Adjuvant Treatment of Melanoma: Recent Developments and Future Perspectives

Adjuvant treatment of melanoma - recent developments and clinical trial outcomes

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ABSTRACT

For early melanoma, surgical excision is the treatment of choice and this strategy is initially curative for the majority of patients. However, only approximately 40-60% of patients who have surgery alone and higher risk stages, will be disease-free after 5 years of follow up, depending on the original III stage of the disease. These patients will relapse either with locoregional or disseminated disease. Adjuvant therapies are required to be able to reduce the recurrence rate on radically operated patients in these different initial stages of the disease.

New treatments have appeared in the landscape of metastatic melanoma and this have opened to new potential scenarios in the adjuvant setting. In particular immunotherapy, immunocheckpoint inhibitors and target therapies have been recently published their potential advantage from the results obtained in the curative setting for stage IV, where the different mechanisms of action could even be potentially more active and more responsive due to the limited subclinical presence of disease in the patients after surgical complete resection.

Currently, interferon alfa (IFN), ipilimumab and more recently antipd1 are immunotherapeutic options are approved for adjuvant treatment of melanoma in US, while in EU only IFN is for clinical use. Other adjuvant treatments have been published and are currently in the phase of approval trough FDA and EMA, based on the results of clinical trials that include PD-1 inhibitors and small-molecule BRAF+ MEK inhibitors.

Actually the first study designed to answer this question is the Keynote 054 (pembrolizumab (MK-3475) versus Placebo after complete resection of high stage III Melanoma) which, through a cross over plan on recurring patients, will be able to define if patients treated in the adjuvant setting will describe a better survival compared to patients treated after recurrence.

A completely new scenario will also become evident from the opportunity to open the therapeutic approach from a neoadjuvant setting: since new therapies are available, patients with macroscopic nodal metastases so far considered operable, might be sent to a medical approach in stage III and surgery proposed only as final resource both to remove disease residuals and to confirm the efficacy of the treatment, while in advanced stage III/IV not operable at the diagnosis of an initially disease advanced situation, patients may partially respond to the new therapies and after obtaining a partial/complete reduction of the disease, become virtually operable from the surgical point of view.

1.Introduction

Melanoma accounts for a small percentage of all skin malignancies, but it is responsible for the majority of deaths due to skin cancers worldwide[1]. Moreover, cause of the increasing aging of the population, the age at death of melanoma patients has steadily increased, with present predictions showing that the number of melanoma cases will increase. Due to the introduction of new systemic drugs, we assist to an increase survival for advanced, unresectable and metastatic melanoma over the last few years. This unprecedented development is related to the introduction of immune checkpoint inhibitors with antibodies against CTLA-4 and PD-1 and targeted therapy with BRAF and MEK inhibitors.

The recent developments and approvals in immunotherapy and targeted agents that have significantly changed the landscape of melanoma therapy in the metastatic setting can represent a great promise for adjuvant and neoadjuvant treatment in high-risk or advanced locoregional disease.

All adjuvant approaches had to be initially tested on an advanced disease therapeutic approach. This review of historical and recent drugs is willing to report on the situation we are facing at the moment where we stand in front of a new era in the therapeutical approach of advanced melanoma patients which is the basis of novel approaches in the adjuvant settings as recent studies come to publication and start their approval pathways through regulatory agencies Worldwide. In this review for an easier comparison between old and recent studies we will consider the 7th American Joint Comitee on cancer (AJCC)[2] and not the 8th [34].

2.STAGE II-III

Melanoma patients with intermediate and thick tumours are offered a sentinel node biopsy (SLNB) to identify lymph node spread as this procedure has prognostic value, stratifying patients in different risk categories[5]. Other parameters such as mitoses and ulceration are also helpful in thinner melanomas[6]. After a positive SLN, the current guidelines recommended a complete lymph node dissection (CLND) of all the involved metastatic basins but in selected patients (to be defined if with very small deposits in the lymph node, as for breast cancer patients or with macroscopic disease due to a virtual situation of microscopic subclinical advanced disease[7]) may be given the choice of avoiding a lymphadenectomy. The final results of the DecoG and MSLT II trials[8,9] may be useful to propose new biologically driven guide lines on N+ patients, but a careful discussion will be needed once all data are supported by longer follow up periods: so far the published data on these 2 trials do not show any survival benefit for the patients undergoing a CLND after the diagnosis of metastatic SLN, but only a reduced risk of nodal locoregional relapse for the patients immediately operated. On the contrary if the subgroups analysis on MSLT II study is brought to a speculative discussion, the opposite seems to be more rational: patients with microscopic deposits in the SLN could benefit from a CLND, while patients with macrometastases would reach the same OS whether operated or not with an immediate CLND. The biological explanation of this different behaviour can be related to the fact that a certain percentage of patients with micrometastases could only have few tumoral cells into the nodes

that are removed with a CLND, while in case of macrometastases, the disease may have also spread heamatogenously, making totally un-useful the proposed CLND.

