The cutting edge of metastatic melanoma therapy

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Practice points

- Approximately 40% of cutaneous metastatic melanomas have an activating mutation in *BRAF* V600.
- Combined *BRAF* and MEK inhibition became the gold standard treatment based on Phase III trials for mutated melanomas.
- Immune checkpoint inhibitors led to breakthroughs in the treatment of advanced melanoma with impressive durable responses in a subset of patients.
- Preclinical models showed potential synergy between targeted therapy and immunotherapy opening new perspectives in strategies aimed to treat melanoma.
- Identification of predictive biomarkers, better understanding of immunobiology and management of potential adverse events are the keys points for the future treatment of advanced melanoma.

The past decade has witnessed impressive new developments for the treatment of melanoma. The discovery of key oncogenic driver mutations, upon which tumor establishment and progression are dependent, changed the prognosis of patients with stage IV disease. Extensive preclinical and clinical studies have shown high response rates and survival benefits over conventional chemotherapies provided by target-specific inhibitors of *BRAF*- or *NRAS*-activating mutations. Recent genomic analyses of melanoma have also given new potentially targetable driver mutations. In addition, the quickened pace of development of immune checkpoint inhibitors for the treatment of melanoma offers the unique opportunity to provide a long-term clinical benefit. In this emerging era, predictive biomarkers for the selection of patients are required to help us develop an optimal therapeutic strategy.

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Aim

Melanoma is the deadliest form of skin cancer and the fifth cause of cancer mortality. Melanoma incidence has been increasing over the last 30 years culminating into a lifetime risk of 1 out of 50 [1]. Approved therapies for patients with metastatic melanoma have recently expanded dramatically. Since 2011, the number of drugs approved from the US FDA surpasses the total approved over the previous 30 years. Most of the recent advances target specific molecular aberrations that are responsible for the malignant transformation of melanoma as well as immune suppression mechanisms. This extensive therapeutic armamentarium has increased the average survival from 6 months to currently above 24 months with further improvements likely in the years to come.

KEYWORDS

- *BRAF* • clinical studies
- immunotherapy
- melanoma • therapy

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Targeting BRAF

In 2002, the cancer genome project achieved its first success in melanoma through the identification of a high frequency of activating mutations in the *BRAF* kinase gene. When the BRAF protein kinase is mutated, it results in constitutive activation of the MAPK pathway leading to cell proliferation. Concomitantly, a new therapeutic strategy was emerging by targeting aberrant proteins of signaling pathways with tyrosine kinase inhibitors. Such inhibitors lead to new therapeutic opportunities as they offer the capacity to selectively target activating mutations driving cancer progression.

The BRAF V600E has a tenfold increase kinase activity compared with the wild-type protein and accounts for 85% of total activating *BRAF* mutations [2]. Other, less frequent, BRAF-activating mutations are observed like BRAF V600K.

Although several agents targeting the MAPK pathway via BRAF inhibition have shown their efficacy in *in vitro* studies, clinical translation was initially unsuccessful. Sorafenib was among the first molecules tested in this context. While it was designed as a pan-RAF inhibitor, sorafenib also inhibits a large spectrum of alternative targets (VEGFR-2, PDGFR, c-KIT and FLT-3). In preclinical studies, sorafenib efficiently inhibited BRAF activity in *BRAF*-mutated melanomas, leading to slower tumor progression in mouse models [3]. However, several studies failed to show clinical benefits. A Phase II trial of sorafenib in 36 treatment-naïve metastatic melanoma patients reported a modest activity with just one partial response (PR) and three stabilizations [4]. Interestingly, the patient who experienced PR had a *BRAF* V600E mutation while the three patients with stable disease were *BRAF* wild-type. Another Phase II trial conducted by Eisen *et al.* showed that only 19% of the patients had stable disease when treated with sorafenib, although no confirmed objective response was recorded in the nonselected population [5]. Moreover, *BRAF* mutation could not successfully predict the clinical outcome in these trials. Addition of sorafenib to a taxol-carboplatin chemotherapy regimen was tested in first and second lines in two randomized Phase III trials [6,7], where no benefit was observed. Sorafenib failed to show any clinical benefits and various hypotheses were brought up in the literature to explain this failure. First, on-treatment biopsies of patients included in the trials showed that sorafenib failed to inhibit

the MAPK pathway adequately. One explanation might be that the dose-limiting toxicity of sorafenib is linked to its activity on pathways other than MAPK and that the dose selected for Phase II and III trials does not result in sufficient decrease in ERK phosphorylation [8,9]. The latter is based on a recent study which pointed that reaching an optimal area under the curve is a predicting factor of response to sorafenib in melanoma patients [10]: recent studies have shown that optimization of sorafenib exposure with dose-escalation strategies in renal cell carcinoma, even after failure of sorafenib 400 mg b.i.d., improves antitumor activity. Sorafenib exposure seems, therefore, directly linked to treatment outcome [11,12]. Goldwasser showed that dose optimization of sorafenib in patients with melanoma results in positive correlation between area under the curve max-objective response and progression-free survival (PFS) [10]. Due to overall lack of efficacy and to the arrival of much more potent compounds, the clinical development of sorafenib is no longer pursued actively.

