Chapter

Novel Therapies in Clinical Development for Advanced Disease

Álvaro Sánchez Arráez, Sonia Maciá and Eduardo Castañón

Abstract

Recent advances in melanoma treatment have supposed a dramatic transformation overcoming the situation that was faced 15 years ago, when advanced melanoma was a fatal disease, with less than five percent of patients being alive after 1 year of diagnosis. However, in spite of the impressive improvement that has been achieved with immunotherapies and targeted therapies that are completely part of the standard landscape for treatment, additional therapeutic advances are still needed. In this chapter, we review those systemic and local treatments which are undergoing clinical development, explaining their mechanisms of action and the already presented either preliminary or final results, most of them in terms of response rate.

Keywords: immunotherapy, targeted therapy, intratumoral, citokines, oncolytic virus, Pattern recognition receptor, new therapeutic targets

1. Introduction

The treatment of metastatic melanoma has evolved dramatically in the past recent years, provoking an important paradigm shift [1], with huge progress in melanoma survival. The development of targeted therapies such as BRAF and MEK 2–6 inhibitors [2–6], as well as the appearance of different molecules targeting program death 1 (PD1) [7, 8] and anti-cytotoxic T-lymphocyte associated antigen (CTLA4) [9–11], has contributed to improving the prognosis of metastatic melanoma, turning melanoma to one of the most responsive tumors to these kinds of therapies.

However, there is still a high percentage of patients who do not respond to first-line immunotherapy. Besides, those patients with *B-RAF* mutant disease who develop progression after both targeted therapy and immunotherapy (regardless of the order of use) face a poor prognosis. Hence, in these two groups of patients, being both considered as patients developing progression to immunotherapy, the disease is still considered an important medical need. Hence, the development of new potential therapies is key, and extensive clinical research is ongoing to develop new treatments which may improve prognosis in all patients.

2. Citokines

a. **Interleukin-2 (IL-2)**

Interleukin 2 is a cytokine that promotes the growth and expansion of T lymphocytes and NK cells [12]. Its antitumor activity has been tested in patients with renal carcinoma and patients with melanoma [13]. However, its toxicity profile (hypotension, capillary leak syndrome...) prevents it from being a standard of care. To try to reduce the toxicity associated with IL2, different strategies have been designed. One of the most developed molecules is Bempegaldesleukin (BEMPEG) [14]. BEMPEG is a pegylated molecule, thereby reducing systemic IL2 exposure. In addition, it has a higher affinity for the IL2 receptor subunit CD122, thereby decreasing the activation of the IL2 pathway that is associated with most serious side effects. However, despite promising results in melanoma patients in the PIVOT-02 [15] study, no increased benefit of BEMPE in combination with Nivolumab versus Nivolumab alone was seen in first-line metastatic melanoma setting (PIVOT IO-001) [16].

b. **Interleukin 12 (IL-12)**

Interleukin 12 is a cytokine mainly produced by monocytes [17]. It is one of the most important stimuli for the activation of NK cells.

In recent years, the role of systemic and intratumoral administration of recombinant IL12 has been studied in different settings [18]. Recently, a new formulation consisting of an IL12 coding plasmid (Tavokinogene telseplasmid or TAVO) has been shown to achieve a sustained concentration of cytokines in the tumor microenvironment [19]. In 2014, data from the OMS100 trial were presented [20]. The results were encouraging in patients with metastatic melanoma with injectable lesions who were exposed to TAVO in combination with electroporation. This study showed a significant response rate and interestingly, cases of maintained responses over time. Years later, very promising data was presented showing that combination of TAVO and Pembrolizumab in patients with metastatic melanoma may be an optimal approach [21].

The phase III KEYNOTE-C87 trial promises interesting results as it evaluates the role of TAVO in combination with Pembrolizumab vs. standard treatment in patients with metastatic melanoma who have already been exposed to prior immunotherapy.

c. **Transforming growth factor beta (TGF-beta)**

Transforming growth factor beta is a cytokine with different roles involved in vascular diseases, autoimmune diseases, and carcinogenesis [22]. It seems that the effect of TGF beta could be dual since it has both tumor suppressor and proinflammatory activity, favoring invasiveness and capacity for metastasis [23].

