicate an active antibodies secretion [69].

Within the tumor there are different levels of maturity of the infiltrating B-cell subsets, with different affinity to antigen BCRs. There are still differences in reports on this matter probably due to differences in the identified markers [58]. CD19 or CD20 or Ig expression evaluation can lead to differences in interpretation of B-cell infiltrates in melanoma and hence their pro- or anti-tumourigenic role. Single-cell sequencing (scRNA-seq) technology was done for infiltrating T-cells in melanoma [70,71] but until 2020 no reports for B-cells in this type of skin cancer. Studies reported in 2020 relying on scRNA-seq for B-cell infiltrating the melanoma have shown that there are the following phenotypes: B-cells switched, activated IgD−, plasma cells, unswitched IgD+ and switched, activated IgD− [72]. In the same year another study has reported that activated, immature and memory B-cells are infiltrating the tumor and rarely, plasma cells were found in melanomas [59].

In melanoma, the double edge sword action of B-cells, is postulated by the finding that various B-cells subtypes can become immune suppressive Bregs, dependent on TME factors [73, 74]. Thus, some TIL that have plasmablast-like phenotype can concomitantly display Breg features [59]. The deregulation in the B-cell subsets is mirrored by the circulating antibodies in melanoma. Hence, high IgG4 levels evaluated in melanoma patients' serum correlate with worse prognosis, while high IgG2 levels correlate with good response to ICI [75]. The differences in the IgG classes are important in melanoma as IgG4 is associated with IL-10- Th2 cells in inflammation impairing anti-tumor action [76]. IgG2 antibodies are mainly antibodies against MAA like TRP1, TRP2 and gp100 or the cancer-testis antigen NY-ESO-1 [75].

Data gathers around the class switching process in the IgG family that is related to the clinical outcome of the patients. RNAseq transcriptomics has demonstrated that there is positive association between high Ig expression / clonality and a good clinical prognosis but RNAseq analysis cannot show the actual isotype [77]. Consequently, intratumoral B-cell IgG repertoires should evaluate both variable region of Igs and their matched isotype. Using long-read sequence methodologies and PCR to evaluate B-cell infiltrates and/or single cell sorted B-lymphocytes these detailed structures of Ig could be revealed.

In melanoma patients there were reported circulating antibodies that are tumor-specific but also other various auto-antibodies were reported [78, 79]. These antibodies are recognizing intracellular antigens and in a recent study published in 2023 cytometry by time-of flight (CyTOF) and several other transcriptomic methodologies were used to identify transcriptomic patterns of B-cell infiltration in tumors [79]. The study has reported a complex evaluation of memory and class-switched B-cells, B-cell phenotypes and their antibody repertoire that sustain the humoral immune response in melanoma [80]. The authors have shown in stage IV melanoma patients that at the circulatory level a decrease of memory B-cell population and an increase of plasmablasts that are secreting antibodies. In contrast, tumors are more frequently infiltrated with memory B-cells and IgD- memory B-cells as compared to cells circulating in blood [80]. Interestingly, the study showed this intra-tumoral increased IgD- memory/naive B-cell ratio compared to circulation, suggesting different processes of humoral responses in TME [80]. More recent studies have pin-pointed the existence of B-cells in small clusters in the TLS of melanomas and that in the germinal centre of these TLS there is a positive correlation between the number and size of antibody generating clones [80,81].

The antibody repertoires in patients' have distinct V, D, J variable region gene [82], some of these genes were preferentially associated with certain antibody isotypes, with specific function and affinity for MAAs [83]. This finding shows that there is a selection of IgD- memory B-cells that secrete a specific antibody at the tumor site, distinct from the one that circulates in the blood. Probably there is an in situ clonal expansion, toward IgG1, IgG2 and IgA1 generation. Melanoma skin metastases are characterized by a positive correlation of gene expression within B-cell, plasma cell and T-lymphocytes (both CD4+ and CD8+) showing that there is a B - T-cell crosstalk that induce the plasma cell differentiation [84]. It is possible that receptor rearrangement and affinity maturation occurs before migration of B-cells to the melanoma site [85].

Various studies, thoroughly reviewed by Chiarutinni, et al., have shown that there is complex regulation of the immune response mediated by diverse B-cell subpopulations, probably immunoregulatory B-cells as well [86]. B-cells within the tumour site can have tissue origin and/or can migrate to through blood vessels. In the tumor, B-cells can form Tumor-Associated Lymphoid Structures (TLS), a site where they can meet APCs and T-cells. This contact leads to affinity maturation and clonal amplification secreting immunoglobulins that can induce cytotoxicity or dampen the immune response. TME can induce B-cells toward differentiating in Bregs through IL-10 that will decrease immune response. Fig. 2 depicts the main mechanisms generated by B-cells within a melanoma.