Based on this initial experience, the development of more specific inhibitors targeting mutant BRAF V600E was started. Vemurafenib, a first in class, has been shown to have a tenfold greater activity against V600E compared with wild-type protein, raising high expectations. Vemurafenib differs from sorafenib in many aspects. First, it is much more selective [13], second it targets the activated form of the kinase (type I inhibitor). Both factors contribute to highly selective and, this time, toxicity-limiting inhibition of BRAF V600E.

In 2010, the first data published from the BRIM-2 Phase II study showed impressive response rates (RRs) in 132 pretreated patients with advanced melanoma that harbored the BRAF V600E mutation treated with vemurafenib [14]. These results opened a new era in melanoma as well as, more generally, in the targeted therapy of solid tumors. After a medium follow-up of 7 months, the RR was 52%. The most common side effects were rash and arthralgia. On the other hand, about 25% of the patients presented cutaneous squamous cell carcinoma. This phenomenon is due to the paradoxical MAPK activation in nonmelanoma cells that are wild-type for BRAF but have an upstream mutation, such as mutations in *KRAS*, *NRAS* or *HRAS* [15,16].

The results of the extended follow-up of the BRIM-3 Phase III study were recently published.

This trial randomized 675 unresectable stage IIIc or IV, previously untreated *BRAF* V600 mutation-positive melanoma patients to receive vemurafenib or dacarbazine. The PFS was significantly longer, 6.9 versus 1.9 m (hazard ratio [HR]: 0.26, $p < 0.001$), as well as the median overall survival (OS), 13.6 versus 9.7 months (HR: 0.70; $p = 0.0008$). This study showed the same efficacy of vemurafenib in V600E as in the V600K mutation regarding PFS and OS. In August 2011, FDA approved vemurafenib based on these data. Although the majority of patients (57%) showed a PR, most of the tumors developed resistance within 6 months, resulting only into a short-term significant improvement of OS [17].

These results were confirmed by another Phase III trial that randomized patients with metastatic melanoma with *BRAF* V600E mutation to receive dabrafenib, also a type I *BRAF* inhibitor or dacarbazine resulting into a similar PFS benefit of 5.1 versus 2.7 months [18]. OS data are difficult to interpret due to the study design that allowed crossover at progression. In May 2013, the FDA approved dabrafenib for advanced melanoma *BRAF* mutated.

Very early in the development of these compounds, the oncology community realized that understanding resistance mechanisms was critical in order to provide strategies that enhance control of resistant variants. Strong translational program was launched to understand the molecular basis of adaptive resistance to *BRAF* inhibition.

In 2010, Nazarian *et al.* used resistant cell lines derived from *BRAF* (V600E) mutated melanoma after treatment with increasing dose of vemurafenib to unveil two interesting mechanisms of acquired resistance: the upregulation of *NRAS* and the induction of *PDGFRb* [19]. The first leads to reactivation of the *MARK* pathway and the latter to the activation of an alternative survival pathway. Moreover, an acquired mutation of *NRAS* (Q61K) was found in one cell line after exon sequencing which was not present in the parental cell line. This mutation leads to activation of *MEK/ERK* via activation of *CRAF*. The mechanism of resistance was confirmed in clinical studies by detecting these mechanisms in 5/12 tumor biopsies of participating patients in Phase II clinical trials treated with vemurafenib at the time of relapse. For the time being, it is unclear whether the *NRAS* mutation pre-exists or arises under the selective pressure of vemurafenib, though mathematical

models based on known mutagenesis rates tend to favor the former. In a limited number of patients, several *MEK1* mutations were found that are associated with acquired resistance (including *MEK1*, Q56 and E203 mutations). Importantly, all relapsed tumor specimens continued to harbor the *BRAF* V600 mutation [20].

So far, there are several mechanisms of resistance that have been identified including activating mutations (*AKT1*, *NRAS*, *MEK1*), elevated expression of selected kinases such as *CRAF* or *COT1*, activation of phosphatidylinositol-3-OH kinase (*PI3K*) via loss of *PTEN* or aberrant splicing of mutant *BRAF* (Figure 1) [21]. The relative frequency of these resistance mechanisms is still not known precisely.

