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Adjuvant Immunotherapy of Melanoma, and Development of New Approaches Using the Neo- Adjuvant Approach in Melanoma

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Abstract

Melanoma is the third most common skin cancer but the leading cause of death from cutaneous malignancies. While early-stage disease is frequently cured by surgical resection with excellent long-term survival, patients with deeper primary lesions (AJCC stage IIB-C) and those with microscopic (IIIA) or clinically evident regional lymph node or in-transit metastases (IIIB-C) have an increased risk of relapse and death–the latter approaching 70% or more at 5 years.

In patients at high-risk of recurrence/metastases, adjuvant therapy with high-dose interferon alpha-2b (HDI) following definitive surgical resection has been shown to improve relapse free and overall survival. Neo-adjuvant chemotherapy and/or radiotherapy have offered the prospect to improve regional recurrence risk and overall survival in several solid tumors. The advent of effective new molecularly targeted therapies for metastatic disease and new immunotherapies that overcome checkpoints of immune response have augmented the range of new options that are in current trial evaluation to determine their role as potential adjuvant therapies, alone and in combination with one another, and the established modality of IFNα. The differential characteristics of the host immune response between early and advanced melanoma provide a strong mechanistic rationale for the use of neo-adjuvant immunotherapeutic approaches in melanoma, and the opportunity to evaluate the mechanism of action suggest neoadjuvant trial evaluation for each of the new candidate agents and combinations of interest. Several neo-adjuvant trials have been conducted in the phase II setting, which have illuminated the mechanism of IFNα, as well as providing insight to the effects of anti-CTLA4 blocking antibodies. These agents (anti-CTLA4 blocking antibody ipilimumab [BMS], and BRAF inhibitor vemurafenib [Genentech]) are likely to be followed by other immunotherapies that may overcome the PD-1

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checkpoint (anti-PD1 [BMS, Merck, Curetech] and anti-PDL-1[Genentech]) as well as other molecularly targeted agents such as the BRAF inhibitor dabrafenibin[GSK] and the MEK inhibitors trametinib [GSK] selumetinib [AZ] and MEK162 [Novartis] in the near future. Evaluation of the clinical role of these agents as adjuvant therapy will take years to accomplish to ascertain the relapse-free survival benefits and overall survival benefits of these agents, but neo-adjuvant exploration may provide early critical evidence of the therapeutic benefits, as well as clarifying the mechanisms of these agents alone and in combination.

Keywords

melanoma; emerging new therapies; immunotherapy; CTLA-4; PD-1, vaccines; adoptive therapy; B-RAF inhibitor; MEK inhibitor; cKIT inhibitor; Bcl-2; HERV-K; micro-RNA; si-RNA; nanoparticles; stem cells

Introduction

With an incidence that has trebled in the Caucasian population in the last two decades, melanoma is an international scourge and the leading cause of cancer death from skin disease. Although surgical resection is curative in early stage disease, patients with advanced disease remain at significant risk of recurrence and death.

Pioneering work over the past 30 years has established high-dose interferon-α (IFN-α) as the mainstay of adjuvant therapy in high-risk operable melanoma, and it is the only adjuvant therapy approved worldwide, following licensure by the US Food and Drug Administration (US FDA) and international regulatory authorities. High-dose interferon-α (HDI),comprising an initial month-long induction phase (20 MU/m^2IV daily for 5 days per week for 4 weeks) followed by the remainder of one year of maintenance phase therapy (at 10 MU/m^2 SC for 48 weeks) has demonstrated the most consistent and largest improvement in relapse free survival (RFS) and in overall survival has shown significant benefit in two independent phase III cooperative group trials as well as several meta-analyses[1–7].

Immune tolerance is a notable feature of advancing melanoma, and events that occur early in melanoma progression appear to alter the host environment, and differ particularly between patients with active measurable disease and those without gross disease [8]; providing a rationale for the neo-adjuvant evaluation of immunotherapeutic approaches to determine the underlying mechanism. Prior neo-adjuvant phase II trials have reported mixed results – although neo-adjuvant HDI for high-risk patients with bulky regional lymph node disease (stage IIIB-C) have shown high clinical and pathological response rates, and trials utilizing biochemotherapy have been attempted.

