

Melanoma adjuvant therapy

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Abstract: The risk of recurrence and death after complete surgical resection of clinically detectable primary cutaneous melanoma ranges from low, intermediate to high risk depending on the stage of disease at diagnosis. This is defined by the depth, ulceration status and mitotic rate of the primary tumor, the presence of regional nodal disease or distant metastasis. For high-risk melanoma, adjuvant therapy aims at eradicating melanoma micrometastases in those patients that carry an unacceptable risk of mortality from melanoma recurrence. The ultimate goal of adjuvant therapy is to provide a potential cure before progression into advanced inoperable stages. After decades of randomized clinical trials, interferon- α alone has been shown to have a significant impact on relapse free survival and at high dosage on overall survival. These survival benefits were seen in comparison to observation (E1684 trial) and the ganglioside GMK vaccine (E1694 trial). Advances in our understanding of melanoma molecular biology and host immunology have given ways to novel targets and therapeutic agents that have demonstrated unprecedented results in the management of metastatic disease and currently are being tested in the adjuvant setting where recent data support a significant clinical impact of adjuvant ipilimumab. Here, we review the standard of care melanoma adjuvant therapy along with the main completed, current and planned clinical trials.

Keywords: Adjuvant; interferon; melanoma; ipilimumab

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Introduction

Malignant melanoma is the fastest growing malignancy in the United States (US) in terms of incidence and it currently represents the fifth most common cancer in men and the seventh most common cancer in women. In the US, about 19 men and 14 women per 100,000 population receive a new diagnosis of melanoma every year. In 2014, it is estimated that 76,100 patients will be diagnosed with melanoma in the US, and about 9,710 will die from this disease (1). Careful surveillance in high-risk individuals, early diagnosis and prompt surgical removal remain the mainstay of management surgically curable melanoma. For high-risk melanoma, adjuvant therapy focuses on clinically invisible disease that may lead to future mortality from melanoma recurrence and presents an opportunity at curing this disease. Furthermore, as rapid advances in the successful

treatment of stage IV melanoma are being made, delaying relapse even without improving survival is of increasing importance. Various modalities including immunological therapy, chemotherapy and radiation therapy have been tested in the adjuvant setting over the past three decades.

Risk stratification in melanoma: TNM classification

There was a recent update to the 2002 American Joint Committee on Cancer (AJCC) TNM—tumor, lymph node and metastasis—staging system in 2009 and newer prognostic factors that have clinical implications were added (2). Stages I and II are clustered as localized melanoma that is limited to the skin. Stage III is described by the presence of lymph node involvement and/or in-transit metastases, while stage IV involves distant metastatic spread.

Tumor depth (Breslow's tumor thickness) is an essential

factor in staging. The survival rates decline with every millimeter increase in tumor thickness; for instance, the 10-year survival rate drops from 92% for T1 tumors (thickness ≤ 1 mm) to 50% with T4 melanoma (thickness > 4 mm). Survival rates of ulcerated tumors are proportionately lower than non-ulcerated melanoma of equivalent T category but are similar to patients with a non-ulcerated melanoma of the subsequent T category. Survival rates with T3b and T4a are similar (68% and 71%, respectively), whereas it falls to 53% with T4b melanoma. High mitotic rate (at least 1 mitosis/mm²) strongly correlates with dropping survival rates. It has replaced the Clark level of invasion as a complementary criterion to ulceration for differentiating T1a versus T1b primary tumor (2).

Involvement of regional lymph nodes or the presence of intralymphatic (satellite or in-transit) metastasis encompasses stage III. Five-year survival rate for any N1 disease (single lymph node involved) is 70% as against 39% for N3 disease (> 4 nodes/matted nodes/in-transit metastases/satellites with metastatic nodes). The new 7th AJCC staging edition has no minimum threshold for lymphatic tumor burden to define the presence of regional nodal metastases. For the same T stage, the nodal sub-classification N1a (micro metastasis) and N1b (macro metastasis) constitute stage IIIA and stage IIIB, respectively. In-transit lymphatic metastases without and with metastatic lymph nodes correspond to N2c and N3, respectively (2). This population of patients without distant spread of primary melanoma who are at high risk for recurrence and death is three times the size of the population with metastatic disease. Therefore, the importance of adjuvant therapy in the context of improving survival rates in melanoma cannot be overstated.