Addressing resistance mechanism through combination therapies

Based on encouraging results from preclinical studies, clinical trials exploring the efficacy of the combination of *BRAF* and *MEK* inhibitors in *BRAF*-mutated tumors were launched confirming a very promising activity. In January of 2014 the FDA approved the use of combination of trametinib and dabrafenib for patients with *BRAF* V600E or V600K mutation based in a Phase I/II trial which showed an improvement of PFS from 5.8 months to over 9 months as well as a higher RR (76 vs 54%) in the combination group versus dabrafenib alone. At American Society of Clinical Oncology Annual Meeting (ASCO) in 2015, data from three clinical trials reported superior activity of the combination. Updated results of the Phase III COMBI-d trial comparing the combination of trametinib (2 mg/day) and dabrafenib (150 mg 2×/day) versus dabrafenib alone (150 mg 2×/day) were presented and soon published in the *Lancet* [22]. Four hundred and twenty-three treatment-naïve patients with an Eastern Cooperative Oncology Group (ECOG) Scale of performance status 0–1 and unresectable melanoma harboring *BRAF* V600E or V600K mutation were randomized in this trial. The results confirming the PFS benefit in favor of the combination (11 vs 8.8 months; HR: 0.67), the RR benefit (69 vs 53%) as well as OS benefit (25.1 vs 18.7 months; HR: 0.71) with 51 versus 42% of patients still alive at 2 years, making the combination the new standard of care in the first-line setting in *BRAF*-mutated advanced melanoma. Interestingly, subgroup analysis for the OS showed that all the subgroup benefit from the combination, independently

Figure 1. Known BRAFi resistance mechanisms.

RTK: Receptor tyrosine kinase.

of age, low density lipoprotein (LDH) level, number of metastasis and mutation genotype. Regarding the side effects, all grade treatment related adverse events (AEs) occurred in 90% in the monotherapy arm versus 87% in the combination arm. The monotherapy arm lead to more cutaneous toxicities such as hyperkeratosis (33 vs 6%), hand-foot syndrome (27 vs 6%) and alopecia (26 vs 5%) whereas diarrhea (18 vs 9%) and pyrexia (52 vs 25%) were more common in the combination arm. Moreover, the incidence of squamous cell carcinoma was observed only in 3% in the combination versus 9% in dabrafenib alone arm. The reduced incidence of cutaneous toxicities is explained by MEK inhibition that blocks the paradoxical activation of MAPK pathway in the skin.

Furthermore, updated data from the coBRIM Phase III trial comparing BRAF inhibitor vemurafenib (960 mg 2/day) with or without the MEK inhibitor cobimetinib (60 mg 1/day) on

days 1–21 of a 28-day cycle in first-line setting confirmed the PFS and OS benefit of the combination (12.3 vs 7.2 months; HR: 0.58 and 22.3 vs 17.4 months; HR: 0.70, respectively) in BRAF V600 mutation-positive tumors after a follow-up of 14.2 months. The improved PFS and overall response rate (ORR) achieved with the combination was accompanied by a high occurrence of side effects such as diarrhea (57 vs 28%), retinopathy (20 vs <1%), increased CPK (30 vs 3%) although treatment-related discontinuation rates in the two arms were similar (13 vs 12%). In this study, tumor samples were analyzed using next generation sequencing for 528 known activating mutations in 17 oncogenic proteins kinases (including RAS/RAF/nonV600 BRAF/ERK). In 46 of 423 (11%) of the tumors, baseline co-existing mutations were found but this did not impact the combination's efficacy regarding PFS and ORR.

Sullivan reported data of a third trial, Phase Ib/II, studying the efficacy of encorafenib

(BRAF inhibitor) and binimetinib (MEK inhibitor) in patients with advanced melanoma carrying *BRAF* V600 mutation and BRAF inhibitors naive [23]. The combination showed 74% of ORR and the median PFS was 11.3 months. Regarding the toxicity, the combination resulted in grade 3/toxicities in 67% of the patients. Pyrexia occurred in 11% of patients but no grade 3–4 was reported.

Based on this data, many centers adopted the combination as standard treatment for BRAF-mutated melanomas. To date, Long *et al.* reported clinical characteristics of long-term survivor patients with dabrafenib plus trametinib treated in a Phase I and II trial. Normal baseline lactate dehydrogenase was associated with prolonged PFS and OS, with OS at years at 62% [24].

Currently novel second-generation BRAF inhibitors are under clinical investigation showing interesting activity. For example, PLX7904, a next-generation RAF inhibitor, which effectively inhibits RAF signaling in *BRAF* V600E melanoma cells, without paradoxical effects in wild-type cells, was successfully tested in vemurafenib-resistant *BRAF* V600E cells that express mutant NRAS and also in *BRAF* V600E splice variant-mediated vemurafenib-resistant cells [25,26].

Another example is BGB-283 which showed promising antitumor activities in preclinical models for not only cancers with B-RAF V600E mutation but also non-V600E *B-RAF* mutation and *K-RAS/N-RAS* mutations. Recently in Phase I trial with patients carrying *BRAF* or *RAS* mutation, BGB-283 showed that among 29 patients treated, three had confirmed PRs and 14 had stable disease. To date, the three patients who had confirmed PRs were diagnosed with melanoma with a *BRAF* V600E mutation, endometrial cancer with a *KRAS* mutation and thyroid cancer with a *BRAF* V600E mutation. Interestingly, all PRs were ongoing with duration of response lasting over 200 days at the time of data cutoff, except for the response for the patient with endometrial cancer, who had a duration of response of 411 days and PFS of 455 days.