The number of positive lymph nodes (LN) and its ratio represent the two most important prognostic factors in stage III melanoma patients[10,11]. The 5-year survival of melanoma patients with LN metastasis ranges from an average of 20-40% when patients present with clinically evident nodal disease, improving to 67% when patients had their LN metastasis identified with SLNB. Patients who have CLND after a positive SLNB show a wide heterogeneity in their prognosis, with 5-year survival rates ranging from 15% in case of multiple positive LNs to 90% in case of a small cellular metastatic deposits in the SLN where the prognosis is even more favorable then that of high risk stage II patients (>4 mm Breslow, ulcerated primary melanoma pts.).

The risk of recurrence in stage III patients is very wide, and no available biomarker for predicting recurrence have been established so far. The best predictor of recurrence is the number of LN involved. Different nomograms based on clinical pathological features have been proposed for predicting which patients with positive SLN are more at risk of relapse[12].

3. BIOCHEMOTHERAPY

Cisplatin and interleukin-2 (IL-2)–based biochemotherapy have been used for stage III melanoma. The trial on 432 high-risk patients assigned to either three cycles of cisplatin, vinblastine, dacarbazine, IL-2, and IFN-α given over a 9-week period or to high-dose IFN-α for 1 year. Results showed that the biochemotherapy regimen significantly prolonged RFS at a median follow-up of 7.2 years [13]. However, there was no significant difference in the OS (5-year rate 56% for both treatment arms; HR 0.98; 95% CI, 0.74–1.31). The biochemotherapy regimen was substantially more toxic, with grade 3 or 4 side effects (consisting primarily of hematologic and gastrointestinal toxicity) observed in 76% of participants. Neurologic, psychiatric, and hepatic toxicities were the most frequent with high-dose IFN-α. Biochemotherapy toxicities were limited to the 9-week treatment period, while IFN-α toxicities were distributed across the year of

treatment. Even though the biochemotherapy regimen was the first to produce a significant improvement in RFS compared with an active control arm, the lack of OS benefit coupled with the failure of this regimen to show an OS benefit relative to chemotherapy alone in patients with stage IV disease has limited its acceptance in the adjuvant setting. [14]

4.Adjuvant CT with/without BCG/INF

Hypothetically, non-detected melanoma micrometastases might be the cause of future relapses and/or may induce tumor tolerance in the host. Different clinical trials comparing patients treated after CLND with immunotherapy with interferon-alpha-2b (IFN-α-2b), bacille Calmette–Guérin vaccine, dacarbazine, or a combination of the last two failed iun showing a higher survival ratein the treatment arm. [151617]

A regimen of IFN-α-2b administered for 1 year at maximum tolerated doses was approved by the FDA in 1995 and later on by EMA for the adjuvant therapy of patients with high-risk (AJCC Stage IIB and III) melanoma: up to now this is still the only approved drug in Europe for melanoma patients in the adjuvant setting.

Adjuvant IFN-α therapies, which could induce TH1 anti-tumor responses, are based on these hypotheses and might be of benefit to some patients with possible micrometastases [18].
IFN-α directly inhibits the proliferation of melanoma cells. Moreover, IFN-α decreases intracellular and secretory levels of VEGF in melanoma cell lines [19], thus reducing microvessel density around the tumour. It has been described to be able to promot tumor immunogenicity and enhances anti-tumor immunity. MHC class I Expression has been analysed by several studies on both melanoma and immune cells when stimulated with IFN-α[20]. The use of IFN-α as an adjuvant therapy in melanoma patients is based on the hypothesis that micrometastatic disease is the cause of future relapses and may induce tumor tolerance in the host. Unfortunately the global efficacy on overall survival is as low as 3%.

During more then 3 decades, low- (LDI), intermediate (IDI)- and high-dose (HDI) IFN-α regimens have been tested in randomized trials in the adjuvant setting[21,22]; these studies greatly differed also in terms of the therapy duration, route of administration and the type of IFNs used.

As just mentioned, the most important discussion is clearly on the dosage: a significant impact on overall survival (OS) was only shown with the high-dose IFN-α2b intravenous regimen (HDI) when compared to observation only (US Intergroup trials E1684: median OS 3.82 vs 2.78 years, p=0.0237) and the GMK vaccine (E1694: OS HR=1.52; P= 0.009).. The outcomes of the E1684 trial in 1995 led to the regulatory approval of IFN by the US Food and Drug Administration (FDA). However, these results were not confirmed in the following E1690 trial that compared HDI *versus* LDI *versus* control. In fact it failed in demonstrating a significant benefit of the HDI, but bearing more adverse events [23]. Furthermore, different randomized trials reported other conflicting results, never offering the real hint to the therapeutic benefit of IFN. The randomized phase III DeCOG trial compared LDI vs LDI plus dacarbazine vs observation in stage III melanoma patients. The Authors found a DFS and OS for the LDI regimen, and, interestingly, a worse therapeutic effect when dacarbazine was added [24].