For advanced disease with metastasis to distant locations, number and location of metastasis and lactate dehydrogenase (LDH) blood levels are key to determining prognosis. One-year survival of patients with M1a disease [distant skin, subcutaneous (SC), and lymph node metastases] is 62%, as compared to 53% for M1b melanomas (lung metastases) and 33% for M1c melanomas (visceral metastases or any distant metastasis with high LDH) (2). Oligo metastatic melanoma that is amenable to surgical removal may still have good survival rates if chosen appropriately (3).

Who should get adjuvant therapy?

Adjuvant therapy has shown maximal survival benefit in AJCC stages IIB, IIC and III. These are patients whose

estimated risk of recurrence exceeds 30% (ranging from 30% chance of recurrence for IIB to 60% chance of recurrence for IIIC) (2).

Immunotherapy with interferon-alpha (IFN- α)

The earliest use of human IFN- α was in the treatment of human cancer with leukemia more than a generation ago in France and osteosarcoma in Sweden based on the suspected viral etiology of these neoplasms. Antitumor effects in one of 11 leukemia patients and indications of improved disease-free survival of children with osteosarcoma spurred studies of IFN- α in other cancers including melanoma under the auspices of American Cancer Society in the USA in the 1980s. Amongst the two families, type I IFN family includes IFN- α , IFN-beta, IFN-epsilon, IFN-kappa and IFN-omega, whereas IFN-gamma constitutes type II IFN singly. Among the IFNs, IFN α -2 has been the most widely studied clinically.

Mechanism

The interferon molecule is thought to induce an immunomodulatory effect primarily and less of a directly cytotoxic or anti-angiogenic effect (4). Research in IFN- α in the neo-adjuvant arena has shown significant influence of IFN- α on Signal Transducer and Activator of Transcription (STAT) signaling and the histopathologic course of events that take place in the tumor. An influx of dendritic cells (DCs) and T lymphocytes into the tumor tissue was noted. The tumor upregulates STAT3 and there is subsequent elaboration of vascular endothelial growth factor (VEGF), tumor growth factor (TGF)-beta and interleukin (IL)-10 among other mediators of immune tolerance. IFN- α was found to down-regulate STAT3 expression in tumor cells. Also, simultaneous induction of STAT1 in the lymph nodes was observed and this correlated with a reversal in T cell signaling defects (5).

High dose IFN- α (HDI): earliest trials

The impetus to study IFN- α in the adjuvant setting for high risk resected melanomas was derived from the evidence of activity of IFN- α in the metastatic setting. The North Central Cancer Treatment Group (NCCTG) trial (6) and the Eastern Cooperative Group (ECOG) trial E1684 were the very first two randomized trials that looked at the benefits of post-surgical adjuvant therapy for high-risk melanoma (7). Both the trials tested HDI [> 10 million units (MU)/dose].

The ECOG E1684 trial utilized a regimen consisting of HDI given intravenously (IV) at 20 MU/m² for 5 consecutive days a week for 4 weeks as the induction phase followed by SC administration at 10 MU/m² thrice weekly for 48 weeks as maintenance. It was initiated in 1984 and at a median follow-up of 6.9 years, 287 patients were studied in total demonstrating a statistically significant difference in relapse-free survival (RFS) and overall survival (OS) in favor of HDI when compared to the observation arm. The estimated 5-year RFS in the treatment arm was 37% [95% confidence interval (CI), 30-46%] versus 26% (95% CI, 19-34%) in the control group. The 5-year OS was 46% (95% CI, 39-55%) and 37% (95% CI, 30-46%) in the treatment and observation arms, respectively. Patients with clinically node-negative but pathologically positive nodes (N1 disease) had the highest impact on survival. The outcomes of this trial led to the regulatory approval by the United States Food and Drug Administration (FDA) in 1995 (7).

High dose IFN- α (HDI): other ECOG and intergroup trials

When weighed against these survival benefits, the toxicity profile of HDI as observed in E1684, with a 67% incidence for grade 3 toxicity, 9% incidence for grade 4 toxicity, and two early therapy-related hepatotoxic deaths, raised concerns over patients' endurance and long-term adherence to the regimen. This factor urged clinician-investigators to study other forms or regimens that varied by dose level, route of administration or duration of IFN- α therapy.