Different formulations are being tested, such as SHR-1701, which has two targets, PD1 receptor ligand (PDL1) and the receptor II of TGF beta [24]. SHR-1701 is

under evaluation in combination with Temozolomide for patients with metastatic melanoma (NCT05106023).

Another formulation currently under evaluation is Vactocertib, an oral inhibitor of the serine/threonine kinase TGFBR1 [25]. Its efficacy is currently being tested in combination with Pembrolizumab in patients with metastatic melanoma.

3. Oncolytic viruses

Oncolytic viruses constitute a very interesting therapeutic weapon since usually they have the capacity to infect only tumor cells, causing them to lyse, and hardly affecting normal cells [26]. Currently, there are different formulations, from viruses with exclusively oncolytic capacity to viruses with the ability to use the machinery of the infected cell to produce different immune stimulators.

Among the most developed viruses already approved, we find Talimogene laherparepvec (T-VEC) [27]. T-VEC is a herpes family virus that is capable of producing GM-CSF. T-VEC provokes not only cellular lysis but also increases the concentration of GM-CSF, thus, favoring a cellular enrichment by dendritic cells and cytotoxic T lymphocytes in the tumor niche. The first results of the OPTIM trial for patients with metastatic melanoma were presented in 2015 and led to TVEC approval by FDA27. Later, it was observed that T-VEC infection could increase the expression of PD1 in the tumor bed, so an attempt was made to show whether adding Pembrolizumab could improve the results of T-VEC injection [28]. However, the phase III study that sought to answer this question was not significant [29]. There are other viruses that have shown efficacy for the treatment of metastatic melanoma, such as the TILT-123 [30] adenovirus (with the ability to produce cytokines such as TNF alpha and IL12), the PVSRIPO 31 virus [31] (a modification of the polio vaccine), Oncos-10232 [32] (adenovirus producing GM-CSF), CAVATAK [33] (enterovirus with oncolytic capacity) or Ad-RTS-hIL-1233 [34] (adenovirus producing IL-12).

4. Intratumoral therapies

One of the greatest advances in immunotherapy is the possibility of intratumoral administration [35]. Although the intratumoral route has been known since the beginning of the 20th century, there are currently many clinical trials using this route [36]. Theoretically, the intratumoral route would allow to use of lower doses of the different agents, obtain a pharmacodynamic profile in real-time, as well as facilitate the combination with different drugs, since a much more manageable toxicity profile is usually seen. In addition, intratumoral therapy has an effect at distant non-injected metastatic sites, in what we know as *abscopal effect* (if the therapy is purely intratumoral) or an anesthetic effect (if the intratumoral strategy is combined with the intravenous one). Currently, there are available results from different molecules administered intratumorally. However, as of today, most positive results from phase II trials with intratumoral agents have not been confirmed in subsequent phase III studies. Interestingly, very positive data in terms of response rates have been seen in the early phases of trials. These therapies face important challenges, starting with the selection of suitable patients, the assessment of response, and the injection

procedure *per se,* which may require the involvement of different departments, such as interventional radiology or surgery. Trials with these kind of agents are very heterogeneous, and characteristics of patients are extremely different among different studies; besides, primary endpoints also differ. Looking retrospectively at the data, it seems that those patients with only cutaneous-subcutaneous disease, achieve the highest benefit, but positive preliminary data have been seen also in mucosal melanoma and overall population, with response rates over 25% in the second line setting, as presented below.

4.1 Pattern recognition receptor (PRR) agonists

Within the PRRs agonists, various molecules have been tested in patients with metastatic melanoma.