Recent scientific advances in our understanding of the molecular mechanisms that drive tumorigenesis and in parallel, induce immune tolerance in melanoma have culminated in trials of molecularly targeted inhibitors of mutated BRAFV600E as well as anti-CTLA4 blocking antibodies that have generated pivotal trial data that established the BRAF inhibitor vemurafenib [9] and the CTLA-4 blocking antibody ipilimumab[10–11] as effective new therapies of metastatic melanoma. Their use in earlier settings of disease and in particular as adjuvant and neo-adjuvant therapies of disease is under intense ongoing investigation. We summarize the data for the use of IFN-α and ipilimumab alone and the basis for combinations of these immunotherapies for adjuvant therapy, and the promise of neo-adjuvant exploration of developmental single agent and combined modality therapies for melanoma.

Materials and Methods

A systematic search strategy was performed using the MEDLINE, EMBASE, Cancerlit, Cochrane, ISI, and Web of Science databases for articles published between January 1, 2002,and May 1, 2012. MeSH headings used included “melanoma, advanced”. “melanoma, adjuvant”melanoma, neo-adjuvant”, “melanoma, interferon” and “melanoma, ipilimumab”.

Indications for Adjuvant Therapy

Adjuvant therapy is indicated in patients at a high risk of recurrence following definitive surgical resection with the intent of treating micro-metastatic disease and reducing the risk of local and distant relapse. The risk of relapse and mortality is dependent on several independently predictive factors which are delineated in the revised 2009 classification on the staging and prognosis of cutaneous melanoma co-published by the American Joint Committee on Cancer (AJCC)and International Union against Cancer (UICC) [12]. These include:

- Primary tumor thickness – single most important micro staging factor predictive of 5- and 10- year survival rates, which decline proportionate to thickness measured in mm.
- Ulceration – an adverse factor associated with poorer survival in every thickness group, such that ulceration of any given T category has a risk of relapse/death that approximates the next higher non-ulcerated melanoma category.
- Mitotic rate – the newest micro staging factor, defined as the number of mitoses per square millimeter of the primary tumor, indicating a more adverse outcome and incorporated as a discriminant of substaging for lesions under 1 mm (T1b).
- Lymph node burden – risk of lymph node involvement increases with tumor thickness by approximately 2–5% for Breslow’s depth ≤ 1.00mm and reaches 34% for T4 lesions [13]. Survival decreases commensurate with increasing lymph node tumor burden – 5 year survival rates for stage IIIA, IIIB and IIIC disease ranging from 78% to 59% and 40% respectively. The importance of accurate assessment of lymph node involvement was underscored by two changes in AJCC 7th edition staging system: a) use of immunohistochemistry and RT-PCR to define nodal disease rather than hematoxylin and eosin (H&E) staining alone, and b) inclusion of micro-metastases (0.1–1.0 mm) in addition to macro-metastases (>1.0 mm) in defining lymph node involvement. However, patients with submicrometastases (<0.1 mm) tend to perform similarly as patients with sentinel lymph node (SLN) negative disease [14]. Blood biomarkers of increased risk of relapse are not generally evaluated, although S100 is one marker that has proven to be of potential use [15].
- Systemic burden of disease – **number** of metastatic sites and **sites** of distant metastases are important prognostic factors. Patients with distant skin, subcutaneous, and lymph node metastases (M1a) disease, pulmonary metastases (M1b) and extra pulmonary visceral metastases (M1c) have 1-year survival rates of 62%, 53% and 33% respectively. Patients with elevated **serumlactate dehydrogenase (LDH) enzyme level** perform as poorly as patients with non-lung visceral metastases suggesting that elevated LDH levels are a marker of a more aggressive phenotype.