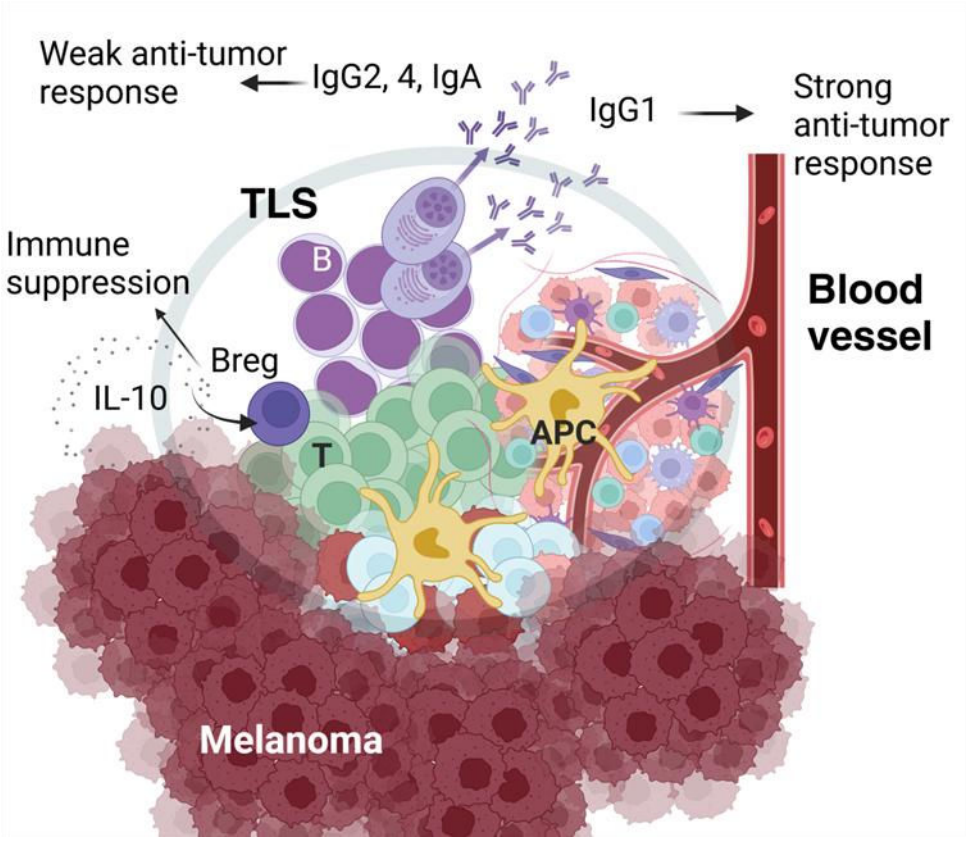

Figure 2: B-lymphocytes dynamics in the melanoma microenvironment. B-cells can have their origin in the skin and/or can arive through the circulation. B-cells establish contacts with T-cells and APC within the tumor-associated lymphoid structures (TLS). Upon immune synapse B-cells suffer affinity maturation and clonal expansion. Upon maturation in plasma cells they can synthetize and secrete tumor-specific IgG1 that sustain a robust immune response. If plama cells will be induce to secrete IgA, IgG2 and IgG4, the generated immune response will be weak. The immune suppressive tumor microenvironment can favour the secretion of soluble factors (IL-10) that can generate B regulatory cells (Breg) that negatively influences immune cell activation and antibody class switching.

Antibodies Against Melanoma Antigens as Clinical and Immune Therapy Markers

For the lesser familiriazed reader in immunology we have to define that there is clear difference between "passive immunotherapy" and "active specific immunotherapy". Thus, passive immunotherapy was recognized starting from the 19th century and it is representing the inoculation of specific antibodies that target pathogens or cancer cells. Active immunotherapy stimulates the host's immune system to respond to a disease or a pathogen [87]. Within our review the vaccination described in various setting is an active immunotherapy.

Antibodies against MAA are found increased in melanoma patients and were proposed as biomarkers for diagnosis, prognosis and as markers for good treatment response. As mentioned above, the anti-tumoral function of antibodies is determined by its isotype and subclass; hence IgG4 has an immune-suppressive action correlating with a poor prognosis in melanoma [2]. Przybyla et al studied the CD8+ cell panel in the peripheral blood mononuclear cells (PBMC) harvested from healthy donors and subjected to MAAs activation. In presence of a peptide pool of several MAAs peptides (Tyr, MAGE-A3, Melan-A/MART-1, gp100, NY-ESO-1) in a quarter of healthy individuals Tyr triggered CD8+ T-cells secreting IFN-γ, while MAGE-A3, Melan-A/MART-1 and gp100 in 10%, 7.5% and 2.5%, respectively. After 3 days of stimulation with the peptides CD8+ T-cells produced granzyme B (GzB) in over 50% of the healthy cells. This report suggests that there is endogenous T-cell immunity against melanoma antigens and this phenomenon was termed by the authors "natural T-cell autoreactivity" [88]. Data gather pleading for an immunity panel, whether immune cells or antibodies, already prone to MAAs activation. The assertion does not contradict any immunology "dogmas" as the melanoma tumor has its origin in a neoplastic transformed self-melanocyte.