a. **TLR9 agonists**

TLR9 is present in the endosome of myeloid cells, B lymphocytes, and dendritic cells [37]. Its functions, although varied, facilitate a pro-inflammatory state in the tumor niche. To date, different intratumoral TLR9 agonists have been tested in patients with metastatic melanoma. Many of them have had negative results, although many others show some signs of activity. CMP-001 has been tested in different scenarios, alone and in combination with an antiPD1 agent [38], not only in patients with metastatic melanoma but also in patients with high-risk locally advanced melanoma in the neoadjuvant setting [39].
On the other hand, the results of the SINERGY-001 trial should be highlighted, which investigated the role of TLR9 agonist SD-101 in combination with Pembrolizumab in patients diagnosed with metastatic melanoma who had previously been treated with antiPD1 [40]. Given these results, the combination of SD-101 + Pembrolizumab is being tested in other tumors.
Another TLR9 agonist is IMO-212 [41]. This is a compound that showed promising results in melanoma in the ILLUMINATE 204 trial in combination with Ipilimumab [42]. However, despite the efficacy in phase 2, the results of phase 3 ILLUMINATE 301 (IMO-212 + ipilimumab vs. ipilimumab) were disappointing [43].

b. **TLR3 agonists**

TLR3 in a receptor located in the endosome capable of recognizing double-stranded RNA [44]. It is mainly expressed on dendritic cells and is responsible for mediating antigen presentation between dendritic cells and lymphocytes. Double-stranded RNA analogs have now been used, as poly I:C-based molecules. BO112, a TLR3 agonist also active against MDA-5 and RIG-I [45], has been tested in different scenarios [46]. The results of phase II testing the efficacy of BO-112 administered intratumorally in combination with pembrolizumab have been encouraging, with 25% response rate in evaluable for response population, which is still better in particular subgroups, such as patients with M1a-N0 disease, who achieved a response rate higher than 70%. Besides, PFS was 16 weeks, which is also a positive result taking into account that all these patients had confirmed progressive disease while on prior immunotherapy [47]. These results need still to be confirmed through randomized trials.

c. **TLR7/8 agonists**

Both TLR7 and TLR8 are receptors located in the endosome [48]. These receptors are capable of recognizing single strands of RNA and triggering the activation of the immune response. Among the most advanced TLR7/8 agonists in development is NKTR-262 [49]. The combination of NKTR-262 administered intratumorally in combination with intravenous BEMPEG is being explored in different tumor types [50]. Although these are preliminary data, it seems that in patients with metastatic melanoma there is some hopeful sign of activity.

d. **STING agonists**

STING pathway activation is triggered by the presence of double-stranded DNA [51]. The activation of this pathway translates into an increase in the response mediated by IFN type I. To date, there are different studies that explore the activation of this pathway using different molecules intratumorally (SYNB1891, CDK-002, BMS-986301, or E7766) [52–56].

4.2 Oncolytic viruses

As previously presented, oncolytic viruses are an important step in the treatment of melanoma. Within the oncolytic viruses administered intratumorally, we have T-VEC (approved by the FDA), PexaVec, and CAVATAK for the treatment of melanoma.

4.3 Other immunity enhancers

Currently, there are different molecules that are being tested and administered with both approaches, intravenous and intratumorally. This is the case with anti-CD40 antibodies. CD40 is a stimulatory signal that enhances the activity of different cells such as macrophages, B and T lymphocytes, as well as antigen-presenting cells. At present, we know encouraging data about the antibodies Selicrelumab [57] (intravenous) and Sotigalimab [58] (intratumoral). Administration of anti-CTLA4 intratumorally has also been investigated with positive signs of efficacy in patients with melanoma [59].

5. Vaccines

Antitumor vaccines have been deeply studied for the past years [60]. Conceptually, it would be based on the administration of selected tumor antigens, as well as other substances that enhance the activation of the immune system (in some cases, dendritic cells, for example, are used per se). This is intended to awaken the acquired response of the host against certain antigens, which would enhance a global response against tumor cells.

Melan A (MART-1), gp100, MAGE, or NY-ESO61 are among the most studied antigens in melanoma [61]. Recently, data from the phase 1/2 trial MM163662 have been presented [62]. In this trial, the role of IO102-IO103 (peptide vaccine composed of IO102 (derived from Indolamine 2,3 dioxygenase (IDO), IO103 (derived from

PDL1), and ISA51 (immunomodulator)) was studied in patients diagnosed with metastatic melanoma. Despite being in the initial phases of research, the data on overall survival, progression-free survival, and response rates are encouraging.