Options for Adjuvant Therapy - IFN

Melanoma is a highly immunogenic tumor, as initially suggested by observations of spontaneous regressions of advanced disease, and more generally evidenced in the identification of lymphocyte responses to primary melanoma and atypical melanocytic nevi, and further documented in the melanoma specific serological and cellular immune responses to antigens including cancer germline antigens coded by X-linked genes (MAGE and NY-ESO-1) and melanoma differentiation antigens. Tumor infiltrating lymphocytes (TILs) are found frequently in atypical nevi, pre-invasive melanocytic lesions (melanoma *in situ*) and early invasive melanoma. The presence of TIL and tumor infiltrating dendritic cells (DC) appear to correlate with prognosis and response to immunomodulator therapies[16–17]. These observations led to early trials utilizing a variety of immunomodulators in metastatic disease. Initial observations of clinical benefit with IFN-α in metastatic disease prompted phase I-II trials utilizing IV, SC, IM routes [18] in which antitumor response rates were observed that were similar to those observed using single-agent chemotherapy (~13–24%) with durable responses that lasted years in up to a third of responding patients. These findings piqued interest in the potential of this agent as an adjuvant therapy for high-risk resectable nodal disease. A flurry of phase III trials were then conducted by US, European, and Australian investigators that pursued different subtypes, dosage, route and schedule of IFN-α used (low dose, ≤3 MU/dose; intermediate dose, 5–10 MU/dose; and high dose ≥10 MU/dose), IFN-α subspecies (IFN-α2a, IFN-α2b and IFN-α2c) as well as the treatment schedule. These are summarized in Table 1 (see Table 1 - **Phase III Studies of IFN-α for Advanced Melanoma**).

Of the phase III trials of IFN-α conducted in the 1980's and 90's, a number are worthy of discussion due to their lasting impact, and the questions that they have raised. The North Central Cancer Treatment Group (NCCTG) trial (20 MU/m^2 thrice weekly I.M. for 12 weeks in patients with stage II-III melanoma) demonstrated improved median disease free survival (DFS) and overall survival (OS) though the results did not achieve statistical significance [19]. Concurrently, the Eastern Cooperative Group(ECOG) tested a regimen consisting of IFN-α 2b I.V. 20 MU/m^2 daily for 5 days for 4 weeks as induction followed by S.C. 10 MU/m^2 thrice weekly for 48 weeks as maintenance versus placebo, in patients with deep primary tumors (>4 mm, T4N0M0) and/or regional lymph node metastases (TxN1–3M0, AJCC stage III) [5]. This randomized phase III study reported statistically significant increases in both DFS and OS. On subset analysis, it became apparent that patients with node-positive disease benefited compared to node-negative patients; although it was noted that patients with deep primary node-negative melanomas (T4N0Mx) were underrepresented. These results led to the approval of IFN-α 2b by the US Food and Drug Administration (FDA) for the adjuvant therapy of patients with high-risk melanoma.

A series of trials have subsequently tested lower doses of IFN-α given for longer periods of time, in hopes that the OS/RFS benefits of HDI could be retained with lesser toxicity through more prolonged therapy [5–7, 20–32]. These alternative regimens included the very-low-dose regimen (1 MU S.C. every other day) tested in EORTC 18871 (stage IIB/III), low-dose regimens (3 MU S.C. thrice weekly) as tested in WHO Melanoma Program Trials 16 (stage III), ECOG trial E1690 (T4N1), the UKCCCR AIM-High trial (stage IIB/III), the Scottish trial (stage IIB/III) and the 2010 German DeCOG study (T3anyN) and intermediate dose regimens tested in trials organized by the European Organization for Research and Treatment of Cancer (EORTC) - EORTC 18952 (stage IIB/III) and EORTC 18991(T3-T4N0M0) – and the Nordic Melanoma Cooperative Group, Nordic IFN trial (stage IIB-IIIB). Aside from the 2008 German DeCOG study which suggested an OS benefit for LDI that has not been seen in multiple other trials of this dosage, none of the trials assessing alternative doses have reported OS benefits and it should be noted that the one outlier trial of

DeCOG was powered to assess the combined regimen of LDI/dacarbazine rather than LDI *per se*.

Over the past 30 years, 17 phase III trials have examined the potential benefits of postoperative adjuvant IFN-α in high-risk operable melanoma patients. Both E1684 and E1694 demonstrated OS improvements with the use of HDI – although subsequent analysis of the survival benefits documented at 7 years in E1684 showed that the differences had diminished on reanalysis past 10 years. This may be due to competing causes of death in the aging patient population, but an analysis with the survival benefits of the HDI regimen in which the causes of mortality were analyzed for E1684 and the subsequent E1690 and 1694 intergroup trials has been proposed, but not yet accomplished. Multiple retrospective analyses and systematic reviews [1] and meta-analyses[2–4] support the conclusion that HDI reduces the risk of relapse by approximately 30%, and has a significant but lesser impact on OS.