Antibodies as Markers for Melanoma Clinical Outcome

Circulatory validated markers in melanoma did not evolve in the last 20 years too much. The AJCC 8th edition shows that the circulating Lactate Dehydrogenase (LDH) levels is the only serum marker validated for melanoma staging and it continued in the last 2021 AJCC edition [89,90]. Therefore, we still do not have a validated set of circulatory markers that can aid staging in melanoma. Elevated LDH correlates with a poor prognosis at diagnosis and its level can monitor response to treatment [91]. Within the circulatory panel of markers, autoantibodies have a good potential to indicate prognosis because they appear early in disease onset and are stable in circulation [91].

Studies have shown that antibodies can be good predictors of the disease outcome. Decreasing antibody responses correlates with advanced stages of melanoma in comparison to the early stages [38]. Probably due to the longer exposure to the tumor, some tolerogenic mechanisms come in action in the advanced stages and hence decrease the antibody production. However, there are still contradictory conclusions on whether circulating antibodies against MAA correlate with good clinical outcome or not [79]. As previously stated IgG4 presence correlates with disease progression, this being an inhibitory IgG subclass [76].

A recent complex study that pursued proteome-wide autoantibody screening and further quantification with protein arrays using proteome-wide human cDNA library (HuPEX) has obtained interesting results when studying the sera harvested from melanoma patients. Thus, the study identified not less than 488 autoantibodies in melanoma patients. Patients that were diagnosed with metastases had an increased level of autoantibodies compared to patients diagnosed with primary tumors. This investigation shows that the "autoantibody landscape" in melanoma, like in many other cancers, can provide new clinical biomarkers [92]. Various auto-antibodies were studied in melanoma patients. For example, antibodies against denaturated collagen were found significantly lower in melanoma patients compared to controls and this finding suggests that auto-antibodies against denaturated type III collagen can be involved also in tumor control [93].

Another group of antibodies that are aberrantly expressed in various cancers, are the antibodies against Cancer-Testis antigens (CT) and these antibodies can become disease markers. Melanoma patients subjected to NY-ESO-1-based cancer vaccine that antibodies against CT can have different epitope usage [94].

Neuronal auto-antibodies were studied in melanoma patients in association with neurological and cognitive dysfunction. These autoantibodies were found in over 22% of melanoma patients, most frequently found were IgA and IgM anti-NMDAR (anti-N-methyl-D-aspartate receptor) antibodies. Antibody-positivity was associated with a significantly impaired cognitive performance, impairments in tests of memory, attention and execution. Neuronal auto-antibodies can be possible biomarkers in melanoma for the prediction of cancer-related cognitive impairment [95].

Melanoma-Associated Retinopathy (MAR) appears in the metastatic stages of melanoma. MAR is actually an auto-immune disorder characterized by autoantibodies directed against retinal proteins, (e.g. acting on cation channel TRPM1 from melanocytes and ON-bipolar cells). In a recent study it was shown that anti-TRPM1 autoantibodies isolated from different melanoma patients have different affinities and concentrations [96]. In 2021 it was reported the first study case of a patient that had resolution of visual symptoms and normalization of electroretinogram of MAR associated with the reduction of specific auto-antibodies after administration of PD-1 inhibitor (pembrolizumab). This report highlights once more the importance of auto-antibody monitoring in immunotherapy [97].

Autoantibodies against the inner ear structures were found also in melanoma patients that proved to have also antiretinal antibodies. Therefore auto-antibodies in melanoma can appear in various organs and can be correlated with the overall clinical prognosis of the patient [98].

Anti-ganglioside antibodies are another type of antibodies, recently studied in melanoma patients as markers of chronic inflammation. In advanced stages, the level of antiganglioside antibodies was found lower compared to earlier stages and were not detected in the control group. Authors argue that tumour ganglioside antigens can trigger an immune response reflected in the generation of auto-antibodies and this event is clear in early stages, while in later stages the tolerogenic mechanisms can lower this generation. Anti-ganglioside antibodies were found associated with other inflammation markers (e.g. IL-8, C reactive protein) sustaining the inflammatory status of the investigated patients [99].