The EORTC 18952 adjuvant IFN trial was designed to investigate also if an antiangiogenic effect could be relevant in the potential benefit of adjuvant IFN[25]. In this trial researchers compared: I) a 4 weeks-induction phase using IFN at 10 million IU/m2/d for 5 days/week for 4 weeks, followed by a maintenance phase with 10 million IU three times a week for 12 months; II) 5 million IU 3 days/week for 24 months; III) observation alone. After the long median follow-up of 11 years, the only difference reported was the distant metastasis-free interval with an HR of 0.95 for the shorter maintenance group *versus* HR of 0.82 for the longer maintenance group (p=0.027).

A metanalysis found that IFNα slightly improved DFS (risk reduction=18%) and OS (risk reduction=11%) in high-risk cutaneous melanoma patients, however, the different studies analysed did not show any differences between low and high dosages[26]. Wheatley *et al.* reported a 5-year absolute benefit of about 3%, with greater efficacy in patients where the primary tumor was ulcerated[27] .

Clinical trials comparing adjuvant HDI to ipilimumab (NCT01274338, NCT01708941, NCT02506153) or to pembrolizumab (NCT02506153) are ongoing, and the results are still pending.

Pegylated IFNα (peg-IFN), which should have a longer half-life through IFN's covalent binding to polyethylene glycol, was tested in the European Organization for Research and Treatment of Cancer (EORTC) trial 18991[28]. The trial tested an induction dosage of subcutaneous peg-IFN at 6 μg/kg/week for 8 weeks, followed by a maintenance dose of weekly subcutaneous injections at 3 μg/kg for up to 5 years. A rather slight improvement of the relapse free survival for the peg-IFN was reported (7-year RFS rate: 39.1% *versus* 34.6%) but authors did not find any differences in OS and distant metastasis-free survival between the treatment and the sole observation group. A pooled analysis of the EORTC trials 18952 and 18991 found that the primary tumor ulceration and the presence of only micrometastases as lymph nodal involvement, could be predictive of IFN efficacy[29]. Moreover, one study reported peg-IFN's association with higher rates of grade 3-4 Adverse Events (47.3% *versus* 25.2%; $p<0.0001$) and treatment discontinuations (54.3% *versus* 30.4%) compared to IFNα[30].

6.VACCINE FOR ADJUVANT TREATMENT

Dendritic cells (DC) are the most efficient antigen-presenting cells of the immune system due to their capacity to activate and prime naive T cells[31]. They play a fundamental role in anticancer immunotherapy due to their role in induction of antitumor immunity. DC can be generated ex vivo, activated, and loaded with tumor antigens before to be injected into the patients.[32] The rationale to include DC vaccination in the adjuvant treatment in stage III patients is that high tumor load causes immune suppression by secretion of immunosuppressive cytokines, and attraction of regulatory T cells and myeloid derived suppressor cells in the tumor microenvironment. The clinical effectiveness of DC vaccination might be improved by increasing the number of antigens. The melanoma differentiation antigens gp100 and tyrosinase were previously selected due to their expression on melanoma cells and have shown to be capable of inducing functional cytotoxic T cells.[33] However, recent findings show that tumor-specific mutations, leading to neoantigens, may drive potent antitumor responses[34]. Carreno and colleagues found that a DC vaccine with carefully selected patient-specific neoantigens, led to an increase in the breadth and diversity of melanoma neoantigen-specific T cells in peripheral blood samples of three stage IV melanoma patients[35]. As most mutated proteins are essentially unique to a tumor, personalized antigen selection might be beneficial in vaccination strategies. A great

challenge will be the identification of the right immunogenic neoantigens, especially in stage III melanoma patients, since only a minimal amount of tumor material might be available[36]. For this reason, and in light of tumor heterogeneity, it might be preferable to combine commonly expressed melanoma differentiation antigens with patient-specific neoantigens in future DC vaccines.

7.Adjuvant vaccine with melanoma antigen GM-2 ganglioside

The GM2 ganglioside is an antigen expressed in the majority of melanomas. The GM2-KLH/QS-21 vaccine induces high immunoglobulin M (IgM) and IgG antibody responses documented in early phase clinical trials at the EORTC melanoma group[37]. The EORTC 18961 trial compared the efficacy of GM2-KLH/QS-21 vaccination versus observation on high risk primary melanoma (with negative SLN). A total of 1,314 patients with a primary tumor > 1.50 mm in thickness were randomly assigned to GM2-KLH/QS-21 vaccination (n = 657) or observation (n = 657). Treatment consisted of subcutaneous injections once per week from week 1 to 4, then every 3 months for the first 2 years and every 6 months during the third year. Relapse-free survival (RFS) was the primary endpoint. Secondary endpoints were distant metastasis-free survival (DMFS) and OS. After a median follow-up of 1.8 years, the trial was stopped at the second interim analysis for futility regarding RFS (hazard ratio [HR], 1.00; $P = .99$) and detrimental outcome regarding OS (HR, 1.66; $P = .02$). After a median follow-up of 4.2 years, 400 relapses, nine deaths without relapse and a total of 236 deaths had been recorded. At 4 years, the vaccination arm showed a decreased RFS rate of 1.2% (HR, 1.03; 95% CI, 0.84 to 1.25) and OS rate of 2.1% (HR, 1.16; 95% CI, 0.90 to 1.51). GM2-KLH/QS-21 vaccination does not improve outcome for patients with stage II melanoma and may induce immune tolerance to tumoral antigens with a consequent worsening of the prognosis.