Survival analysis in E1684 suggested that the reduction in relapse risk occurred early in the course of this treatment – underscoring the importance of the HDI regimen's induction phase. The utility of a truncated course of therapy has prospectively been evaluated in 2 studies – the Hellenic He13A/98 study reported in 2009 (1 month of modified induction therapy only vs. 1 year of modified induction and modified maintenance HDI), and the more recent E1697 (1 month induction vs. observation) [33–34]. Although the former study initially suggested that the month-long modified induction regimen tested in 13A/98 was not inferior to the extended course of therapy in terms of OS/RFS, the limited numbers of patients enrolled (364 patients), lack of an observation control arm and the modified lower doses of induction IFN-α used (15MU/m^2 vs 20 MU/m^2) and maintenance (10 MU flat dosage vs. 10 MU/M2) for the critical induction phase and maintenance cannot be ignored. At the 2011 ASCO meeting, E1697 was reported due to its early termination due to futility analysis following the interim analysis at 1150 patients' enrollment, of a planned 1420. The lack of any significant and durable difference from the therapy with induction at full dosage as compared with observation in this trial forces us to conclude that truncated regimens do not achieve the RFS/OS benefits that have been repeatedly documented with the 1 year HDI regimen.

The antipodal argument of whether an extended duration of therapy has greater benefit (dose-response relationship) has been explored in E1690, WHO 16, EORTC 18952, 18991 and the Nordic IFN trial [22–24]. EORTC 18952 and Nordic IFN trial utilized IFN-α2b while EORTC 18991 explored the use of pegylated IFN in predominantly high risk patients (74–100% stage III or greater). The use of pegylated form was spurred by the observation that the undisputed efficacy of IFN, the adverse effect profile and high treatment costs affected patient compliance. Pegylated IFN-α – which allows less frequent administration with no tradeoff in potency - had been trialed in infectious hepatitis with success.

The ECOG-Intergroup trial E1690 in the US, and the WHO trial 16 in Europe tested whether 2 or 3 years of lower dose IFN (3MU TIW) might improve relapse-free or overall survival. Both of these trials were ultimately negative. EORTC 18952 reported adjuvant intermediate dose IFN had a negligible effect on distant metastasis-free interval (DMFI), distant-metastasis free survival (DMFS) and OS. However, when analyzed by stage, patients with stage IIB/C disease appeared to benefit from incremental therapy compared to stage III patients suggesting that a low pre-operative tumor burden predicted improved IFN response. However, the Nordic IFN trial and EORTC 18991 suggested that IFN at the intermediate dosage for 1 year significantly improved RFS with no OS benefit and no added benefit with additional years of therapy. Sub-group analysis from EORTC 18991 suggested that patients with ulcerated primaries and microscopic nodal metastases benefited disproportionately in

terms of RFS/DMFS/OS– and this is to be prospectively evaluated in EORTC 18081 (adjuvant pegylated IFN for 2 years vs. observation in ulcerated node-negative patients).

Indications for Investigation of the Neo-adjuvant Setting of Therapy

Neo-adjuvant therapy has been considered for patients with locally advanced disease for whom immediate surgery is not feasible with the aim of down-staging disease to allow definitive resection and/or improving disease control locally. Neo-adjuvant therapy has been shown to improve survival in patients with bladder, breast, cervical and esophageal cancers [35–37] and has improved surgical outcomes in breast, laryngeal and rectal cancers.

Although neo-adjuvant options typically comprise radiotherapy and/or chemotherapy, there is great interest in the pursuit of neo-adjuvant settings for investigation of immunotherapy and molecular inhibitors of tumor drivers, given the unique immunogenicity of melanoma as delineated above and the limited utility of radiotherapy and chemotherapy in these patients. This is described in detail elsewhere [38]. Relevant to this consideration, the host immune response differs markedly between early and advanced melanoma. Melanoma progression is associated with a gradual shift from T_{H1}, mixed T_{H1}/T_{H2} to a T_{H2}/Treg predominant response [8, 41] – while the former promotes anti-tumor responses by cytotoxic T-cells (CTL) the latter is associated with a down-regulation of anti-tumor immunity and development of tumor-induced immune evasion suggesting that neo-adjuvant immunomodulatory therapy may be useful in this population.