On the other hand, another example of a multi-epitope vaccine was used in trial 18,174 in combination with Pembrolizumab [63]. In this case, the vaccine contained gp100, MelanA/MART-1, two tyrosinase peptides, MAGE-A3 and MAGE-A1,2,3,6 [64]. Despite more modest results, overall survival data in patients who had not been exposed to prior PD1 therapy are promising.

Another different approach was carried out in the phase 2 trial GCO 14–0780 for patients diagnosed with high-risk melanoma and who were treated with complete surgery. This trial studied the efficacy of a poly ICLC-matured dendritic cell vaccine in combination with a peptide vaccine containing NY-ESO and Melan A. This strategy was compared with the administration of Montanide ISA 51 VG and poly ICLC as an adjuvant of the NY-ESO/Melan A vaccine. Results presented at AACR in 2022 showed different degrees of immunization. The effect on relapse-free survival remains to be studied.

On the other hand, there is also a strategy for the development of vaccines based on RNA technology. This is the case of BNT111 [65]. It is a vaccine with RNA encoding for MAGE-A3, NY-ESO1, tyrosinase, and TPTE (putative tyrosine-protein phosphatase). In the phase 1 MERIT study, BNT111 was injected at the lymph node level in patients with metastatic melanoma. The toxicity profile was favorable, so it is currently under development in combination with antiPD1 blockade.

Finally, there is also a vaccination approach against the activity of certain proteins. This is the case of UV1, a vaccine against the catalytic subunit of reverse telomerase (hTERT) [66]. In phase I UV1/hTERT-MM-103 trial, UV1 vaccination was used in combination with Pembrolizumab in patients with metastatic melanoma [67]. The data presented showed interesting results in terms of overall survival and response rate.

6. New therapeutic targets

6.1 Exhausted T cell

Exhausted lymphocytes are defined as lymphocytes with diminished effector functions, as well as compromised cytokine expression [68]. Reversing this state has become a very interesting therapeutic approach since it could be causing both resistance and refractoriness to treatment in some patients. Over the years, certain proteins have been discovered that, when expressed on the surface of lymphocytes, could be contributing to this cellular exhaustion [69]. This is the case with proteins such as LAG3 (lymphocyte activation gene 3) [70] or TIM3 [71] (T cell immunoglobulin domain and mucin domain protein 3). Data on Relatlimab (anti-LAG3) in combination with Nivolumab in patients with metastatic melanoma have recently been presented [72]. The positive first-line results of the combination of Relatlimab with Nivolumab versus Nivolumab in patients with metastatic melanoma who had not previously received any line have led to the approval of the combination by the FDA.

There are also drugs that try to block TIM3, although they are less developed. An example of these would be TSR-022 [73] or MBG-453 [74], which could have a promising role in the treatment of melanoma.

6.2 Bispecific antibodies

There are some drugs under development that are capable of binding to two different domains [75]. This is the case of the antiPD1/antiLAG3 [76] or antiPD1/antiTIGIT [77] antibodies. With this approach, the aim is to reduce the "off tumor" side effects while maintaining or even improving efficacy.

6.3 Other therapies

One of the most important discoveries for patients with uveal melanoma has been the development of Tebentafusp [78]. It is composed of a fusion protein containing the human T cell receptor (TCR) specific for the gp100 antigen. At the same time, it is bound to an antibody fragment against CD3. Despite the fact that the drug is currently restricted to those patients with HLA A2:01, it has meant a radical change for a pathology in which there was not an effective alternative [79].

There are currently other trials using TCRs from patients diagnosed with melanoma and who are considered responders to immunotherapy. These TCRs are being tested in patients with different solid tumors (NCT04729543).

7. Conclusions

The treatment of melanoma has dramatically changed over the past few years. The scenario has shifted from barely having drugs available, to having hundreds of trials available for this population. In the future, it is conceivable that just as targeted therapies, it is very likely that we will know the mechanisms of immunoresistance underlying each patient and thus be able to personalize immunotherapy cancer treatments even more.

Author details

Álvaro Sánchez Arráez[1], Sonia Maciá[2*] and Eduardo Castañón[1]

1 Clínica Universidad de Navarra, Spain

2 Highlight Therapeutics, Spain

*Address all correspondence to: smacia@yahoo.com

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