Targeting *NRAS*-mutated melanoma

Twenty percent of melanoma patients are harboring *NRAS* mutations and are showing a more aggressive clinical behavior compared with patients harboring *BRAF* mutations or wild-type [27]. Mutations in codon 61 impair the enzymatic activity of RAS to cleave GTP to

GDP, whereas mutations in codons 12 and 13 prevent the association of RAS with GTPase, resulting in constitutive, oncogenic signaling of RAS. Due to the very high affinity of GTP for NRAS and due to the very high cellular GTP concentration, to this day, there is no small molecule that has been shown to inhibit its action directly, though allosteric inhibitors are being worked up. Up till now, trials have been focusing on downstream blockade using MEK inhibitors.

In 2013 Ascierto *et al.* published a Phase II trial of 71 patients with advanced melanoma harboring BRAF or NRAS mutation treated with a highly selective allosteric MEK1/2 inhibitor binimetinib (MEK 162). Six out of the 30 patients (20%) with *NRAS* mutation achieved PR whereas 63% achieved disease control, suggesting for the first time the need for exploring the clinical benefit in the *BRAF* wild-type *NRAS*-mutated population [28].

Since NRAS protein constitutively activates downstream signaling pathways such as MARK and PI3K/AKT/mTOR, several combinations are being tested.

Recently, it has been shown that the combination of metformin, which indirectly inhibits the PI3K/mTOR pathway, has synergistic antitumor activity with MEK inhibitor trametinib in *NRAS*-mutant melanoma *in vitro* and *in vivo* and that cotargeting MEK/PI3K/mTOR pathway is more efficient than targeting MEK/mTOR alone. Another data showed that coinhibition of MEK and POLO-like kinase 1 (PLK1), a cell cycle regulator which is overexpressed in *NRAS*-mutant melanoma, has interesting cytotoxic activity.

Other targeted therapies

Preclinical evidence supports the existence of multiple feedback-regulated pathways leading to upregulation of different oncogenes after single BRAF and MEK inhibition such as ERK, CRAF, PDGFRB and IGF1RB [29]. SCH772984, a specific inhibitor of ERK1/2 activity, showed efficacy in BRAFi and MEKi-resistant *RAS* - or *BRAF* -mutant melanoma cells. Interestingly, SCH772984 was also effective in single-agent BRAF or MEK inhibitor resistance. In addition, Shi *et al.* analyzed tumor samples from patients with acquired resistance to BRAF inhibitors; 22% of specimens exhibited increased AKT activity, suggesting a strong rationale to design trials testing the combined inhibition of the MAPK and PI3K pathways [30].

Kwong *et al.* introduced a very elegant strategy to identify targets that have synergistic effects when coinhibited along with MEK in an *NRAS*-mutated melanoma model. First of all, after comparing the antitumoral effect between an inducible mouse model of *NRAS*-mutant melanoma and pharmacological MEK inhibition (selumetinib), he showed that only genetic extinction of *NRAS* results in tumor regression. Furthermore, partial genetic extinction of *NRAS* activity resulted in the induction of apoptosis but not cell-cycle arrest, similar to the effect seen with MEKi treatment. Next, with the help of reverse-phase protein array, he identified a group of genes whose expression showed statistically significant regulation by *NRAS* extinction but no change or a change in the opposite direction after selumetinib treatment. In addition, with the help of genomic technologies and bioinformatics models, Cyclin-dependent kinase 4 (CDK4) was identified as a key target driver of the molecular differences correlating with the different phenotypic consequences (tumor regression vs tumor stasis) [31]. CDK4 controls the passage through the G1 checkpoint of the cell cycle by phosphorylating the retinoblastoma protein. Mutation in CDK4 generates a dominant oncogene. As a proof of concept, the selective inhibition of both MEK and CDK4/6 leads to tumor regression in an inducible mouse model of *NRAS*-mutant melanoma (Q61K). This strategy opens new possibilities for combinations in melanoma. Recently, a preliminary report of a Phase Ib/II trial presented at the 2014 ASCO annual meeting, demonstrated promising results in patients with *NRAS*-mutant melanoma that were treated by binimetinib (MEKi) and a highly selective CDK4/6 inhibitor, LEE011. 14 patients with median 2 prior lines were treated: six achieved PR whereas six had experienced stable disease. Common treatment-related toxicities were CPK elevation, edema and rash [32].

Furthermore, preclinical data support that in BRAF inhibitor resistant melanoma cell lines, ERK inhibition is more potent than MEKi in combination with PI3K/m TOR inhibition. In this context, ERK inhibitors are under development. To date, several newly developed inhibitors that either target mutated kinases or protein overexpression are under investigations. For example, preclinical data have shown very interesting antitumor activity after inhibition of murine double minute 2 (MDM2) and MDM4 – two ubiquitin ligases which have been shown

to be the major negative regulators of the tumor suppressor p53 (known also as ‘guardian of the genome’). Preclinical studies have implicated an importance of the MDM2–MDM4 hetero-complex in cancer. Contrary at MDM2 which is rarely expressed in melanoma, MDM4 is often upregulated. Although pharmacological MDM2 inhibition has no antitumoral impact, inhibition of the MDM4–p53 interaction leads to restoration of p53 function in melanoma cells, and causes increased sensitivity to cytotoxic chemotherapy and to inhibitors of the BRAF (V600E) oncogene.