E1690

In this trial, the ECOG and the US Intergroup re-used the E1684 HDI regimen and compared its efficacy against a low-dose regimen of IFN- α 2b (LDI) at 3 MU SC thrice weekly for two years and a third arm consisting of patients who were observed without therapy (Obs). Accrual of patients in E1690 was completed between 1991 and 1995, and at 4.3 years median follow-up, the 5-year estimated RFS rates were 44% for HDI, 40% for LDI, and 35% for the Obs arm, respectively (8). The effect of HDI on RFS alone was able to reach statistical significance ($P=0.03$). Neither HDI nor LDI was found to establish OS benefit compared with Obs (52% high dose *vs.* 53% low dose *vs.* 55% observation). However, improved OS of the E1690 Obs arm was noticed when compared to E1684 Obs arm (median 6 *vs.* 2.8 years). Unlike E1684, subjects in E1690 did not require elective lymph node dissection (LND).

More importantly, a retrospective analysis revealed some cross over from the observation arm at regional nodal recurrence to IFN- α salvage therapy that may have impacted the survival analysis in E1690.

E1694

The US Intergroup undertook the E1694 trial and compared HDI with a ganglioside vaccine, considered the most optimal vaccine candidate at that time. The GMK vaccine consisted of purified ganglioside GM2 coupled to keyhole limpet hemocyanin (KLH) and combined with the QS-21 adjuvant. It was hypothesized that vaccination kindled antibodies against GM2 capable of exclusively attaching to GM2 and knocking off malignant melanocytes *in vitro* via complement or antibody-based cell-mediated cytotoxicity. HDI was superior compared to GMK with improved RFS (HR =1.47, $P=0.001$) and OS (HR =1.52, $P=0.009$) (9).

E2696

The ECOG led a randomized, phase-II trial E2696 that recruited 107 patients with surgically resected stage IIB, III and IV disease that was conducted between 1998 and 2000 (10). The intent was to study the anti-GM2 antibody response to GMK vaccine in the presence versus absence of IFN. The study compared three arms—arm A (GMK plus concurrent HDI), arm B (GMK plus sequential HDI), and arm C (GMK alone). The combined approach reduced the risk of recurrence when compared to GMK alone (HR =1.96 for C versus B and HR =1.75 for C versus A).

Interferon- α (IFN- α)—other tested doses, routes and duration

The search for less toxic and more efficacious regimens led to multiple trials using other dosing ranges, routes of administration, duration of therapy and formulations. While the Sunbelt Melanoma Trial looked at LND versus LND plus standard HDI (11), the Italian Melanoma Group trial studied a shorter course of a more intense course of interferon than the standard regimen (12), with no statistically significant differences seen.

Hellenic trial

The Hellenic Oncology group intended to test the hypothesis that the intravenous induction phase of the HDI regimen was the critical component of the regimen and was sufficient in exerting the therapeutic impact of

HDI in high-risk melanoma (13). In the phase III He 13A/98 study, patients were randomized between 1998 and 2004 to a modified induction phase of 15 MU/m² HDI only versus the same induction phase followed by a modified maintenance phase of 10 MU (not per m²) thrice weekly for a year. With 182 patients per arm and a median follow-up of 5.25 years, the analysis in 2009 revealed no statistically significant difference in either median RFS or OS. However, the study was criticized for the modified regimen used and the relatively small sample size to allow it to demonstrate a clinically significant difference.

E1697

US Intergroup study E1697 tested a similar hypothesis amongst patients with resectable intermediate risk melanoma (\geq T3 or any thickness with microscopic nodal disease N1a-N2a). Between 1998 and 2010, the study recruited 1,150 patients and randomized them to either four weeks of HDI (20 MU/m²/day for five days weekly) versus observation (14). In 2010, a third interim analysis deemed the study futile and was thereafter closed. When presented to American Society of Clinical Oncology (ASCO) in 2011, the study reported no impact on either RFS or OS with this four-week long regimen. The results of this trial supported the E1684 HDI 1-year regimen as the standard for high risk melanoma.