A complex auto-antibody signature was identified in melanoma patients. Primary melanoma patients were tested with a high-throughput microarray platform expressing MAAs. 10 autoantibody that can serve as biomarkers, displaying a sensitivity of 79% and a specificity of 84% could detect primary melanoma [100]. Although it looks hardly acceptable for an unauthorized eye 79% sensitivity and 84% specificity, there are methylation markers in oncology that display the same sensitivity and specificity [101]. A prior clinical study regarding the monitoring of adjuvant ganglioside GM2-KLH/QS-21 vaccination (EORTC18961 trial) has shown that the auto-antibodies levels in melanoma patients could monitor the vaccination efficacy. Almost 1,000 patients were enrolled in this clinical trial and a prognostic serum antibody response against MAAs were reported for stage II melanoma patients. Moreover, the presence of antibodies was correlated with a good clinical outcome after this vaccination [102]. Earlier studies (1999) have shown that IgM is the antibody class that is generated upon this type of vaccination, but then in 2018 a published study proved that a set of antibody responses are correlated with a beneficial outcome for GM2-KLH/QS-21 vaccination [102,103]. However, the GM2-KLH/QS-21 vaccination is still not translated in clinical application.

Autoantibodies can be surrogate markers for melanoma-specific T lymphocytes, hence post-surgery of the primary tumour, T-cells were found are directed against MAAs and their associated humoral responses elevated. Hence, T-lymphocytes were found directed against Melan-A/MART-1, Tyrosinase, NA17-A and p53 [104]. Recently, it was shown that after immune-therapy T-cell clones against melanoma neoantigens, are frequently found in circulation corroborated with specific antibodies. This finding correlated with an intense immune cell infiltration within the tumor [105].

Our study developed on PEPperCHIP® Melanoma Antigen Microarrays analyzed auto-antibodies against MAA in untreated melanoma patients. The array is printed with sequences spanning the structure of 21 MAA. The printed peptide sequences spotted on the array have a length of 15 aminoacid peptides with peptide-peptide overlap of 13 aminoacids, so that there are 4,125 peptides printed in duplicate to identifying the antibodies against specific MAA sequences. In Fig. 3 we present as example of the peptide array after analyzing serum harvested from a stage IV melanoma with no treatment. It can be observed that there are specific antibodies that are synthetized and secreted by B-cell clones, in our case the highest circulating concentrations are of antibodies against specific sequences from MART and MAGE antigens.

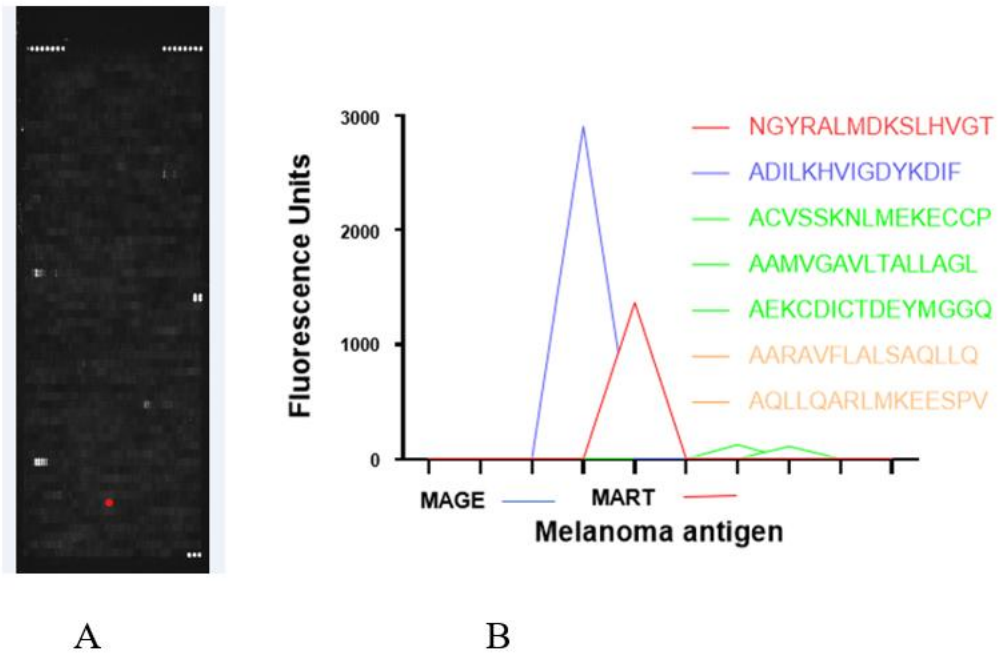

Figure 3: Melanoma associated antigens antibodies analyzed with peptide arrays. A. General view of the peptide array showing the clear antibody detection for specific peptides comprised in the MAA antigens (laser 535nm). B. Florescence units (after background subtraction) for the main imuno-dominant peptides (sequence NGYRALMDKSLHVGT) from MART-1 antigen and (sequence ADILKHVIGDYKDIF) from MAGE antigens in comparison to lower antibodies concentrations for tyrosinase (sequences ACVSSKNLMEKECCP, AAMVGAVLTALLAGL, AEKCDICTDEYMGGQ) and BAGE (sequences AARAVFLALSAQLLQ, AQLLQARLMKEESPV).