Furthermore, the MDM2 antagonist MI-319 in conjunction with the multikinase inhibitor sorafenib showed an interesting cytotoxic effect *in vitro* determined primarily by the activity of the serine/threonine protein kinase GSK-3 β . Interestingly, p53 reactivation in association with BRAF V600E inhibition or with a senescence-inducing Aurora A kinase inhibitor led to additive suppression *in vivo* [33,34].

Hence, it is becoming increasingly clear that genetic landscape of melanoma impacts our treatment modalities and that it will guide our choice of therapeutic strategies.

Immunotherapy

The immune system is a very complex and potent network of interacting cells that is required to make critical decisions as to activate or not when exposed to an antigen. These decisions are influenced by many factors and a fine balance is needed to get adequate immune protection while preventing autoimmunity. To this end, immune cells have numerous costimulatory and coinhibitory receptors. The later, also termed ‘immune check points’, have been targeted by blocking monoclonal antibodies that led to a revolution in the treatment of stage IV melanoma.

CTLA-4 is the prototypic immune-checkpoint receptor acting as a physiological brake on the T-cell activation by dendritic cells. It has been targeted by a blocking monoclonal antibody (ipilimumab), resulting in an improved immune response against the tumor.

The canonical mode of action is that ipilimumab enhance T-cell activation by blocking the negative signals resulting from CTLA-4 engagement by B7.1. In line with this mechanism, Kvistborg *et al.* analyzed the antigenic specificities of T cells from melanoma patients before and after treatment with ipilimumab. Remarkably, whereas existing responses against

tumor antigens was not affected by ipilimumab therapy, a large number of new antigenic specificities arose on treatment [35]. Several reports have shown that anti-CTLA4-therapy has other mechanisms of actions such as depletion of immune-suppressive T cells (Tregs) in the local tumor microenvironment [36]. This diversification of the antigenic repertoire could be at the origin of the long-term benefit observed with ipilimumab.

At ASCO 2010, Hodi *et al.* presented data from a placebo-controlled Phase III randomized trial of previously treated patients with metastatic melanoma. Among 676 patients, the ipilimumab alone arm compared with glycoprotein 100 (gp100) vaccine alone improved survival rates (10.1 vs 6.4 months) [37]. Although the RR was only 10%, when objective responses were observed, 17% in the combination and 60% in the ipilimumab monotherapy were maintained for at least 2 years. Based on this data ipilimumab was approved by the FDA in March 2011 for pretreated metastatic melanoma, opening a new era cancer immunotherapy.

Furthermore, a recent pooled analysis of long-term survival of 1861 patients with advanced melanoma who have been treated with ipilimumab in different clinical trials, showed that 22% (n = 254) of these patients presented durable objective tumor responses and were still alive after a 3-year follow-up regardless the dose or line of treatment [38]. In addition, patients that were alive at year 3 had a very high survival probability extending up to year 7 and more. Interestingly, complete responses were achieved many months after the last dose of ipilimumab suggesting, in line with preclinical data, that anti-CTLA4-inhibition lead to sufficient and durable expansion of tumor-reactive T cells.

Several trials have attempted to explore the potential benefit of combining ipilimumab with chemotherapy. Although the results seem promising with some gain in OS, the toxicity was also higher putting doubts on its clinical utility. For example, the combination of dacarbazine with ipilimumab resulted to 20.8% of 3-year OS versus 2.2% among patients treated with chemotherapy alone [39]. However, toxicity was also significantly higher as expected from the combination regimen with grade 3 or grade 4 side effects occurring in 56% of patients treated with the combination and in 27% of patients treated with chemotherapy alone. This significantly higher rate of AEs observed in the combination arm might be attributed to the administration of a higher

dose of ipilimumab (10 mg/kg). To conclude, high-grade toxicities compromise the effectiveness of this combination and its clinical use.

Recently, our therapeutic armamentarium against melanoma has continued to broaden and a second inhibitory checkpoint receptor was added. The programmed cell death protein-1 (PD-1) receptor. Its activation via PD-L1, which can be expressed by tumor or tumor stroma cells, triggers apoptosis and downregulation of T-cell effector functions. Blocking the ligand or the receptor results in a potentiated antitumor immune response in melanoma and other tumor types [40,41].

Based on the preliminary report of the randomized Phase III trial CheckMate-037 evaluating nivolumab compared with the physician's choice of chemotherapy (DTIC or carboplatinum/paclitaxel) in previously treated patients with ipilimumab and, in case of BRAF mutation, a BRAF inhibitor, nivolumab was granted an accelerated approval in this setting. In this trial, nivolumab leads to an improved objective RR based upon RECIST criteria (32 vs 10%) compared with chemotherapy. Furthermore, 82% of nivolumab responders had more than 50% reduction of target lesion burden compared with 60% of chemotherapy responders. Clinical activity was observed for nivolumab regardless of prior response to BRAF inhibitors or anti-CTLA4. Treatment toxicity was manageable and grade 3/4 side effects were higher in the chemotherapy group (9 vs 31%) [42].