Other trials investigated less intensive regimens in terms of dosing of IFN- α . These included the very low dose (1 MU SC every other day) as in European Organization for Research and Treatment of Cancer (EORTC) 18871 (stage IIB, IIIA) (15), low dose (\leq 3 MU SC thrice weekly) tested in WHO melanoma trial 16 (stage III) (16), E1690 (T4, N1) (8), UKCCCR AIM-High trial (stage IIB/III) (17), Scottish trial (stage IIB, III) (18) and the 2010 German DeCOG study (T3anyN) (19). Intermediate dose regimens (5-10 MU/m²) were tested in the EORTC 18952 (T4 N1-2) (20) and EORTC 18991 (TxN1) (21) studies. Although, these trials showed benefit in RFS for the IFN arms, this impact appeared to be lost with time. Support to this observation also comes from the French multicenter trial that indicated that the effect of IFN- α on RFS was lost on cessation of treatment (22).

EORTC 18952

This trial (20) enrolled 1,388 patients with stage IIB/III disease to four weeks of induction with 10 MU IV five times a week, followed by one of two maintenance regimens, i.e., SC 10 MU three days a week for one year versus SC 5 MU three days a week for two years. The third arm

was an observation control arm. It was conducted from 1996 to 2000. At 4.65 years median follow-up, the results demonstrated a statistically insignificant 7.2% increase in distant metastasis-free interval (47% versus 43% and 40% respectively) and a 5.4% increase in OS (53% in the two-year arm compared to 48% each in the one-year and observation. The increase in OS was observed only in patients treated for 25 months with 5 MU IFN- α 2b and not in those treated for 13 months with 10 MU IFN- α 2b. These results suggested that the duration of therapy might be more important than dose.

DeCOG

In 2008, a randomized Phase III Dermatologic Cooperative Group (DeCOG) trial (23) looked at a combination of LDI/dacarbazine (DTIC) *vs.* LDI alone. Analysis at four years median follow up revealed surprisingly that the low-dose IFN group showed an improvement in DFS (HR =0.69) and OS (HR =0.62). However, the trial was aimed at finding whether DTIC adds any benefit to IFN- α and not whether LDI was superior to observation. These results do not match with the earlier trials that tested LDI, i.e., the Austrian (AMCG) trial and French (FCGM) trial (22,24) that showed no OS benefit for LDI. Unlike in North America, LDI therapy has been approved as an adjuvant therapy for stage II patients by the European Medicines Agency (EMA) in Europe. Regional differences exist in Europe in the adjuvant use of IFN. HDI regimens are not commonly used in Europe as much as in the USA.

Pegylated-interferon

EORTC 18991

Pegylated IFN- α as tested in this trial achieved regulatory approval for use as adjuvant therapy of high-risk melanoma with lymph node metastases (21). The covalent bonding of the interferon molecule with a polyethylene glycol moiety results in sustained absorption and longer half-life. The EORTC 18991 trial looked at the efficacy and safety of peg-IFN- α 2b versus observation amongst 1,256 patients recruited from 2000 to 2002 with resected AJCC stage III melanoma. The regimen comprised induction dose of peg-IFN SC 6 mcg/kg a week for eight weeks followed by maintenance dose of once weekly SC injections at 3 mcg/kg for up to five years. At 7.6 years median follow-up, the group released data, which showed an improved RFS in the treatment arm (HR =0.87, 95% CI, 0.76-1.00, P=0.05) with no difference in OS/DMFS between observation and

treatment group. Sub-set analysis indicated that subjects with microscopic nodal metastasis and ulcerated primary tumor had the greatest benefit in terms of RFS, OS and DMFS. During the study, peg-IFN was discontinued for toxicity in 37% of patients.

Interferon- α (IFN- α)—meta-analyses

At least four different systematic reviews and meta-analyses on adjuvant therapy have been published from 2002 through 2010. The largest was a 2010 meta-analysis from Mocellin *et al.* (25) that included RCTs published between 1990 and 2008 covering 8,122 patients, of whom 4,362 subjects had received IFN- α . In twelve out of fourteen studies included, IFN- α was tested against observation, and 17 different comparisons were established. In sub-group analysis, no specific regimen, dosing, formulation, study design or staging provided any difference in overall hazard ratio estimates. Four out of 14 comparators revealed a statistically significant OS benefit with IFN- α . The review concluded that adjuvant IFN α therapy demonstrated a statistically significant 18% risk reduction for recurrence (HR =0.82, 95% CI, 0.77-0.87, P<0.001) and 11% risk reduction for death (HR =0.89, 95% CI, 0.83-0.96, P=0.002).