8.MAGE –A3

MAGE-A3 is expressed on approximately 60% of melanoma specimens as a tumour specific protein. The DERMA (ADjuvant ImmunothERapy with MAGE3 in MelanomA) is a double-blind, randomized, placebo-controlled phase III study of recombinant MAGE-A3 with AS15 antigen-specific cancer immunotherapeutic (ASCI) in stage IIIB/C patients with MAGE-A3–positive. The study was based on an EORTC phase II study of patients with stage IV M1A disease that identified a superior survival benefit for patients receiving the MAGE-A3 vaccine treated with the AS15 rather than the ASO2B adjuvant (HR 0.55; 95% CI, 0.28–1.06) [38].
Furthermore, a genetic predictor identified a group of patients receiving MAGE-A3 with AS15 ASCI with a better OS (HR 0.27; 95% CI, 0.08–0.89). The DERMA study screened 3,914 patients and randomly assigned 1,344 patients 2:1 to 13 intramuscular injections of vaccine or placebo. The latter failed to meet its coprimary endpoint of DFS in either the overall population of patients studied or in those with the potential predictive gene signature.
To further investigate the role of the Gene Signature (GS) in predicting a response to MAGE-A3 immunotherapeutic the PREDICT study was conducted in 49 centers in Europe and the United States on advanced melanoma patients. Because of frequent rapid progression in M1b-c melanoma, patients naive to previous systemic treatment with non-resectable stage IIIB-C and IV-M1a melanoma were included. This phase II study was not controlled, as placebo administration is unethical in this population and no highly effective treatment was available at the time of study design.
The OS of MAGE-A3-positive patients with unresectable stage IIIB-C/IV-M1a demonstrated an overall 1-year OS rate of 83.5%. 1-year OS rates did not change when stratified in GS− and GS+ patients, indicating that in this study, GS was not predictive of outcome. Unexpectedly, the objective response rate was lower in this study than in other studies carried out in the same setting with the MAGE-A3 immunotherapeutic.

The MAGE 3 vaccine will probably no more undergo clinical investigations in the future, both in the adjuvant or therapeutic settings.

9.BEVACIZUMAB

Since Bevacizumab has reported to have some activity in patients with advanced melanoma, it has been valuated in the adjuvant setting. In a phase III multicenter trial conducted in the United Kingdom, 1,343 patients with resected stage IIB, IIC, or III disease were randomly assigned to receive 1 year of bevacizumab treatment (7.5 mg/kg every 3 weeks) or to observation. [39] Results showed no significant difference in OS at a median follow-up of 25 months, (HR 0.97; 95% CI, 0.78–1.22; p = .76). However, there was a significant increase in the DFS (1-year and 2-year disease-free rates 77% vs. 70% and 59% vs. 57%, respectively; HR 0.83; 95% CI, 0.70–0.98). Interpretation of the trial and the potential role of bevacizumab will require further follow-up to assess the 5-year OS rate. Until then, the use of bevacizumab in the adjuvant setting for patients with advanced melanoma is not recommended.

10.RADIATION THERAPY AS ADJUVANT TREATMENT

Adjuvant radiation treatment following CLND in the melanoma patient population has been suggested and investigated in order to gain regional control and consequently to improve survival[40]. Disease-free, survival rates and complications drive important issues on the therapeutic post CLND discussions. Historically, melanoma has been thought to be a relatively radioresistant tumour. Nowadays, radiation delivered according to the hypofractionated schedule is the most used, although there are no data to confirm that this schedule improves the therapeutic impact. Almost all the reviewed studies were retrospective, which could have led to an underestimation of the true incidence of the treatment toxicity and morbidity. Improved Loco regional control, but not OS has been reported when performing adjuvant radiotherapy after CLND for metastases of melanoma[41]. A recent study is describing an improvement in a subgroup of patients with a particular gene expression signature who would probably benefit from adjuvant radiotherapy[42]. The available data indicate the need for improved regional control rates in patients with extranodal extension, multiple involved nodes (more than three) and patients with large involved nodes (larger than 3 cm)[43]. The complications seem manageable and consist mainly of fibrosis and edema. This treatment is mentioned in most guide lines where a discussion case by case is suggested after CLND.