Options for Neo-adjuvant Therapy

Several phase II studies have investigated concurrent chemotherapeutic and biochemotherapeutic (BCT) approaches for treatment of patients with resectable loco-regional or recurrent disease (IIIC/IVA) [42–44] and are summarized in Table 2 (Table 2 – **Studies of Neo-adjuvant Approachesin Melanoma**). These generally consisted of 2 or more cycles of chemotherapy (dacarbazine, cisplatin, and vinblastine) combined with immunomodulatory agents such as IL-2 and IFN-α and were associated with objective responses though complete responses (CR) were rare. Although apparently active in the neo-adjuvant phase II setting, two phase III trials (E3695/EORTC 18951) that pitted BCT against single agent chemotherapy demonstrated no significant benefit in either PFS/response rate with significant attendant toxicity [45–46]. It must be noted however that the doses of IL-2/IFN-α administered in these studies were low and potentially sub-optimal. Furthermore, the use of multiple chemotherapeutic agents contributed to the significant complexity of these regimens, and the number of adverse reactions noted.

Given the significant early separation of hazard curves observed in E1684 with patients on adjuvant HDI, Moschos et al performed a neo-adjuvant study in patients with stage IIIB/C disease who underwent initial biopsy followed by induction HDI (I.V. 20 MU/m^2 5 days a week for 4 weeks) followed by completion lymph node dissection and subsequent maintenance HDI (SC 10 MU/m^2 3 days a week for 48 weeks) [47]. Of the 20 patients enrolled, 3 had pathologic CRs whilst 8 had partial responses (PR) for a total objective response rate of 55%. Responders had significantly greater intra-tumoral (CD3+/CD11+) monocyte-derived dendritic cell subpopulations and evidence of abrogation of immune tolerance (p-STAT1 up-regulation and p-STAT3 down-regulation) [48]. Despite the striking findings in this neoadjuvant application of the induction portion of the HDI regimen, a large clinical evaluation of one month of induction therapy alone has shown no durable benefit when compared to observation for stage IIA melanoma patients [34].

Ipilimumab (Medarex Inc/Bristol-Myers Squibb) is a fully humanized immunoglobulin G1κ monoclonal antibody that competitively inhibits CTLA-4. Recently, two phase III trials have

been published that have evaluated ipilimumab in metastatic melanoma patients in different settings (first vs. second line), at different doses (10 mg/kg vs. 3 mg/kg) and against different comparators (gp100 peptide vaccine vs. dacarbazine) [10–11]. Although both the earlier phase III MDX010–20 trial and more recently published CA184-024 trial were associated with relatively low response rates of around 10%, the agent's *raison d' etre*(and regulatory approval) lay in producing durable CRs that together with the plateauing of the survival curves suggested a potential cure in a notoriously difficult to treat population. Data from this and other trials assessing the use of CTLA-4 inhibition in melanoma are summarized in Table 3 (Table 3 – **Phase II/III Studies ofCTLA-4 Blockadein Melanoma**) [10–11, 49–54].

Given the durable responses seen, investigators were excited to assess the potential of this agent in the neo-adjuvant space. An ongoing trial at the University of Pittsburgh is assessing the efficacy of neo-adjuvant pre-operative ipilimumab in patients with node-positive disease (IIIB-C). Following pre-treatment biopsies, induction ipilimumab (I.V. 10 mg/kg) was given on days 1 and 21 followed by definitive lymphadenectomy after at least 2 weeks. 2–4 weeks following surgery, two further doses of maintenance ipilimumab (I.V. 10 mg/kg) were given at 3 weekly intervals. Preliminary data was presented at ASCO 2011 following the enrollment of 17 of 28 planned patients, of whom 16 were suitable for analysis [55]. At a median follow-up of 7.9 months, the PFS probability was 84.4% at 6 and 63.3% at 12 months. More recent 14 month median follow-up data presented at ASCO 2012 was notable for median PFS 15.5 months and PFS probability was 82.4% at 6 and 53% at 12 months respectively [56]. Correlative analyses and detailed histopathological assessment were performed at baseline, 6 weeks, 3, 6, 9, 12 months both to characterize the tumor-microenvironment and immune interactions pre- and post- administration of ipilimumab and evaluate the effect(s) of ipilimumab on these interactions. Authors reported significant increases in the frequency of circulating FoxP3+T_{reg} cells that paralleled significant decline in circulating myeloid-derived stem cells.