EORTC 18071

Eggermont *et al.*, reported the results of the phase III EORTC 18071 trial stage III melanoma patients (N=951) who were randomized 1:1 to receive either adjuvant ipilimumab at 10 mg/kg or placebo following complete surgical resection. After a median follow-up of 2.7 years, 46.5% and 34.8% (P=0.0013) of patients were relapse free in the ipilimumab and placebo treatment arms, respectively. Grade 3/4 AEs occurred in more patients receiving ipilimumab compared with placebo included gastrointestinal (15.9% *vs.* 0.8%), endocrine (8.5% *vs.* 0%), and hepatic events (10.6% *vs.* 0.2%). It is noteworthy that the dose level of ipilimumab used in this trial is higher than the current dose level (3 mg/kg) approved by the U.S. FDA for inoperable metastatic melanoma (26).

AVAST-M

Corrie *et al.*, reported the results of a preplanned interim analysis of the phase III AVAST-M trial that tested adjuvant bevacizumab versus observation in stage II/III resected melanoma patients (N=1,343). At a median follow-up of 25 months, overall survival and distant metastasis-free survival were similar among treatment arms. A modest improvement

in the disease free interval (DFI) was observed [HR: 0.83; 95% CI: 0.70-0.98; P=0.03]. Longer follow-up is needed to better assess the DFI benefit seen and to evaluate the effect on the primary endpoint of overall survival at 5 years (27).

US Intergroup E1609

The randomized US intergroup phase III trial (E1609) is investigating standard high-dose interferon alpha-2b *vs.* ipilimumab given at 3 or 10 mg/kg. It is expected to complete target accrual in August 2014. This trial is key at informing the field in regards to the clinical efficacy of ipilimumab versus interferon alpha-2b as well as the risk-benefit ratio of the 2 dose levels of ipilimumab (10 and 3 mg/kg) in the high risk adjuvant setting (28).

Predictors of benefit and prognostic markers

The remarkably consistent but modest beneficial effect of adjuvant IFN α has been well demonstrated, but it also comes at the expense of significant toxicity and cost. Hence, there is a need to focus treatment on patients who are most likely to benefit from adjuvant IFN- α therapy as was noted in some of the trials. After the Wheatley meta-analysis and later a sub-set analysis of EORTC 18991 revealed specific benefit seen amongst patients with ulcerated primary tumors, some focus has been placed on targeting such patient groups in clinical trials including the on-going EORTC 18081. This trial will study adjuvant peg-IFN for two years *vs.* observation in patients with an ulcerated primary cutaneous melanoma with T[2-4]b, N0, M0 melanoma (29).

Gogas *et al.* (30) in 2006 had reported a prospectively validated analysis of autoimmunity as a biomarker associated with IFN- α benefit. Also, our group at the University of Pittsburgh and ECOG studied these data from the E2696 and E1694 trials to further understand the newly found association between autoimmune related side effects and improved outcomes with IFN- α . A landmark analysis of E1694 revealed a trend towards a survival advantage associated with HDI-induced autoimmunity in stage III patients treated with HDI. In both trials, the presence of autoantibodies in sera of patients was significantly more frequent in the HDI arm compared to the vaccine arm. But the development of autoimmunity occurs over a period of up to one year and therefore cannot be used as baseline or early on-treatment predictor of IFN- α therapeutic benefit (31). We are currently testing the immunogenic predictors of autoimmunity associated with IFN- α in the

context of the E1697 trial as potential predictors of IFN- α therapeutic benefits. The expression of methylthioadenosine phosphorylase (MTAP) that plays a significant role in the activity of STAT1 has shown an association with improved OS and RFS as noted in a retrospective study from Meyer *et al.* (32). Accumulating data reinforces the importance of the relative balance of pSTAT1/pSTAT3 in the tumor microenvironment (TME). Serum markers of interest include S100B, melanoma-inhibiting activity (MIA) and tumor-associated antigen 90 immune complex (TA90IC) (4). Tarhini *et al.*, demonstrated that a high or increasing serum level of S100B is an independent prognostic marker of risk for mortality in patients with high-risk disease, as tested in the context of the E1694 trial (31).