Antibodies Induced by Immune-Therapy

The majority of recent studies focus on antibodies induced in melanoma patients subjected to immune therapy. With the development of ICI, autoantibodies were analyzed as markers for efficacy. Hence increased levels of autoantibodies against melanoma antigens: gp100, MelanA/MART1, TRP1/TYRP1 and TRP2/TYPR2 were found correlated with antibodies against antigen NY-ESO-1 [79]. Thus, the presence of these antibodies can be good indicators for therapy efficacy. The study has shown that auto-antibodies against NY-ESO-1 and TRP1 are IgG1 and IgG2, while the antibodies against TRP2 are IgG2 and their presence is associated with good clinical outcome after ICI, while no significance was registered when the sub-classes IgG3 and IgG4 were identified [79]. In another study, a correlation between pre-treatment IgG2, the response to therapy and the prolonged overall survival was reported in ICI melanoma patients. Therefore, IgG sub-classes can be good predictors of clinical success of immune therapy in metastatic melanoma patients [75].

Immune-therapy adverse effects were also studied in relation to auto-antibodies levels in patients receiving PD-1 alone or combined anti-PD-1/anti-CTLA-4. This recent study showed that neurologic immune-related Adverse Events (irAE-n) were associated with higher percentage of circulating CD8+ Effector Memory cells (EM-)1 and Central Memory (CM) T-cells when compared to patients without irAEs. A marked decrease in the B-lymphocytes displaying IgD-CD11c+CD21low and IgD-CD24+CD21 high phenotypes were found in melanoma patients displaying positivity for autoantibody related to irAE-n [106]. In 2023, Müller-Jensen, et al., have investigated irAE-n upon ICI by analyzing the neuronal autoantibody profiles. In a small cohort of patients' serum samples a large panel of neuromuscular/brain autoantibodies was analyzed. From this large panel of autoantibodies, the identification of neuromuscular auto-antibodies, anti-titin, anti-skeletal muscle, anti-heart muscle, anti-LRP4, anti-RyR and anti-AchR, could detect with high sensitivity and specificity the initiation of myositis, myocarditis or myasthenia gravis upon ICI treatment. Thus, this recent study shows the neuromuscular autoantibodies as prediction biomarkers for irAE-n in ICI-treated patients [107].

There are still debates regarding if the presence of auto-antibodies is a sign of immune-therapy toxicity or a sign of therapy efficacy. Using the HuProt Human Proteome Microarray the autoantibodies pattern was studied in almost 1,000 patients. The authors point out that there are still debates regarding if autoantibodies are just molecular bystanders within immune-therapy or they actually induce systemic or organ specific irAE. Autoantibodies associating severe irAE were identified as immune-related pathway antigens [108].

Inflammatory arthritis is another irAE upon ICI-treatment (irAE-IA) in melanoma patients. Gatto, et al., have reported that patients with irAE-IA have higher circulating percentages of CD19+ B-cells as compared to those that did not experience this type of irAE. The same finding was reported for transitional CD19+CD10+CD24high CD38high B-cells and this population increased before the irAE-IA and decreased in the quiescent stages. Autoantibodies, like rheumatoid factor, anti-citrullinated protein antibodies and antibodies against joint-related proteins were also found elevated in the tested group [109].

ICI - patients were monitored for several parameters as response to 2 months of treatment and besides β-2 microglobulin (B2-MG), neopterin (NPT), IL-6, IL-18, HLA-DRB1, autoantibodies were evaluated. In melanoma patients increased level of B2-MG, NPT, IL-6 and IL-18 were associated with early disease progression. The appearance of irAE was an indicative of good clinical outcome and prolonged PFS. Several auto-antibodies were analyzed: antibodies to extractable nuclear antigens, anticardiolipin antibodies, anti-MCV antibodies, anti-thyroid peroxidase autoantibodies (anti-TPO), antibodies to thyroid stimulating hormone receptor, antibodies to β-2-glycoprotein, anti-neutrophil cytoplasmic antibodies IgG, antinuclear antibodies, anti-mitochondrial antibody, anti-liver kidney microsomal antibody and anti-smooth muscle antibody. A higher level of B2-MG was found as predictor of shorter PFS in melanoma. From the complex panel of studied antibodies in melanoma, anti-TPO antibodies were detected in patients developing autoimmune thyroiditis. Antibodies to extractable nuclear antigens were associated to irAE hepatitis but not to other irAE like rash, pneumonitis and colitis. No other significant association between the antibodies and clinical parameters of patients with melanoma was reported in this study [110]. Earlier studies have shown in ipilimumab-treated melanoma patients that irAE development is linked to the occurrence of auto-antibodies. Autoantibodies were identified in almost 20% of patients and these individuals with antithyroid antibodies had significantly more thyroid dysfunction upon therapy. Similar to the study presented above, patients with autoantibodies had better survival and an improved therapy response [111]. ICI that triggers irAE hypophysitis is associated with high levels of anti-GNAL and anti-ITM2B autoantibodies, while irAE pneumonitis associates anti-CD74 autoantibody presence [112].