11.TRIAL IN RESECTED STAGE II

12.NEW DRUGS IN STAGE III

It is well known that T-cell responses are regulated through a complex balance of inhibitory and activating signals and that the tumour itself can dysregulate these pathways, leading therefore to an impairment of the immune system activities. The relevant new concept that was developed following the failure of cytokine-based immunotherapy and the increasing evidence of the clinical activity of different target therapies in several cancer types was constituted by the potential of targeting these inhibitory and activating immunological synapses as a new tool to promote the immune response[44]. Until now, two main types of immune modulating drug antibodies have been developed and used in the treatment of advanced metastatic melanoma, the first targeting the CTLA-4 antigens, the other the PD-1/PD-L1 pathway (Table1).

12.1IPILIMUMAB IN STAGE III

The European Organisation for Research and Treatment of Cancer (EORTC) concluded the 18071 study on adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma patients[45]. It is the first trial with an immune checkpoint inhibitor used postoperatively after lymph node dissection, showing significant improvement in recurrence-free survival and overall survival (5-year RFS rates 40.8% vs. 30.3% and 5-year OS rates 65.4% vs. 54.4%, respectively).

Despite clear benefits in reduction of risk of death, the use of adjuvant ipilimumab has not reached a global use: only FDA has approved the drug, while EMA did not activate the discussion on the same clinical indication. Considering the significant adverse event rates (grade 3–4 immune-related adverse events occurred in 41.6% of patients treated with ipilimumab as compared to 2.7% in placebo arm), resulting in only 42% of patients receiving more than four

doses of ipilimumab, the survival benefit of ipilimumab over placebo was generally consistent across subgroups. This benefit was observed not only in patients with microscopic involvement in the SLN but also in patients with macroscopic or palpable nodes. Similarly, in contrast to interferon alfa, for which ulceration is the overriding determinant of activity, ipilimumab prolonged survival among patients with nonulcerated melanoma and among those with ulcerated melanoma and with no difference in terms of metastatic nodal involvement. The main topic for discussion has to be the dosage for IPI adjuvant administration: the EORTC study was designed to propose the same dosage of 10 mg/m^2 for 3 years following the very first findings and study results in advanced melanoma[46], but later the dosage of 3 mg/m^2 was defined as efficacy as the higher dosage in this more advanced melanoma patients setting, so it appears irrational to approve for adjuvant use a drug that should be used at a more toxic, prolonged and expensive schedule than the same for an advanced disease indication.

In conclusion, it should be acknowledged that considering the low number of patients who had received the induction and mantainance phase of ipilimumab in the trial, the severe toxicity in a large number of patients, plus the lack of difference in DFS between the two dosage (3mg/kg and the 10 mg/kg) reported in the ASCO abstract[47] (cit) had raised doubts on the usage of this drug as potential new candidate worldwide as adjuvant therapy in melanoma.

12.2ANTI PD-1 IN STAGE III

Anti PD1 have beenapproved as first line treatmetn for metastatic melanoma.

Nivolumab and ipilimumab value in the adjuvant setting has been evaluated in a randomized, double-blind, phase 3 trial, randomly assigned 906 patients (≥15 years of age) who were undergoing complete resection of stage IIIB, IIIC, or IV melanoma to receive an intravenous infusion of either nivolumab at a dose of 3 mg per kilogram of body weight every 2 weeks (453 patients) or ipilimumab at a dose of 10 mg per kilogram every 3 weeks for four doses and then every 12 weeks (453 patients)[48]. The period of treatment was up to 1 year or until disease recurrence, a report of unacceptable toxic effects, or withdrawal of consent. The 12-month rate of RFS primary endpoint was 70.5% in the nivolumab group and 60.8% in the ipilimumab group. Grade 3 or 4 adverse events that investigators deemed to be related to a trial drug were reported in 14.4% of the patients in the nivolumab group and in 45.9% of those in the ipilimumab group. There were 2 deaths (0.4%) from toxic effects (marrow aplasia and colitis, both of which

occurred more than 100 days after the last dose) in the ipilimumab group and no treatment-related deaths in the nivolumab group. The pathway for approval is under way through the regulatory agencies Worldwide (a part from the USA) and they will be approved by the time this article will be published.

Concerning Pembrolizumab, Keynote 054 tried to assess whether post-resection adjuvant therapy with pembrolizumab improves recurrence-free survival (RFS) as compared to placebo for high-risk participants with melanoma (Stage IIIA [> 1 mm metastasis], IIIB and IIIC). Participants were stratified for stage of disease and region and then were randomly assigned to receive either pembrolizumab or placebo. 1019 stage IIIA-C melanoma patients were enrolled in the study.[49]

Patients were randomized to 200 mg of pembrolizumab (n = 514) or placebo (n = 505) intravenously every 3 weeks for a total of 18 doses (approximately 1year) or until disease recurrence or unacceptable toxicity. Regarding - BRAF status, 40.9% had a V600E or V600K mutation, 6.8% had another mutation, 45.3% were wild-type, and the status was unknown for 7.0%. The primary endpoint (RFS rate) was 71.4% in pembrolizumab arm, (CI: 95%, 66.8–75.4) versus 53.2% in placebo arm (CI: 95%, 47.9–58.2). An RFS benefit with the PD-1 inhibitor was observed across patients with either stage IIIA, IIIB, or IIIC disease. Grade 3–5 treatment-related adverse events occurred in 14.7% of the pembrolizumab arm versus 3.4% of the placebo group.

