Combination therapies in advanced melanoma

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Practice points

- In recent years, the advent of new-generation drugs, in particular targeted agents and monoclonal antibodies, has changed the treatment scenario for certain neoplastic diseases. An important aspect of this is the potential for novel combination therapy approaches.
- The approval in 2011 of ipilimumab, an anti-CTLA4 monoclonal antibody, and vemurafenib, a BRAF inhibitor, completely changed melanoma management, with both drugs being shown to improve overall survival.
- Given their different characteristics, the potential to combine these two drugs in patients with advanced BRAF-mutated melanoma has been a topic of much interest, with some emerging data suggesting that sequential treatment with ipilimumab first followed by vemurafenib may be preferable to the opposite order.
- Another novel approach is the combination of a BRAF inhibitor with a MEK inhibitor (e.g., trametinib), the rationale for which coming from evidence that the most important mechanisms of resistance to BRAF inhibitors are related to the reactivation of the MAPK pathway. Combination therapy with the BRAF inhibitor dabrafenib and trametinib has demonstrated superiority compared with dabrafenib alone, and several other trials of combined MEK and BRAF inhibition are ongoing.
- Another possibility for increasing the effectiveness of these compounds is to combine them with agents that target molecules that are upregulated in the resistance processes (e.g., a trial of triple-combination therapy consisting of a BRAF inhibitor, a MEK inhibitor and an inhibitor of cyclin CDK4/6 [LEE011] is ongoing).
- Combined approaches to these novel agents with traditional techniques, such as surgery, radiotherapy and/or chemotherapy, also seem to be potentially useful and are being investigated.
- The possibility of using other forms of treatment that impact on the immune system is another opportunity for a combined approach (e.g., the combination of ipilimumab plus bevacizumab has shown promise).
- New and emerging immunotherapeutic treatments (e.g., the anti-PD1 monoclonal antibodies nivolumab and MK-3475 [formerly lambrolizumab]) have also been associated with a significant improvement in survival, and the combination of these drugs with other novel agents, such as ipilimumab, represents an exciting approach.
- Targeted agents in combination or in sequence with new immunotherapeutic compounds represent the future, not only for the treatment of melanoma, but also for the treatment of cancer in general.

SUMMARY Until recently, melanoma represented a significant clinical challenge to oncologists. However, the approval in 2011 of ipilimumab, an anti-CTLA4 monoclonal antibody, and vemurafenib, a BRAF inhibitor, completely changed melanoma management, with both drugs being shown to improve overall survival. The advent of these two drugs, together with the ongoing development of other targeted agents (e.g., MEK inhibitors) and immunotherapeutic compounds (e.g., anti-PD-L1), is providing the opportunity to trial new combination or sequential therapy approaches. Combined approaches of these new agents

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SUMMARY (CONT.) with surgery, radiotherapy and/or chemotherapy are also being assessed. Targeted agents in combination or in sequence with new immunotherapeutic compounds may represent the future, not only for the treatment of melanoma, but also for the treatment of cancer in general.

KEYWORDS

- combination
- immunotherapy
- melanoma • sequencing
- target therapy

Combination therapy represents a valuable strategy in the treatment of a wide range of diseases, including cardiovascular disease, infection in transplantation and cancer. TB and HIV are two of the most striking examples in which the use of combination therapy has revolutionized patient care. In particular, the highly active antiretroviral therapy AIDS cocktail has transformed AIDS from an acute deadly infection into a chronic illness. Today, a patient with an HIV infection can survive for 20–30 years, an outcome that is similar to those of many other chronic diseases. In oncology, a combination of surgery, radiotherapy and/or chemotherapy has long represented an important aspect of the care of patients with many types of cancer. However, in recent years, the advent of new-generation drugs, particularly targeted agents and monoclonal antibodies, has changed the scenario for certain neoplastic diseases. An important aspect of this is the potential for novel combination therapy approaches.

In recent years, melanoma has become a model for testing new agents that are important to the wider oncology community [1–3]. For approximately the previous 40 years, melanoma represented a significant clinical challenge to oncologists. Indeed, until 2011, outcomes relating to the treatment of melanoma were completely unsatisfactory: median overall survival (OS) was 6.2 months, median progression-free survival (PFS) was 1.7 months, only 14.5% of patients were disease free at 6 months and only 25% of patients were alive at 1 year [4]. These unacceptable outcomes helped encourage experimental and clinical research and have led to the development of numerous molecules that are active against this disease.