There are also studies that show no association between auto-antibodies presence and immune therapy. Immune therapy involving autologous peptide-loaded DC vaccination was studied in stage IV melanoma patients. The authors show that there was no correlation between autoantibody presence after vaccination and patients' survival, in contrast DCs specific phenotype did correlate with patient's survival [113]. In almost 1,000 melanoma patients subjected to nivolumab, ipilimumab or ipilimumab combined with nivolumab the analyzed autoantibodies levels were studied in correlation with clinical outcome and the presence of irAE. The study showed that baseline serum auto-antibodies pattern can predicted recurrence and severe toxicity to therapy [114].

Therefore, as numerous studies already stated, evaluating serological response via antibodies profile represent both a traditional and a novel challenge in melanoma immuno-oncology that provide further insights into mechanisms of disease and /or resistance to immunotherapies (e.g. ICI). Robust efforts are in progress aimed to novel biomarkers discovery, combined or optimized panels of biomarkers that will further aid prognosis and therapeutic decision-making process. Currently, costs related to the potential biomarkers transition toward clinic are envisaged in relation to methods standardization especially in ICI drug quantification and efficacy monitoring. Moreover, one should encompass costs related to sampling, sample processing and data analyses that are further associated with multi-omics profiling costs. Thus, integrating sampling and analysis costs is part of a further enlarged exploration joining clinical (e.g., imaging) and preclinical attempts (e.g., molecular, genetic etc.) in melanoma approaching [115]. Certain limitations must be addressed to facilitate the introduction of such tests into the clinic. Although the results obtained are statistically significant, the number of patients tested must be increased so that extensive multivariable analyses can be performed. Second, it is necessary to introduce a validation cohort that would test the hypothesis of the prospective study and support its applicability. Additionally, it must be taken into account that in patients diagnozed with uveal and mucosal melanoma, immunotherapy is less effective and lower response rates influence the parameters of the study [75].

CAR-T-cell Therapy Relying on Melanoma Antigens

The design of CAR-T-cells opened a new door in the therapeutical approaches in hematological malignancies at first and then, it was driven also towards solid tumors. A major breakthrough was obtained when experimental data showed that TIL-induced tumor rejection when they are directed to MAA. T-cell reactivity was identified as being against MART-1, tyrosinase and gp100. But when this therapy was tested in patients this CAR-T-cells induced high toxicities [116]. The discovery of neoantigens generated by tumor-specific mutations, pin-pointed that the targets in clinically setting is much more complex [117]. Moreover, targeted therapy and check-point inhibitors in melanoma somewhat shadowed the translation of CAR-T therapy into clinic, a therapy that in comparison to the others is more difficult to achieve [118, 119]. But then again, as resistance to immune therapy was clinically observed in patients, additional therapies were searched. Data published beginning with 2018 - 2019 showed that as CAR-T-cell therapy was successful in hematological malignancies, engineered cells toward specific melanoma antigens could hold a new therapeutical promise [120-123]. In 2021 the results obtained with CAR-T-cell were already promising although one of the hurdles was the emergence of resistance due to antigen loss [124]. Therefore, in order to eradicate melanomas containing antigen escape variants, melanoma-specific CD4+ T-cell combined with OX40 co-stimulation or CTLA-4 blockade displayed positive results published in 2023 [125]. In the same year the intracellular proteome was studied as a source for neo-antigens targets. Actually, these intracellular molecules are peptides presented in MHC class I restriction by melanoma cells. Against MHC-peptides complexes, new therapeutic agents can be developed, such as TCR-like antibodies, TCR engineered T-cells and CAR-T-cells. In this light, anti-gp100/HLA-A2 TCR-based T-cell proved clinical efficacy and hence was approved by the FDA for metastatic uveal melanoma [126]. Results published in 2021 regarding engineered CAR-T-cells targeting specific high-molecular-weight melanoma-associated antigen (HMW-MAA) have shown efficient killing of melanoma cells expressing intensively HMW-MAA, but the ones with low expression were not damaged. The study underlines that differences in molecular mechanisms can strongly influence the efficacy of therapy in clinical settings [127]. In 2023 a CAR- T-cell targeting Chondroitin Sulfate Proteoglycan 4 (CSPG4), combined with a Chimeric Co-Stimulatory Receptor (CCR) was designed. This CAR-T-cell displayed good anti-tumor response both *in-vitro* and *in-vivo* [128].