The 1-year RFS rate was 77.1% in the PD-L1–positive group, (CI: 95%, 72.7–80.9) in the active arm and 62.6% (CI: 96%, 57.7–67.0) in the placebo one (HR, 0.54; CI: 95%, 0.42–0.69; P < .001). The 18-month RFS rates were 74.2% versus 54.5%, respectively.

Among PD-L1–negative patients, the 1-year RFS rates were 72.2% (CI: 95%, 58.6–82.0) in the pembrolizumab arm versus 52.2% (CI:95%, 38.2–64.5) in the placebo group (HR, 0.47; CI:95%, 0.26–0.8; P = .01). The 18-month RFS rates were 60.6% versus 52.2%, respectively [37].

In BRAF V600E positive patients, the 1-year RFS rate was 72.5% with pembrolizumab versus 58.6% with placebo (HR, 0.57; CI: 99%, 0.37–0.89; P = .0009). The 18-month RFS rates were 69.2% versus 52.4%, respectively [37].

On the other hand, among BRAF wild-type patients, the 1-year RFS rate was 73.0% with pembrolizumab versus 59.7% with placebo (HR, 0.64; CI: 99%, 0.42–0.96; P = .0039). The 18-month RFS rates were 66.7% versus 48.8%, respectively.

The SWOG S1404 is a Phase III trial comparing high-dose IFNα with pembrolizumab (at doses of 200 mg) in patients with high-risk resected melanoma (stages III A-C and IV with no evidence of disease) for 52 weeks [50]. Primary outcomes include OS and RFS (ClinicalTrials.gov number: NCT02506153). This trial will offer new elements regarding the adjuvant treatment topic in melanoma.

12.3 BRAF INHIBITORS IN STAGE III

Approximately 50% of melanoma tumors have an activating mutation in the BRAF oncogene, which results in the constitutive activation of the MAP kinase signaling pathway[51]. Potent selective antagonists of mutant BRAF (vemurafenib and dabrafenib) are regarded as standard of care for metastatic BRAF mutant melanoma[52] [53] Of at least as much clinical importance, however, is the fact that approximately 90% of patients whose cancers carry the BRAF mutation have some tumor shrinkage with targeted inhibitors and that these responses occur very rapidly. This rapid response rate can provide palliative relief for patients with significant tumor-related symptoms but usually lasts 6 to 9 months when often a fast recurring disease appears and other therapies need to be considered [54] [55] [56] [57] [58].

Activated BRAF phosphorylates MEK, the next downstream target in the MAP kinase pathway. Trametinib and Cobimetinib are selective MEK inhibitors and both have been shown to improve median progression free and overall survival in comparison to chemotherapy in BRAF mutant melanoma[6264],[59] Dual inhibition of both BRAF and MEK pathways in BRAF mutant disease is now proven to be significantly more potent than single agent inhibition in advanced melanoma patients.

12.4Single drug adjuvant treatment with BRAF inhibitor

The BRIM 8 study was designed as a double bling placebo controlled study of adjuvant vemurafenib in patients with completed resected BRAF V600+ mutant melanoma at high risk for

recurrence. Results from this study showed a clinical benefit of the treatment arm in stages IIC-IIIB (HR=0.54, p<0.001) but not for stage IIIC (HR=0.80, p=0.26), when survival estimates curves are not significantly different. Considering the study design, the primary DFS endpoint was not met in patients with resected stage IIIC *BRAF*$^{V600+}$ melanoma, although one year of adjuvant vemurafenib showed a numerical DFS benefit in patients with resected stage IIC/IIIA/IIIB disease.

The role of BRAF inhibitor alone should be put in the context of recently reported trials.

The placebo-controlled COMBI-AD study showed that combination adjuvant treatment with a MEK and a BRAF inhibitor is able to reduce the risk of recurrence in patients with resected stage III *BRAF*$^{V600+}$ melanoma (HR 0·47, 95% CI 0·39–0·58; p<0.001) (referenza). In addition, a recent head-to-head adjuvant study (CheckMate 238) comparing nivolumab versus ipilimumab in patients with resected stage IIIB/C–IV melanoma showed that nivolumab significantly reduced the rate of recurrence or death (HR 0·65, 95% CI 0·51–0·83, p<0.001) with a lower incidence of grade 3/4 events (25·4% *vs* 55·2%) and AE-related discontinuation rate (9·7% *vs* 42·6%)[56]. Based on these results, it is clear that combination adjuvant treatment with BRAF and MEK inhibitors or single-agent nivolumab provide more favourable DFS and survival outcomes in patients with melanoma who are at high risk of recurrence. Although, we cannot exclude a role for single-agent BRAF inhibitors within certain disease substages (IIC) of this patient population, although there are no ongoing or planned studies to explore this.