Over the years, there have been several attempts to improve the OS of melanoma patients with advanced disease. Combinations of different chemotherapeutic treatments and combinations of these agents with various biologic response modifiers have resulted in a greater number of objective responses (ORs), but have largely failed to improve upon the survival achieved with dacarbazine monotherapy. One of the first examples of combination

therapy in melanoma was that of dacarbazine with tamoxifen, which, although initially suggesting superiority compared with dacarbazine alone [5], has never been confirmed as providing a meaningful clinical improvement. Even the combined approach of chemotherapy with new small molecules, such as oblimersen and sorafenib, has failed to show a benefit, despite initial promise [6–9]. In particular, sorafenib, an inhibitor of multiple kinases including BRAF, a protein that is mutated in 40–50% of melanoma [10], seemed to be a targeted agent that could mark a turning point in the treatment of melanoma, given the PFS of 307 days (>10 months) reached in a Phase I study in combination with carboplatin and paclitaxel [7]. However, sorafenib did not improve outcomes (PFS and OS) when added to carboplatin plus paclitaxel in randomized Phase III studies of patients with metastatic melanoma [9,11].

Novel agents in melanoma management

In 2011, the story of melanoma management was changed completely with the approval of two drugs that were found to be effective in improving the OS of melanoma patients: ipilimumab [12] and vemurafenib [13]. These two innovative products have different characteristics. Ipilimumab, an anti-CTLA4 monoclonal antibody, is an agent that potentiates the immune system. In a three-arm, pivotal Phase III study, ipilimumab resulted in improved OS (10.1 vs 6.4 months in the control arm) when given as a second-line therapy in patients with unresectable stage III or IV melanoma [12]. An important characteristic of ipilimumab is its significant impact on OS and reduced effect on surrogate markers of survival (OR and PFS). This was why in the two Phase III trials assessing first-line [12,14] and second-line treatment, the original primary end points (PFS and best overall response rate [ORR], respectively) were modified. Moreover, a recent meta-analysis of 4846 patients treated with ipilimumab as part of a clinical trial or expanded-access program confirmed that patients who were still alive after 3 years (21%) were likely to survive longer term, indicating that there is a plateau in OS

beginning at approximately the third year and extending through to 10 years [15].

In general, the efficacy of ipilimumab is not affected by mutational status [16], although there is some evidence that *NRAS* mutations are associated with an improved outcome to immunotherapy [17]. Vemurafenib, on the other hand, is the first example of an effective molecular-targeted therapy in melanoma. Despite the failure of sorafenib as a *BRAF* inhibitor in melanoma, studies with vemurafenib have confirmed that the original concept of targeting *BRAF* is a valid strategy. Treatment with vemurafenib has resulted in a median OS of 13.6 months compared with 9.7 months with dacarbazine alone, a PFS of 6.9 months and a 57% ORR in previously untreated patients with *BRAF* V600E-mutated metastatic melanoma [18]. Vemurafenib has also been shown to have an effect at the level of the immune system, since it increases tumor-infiltrating lymphocytes [19], increases the expression of antigens associated with melanoma [19], enhances antigen presentation [20] and reduces the number of myeloid-derived suppressor cells in the tumor [21]. A second *BRAF* inhibitor, dabrafenib, has also recently been approved by the US FDA (in May 2013) for the treatment of advanced melanoma in patients with the *BRAF* V600 mutation. Dabrafenib has demonstrated equivalent efficacy to vemurafenib (PFS of 6.1 months and ORR of 50%) with a different safety profile (no photosensitivity reported and lower incidence of squamous cell carcinoma [SCC]/keratoacanthoma, but grade 2–3 fever was reported in 11% of patients) [22]. Another new agent is trametinib, a MEK inhibitor that targets MEK, an essential intermediary kinase protein within the MAPK pathway. Trametinib was approved in 2013 for the treatment of melanoma with *BRAF* V600E or V600K mutations.