Fast forward in the story of CAR-T-cell therapy in skin melanoma and in 2024, Amtagvi (Lifileucel) was FDA-approved as being the first CAR-T-cell therapy in solid tumors, namely in metastatic skin melanoma [17,129,130].

Nowadays the search is to optimize CAR-T-cells in melanoma designing them towards specific antigens. Thus, TYRP1 antigen was used to design a specific highly sensitive CAR-T-cell for cutaneous and rare melanoma subtypes resistant to immune

checkpoint blockade. Data regarding the efficacy and safety profile can further lead to the initiation of phase I clinical trial [131,132]. CAR-T-cell targeting the intracellular oncoprotein PRAME (Preferentially Expressed Antigen in Melanoma) is another very recent approach. Thus, a CAR T-cell expressing six single-chain variable fragments recognizing the PRAMEp301/HLA-A∗24:02 complex was reported with positive experimental results [133]. Investigating patients subjected to this therapy it was shown that responsive patients had baseline tumors augmented in tumor-reactive TILs and after therapy, effective cells preferentially infiltrated tumors post-therapy, suggesting a functional reinvigoration. The study highlights the dynamics of tumor-specific clonotypes accompanying a good clinical response to this therapy [134]. New players are entering the chimeric antigen receptors area, specifically CAR natural killer cells with their fine-tuning capacity of cytotoxic activity [135] or CAR macrophages that would rewire tumor-associated macrophages [136].

Limitations of the CAR-T-cell Therapy

There are still various limitations of the approved CAR-T therapies like major side-effects, low anti-tumor activity, tumors antigen escape, restricted trafficking of the cells and last, but not least limited tumor infiltration [137]. Because they can trigger side-effects like cytokine-release syndrome, immune neurotoxicity syndrome, hypo-gammaglobulinaemia and cytopenias alleviating the side-effects and implementing new stages of manufacturing the CAR-T-cells can expand their clinical implementation beyond the haematological malignancies [138,139]. Another limitation is the tumors antigen escape phenomenon. In solid tumors CAR-T-cell therapy decreases the tumor antigen expression, for example targeting with CAR-T-cell therapy IL13Ra2 in glioblastoma tumor recurrences had a decreased IL13Ra2 expression [140]. Trying to overcome this antigen escape, a recent study has shown the good pre-clinical results of CAR-T-cell therapy aiming at a melanoma antigen TYRP1 that is more specific to melanosomes [141]. The heterogeneity of TAA is another major limitation in solid tumors [142] in both intensity and distribution. Overcoming this limitation can be done by using dual-antigen CAR T-cells to simultaneously target multiple TAA and there are clinical studies on-going on dual targeting with CAR-T-cells in leukaemias and various solid tumors but not in melanomas. Another important limitation in solid tumors is the reduced capacity of CAR-T-cells to traffic and to infiltrate solid tumors. This is due one one hand due to the immuno suppressive TME and to the physical tumor stroma barriers on the other. Increasing penetrability into the tumor can be done by direct intra-tumoral inoculation, that will also limit the by-standers unwanted toxicities. TME interactions can alter CAR-T-cells functions. The high immunesuppressive TME leads to poor T-cell expansion and short-term T-cell persistence of this therapy. Therefore, a combination of CAR-T therapy and ICI would provide a sustained T-cell persistence and functionality [143]. Regarding the economic part of this therapy, manufacturing processes, high standardization and technological conundrums that still need to be solved hinder a large scale clinical application of CAR-T-cells [118,139]. Therapies developed using CAR-T-cells in solid tumors still need a complex workforce to overcome limitations and implement these treatments.

B-lymphocytes as Antibodies Producing Cells in Melanoma Therapy

There are various studies that are focusing on B-cells as therapy agents in melanoma as antibodies producing cells. There are specific B-cell subsets that are subjected to complex processes like differentiation, migration, antibody expression, maturation, class switching and secretion. As tumor cells, more specifically melanoma cells, can escape host's immune responses, B-lymphocytes are not totally efficient to neutralize tumor antigens [144].