Complete results from this trial and other trials evaluating neoadjuvant ipilimumab in prostate cancer (NCT01194271) and urothelial carcinoma (NCT00362713) are eagerly awaited to inform the scope of this discussion in the near future. At this time, there are no approved neo-adjuvant treatment options for patients with unresectable melanoma and the optimal treatment remains enrollment in a clinical trial.

Conclusions

Aggregate data from European and US intergroup studies (E1684, E1690, E1694, EORTC 18952, and EORTC 18991) suggest that adjuvant IFN-α improves RFS by about 30% with a lesser impact on OS that dissipates after 10 yrs. The European experience with pegylated IFN-α suggests that at the 3–6 μ g/kg/week dose approved for adjuvant therapy in the United States and Europe, pegylated IFN-α improves RFS in stage III disease by approximately 6.7% over the 4 year observation period in EORTC 18991 though this effect was sustained at a median follow-up of 7.6 years as updated at ASCO 2011 [57]. Although treatment with pegylated IFN improved DMFS, the difference was not statistically significant possibly because the survival benefit observed in a select minority (ulcerated node-negative) was attenuated by the majority of patients and the results of EORTC 18081 are eagerly awaited to answer this question.

Although hampered by a relatively low response rate and high incidence of grade 3/4 adverse events observed in the phase III trials in patients with metastatic melanoma, the promising RFS/OS benefit seen with ipilimumab have prompted studies of adjuvant and neo-adjuvant approaches both by the EORTC and the U.S. intergroup. Early data from a

UPCI study of neo-adjuvant ipilimumab in 30 stage III patients presented at ASCO 2011 and 2012 reported PFS improvements and evidence of significant modulation of host suppressor/effector immune responses. Final results from this study would greatly influence the design and conduct of further neo-adjuvant phase III trials utilizing ipilimumab.

The comparative genomic hybridization and systematic oncogene mutation analyses performed by Bastian and colleagues has led to the recognition of distinct molecular sub-types in melanoma – resulting in a shift in taxonomy from one based on clinical and histologic characteristics to a modern one based on the molecular heterogeneity observed with direct implications for therapeutic targeting [58]. It is now known that melanomas from sun-damaged skin, non-sundamaged skin, mucosal or acral surfaces harbor distinct molecular phenotypes. Specifically, activating mutations in B-RAF are present in approximately 40 to 60% of advanced melanomas especially those arising from non-sun damaged skin and results in activation of the RAS/RAF/MEK/ERK pathway providing a constitutive growth signal. Use of oncogenic BRAF inhibitors such as vemurafenib have shown dramatic results with CR rates of 48% in all subgroups including the high-risk M1c and elevated LDH cohorts in the phase III registration trial compared to DTIC [9].

Emerging evidence suggests that different melanoma subtypes may each be driven by diverse mechanisms of progression, associated with differing mechanisms of tumor escape and specific immunosuppression, innate immune cell activation and altered T-cell trafficking into tumor sites that in turn modulate response to immunotherapies. For example, Boni et al have reported that BRAF mutations contribute to immune escape – with BRAF inhibition resulting in increased recognition by antigen-specific T cells and reduced tumor-mediated immune tolerance [59] suggesting that BRAF-inhibition may be synergistic if combined with immuno-modulatory therapies such as IFN-α, IL-2 or ipilimumab. Additional mechanisms of MAPK pathway induced immunosuppression are emerging (Ferrone, Zarour, Kirkwood unpublished work) that may lend to combined modality immunotherapy for the future.