Combined & sequential ipilimumab & *BRAF* inhibitor therapy

Given the impact of ipilimumab and vemurafenib on the survival of patients with advanced melanoma, together with their different characteristics (ipilimumab has a slow onset of action but with the possibility for long-term survival in 20% of cases, while vemurafenib has an immediate impact but with a median duration of response of 6–8 months), the potential to combine these two drugs in patients with *BRAF*-mutated melanoma has been a topic of much interest (Table 1). Unfortunately, a Phase I study

of these two drugs in combination showed an increase in liver toxicity such that the approach was not considered feasible [23]. As such, an alternative strategy is sequential therapy; the sequencing of vemurafenib and ipilimumab might offer a better way for patients to benefit from the effects of both drugs [24,25]. Several experiences confirm that sequential treatment with the two agents results in a greater efficacy compared with single-agent treatment, with a median survival time of approximately 20 months [24–26]. However, the finding that approximately 40% of patients progressing after *BRAF* inhibitor treatment have a rapidly progressive disease, with death occurring within 1–2 months [27], means that subsequent treatment with ipilimumab in these patients may not be possible, since the patient would be not able to receive the four cycles of ipilimumab treatment that are necessary to obtain a meaningful benefit. Such data suggest that patients with advanced melanoma and who are positive for the *BRAF* V600 mutation would benefit most from a sequential treatment regimen of ipilimumab first and then *BRAF* inhibitor therapy after progression on ipilimumab [25]. The ECOG–ACRIN EA6134 randomized Phase III trial of dabrafenib plus trametinib followed by ipilimumab plus nivolumab at progression versus ipilimumab plus nivolumab followed by dabrafenib plus trametinib at progression in patients with advanced *BRAF*^{V600}-mutant melanoma should provide further information on which treatment should be the first-line choice in sequential therapy.

Other ipilimumab combination therapies

The importance of new treatment strategies for improving the impact of ipilimumab on OS in the wild-type (WT) population is very clear. A combined approach with traditional techniques such as surgery, radiotherapy and/or chemotherapy seems potentially useful. Surgical excision of progressing lesions in a general context of response or stable disease also provides the opportunity to allow histological evaluation of the lesion, with the possibility of discovering a false assessment of progression in accordance with the new immune-related response criteria [28]. In addition, treatment with radiotherapy after induction with ipilimumab has been shown to enhance the efficacy of ipilimumab, with responses distant from the site of irradiation (abscopal effect) [29]. This effect is clearly mediated by the immune system being activated by the further effects of

Table 1. Ipilimumab plus vemurafenib combined or sequential therapy.

Study (year)	Design and patients	Results	Ref.
Ribas <i>et al.</i> (2013)	1 month of single-agent vemurafenib 960 mg twice daily followed by four infusions of ipilimumab 3 mg/kg every 3 weeks and concurrent twice-daily doses of vemurafenib (n = 6) or reduced-dose vemurafenib 720 mg twice daily with a full dose of ipilimumab (n = 4)	Dose-limiting hepatic toxicity; grade 3 elevations in aminotransferase levels developed in four patients 2–5 weeks after the first infusion of ipilimumab in combination with vemurafenib. Elevations in aminotransferase levels (grade 3 in two patients and grade 2 in one patient) developed within 3 weeks after starting ipilimumab. After the toxic effects were reviewed, the remaining two patients in the second cohort received vemurafenib alone. In addition, two patients had elevations of grade 2 or 3 in their total bilirubin levels with concomitant grade 3 elevations in aminotransferase levels	[23]
Ascierto <i>et al.</i> (2012)	Retrospective analysis of <i>BRAF</i> mutation-positive patients treated with vemurafenib 960 mg or dabrafenib 150 mg twice daily and ipilimumab 3 mg/kg every 3 weeks for four doses as part of a clinical trial or expanded access program. Ipilimumab was followed by a <i>BRAF</i> inhibitor (n = 6) or a <i>BRAF</i> inhibitor was followed by ipilimumab (n = 28)	Of the 28 patients receiving the <i>BRAF</i> inhibitor first, 12 (43%) had rapid disease progression resulting in death and were unable to complete ipilimumab treatment. Median OS for rapid progressors was 5.7 months (95% CI: 5.0–6.3) compared with 18.6 months (95% CI: 3.2–41.3; $p < 0.0001$) for those patients who were able to complete the ipilimumab treatment	[24]
Ascierto <i>et al.</i> (2013)	Retrospective analysis of <i>BRAF</i> mutation-positive patients treated with vemurafenib or dabrafenib and ipilimumab 3 mg/kg every 3 weeks for four doses as part of an expanded access program. Ipilimumab was followed by a <i>BRAF</i> inhibitor (n = 48) or a <i>BRAF</i> inhibitor was followed by ipilimumab (n = 45)	Median OS was 14.5 months (range: 11.1–17.9 months) in patients receiving ipilimumab first and 9.7 months (range: 4.6–14.9 months) in patients receiving the <i>BRAF</i> inhibitor first ($p = 0.01$). Among the 45 <i>BRAF</i> inhibitor-first patients, 18 (40%) had rapid disease progression (median OS: 5.8 months) and were unable to complete all four induction doses of ipilimumab, while the remaining 27 (60%) had slower disease progression (median OS: 19.3 months) and were able to complete the therapy with ipilimumab	[25]
Ackerman <i>et al.</i> (2012)	Retrospective analysis of <i>BRAF</i> mutation-positive patients treated with vemurafenib as part of a clinical trial (2009–2012; n = 43). 16 patients received immunotherapy (IL-2, n = 11; ipilimumab, n = 4; IL-2 then ipilimumab, n = 1) prior to vemurafenib. Ten patients received ipilimumab after disease progression on vemurafenib	Median OS was 19.3 months overall and 31.2 months in the 16 patients who received immunotherapy first. Of the ten patients receiving ipilimumab after vemurafenib, five died within 3 months of the last vemurafenib dose, and all ten had disease progression at 6 months, with a median PFS of 0.7 months and an OS of 2.2 months	[26]