Adjuvant radiation therapy (RT)

In melanoma, RT is rarely indicated at the primary tumor setting. Contrary to past belief that melanoma is a relatively radio-resistant tumor, *in vitro* studies of melanoma cell lines have showcased radiation responsiveness, although this may widely differ within the same tumor and that melanoma may require higher than standard doses per radiation fraction for effective cytodestruction (33).

Despite wide excision of primary tumor and complete LND, the risk of local relapse for stage III is 15% to 20% and is even higher at about 30% to 50% for patients with high-risk features: extracapsular lymph node extension, positive margins, involvement of four or more nodes, bulky disease (exceeding 3 cm in size), cervical lymph node location, and recurrent disease (33). In these cases, adjuvant RT may be considered valuable for local disease control. Data from multiple nonrandomized trials is available, but inconsistent. Nevertheless, a few of them have shown some positive strides in the adjuvant setting and have generally concluded that adjuvant RT was associated with improved local and regional control rates without OS benefit. As in prostate and breast cancer, hypofractionation of RT appears to be as efficacious as standard radiation dosing in melanoma (33).

A retrospective study published in 2003 by Ballo *et al.*, from MDACC involving 160 patients who had surgery followed by RT (30 in 6 Gy fractions two times per week) demonstrated ten-year local, regional, and locoregional control rates of 94%, 94%, and 91%, respectively. Other results shown were ten-year disease-specific, disease-free, and distant metastasis-free survival rates of 48%, 42%, and 43%, respectively (34). Another retrospective study was published by Agrawal *et al.* in 2009 and looked at patients from Roswell

Park and MDACC with clinically advanced, regional lymph node-metastatic disease (n=615) (35). It compared surgery plus adjuvant RT with surgery alone and demonstrated a reduction in the regional recurrence rate (10.2% versus 40.6%); furthermore, adjuvant radiotherapy was significantly associated with 5-year regional control (P<0.0001), distant metastasis-free survival (P=0.0006), and disease-specific survival (P<0.0001). Retrospective data from Strojjan *et al.* (36) published in 2010 showed considerable improvement in local relapse control at 2 years by using adjuvant radiotherapy (60 in 2 Gy fractions five times a week) as compared to surgery alone (78% vs. 56%; P=0.015) among patients with regionally advanced melanoma to the neck and/or parotid. A more recent randomized trial, ANZMTG 01.01/TROG 02.01 (Australia New Zealand Melanoma Trial Group/Trans-Tasman Oncology Group) (37) compared adjuvant radiotherapy (48 Gy in 20 fractions) with observation, following lymphadenectomy among 217 patients with nodal metastases and a high risk of recurrence (based on number of nodes involved, extranodal spread, and maximum size of involved nodes). After a median follow-up of 40 months, the risk of lymph-node field relapse was reduced in the adjuvant radiotherapy group (20 relapses in RT vs. 34 in observation; HR =0.56, 95% CI, 0.32-0.98; P=0.041). However, there were no statistically significant differences in RFS or OS (38-43). Recent guidelines from the National Comprehensive Cancer Network support the application of adjuvant radiotherapy, based on lower level evidence.

Trials testing other adjuvant agents

Chemotherapy

Agents like DTIC, BCG and levamisole have been tested in the adjuvant setting (44-47). In summary, chemotherapy with or without combination therapy with other modalities has not demonstrated any improvement in either DFS or OS in any RCT to date.

Biochemotherapy (BCT)

The South West Oncology Group (SWOG) (48) intergroup study S0008 was a phase III adjuvant melanoma study in high-risk, node-positive patients that assessed whether a BCT regimen administered over nine weeks was more effective than the standard 52-week HDI regimen. The BCT regimen consisted of three cycles of cisplatin, vinblastine, DTIC combined with low doses of IL-2 and

IFN- α . At a median follow-up of about six years, there was significant improvement in RFS for BCT compared with HDI (median 4.0 *vs.* 1.9 years), but no improvement in OS and higher grade III/IV toxicity for the BCT group than the HDI group (76% *vs.* 64%). It was observed that patients on the HDI arm were more frequently followed during therapy as clinically indicated with IFN α , while BCT patients were seen every three months following completion of the 9-week BCT regimen. It is not clear whether this imbalance of early follow up between the HDI and BCT arms may have affected the RFS outcome. Multiple other agents that have been tested in small nonrandomized trials include vitamin A, megestrol acetate, BCG, corynebacterium parvum and transfer factor with no demonstrable benefits.