In September 2014, pembrolizumab, another PD-1 blocking antibody was approved by the FDA for the treatment of metastatic melanoma in patients with progressive disease after ipilimumab and a BRAF inhibitor for tumors with a *BRAF* V600 mutation based on results of a Phase I study that included 411 patients. Results from this trial were updated for survival at the 2014 ASCO meeting. Patients with progressing advanced melanoma were treated with pembrolizumab either at the recommended dose of 10 mg/kg every 2 weeks, 2 mg/kg or 10 mg/kg every 3 weeks. The RR was approximately 34% in the three groups. Interestingly, the ORR using RECIST criteria were 40% for ipilimumab naive patients and 28% for ipilimumab pretreated patients. OS at 1 year was 69%. Toxicity was low with 12% of patients experiencing grade 3–4 AEs and only 4% discontinuing the treatment due to a drug-related side effect [43].

Clinical activity has also been observed with anti-PD-L1 monoclonal antibodies. For example, MPDL3280A showed 29% ORR in a Phase I study including 45 patients with advanced melanoma, though the activity seems lower compared with PD-1 antibodies in this disease [44].

As mentioned above, CTLA-4 blockade acts in the early T-cell priming phase whereas PD-1/PD-L1 acts in the effector phase involving antigen experienced T cells. Preclinical data suggest synergy of antitumoral activity in case of combination. In a Phase I study with advanced melanoma patients, in a separate cohort the combination of ipilimumab and nivolumab once every 3 weeks for four doses followed by nivolumab every 3 weeks through week 24, achieved objective responses in 42% of the 53 patients.

In the 2015 ASCO Annual meeting two trials confirmed that the combination of nivolumab with ipilimumab significantly prolongs PFS comparing to ipilimumab (CheckMate-069 and 067) and that PFS is numerically higher than nivolumab (CheckMate-067, exploratory end point). In the Phase II trial CheckMate-069 142 treatment-naïve patients were randomized to receive four cycles of ipilimumab 3 mg/kg every 3 weeks with or without for nivolumab (1 mg/kg) every 3 weeks followed by nivolumab 3 mg/kg every weeks until progression. The checkpoint inhibitor combination had an overall RR of 59% with a 22% complete RRs in a subgroup of BRAF V600 wild-type patients [45]. However, almost half of the patients experienced grade 3–4 AEs versus 22% in the ipilimumab monotherapy arm, though a large fraction of these AEs are related to asymptomatic abnormal lab values.

The second randomized Phase III trial, CheckMate-067, presented by J Wolchok, also confirmed the advantage of the CTLA-4 and PD-1 inhibition combination over the respective single agents 945 previously untreated patients with unresectable stage III or IV melanoma were randomized to receive ipilimumab alone (3 mg/kg every 3 weeks for 4 cycles), nivolumab alone (3 mg/kg every 2 weeks until progression) or a combination (same schedule as CheckMate-069) [46]. The patients were stratified according to PD-L1 expression (cutoff 5%), BRAF status and AJCC stage. The primary co-endpoints were PFS and OS benefit while secondary end points were ORR, PD-L1 as biomarker and safety.

While OS data are expected for late 2016, the combination arm leads to an improvement of median PFS of 11.5 versus 2.9 months with ipilimumab and 6.9 months with nivolumab alone. Of note, the study was powered to compare the combination arm or the nivolumab arm to the ipilimumab arm only. The ORR was 57.6, 19 and 43.7%, respectively. The correlation between nivolumab activity and PD-L1 expression remained a challenge: the PFS in patients with tumors PD-L1 positives was 14 months in both combination and monotherapy by nivolumab arm, while in ipilimumab arm was only 3.9 months. However, the PD-L1-negative cohort, the combination achieved a PFS of 11.2 months, numerically superior to nivolumab alone (5.3 m) or ipilimumab alone (2.8 m). Even with a lower cutoff of 1%PD-L1 expression the mPFS was the same in the two arms containing nivolumab (12.4 months) and the PD-L1-negative cohort continue to benefit more from the combination (11.2 vs 2.8 m with ipilimumab or nivolumab alone).

Interestingly, the ORR was higher for patients with PD-L1-positive tumors with 72% ORR in the combination arm comparing to 57 and 21% in nivolumab and ipilimumab arm, respectively (although the PFS was the same). In the PD-L1-negative melanomas the combination leads to an impressive 55% of ORR comparing to 41% in the nivolumab and 18% in the ipilimumab arm.