OS: Overall survival; PFS: Progression-free survival.

irradiation. Recently, the effect of radiotherapy after progression to ipilimumab was reported in a small number of advanced melanoma patients [30]. Regression of a nonradiated lesion was observed in 11 out of 21 patients (52%), with a median OS of 22.4 months compared with 8.3 months for patients without this effect after radiotherapy. Another possible combination of ipilimumab with locoregional treatment is using electrochemotherapy (ECT). ECT uses a surgical technique based on the principle of electroporation, which allows some antineoplastic drugs, such as bleomycin and cisplatin, in order to better penetrate into the tumor cell and thereby increase efficacy. Such a treatment is indicated in locoregional therapy and is of interest because of its ability to attract cells of the immune system into the tumor compartment [31], as well as

probably increasing the release of tumor antigens. Preliminary data from a retrospective analysis in a small cohort of 15 patients showed the presence of responses that were distant from the site of ECT treatment [32]. Prospective studies will assess the possible role of this combination approach.

Chemotherapy provides another option for combination with ipilimumab. At present, the addition of dacarbazine to ipilimumab does not seem to considerably increase efficacy. In fact, in a pivotal first-line study [14], while dacarbazine plus ipilimumab was superior to treatment with dacarbazine alone, it did not show increased activity compared with ipilimumab monotherapy [12]. Another combination approach for ipilimumab using fotemustine seems more promising. Indeed, an Italian study showed a

promising disease control rate (DCR) of 45.5% in a population of 82 patients (and 50% in patients with brain metastases) [33]. A Phase III study conducted to confirm the efficacy of this combination is ongoing. Moreover, in the future, new chemotherapeutic agents could be used in order to enhance the activity of anti-CTLA4 treatments. For instance, this could be the case with nab-paclitaxel, which recently provided interesting results in a Phase III study in which it was compared with dacarbazine [34]. In this study, the PFS (primary end point) was 4.8 months for nab-paclitaxel compared with 2.5 months for dacarbazine. We are awaiting the analysis regarding the OS. Considering that classical chemotherapeutic agents do not seem to have a more prominent role, especially in combination with novel agents, nab-paclitaxel could be a promising candidate for new combination studies.