Immunotherapy with vaccines

Vaccines in melanoma can be grouped based on the type of antigens incorporated- peptide, ganglioside, and whole cell/cell lysate. Examples of the peptide vaccines include MART-1/Melan-A, gp100, and tyrosinase-these are melanocyte lineage antigens identified by cytotoxic T lymphocytes with the help of HLA-A2 eliciting a direct cytotoxic T cell response.

The MAGE antigens constitute a family of tumor antigens that are expressed in a few cancers including melanoma, but are not expressed in normal tissues other than testis and placenta. Adjuvant MAGE-A3 protein is being tested in a randomized phase III trial (DERMA) based on promising results from a previous study in advanced melanoma (49). The melacine vaccine trial conducted in the USA showed some promise initially, but failed to sustain it. Similarly, an Australian study using vaccinia viral lysates in high-risk subjects following resection showed a statistically insignificant increase in RFS (50). Gangliosides are sialic acid-containing glycosphingolipids that are overexpressed on the surface of melanoma cells. The E1694 trial that tested GM2 with BCG and with KLH and a QS21 adjuvant (GMK) has already been quoted earlier with no therapeutic impact for the vaccine (9).

A phase III trial for resected stage III/IV melanoma compared a polyvalent vaccine known as Canvaxin against BCG vaccination. DFS and OS were worse in the Canvaxin group likely due to vaccine-induced immunosuppression (51).

Modalities of the near future: under trial

Immunotherapy with anti-CTLA4 antibodies

CTLA-4 competes with CD28 for binding B7 on antigen

presenting cells and serves as a key inhibitory checkpoint in self-regulating the adaptive immune response. The role of the anti-CTLA-4 monoclonal antibodies were first studied in advanced disease and later brought into trials conducted in the adjuvant setting.

Ipilimumab (Medarex Inc/Bristol-Myers Squibb) is a fully humanized immunoglobulin G1 kappa monoclonal antibody that barricades the CTLA-4. It has achieved the FDA and European regulatory approval for treating advanced melanoma as both first- and second-line options. The phase III MDX010-20 trial compared ipilimumab alone (3 mg/kg dose), ipilimumab plus a peptide vaccine, and vaccine plus placebo (52). The trial showed a statistically significant survival benefit for ipilimumab as compared to the Gp100 peptide vaccine comparator. The next positive phase III study CA 184-024 compared ipilimumab (at 10 mg/kg) plus dacarbazine to dacarbazine with placebo (53). Adjuvant ipilimumab trials that are underway include the US Intergroup E1609 trial testing ipilimumab at 3 or 10 mg/kg versus HDI and the EORTC 18071 investigating ipilimumab at 10 mg/kg against placebo.

Immunotherapy with anti-programmed death 1 (PD-1) and anti-PD-L1 agents

PD-1 physiology plays a significant role at the tumor end of the T-cell interaction as opposed to CTLA-4 blockade that happens during the initial phase of antigen presentation. The PD-1 receptor is expressed by T cells during long-term antigen exposure and regulates the effector phase of T-cell responses. PD-1 and PD-L1 blockade are subjects of ongoing research and multiple phase I-III trials. In the near future, these agents will be part of trials testing adjuvant therapy.

Targeted therapy with inhibitors of BRAF

Around 40-50% of melanomas have been observed to have activating mutations in the B-rapidly accelerated fibrosarcoma gene (*BRAF* gene). Here, 80-90% of cases are V600E mutations in which glutamic acid has substituted for valine at the V600 locus. BRAF phosphorylates regulatory serine residues on MEK1 and MEK2 and hence, mutation of BRAF activates the RAS/RAF/MEK/ERK pathway leading to tumor proliferation. Vemurafenib and dabrafenib have both achieved regulatory approval based on significant phase III trial impact on RFS and OS (54). Trials are underway testing both vemurafenib (monotherapy) and dabrafenib (in combination with trametinib) as adjuvant

therapy in high-risk melanoma.