Regarding the toxicity profile, although the combination was clearly more toxic with 55% of patients presenting grade 3–4 side effects compared with 16.3% in the nivolumab and 27.3% in the ipilimumab arm, no treatment-related death was reported, while there was one treatment-related death associated with each of the monotherapies. Treatment-related discontinuations were 36.4% in the combination arm, 7.7% in the nivolumab and 14.8% in the ipilimumab arm. To date 38% of patients discontinue the treatment due to toxicity, however, the RR was 68% and half of those responses occurred after the stop of the treatment. The most common side effect for the combination was skin toxicity including pruritus and rash (33 and 28%, respectively) as well as gastrointestinal toxicity (diarrhea 44%, colitis 11%). The 18 and 5% of patients presented grade 3–4 hepatic and endocrine toxicity in the combination arm, 2.6 and 0.6% in the nivolumab while 1.6 and 2.3% in the ipilimumab arm. Although incidence of side

effects was highest in the combination arm, these toxicities were consistent with those observed in CheckMate-069, and were manageable using established algorithm leading to high-resolution rates for the majority of grade 3–4: 85–100% for the combination, 50–100% for nivolumab and 83–100% for ipilimumab arm. It should be noted that the range of immune-related AE is very broad and that rare toxicities are often below the reported cutoffs, yet can affect a sizable patient population. Close attention to these rare events should be made in future studies, including post-marketing Phase IV.

At this time PD-L1 expression is a poor biomarker in melanoma. It is unclear if this is due to technical issues or due to heterogeneity in its expression leading to a high percentage of false-negative results. Additionally, PD-L1 status is dynamic and negative patients might become positive during the course of the disease. All these aspects make it currently very difficult to use PD-L1 as a strict biomarker that would deny treatment to a given melanoma patient population.

More recently, Tumeh *et al.* reported that the level of pre-existing CD8⁺ T cells located at the invasive tumor margin was associated with expression of the PD-1/PD-L1 levels in samples of 46 patients with advanced melanoma obtained before and during treatment therapy with pembrolizumab [47]. In parallel, the patients that responded to treatment showed that the proliferation of intratumoral CD8⁺ T cells directly correlated with radiographic reduction in tumor size. Furthermore, they established a predictive model based on CD8 expression at the invasive margin and validated the model in an independent cohort of 15 patients. This study identified pre-existing CD8⁺ T cells as a potential predictive biomarker.

Recently, FDA approved oncolytic virus therapy for the treatment of melanoma lesions in the skin and lymph nodes based on results of the OPTiM trial. Four hundred and thirty six patients randomly assigned in a 2:1 ratio to be treated with intralesional talimogene laherparepvec (T-VEC) or subcutaneous GM-CSF [48].

T-VEC is a herpes simplex virus type 1-derived oncolytic immunotherapy which eliminates cancer cells via directly cancer cell destruction since herpes simplex virus has been genetically modified to selectively replicate in the tumor cells and secondly by enhancing systemic antitumor immune response via the expression of the gene for GM-CSF.

The overall RR (26.4 vs 5.7%) and also median time to treatment failure (8.2 vs 2.9 months) were statistically better with T-VEC, with a trend for OS benefit (23.3 vs 18.9 months, HR: 0.79; *p* = 0.051). One of the more important thing in this trial is that 11% of the patients had complete response. To date the most frequent AEs were chills, pyrexia, injection-site pain and flu-like symptoms. No treatment-related deaths were reported. Currently, the combination of T-VEC with checkpoint inhibitors is ongoing [49].

Conclusion

Over the last few years, high-quality translational programs have profoundly impacted the clinical landscape of melanoma. These programs have allowed designing smaller, hypothesis-driven Phase II trials, which lead to statistically significant results on smaller cohorts and to a more restrictive use of larger Phase III trials. Due to the rich treatment pipeline ahead of us, both in the targeted therapy as well as in the immunoncology side, the number of possible combinations is quickly becoming intractable. For this reason, preclinical research will be required to select combinations based on strong rational bases and to propose smaller trials with a well-defined, biomarker-driven patient selection. Keeping up with the increased pace of translational innovations will be a welcomed challenge for the clinical trial design in the years to come.

Future perspective

The past 5 years have witnessed tremendous advances in our understanding of the mutational landscape of melanoma. This progress allowed to set BRAF/MEK dual inhibition as the regimen of choice for BRAF-mutated melanoma. However, this deeper understanding has also revealed the heterogeneity and the molecular complexity of melanoma, highlighted by a recent publication of the TCGA consortium which established a genomics based classification scheme for cutaneous melanoma using six distinct profiling strategies, whole genome, exome, mRNA and miRNA sequencing as well as DNA methylation and reverse-phase protein expression profile [2]. Four subtypes were identified based on the presence or the absence of *BRAF* mutations, *RAS* mutations and *NF1* mutations as well as a fourth subtype called ‘triple wild-type’ characterized by focal amplifications and frequent *KIT* gene mutations. Although there is no clear

prognostic correlation between these subtypes, there were survival differences in subclasses based on tumor transcriptomic profiles referred to as 'MITF-low', 'keratin' and 'immune'. The latter signature is related to lymphocytes infiltration as well as to lymphocyte-specific protein tyrosine kinase (LCK) expression – a protein that is linked to T-cell proliferation response.