Combination therapy with conventional cancer agents is certainly not the only way to enhance the effects of treatment with ipilimumab. The possibility of using other forms of treatment that impact on the immune system is another opportunity for a combined approach. For example, VEGF is able to mediate a mechanism of immunosuppression [35], which would be inhibited by treatment with bevacizumab. The combination of ipilimumab plus bevacizumab in a Phase I study of 22 patients has provided interesting results, with a DCR of 64% and a 1-year survival rate of 72% [36]. A similar combination approach of bevacizumab plus MPDL3280A (an anti-PD-L1) is currently being evaluated (NCT01633970). The combination of ipilimumab and GM-CSF has also been recently investigated in a Phase II trial in which 245 patients were randomized to receive ipilimumab and GM-CSF in combination or ipilimumab alone [37]. The survival rate after 1 year of treatment in the combination arm was 68.9% compared with 52.9% in the monotherapy arm, while the median OS in the combination arm was 17.5 months compared with 12.7 months in the group of patients that only received ipilimumab. It was interesting to note a reduction in gastrointestinal and pulmonary side effects observed in the combination arm of the study. The combination or sequential therapy of denileukin diftotox with ipilimumab may also be of interest, with previous experience being promising in patients who were treated with ipilimumab after denileukin diftotox progression [38].

The potential for combining ipilimumab with other immunotherapies is also being investigated. Nivolumab and MK3475 (formerly lambrolizumab) are anti-PD1 monoclonal antibodies. PD1 is another checkpoint blockade molecule of the immune system similar to CTLA-4, but with a different mechanism of action [39,40], and both nivolumab and MK3475 have been shown to have a significant positive effect on outcomes in melanoma. In a Phase I/II study, nivolumab was associated with a median survival duration of 16.8 months, with 62% of patients surviving at 1 year and 43% at 2 years [41]. Similarly, in a Phase I study of MK3475 (pembrolizumab), approximately 34% of patients had durable ORs and improved survival [42]. Combining ipilimumab with nivolumab represents an exciting approach that has already provided interesting preclinical data [43]. In a recent Phase I study, this combination resulted in exceptionally promising data, with 53% of patients having a rapid OR and 41% of patients showing a reduction of tumor mass of >80% [44]. In addition, 82% of patients were still alive at 1 year. These are remarkable results if we consider that this is immunotherapy. These new compounds represent novel opportunities for combination approaches in melanoma together with other possible agents such as talimogene laherparepvec, an oncolytic herpes simplex virus type 1 engineered to replicate selectively in tumor cells and express GM-CSF, which recently demonstrated an improvement in durable response rate versus control (GM-CSF; 16.3 vs 2.1%, respectively) [45], or in combination with other vaccines, such as MAGE-A3 [46].

BRAF inhibitor combination therapies

The advent of vemurafenib and other molecules that are active against targets in different melanomas with other mutations, such as those in *NRAS* (found in 10–15% of patients with advanced melanoma) has led to a revolution in the treatment approaches for the patient with advanced melanoma [47,48]. This population can now be split into two different groups: those who have a mutated melanoma (*BRAF*, *NRAS* or *cKIT*) and the WT group. However, while the advent of BRAF inhibitors has certainly improved the survival of patients with melanoma, it is equally true that there are new problems, such as the occurrence of SCC and keratoacanthoma due to a paradoxical effect of BRAF inhibitors that, in the presence of *BRAF*

WT and with the *NRAS* mutation (as often occurs in the skin), they can enhance rather than inhibit MAPK activity, thereby increasing cellular proliferation. Moreover, as mentioned previously, it has been reported that approximately 40% of patients progressing after BRAF inhibitor treatment have a rapidly progressive disease, with death occurring within 1–2 months [27]. However, it is also true that these observations are from clinical trials in which BRAF inhibitor treatment was stopped at the time of disease progression. This observation forms the basis for continuing treatment beyond progression with these drugs. Previous experience has suggested that the treatment of patients with vemurafenib beyond disease progression can result in an increase in OS [49]. This may be important when considering a combined approach with the classical cancer therapies of surgery, radiotherapy or chemotherapy. Indeed, in patients with an isolated progression, additional treatment by simple surgical excision or local radiotherapy (in the context of a good general response to treatment) may be effective. Such treatment could remove the resistant clones, thus permitting continued BRAF inhibitor treatment. For example, the combination of vemurafenib with radiation treatment has greatly improved the effectiveness of treatment of brain metastases in malignant melanoma compared with either single-agent therapy, with 6-month local control, freedom from new brain metastases and OS values of 75, 57 and 92%, respectively [50]. The possibility of combining vemurafenib with chemotherapy is also currently being studied in an ongoing trial assessing the effectiveness of adding fotemustine to treatment after progression on vemurafenib (EudraCT no. 2012-004172-18).