As prior stated, antibody isotypes (e.g. IgG4 and IgA) can support immune evasion. Probably in the melanoma context, IgG4 restricts Fc-mediated functions at the tumor site. Cells that infiltrate melanoma tumors express a panel of Fc receptors, thus these are seminal for an effective anti-tumoral action. Therapeutic antibodies are designed to induce their effect through Fabs, so that the T-cell inhibitory signals were removed [145]. New approaches like engineered antibodies that are insensitive to immunosuppressive TME, antibodies that display high affinity to activatory Fc receptors can induce the stimulation of anti-tumoral effector cells [146]. In the light of using antibodies as therapy drugs, the logical therapeutical endeavour was to use B-lymphocytes. First of all, B-cells that have immune-suppressive effect in melanoma can be therapy targets, for example Bregs or IL-10-producing B-cells population should be dampened. Tumor antigen-specific mature memory B-cells that have vigorous anti-tumoral action should be activated, for example in adoptive therapy using particular B-cell subsets. Recently, subsets of B-cells expressing PD-L1 were shown to be elevated in melanoma patients and were found associated with tumor stage and bone metastasis [147]. Targeting this type of B-cells can be another strategy to dampen antitumoral functions. Hence, to better understand the B-cells role in cancer with focus on cutaneous melanoma, both their immunophenotype and functional status must be approached; these efforts would allow a better depiction of infiltrating B-cells and discriminate between resident and

transient subsets of B-cells. For instance, as mentioned previously, the fact that some B-cell subsets express immune checkpoint molecules, such as PD-L1, raises the hypothesis to involve specific checkpoint inhibitors as a strategy to revive the antitumor functions of B-lymphocytes. Moreover, targeting certain immunosuppressive cytokines appending to TME e.g., such as IL-10, VEGF or TGF-ß could re-boost B-cells for tumor rejection by allowing antibody class switching to antitumor isotypes (e.g., IgG1) [86]. The density of B-cells in TLS was found increased in immune checkpoint therapy responders versus non-responders and is correlated with improved survival, conferring B-cells from these areas a biomarker power for measuring the therapy efficacy. Other key point to be addressed in deciphering B-cell roles tackles the manner of interaction with other immune cells in the dermal network. Moreover in order to emerge B-cell targeted therapies, secretory functions of antibodies and cytokines must be addressed as these features still remains largely unexplored in B-cell landscape [42,148].

There is an array of immune molecules with immunosuppressive functions (e.g. IL-10, VEGF, TGF-ß and so on) that comprise TME and all these molecules contribute to the immune-suppressive action upon B-cells [134,135]. If these molecules are inactivated, B-cells are allowed to undergo class switching to activatory antibody isotypes, namely IgG1.

Despite continuous inquires in tumor immunology, the precise role B-cells in antitumor immunity is still to be defined as different T-cell subsets collate various functions, like regulatory B-cells and B-cells population from TLS [42]. With the last years focusing on T-cells in melanoma, B-cells should gain their position as well in the anti-tumoral processes. Through T - B combined actions, stimulation of tumor-neutralizing T-cells can induce an enhanced antibody responses against cancer antigens [54]. Furthermore, a concentrated action were T and B-cells have a conjoint action could improve the already approved immune-therapy efficacy or even to overcome the resistance to immune therapy [149].

Conclusion

The research in the domain of antibodies against melanoma antigens is effervescent. An important issue in this domain is the longitudinal stability of antibody repertoires in a tumor that is changing its molecular pattern during various phases of progression or even more during immune therapies. Antibodies make an important class of biomarkers whether for clinical evaluation or for therapy monitoring, especially in the immune therapy context. There are specific antibody signatures that can prognosticate melanoma recurrence and/or the development of toxicity during immunotherapy. Studies still go on to establish if the presence of this antibodies are or not directly involved in the pathophysiology of immune therapy toxicity. However, it should be underlined that auto-antibodies are biomolecules typically associated with an autoimmune condition where auto-antibodies expression become useful tools for diagnostic purposes. However, in many types of cancer recent data assigns auto-antibodies term to the corresponding auto-antigens that are specific molecules for cancer cells and not produced by normal ones. Although this assertion signifies a new facet of the autoantibody concepts, these tumor autoantibodies are still in their infancy regarding their role, in either inhibiting or favoring tumor development. Many unsolved issues related to tumor autoantibodies are still to be explored by the researchers in terms of autoantibodies specificity, their structural and epitope identity as well as their prospective role for early detection of cancer, tumor staging, therapy monitoring and so on. In melanoma, a type of cancer recognized for its high mutational burden and therefore a high amount of corresponding neoantigens such auto-antibodies could be endorsed as important biomarkers for early detection and therapy efficacy monitoring. In addition, autoantibodies may also represent a by-product of the tumor's inflammatory milieu, but their role in cancer management appears to be much more complex than one might expect.

As T-lymphocytes have come into the spotlight due to last year’s immune therapies development in melanoma, another importanT-cell, slightly neglected in this skin cancer, B-cell and its antitumor functions can be important in developing the next generation of immuno-oncology therapies. Evaluating B-cells as players that are both generators of antibodies and antigen presenting cells can widen the immune-based therapies in melanoma.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Conceptualization, M.N.; writing-original draft preparation, E-G.D.; S.Z.; writing-review and editing, M.N.; C.C.; E G.D.; S.Z. All authors have read and agreed to the published version of the manuscript.

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