The treatment with single drug BRAF inhibitor/s side effects may be one important aspect to consider for not approving the treatment in the adjuvant settings: these include a variety of different effects with the majority occurring on the skin and appendage[60,61,62]. The most common adverse events recorded in the BRIM-3 registration trial included arthralgia, fatigue, nausea, rashes, photosensitivity and cutaneous squamous cell carcinoma (cSCC) or keratoacanthoma (KA)[63]. The dose was modified or interrupted due to adverse events in 38% of patients treated

with vemurafenib and permanently discontinued in only 7% of the patients treated. When transferring these treatment/s from advanced melanoma patients into a concept of adjuvant therapy all these aspects have to be considered: globally a high percentage of patients would not benefit from any adjuvant therapy as already cured by surgery: a treatment with important side effects and an alteration of the quality of life with no selection on the patients who may really benefit form a treatment is not going to be easily accepted by most patients.

12.5Combination target therapy

The results of the fase III COMBI-AD adjuvant study Adjuvant Dabrafenib plus Trametinib in Stage III *BRAF*-Mutated Melanoma have been positive and will certainly modify the clinical practice in the next future. This was a double-blind, placebo-controlled, phase 3 trial, enrolling 870 patients with completely resected, stage III melanoma with *BRAF* V600E or V600K mutations that were randomly assigned to receive oral dabrafenib at a dose of 150 mg twice daily plus trametinib at a dose of 2 mg once daily (combination therapy, 438 patients) or two matched placebo tablets (432 patients) for 12 months. Primary end point was the RFS and showed the 58% in the combination-therapy group versus 39% in the placebo arm group ($P<0.001$). The main secondary end point was overall survival and formally did not reach the forecasted results but in any case the 3-year overall survival rate was 86% in the combination-therapy group and 77% in the placebo group ($P = 0.0006$), instead of the prespecified interim analysis boundary of $P=0.000019$. The safety profile of dabrafenib plus trametinib was consistent with that observed with the combination in patients with metastatic melanoma. [64] The pathway for approval in the adjuvant setting is under way through the regulatory agencies Worldwide and might be approved by the time this article will be published.

13.FUTURE DEVELOPMENT

The introduction of the adjuvant setting would change our way of looking and studying the disease, swithching from the main goal that was disease free survival to a longer vision of the disease. Already in the era of immunotherapy we have assisted to longer melanoma specific survival even despite quick relapse free survival or even progression after first relapse.

The use of PD-1 inhibitors in the adjuvant setting has been a major discussion topic in last years. Some of the challenges of assessing these drugs in the adjuvant setting include choosing a comparator arm, due to the standard of care being unclear, patient selection, unexpected toxicity, and deciding how long to treat the patient. Another challenge is presented by the selection of a meaningful primary endpoint, whether that is OS or RFS.. An interesting question on the role of adjuvant therapies has to be raised: is it important to start a treatment in the adjuvant setting which means to treat a robust percentage of patients who are already potentially cured with surgery or, at the end, the survival obtained will be the same in case patients are treated only after recurrence of their disease?

There are some data suggested that due to the primed immune system you get more toxicity in the adjuvant setting than in the advanced disease setting [39]. A number of important questions have found response by trials mentioned above, but remain many other questions that need to be settled regarding the use of anti–PD-1 blockade in the adjuvant setting. For example if PD-L1 expression in resected melanoma tumors serve as a biomarker for successful adjuvant treatment, and if there are other novel biomarkers that could be used in order to differentiate between patients who will derive the most benefit from treatment, without exposing to unnecessary ineffective and toxic treatment this population. In conclusion, a major advantage of immunotherapy is the possibility to discontinue treatment and maintain antitumor responses. The immunological 'memory' induced by the immunotherapy agent offers the potential for long-lasting, possibly life-long, therapeutic responses.

For sure the identification of the patietns is a matter of debate and will be one of the first issue to be solved. The American Joint Committee on Cancer (AJCC) has reported in its 8th edition a melanoma-specific survival (MSS) for all stage sub- groups higher than those reported in the seventh edition.

The higher survival of patients in the more contemporary cohort examined is likely a consequence of the widespread use of sentinel lymph node (SLN) biopsy; the requirement of SLN biopsy for patients with T2 through T4 primary melanoma to be included in AJCC staging;

and, to a lesser extent, newer imaging technologies that improve the detection of clinically occult metastatic disease.

Despite this, there is still a marked prognostic heterogeneity within the same stage and a prognostic hierarchy between the different stages seems to lack.