• Combined inhibition of BRAF & MEK

Previously, it was believed that inhibition of the MEK protein (immediately downstream in the MAPK signaling pathway) might be superior to inhibition of the BRAF protein in melanoma treatment. This observation was born from pre-clinical studies in *BRAF*-mutated melanoma cell lines, in which better growth inhibition was shown with MEK inhibitors compared with BRAF inhibitors [51]. The most-studied MEK inhibitor is trametinib, which has been shown to be superior to chemotherapy (dacarbazine or paclitaxel) with regards to PFS and OR in a recent Phase III study [52]. However, comparisons of Phase III studies in the *BRAF*-mutant

population suggest that BRAF inhibition seems to give a slightly superior benefit (PFS and ORR) compared with MEK inhibitors [15,28,31]. A different approach is the combination of a BRAF inhibitor with a MEK inhibitor, the rationale for which coming from evidence that the most important mechanisms of resistance to BRAF inhibitors are related to the reactivation of the MAPK pathway (Table 2) [53]. Combination therapy with dabrafenib and trametinib has demonstrated superiority compared with dabrafenib alone in a Phase I/II trial involving *BRAF*^{V600}-mutant metastatic melanoma patients [54]. With combined therapy, PFS was 9.4 months compared with 5.8 months with dabrafenib alone, ORR was 76% compared with 54% and 79% of combination-treated patients were alive at 1 year. These results should be viewed as excellent for the monotherapy arm as well as combined therapy, given that, until very recently, the median PFS value was just 1.7 months [4]. Another interesting point regarding the combination therapy is that the increase in efficacy was accompanied by a reduction in side effects, especially the occurrence of SCC of the skin. This combination approach thus significantly reduced the previously described paradoxical effect of enhanced MAPK activity. However, fever was more frequent with the combination therapy, being observed in 71% (grade 1–4) of patients (vs 26% with dabrafenib alone). Two further Phase III trials are currently investigating the dabrafenib/trametinib combination: the COMBI-d trial (dabrafenib/trametinib vs dabrafenib; ClinicalTrials.gov identifier: NCT01584648) and the MEK COMBI-v trial (dabrafenib/trametinib vs vemurafenib; NCT01597908).

Recently, data from another interesting combination of a BRAF inhibitor and a MEK inhibitor (vemurafenib plus cobimetinib) have been reported [55]. These data demonstrated an ORR of 85%, with the median PFS not yet being reached after a median follow-up of 10 months. Finally, another combined BRAF/MEK inhibition study is ongoing, assessing LGX818 plus MEK162. This combination is very interesting because it would seem that LGX818 is a much more potent BRAF inhibitor than either vemurafenib or dabrafenib, and so is capable of a more durable response (~10 months in a Phase I trial) and has a more favorable safety profile (absence of photosensitivity and fever and lower incidence of SCC) [56].

Table 2. BRAF inhibitor plus MEK inhibitor combination therapy.

Study (year)	Design and patients	Results	Ref.
Flaherty <i>et al.</i> (2012)	Open-label study of patients with metastatic melanoma and <i>BRAF</i> V600 mutations (n = 162) randomized to combination therapy with dabrafenib 150 mg plus trametinib 1 or 2 mg (150/2 group) or dabrafenib monotherapy	Median PFS in the combination 150/2 group was 9.4 months compared with 5.8 months in the monotherapy group (hazard ratio for progression or death: 0.39; 95% CI: 0.25–0.62; p < 0.001). ORR was 76% with combination 150/2 therapy compared with 54% with monotherapy (p = 0.03). Dose-limiting toxic effects were infrequent in patients receiving combination therapy (cutaneous squamous cell carcinoma occurred in 7% of patients receiving combination 150/2 and in 19% receiving monotherapy, whereas pyrexia was more common in the combination 150/2 group than in the monotherapy group [71 vs 26%])	[54]
McArthur <i>et al.</i> (2013)	Phase Ib dose-escalation and expansion study of patients with <i>BRAF</i> ^{V600} -mutated melanoma who were BRAF inhibitor naive or had disease progression on vemurafenib (n = 115). Patients in the dose-escalation portion received vemurafenib 720 or 960 mg twice daily continuously and cobimetinib 60, 80 or 100 mg once daily 14 days on/14 days off (14/14); 21 days on/7 days off (21/7); or continuously (28/0). Two dose levels were expanded: vemurafenib 720 and 960 mg + cobimetinib 60 mg once daily 21/7	BRAF inhibitor-naïve patients had a 73% confirmed response rate; median PFS is currently immature. Dose-limiting toxicities were observed in four patients in the vemurafenib 960 mg twice daily + cobimetinib 60 mg once daily 28/0 cohort, including mucositis (n = 1) and arthralgia (n = 1)	[55]
Kefford <i>et al.</i> (2013)	Ongoing Phase Ib/II study of LGX818 and MEK162 in BRAF inhibitor-naïve and -pretreated patients with <i>BRAF</i> -mutant tumors. To date, doses have included LGX818 once daily + MEK162 twice daily dosed at 50 + 45 mg, 100 + 45 mg, 200 + 45 mg and 400 + 45 mg, respectively (n = 20)	No dose-limiting toxicities have been observed at the dose levels administered to date. No events of fever, hand–foot–skin reactions, hyperkeratosis or squamous cell carcinoma have been observed	[56]