The next wave of clinic trials in melanoma will test rational combinations designed to exploit molecular chinks in the immunomodulatory armor of various sub-types of melanoma aiming to improve the durable OS/RFS benefits of adjuvant therapy. Neo-adjuvant study designs - in which tumor tissue is obtained both before and after introduction of study agent(s) – are crucial to dissecting the panoply of molecular and immunological interactions and furthering our understanding of anti-tumor responses so as to better tailor therapies to those most likely to benefit. These are detailed in a FDA recent publication that defined criteria for pathologic complete response (pCR) as an intermediate endpoint in clinical trials that is likely to spur interest in designing neo-adjuvant trials aimed at evaluating clinically active agents in high risk patient populations and potentially allowing accelerated approval if benefit is noted [60]. For now, the optimal management of patients with high-risk disease in the adjuvant setting is HDI whilst neo-adjuvant patients are best treated with enrolment into a clinic trial.

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Table 1

Phase III Studies of IFN-α for Advanced Melanoma

Study Reference	Number of Patients Eligible for Analysis	TNM Stage	Therapy and IFN Sub-species	Dose and Schedule – Treatment Arm	Median Follow-up at Time of Reporting (yrs)	DFS/RFS	OS	% Node positive
High Dose								
NCCTG 83-7052[19]	262	II–III (T2-4N0M0/TanyN+M0)	IFN-α2a vs. observation	I.M. 20 MU/m^2 thrice weekly for 4 months	6.1	NS	NS	61
ECOG E1684[5]	287	II–III (T4N0M0/TanyN+M0)	IFN-α2b vs. observation	I.V. 20MU/m^2 5 days a week for 4 weeks → then → SC 10MU/m^2 3 days a week for 48 weeks	12.6	HR: 1.38 (HDI vs. obs) (S)	HR: 1.22 (HDI vs. obs) (S at 6.9 yrs but NS at 12.6 yrs)	89
ECOG E1690[6]	642	II–III (T4N0M0/TanyN+M0)	IFN-α2b - high dose vs. low dose vs. observation	High Dose: I.V. 20MU/m^2 5 days a week for 4 weeks → then → S.C. 10MU/m^2 3 days a week for 48 weeks Low Dose: S.C. 3MU/m^2 2 days a week for 2 years	4.3	HR: 1.28 (HDI vs. obs) (S) 1.19 (LDI vs. obs) (NS) RFS: 44% (HDI) vs. 40% (LDI) vs. 35% (obs).	HR: 1.0 (HDI vs. obs) (NS) 1.04 (LDI vs. obs) (NS) OS: 52% (HDI) vs. 53% (LDI) vs. 55% (obs).	74
ECOG E1694[7]	774	II–III (T4N0M0/ TanyN+M0)	IFN-α2b vs. GMK vaccine	I.V. 20MU/m^2 5 days a week for 4 weeks → then → S.C. 10 MU/m^2 2 days a week for 48 weeks	2.1	HR: 1.49 (HDI vs. GMK) (S) RFS: 25% (HDI) vs. 39% (GMK)	HR: 1.38 (HDI vs. GMK) (S) OS: 78% (HDI) vs. 73% (GMK)	77
ECOG E2696[20]	107	II–IV	GMK vaccination with concurrent HDI (Arm A) vs. GMK vaccination with HDI beginning D28 (Arm B) vs. GMK vaccination alone (Arm C)	HDI: I.V. 20MU/m^2 5 days a week for 4 weeks → then → S.C. 10MU/m^2 3 days a week for 48 weeks GMK vaccination: GM2-KLH/ QS-21 on D1, 8,	2.4	HR: 1.75 (C vs. A) (S) 1.96 (C vs. B) (S) RFS: Not reached (A) vs. 30.72 mths (B) vs. 14.85 mths (C)	HR: not reported OS: Not reached (A, B or C)	Not Available