Within stage III, a new subgroup (stage IIID) has been added with respect to the previous edition. Consequently, the prognosis of stage IIIA patients has improved, while a higher risk subgroup of stage III patient has been identified.so the Anyway, no one of these trials has focused on stage IIC patients who may deserve of adjuvant therapy as well as stage III ones (Fig. 2). If their results would be the base for adjuvant therapy indications, there is the risk of an unfair limitation to the clinical practic Anyway, no one of these trials has focused on stage IIC patients who may deserve of adjuvant therapy as well as stage III ones (Fig. 2). If their results would be the base for adjuvant therapy indications, there is the risk of an unfair limitation to the clinical practice

Combination therapies are at the moment under consideration .

Programmed cell death-1 (PD1) is an immune inhibitory receptor expressed by activated T and B cells that binds to the two known ligands PDL1 and PDL2. PDL1 is expressed by a wide variety of tissues, and also on human tumors, including melanoma. When PD1 binds to its ligands, it negatively regulates T-cell function [65]. This mechanism is used by several tumors to escape the immune system control. According to recent studies IFN could regulate PD1/PDL1-2 expression resulting in a controversial pro-tumor escape effect . In fact, CD8+ T cells matured in the presence of IFN-α showing higher levels of PD1 and a relatively poor ability to inhibit tumor growth efficiently[66]. Moreover, IFN-α has been reported to increase PDL1 cellular expression on hepatocytes[67], and IFN-γ on tumor cells[68] . These lines of evidence support the hypothesis that IFN-α-mediated anti-tumor activity would be significantly enhanced through PD-1 blockade. The combination of the anti-PD1 pembrolizumab and pegylated IFN-α2b was recently tested in a phase I clinical trial and was well tolerated with no dose limiting toxicities and mostly grade 1 adverse events. Enrolled patients were affected by recurrent inoperable stage III and IV melanoma and previous treatments included adjuvant IFN, vemurafenib, chemotherapy, and radiotherapy. Six out of 12 patients were evaluated for clinical responses at week 12 with 1

complete response, 4 stable disease, and 1 progressive disease with mixed clinical responses[69]. In stage II, we do not have studies at the moment with new adjuvant treatments, although those patients represent a big percentage of the total amount of potential candidates to an adjuvant treatment. In the latter, to decrease DFS while increasing the OS will globally impact even more than in stage III on the global survival of melanoma patients.

Not completely similarly to advanced disease we shall now face a new difficult decision to be taken at least in half of the patients: which therapy to adopt in the adjuvant setting for patients presenting BRAF mutation? In metastatic disease we shall always have the chance to propose both treatments and the main issue is to decide which to propose as first line, and in this advance disease patients population the choice is usually justified or motivated on the basis of an empiric feeling: offer a target therapy to patients with bulky, aggressive disease, and a immunotherapy to more chronic low aggressive metastatic progression of melanoma. Can we apply the same philosophy to the adjuvant setting? Probably not, but more than this, in the adjuvant setting we shall be able to offer only one treatment schedule, so the choice will be at this stage more a patient to patient discussion then a decision driven from clinical or biological issues: patients with easy accessibility to the hospital willing to receive ev. Injection every 3 weeks will be offered immunotherapy, while older, less geographically accessible patients with less sun exposure risk may prefer the oral approach of target therapy.

The large spectrum of prognosis in stage III patients is opening a huge request of biomarker to evaluate the risk of progression and to identify whom out of all the patients will develop a disease progression. Many clinical and pathologic prognostic factors have been evaluated. Clinically age and phenotype (like mole count) has been demonstrated to play a role in survival in positive SLN patients[70]. Pathologic features of primary like Breslow thickness, ulceration, mitoses and regression have been associated to prognosis as well as the number of lymph node excised and the number of positive ones[5,6,71]. Genetic biomarkers are now under evaluation. The urgent need of biomarker has increased after the discovery of efficacy in the adjuvant therapy in terms of increasing survival. To be able to detect which patients are at risk of progression and so to candidate only those ones to adjuvant therapy would be the next goal of melanoma research.

Last topic under study will be the concept of neoadjuvant therapies: this approach could be proposed in different scenarios i.e. for stage III palpable nodal disease and for stage IV melanoma patients. Years ago the only possibly effective therapy was surgery. Now 2 studies demonstrate that surgery is no more the gold standard and new hypothesis are under investigation from the medical point of view. In stage IV on the contrary, surgery could be seen as a confirmation of efficacy of medical treatments where both a complete response or a partial response have been reached, offering to patients the opportunity of interrupting their medical treatments once the condition of no evidence of residual disease has been confirmed.

14.CONCLUSION

Melanoma clinical research during the last 10 years has driven the most important changes in treatment approaches seen in oncology since ever. From being an orphan and neglected disease it has moved to the most pioneering tumor from which so many other cancer types are learning and developing new strategies[72]. Advanced disease has demonstrated an impressive treatment efficacy improvement passing from 5% to more then 50% survival benefit at 5 years. From these results a new set of trials have been developed in the adjuvant setting and the results of the first studies have been recently offered to the scientific community to become common practice as soon as regulatory agencies will permit their clinical use.

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