Neoadjuvant therapy

Therapy offered to individuals with disease that is difficult to resect surgically, in an attempt to downstage the disease prior to undergoing treatment aimed at cure or local disease control is termed 'Neoadjuvant therapy'. Given the unique immunogenicity of melanoma, there is greater interest in pursuing immunotherapy in the neoadjuvant setting. Neoadjuvant therapy gives a subtle picture of how the patient will respond to adjuvant therapy after their tumor is resected surgically post-neoadjuvant therapy.

Multiple phase II studies have been done to investigate chemotherapeutic and biochemotherapeutic regimens in the neoadjuvant setting for IIIB/IVA disease. The regimens usually included three or more cycles of chemotherapy (dacarbazine, cisplatin and vinblastine) plus immunomodulators like IL-2 and IFN- α and objective responses were seen. However, the phase III trials namely, E3695 and EORTC 18951 that compared them against single-agent chemotherapy demonstrated no significant benefit in either PFS or response rate along with significant toxicity (55,56). Moschos *et al.* tested the effect of HDI in patients with stage IIIB/C disease who underwent biopsy followed by induction HDI (IV 20 MU/m² 5 days a week for 4 weeks) followed by completion LND and subsequent maintenance HDI (SC 10 MU/m² 3 days a week for 48 weeks). Amongst 20 patients studied, 3 had pathologic CRs and 8 had partial responses (PR) for an objective response rate of 55%. Responders had significantly greater intratumoral (CD3+/CD11+) monocyte-derived dendritic cells and evidence of withdrawal of immune tolerance (57).

Neoadjuvant ipilimumab was studied by Tarhini *et al.*, among patients with locally and regionally advanced melanoma. After pretreatment biopsies, induction ipilimumab (IV 10 mg/kg) was given on days 1 and 21 followed by radical regional lymphadenectomy after at least 2 weeks. Two to 4 weeks after surgery, two more doses of maintenance ipilimumab (IV 10 mg/kg) were given at 3-weekly intervals. At 14-month median follow-up, data presented at ASCO 2012 revealed a median PFS of 15.5 months. Besides, significant immunomodulatory findings were reported such as the downregulation of myeloid derived suppressor cells in the circulation and the TME as well as the induction of tumor specific T cell responses and T cell memory, that were associated with clinical benefit (58).

For the adjuvant treatment of high risk melanoma,

HDI (stage IIB-III) and peg-IFN (stage III) currently have US FDA approval. Overall, IFN α has significant immune modulating activity and anti-tumor effects that translated into both RFS (E1684, E1690, E1694) and OS (E1684 and E1694) benefits seen with HDI making this regimen unique in comparison to other tested IFN- α regimens. Peg-IFN has demonstrated RFS benefits that appear to be mostly confined to the microscopic nodal disease population and no overall impact upon OS. Subgroup analysis of EORTC 18991 trial suggested a survival benefit with peg-IFN in microscopic nodal disease with ulcerated primary. This regimen may therefore be applicable for patients who cannot undertake HDI.

Conclusions

Statistically significant reduction in the risk of melanoma recurrence and death has been shown only with HDI when compared to observation (E1684) and the GMK vaccine (E1694), however, it is associated with significant toxicity and cost. Not long ago, peg-IFN received regulatory approval as adjuvant therapy in node positive disease based on the RFS benefit seen in the EORTC 18991 trial. The latest and comprehensive meta-analysis of adjuvant IFN α trials, IFN- α was associated with an 18% risk reduction in recurrence and 11% risk reduction in mortality (25). Ongoing adjuvant trials are testing ipilimumab CTLA4-blockade therapy (EORTC 18071 and E1609), BRAF inhibitors (BRIM-8 and COMBI-AD) and MAGE-A3 vaccine (DERMA). While recent updates indicate that the DERMA trial did not reach its primary endpoint of RFS, the results of EORTC 18071 are awaited in the first or second quarter of 2014. Adjuvant trials utilizing anti-PD1 antibody therapy are in the planning phase. Research in identifying specific populations that benefit the most from adjuvant systemic therapy is ongoing and is supplemented by attempts to identify therapeutic predictive biomarkers.

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