ORR: Overall response rate; PFS: Progression-free survival.

Other MEK inhibitor combination therapies

Although the combination of BRAF inhibitor and MEK inhibitor is very effective, the problem of resistance developing remains. For this reason, the possibility of combining MEK inhibitors with other active drugs is being evaluated. Studies are underway for combinations of MEK inhibitors with ipilimumab (NCT01767454, NCT01940809) and anti-PD-L1 (NCT01656642). It will be very important to verify the feasibility of these combinations, especially since previous studies of vemurafenib in combination with these agents demonstrated prohibitive hepatic toxicity when both agents were used at full dose [57]. However, dose-escalation studies were not performed.

Another possibility for increasing the effectiveness of these compounds is to combine them with other agents that can affect the target molecules that are upregulated in the resistance processes. For instance, in a preclinical model, the upregulation of EGFR [58] or phospho-ErbB3 [59] has been described after treatment with a BRAF inhibitor plus a MEK inhibitor, with the efficacy of

the combination being increased by adding a compound that is capable of downregulating the process that is activated by the mechanisms of resistance [59]. Inhibiting the CDK4 cyclin seems to offer the possibility of treatment in melanoma [60]. The p16–cyclin D–CDK4/6–retinoblastoma protein pathway (CDK4 pathway) is deregulated in 90% of melanomas and could be a good therapeutic target because CDK4 may promote cell-cycle progression and inhibit both cell senescence and apoptosis. A trial of triple-combination therapy with the compounds LGX818, MEK162 and LEE011 (an inhibitor of cyclin CDK4/6) is ongoing. In preclinical studies, LEE011 seems to further enhance the efficacy of combined BRAF/MEK inhibition [NOVARTIS, DATA ON FILE].

MEK162 was the first MEK inhibitor to be tested on patients with *NRAS*-mutated melanoma [21], a population representing 10–15% of melanoma patients. In a Phase II study, MEK162 showed an ORR of 22%, with a DCR of approximately 60% and a PFS of 3.8 months. There are currently several ongoing Phase III studies that are exploring the efficacy of MEK

inhibitors versus dacarbazine treatment in the *NRAS*-mutated population. Combination studies with immunotherapy are also under discussion. However, while the BRAF inhibitors have a potentiating effect on the immune system, MEK inhibitors have a possible reverse effect, reducing the secretion of cytokines [61,62] and reducing the activity of T lymphocytes [20] and dendritic cells [63]. For these compounds, sequencing with immunotherapeutic agents (e.g., ipilimumab, nivolumab and MK3475, among others) is perhaps more plausible than combined therapy.

Conclusion

In general, the data that have recently emerged appear to further suggest a revolution in the treatment of patients with advanced melanoma. The immune escape mechanisms of melanoma are now recognized as a new feature of the cancer, and the currently available treatments seem to be remarkably effective. Indeed, the new and emerging immunotherapeutic treatments have shown significant improvements in survival and will likely lead to changes in cancer treatment guidelines in the future, especially in combination and/or sequence.

Future perspective

Targeted agents in combination or in sequence with new immunotherapeutic compounds represent the future, not only for the treatment of melanoma, but also for the treatment of cancer in general. This is a real therapeutic revolution that is occurring across the entire spectrum of oncology. The potential for many cancers to become a chronic disease is perhaps not so far away.

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