Based on this molecular classification the authors suggested potential therapeutic strategies. For example, for the NF-1-mutant subtype that is functionally closest to the RAS mutation subtype, MEK inhibitors may be a good choice since preclinical studies have shown activity in this context. Moreover, these findings suggest that the immune subclass may respond better to immunotherapies such as immune checkpoint inhibitors. Of importance is the mutational load of the different subtype that appears to be quite variable, with the NF1 subtype harboring the highest number of mutations.

An important axis of research is the so-called 'triple-negative melanoma', that is, BRAF/NRAS/NF1 wild-type. So far, a number of potential driver mutations (KIT, PDGFRA, RTKs and KDR) were identified supporting the use of imatinib, regorafenib, pazopanib based on specific mutations.

Immunotherapy and checkpoint inhibitors lead also to significant survival benefit for patients. The past few years, it became clearer that successful immunotherapy is strongly relying on somatic mutation presented by class I MHC, the so-called neoantigens. Recently, Snyder *et al.* have generated interesting data regarding the potential role of mutational load in melanoma tumors in relation to the clinical benefit from immunotherapy with CTLA-4 inhibitors [50]. The authors used whole exome sequencing to identify somatic mutations on tumors from 25 melanoma patients who were treated with ipilimumab or tremelimumab, another CTLA4 inhibitor. In addition, they predicted neoantigens generated from these mutations based on a bioinformatics analysis. Results showed a significant difference in mutation burden between the patients that presented a clinical benefit with CTLA4 inhibition and those with marginal or no benefit. This higher mutational load (cutoff of 100 mutations) was associated with a significant improvement in OS in the discovery set, but not in the validation set where there was only a trend toward improved survival. On the other hand, the neoantigen

landscape identified was present in tumors samples in both the discovery and validation sets with a strong response to CTLA-4 blockade suggesting that the mutational load in itself may be insufficient to impact the clinical benefit from CTLA-4 inhibitors but that it is the quality of mutations that is predictive. As stated above, the mutation load of the different TCGA subtypes seems quite different arguing that each subtype might respond differently to immunotherapies.

Recently, Kreiter *et al.* demonstrated that in three independent murine tumors models (including melanoma, breast and colon cancer), the mutasome is predominantly recognized by CD4 T cells. Furthermore, injection of RNA pentasome leads to survival benefit in these murine models. These findings indicate that MHC class II presented epitopes may be more abundant than previously appreciated leading to a new approach of individualized immunotherapy with the use of a unique poly-neo-epitope mRNA cancer vaccine that can target both class I and class II presentation.

What is more, preclinical models showed that oncogenic BRAF (V600E) renders the microenvironment immunosuppressive via induction of IL-1 in melanomas [51]. Interestingly, Knight *et al.* showed that BRAF inhibition leads to microenvironment changes by enhancing the ratio of CD8/CD4 T-cells in tumor and decreasing immune suppressive tumor CCL2 expression in a BRAF V600E-mutant mouse model. In addition, the combination of BRAF inhibitor and anti-CCL2 or agonistic anti-CD137 antibodies showed significant antitumor activity in this model [52]. Furthermore, *in vivo* study in melanoma model showed that vemurafenib increase the TIL infiltration as well as the antitumor activity of adoptively transferred T cells [53] and modification of tumor infiltrating lymphocyte population as well as PD-L1 expression was seen in tumor biopsies from patients treated with MAPK inhibitors [54]. All these elements raise new questions on the potential synergy between targeted therapy and immunotherapy which should open new therapeutic opportunities.

Importantly, the optimal sequence of treatment remains to be determined in the BRAF-mutated patient population: it is still unclear whether BRAF/MEK inhibitor doublet followed by immunotherapy or the opposite is more effective. Randomized trials are ongoing to answer this important question (NCT01673584 are completed and the results are pending,

NCT02224781 and NCT01783938 are still ongoing).

Furthermore, although the discovery and the development of small molecule and checkpoint inhibitors change the hallmark of melanoma therapy, the place of chemotherapy remains unclear. Although it is considered inactive in this disease with RRs less than 10%, recent studies showed that the combination of TZM plus ipilimumab lead to 28% RR and the combination of TZM plus veliparib, an inhibitor of DNA repair enzymes, showed an interesting activity in the low ERCC1 expression subgroup [55,56].

Last but not least, we should not underestimate the impact of microenvironment. Weeraratna *et al.* reported that aged fibroblasts secrete a Wnt antagonist, sFRP2, which regulates β -catenin that normally blocks the invasion of melanoma cells. In addition, β -catenin loss

promotes oxidative stress in melanoma cells, rendering them more resistant to targeted therapy (vemurafenib) [57].

Thus, based on the available relevant preclinical and clinical evidence, we anticipate the future treatment of melanoma to be a combination of inhibition of RAF/MEK/ERK pathway and a genotype-dependent immunotherapy leading to a new era of personalized immunotherapy.

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