Study Reference	Number of Patients Eligible for Analysis	TNM Stage	Therapy and IFN Sub-species	Dose and Schedule – Treatment Arm	Median Follow-up at Time of Reporting (yrs)	DFS/RFS	OS	% Node positive
				15, 22 then weeks 12, 24,36				
Italian Melanoma Intergroup[21]	330	III (TanyN1-3M0)	Intensified IFN-α2b (IHDI) every other month vs. IFN-α2b for 1 yr	IHDI: I.V. 20MU/m^2 5 days a week for 4 weeks every other month for 4 cycles Standard HDI: I.V. 20MU/m^2 5 days a week for 4 weeks → then → S.C. 10MU/m^2 3 days a week for 48 weeks	5.0	Median RFS: 47.9 mths (IHDI) vs. 35.6 mths (HDI) (NS) 5 year RFS: 45.8% (IHDI) vs. 44.3% (HDI)	Median OS: 88.7 mths 5 year OS: 60.1% (IHDI) vs. 82.6% (HDI) (NS)	100
Intermediate Dose								
EORTC 18952[22]	1388	II–III (T4N0M0 or TanyN+M0)	IFN-α2b for 1 yr vs. 2 yrs vs. observation	Induction: I.V. 10MU 5 days a week for 4 weeks Maintenance: S.C. 10MU 3 days a week for 1 year *OR* S.C. 5MU 3 days a week for 2 years	4.7	DMFI: HR: 0.93 (13mth vs. obs) (NS) 0.83 (25mth vs. obs) (S)	DMFS: HR: 0.95 (13mth vs. obs) (NS) 0.85 (25mth vs. obs) (NS)	74
EORTC 18991[23]	1256	III (TanyN+M0)	PEG IFN-α2b vs. observation	Induction: S.C. 6μg/kg/week for 8 weeks Maintenance: S.C. 3μg/kg/week for 5 years	7.6	34.8 mths (IFN) vs. 25.6 mths (obs); S	Not reported	100
Nordic IFN[24]	855	IIB-IIIB (T4N0M0 or TanyN1-2M0)	IFN-α2b for 1 yr vs. 2 yrs vs. observation	observation (A) vs. S.C. 10MU 5 days a week for 4 weeks then S.C. 10MU 3 days a week for 1 year (B) vs. S.C. 10MU 5 days a week for 4 weeks then S.C. 10MU 3	6.0	23.2 mths (A) vs. 37.8 mths (B) vs. 28.6 mths (C) IFN vs. obs& IFN 1yr vs. obs(S);	56.1 mths (A) vs. 72.1 mths	81

Study Reference	Number of Patients Eligible for Analysis	TNM Stage	Therapy and IFN Sub-species	Dose and Schedule – Treatment Arm	Median Follow-up at Time of Reporting (yrs)	DFS/RFS	OS	% Node positive
				days a week for 2 years(C)		IFN 2yr vs. obs (NS)	(B) vs. 64.3 mths (C) (NS)	
Low Dose								
Austrian Melanoma Cooperative Group (AMCG)[25]	311	II (T2-4N0M0)	IFN-α2a vs. observation	S.C. 3MU 7 days a week for 3 weeks → then → S.C. 3MU 3 days a week for 1 year	3.4	RFS/ DMFS not reported Rate of relapse: (24.0% LDI vs. 36.3% obs)	Not available	0
French Melanoma Cooperative Group (FCGM)[26]	499	II (T2-4N0M0)	IFN-α2a vs. observation	S.C. 3MU 3 days a week for 18 months	>3	HR: 0.74 (LDI vs. obs) (S)	HR: 0.70 (LDI vs. obs) (S)	0
WHO Melanoma Program Trial 16[27]	444	III (TanyN+M0)	IFN-α2a vs. observation	S.C. 3MU 3 days a week for 36 months	7.3	NS	NS	100
Scottish Melanoma Cooperative Group[28]	96	II–III (T3-4N0M0/ TanyN+M0)	IFN-α2a vs. observation	S.C. 3MU 3 days a week for 6 months	>6	NS	NS	Not Available
EORTC 18871/DKG 80-1[29]	728	II–III (T3-4N0M0/ TanyN+M0)	IFN-α2b vs. IFN-γ vs. ISCADOR M® vs. observation	IFN-α2b: S.C. 1MU every other day for 12 months IFN-γ: S.C. 0.2mg every other day for 12 months	8.2	NS	NS	58
UKCCCR/AIM HIGH[30]	674	II–III (T3-4N0M0/TanyN+M0)	IFN-α2a vs. observation	S.C. 3MU 3 days a week for 24 months	3.1	NS	NS	Not available
DeCOG[31]	840	III (T3anyN+M0)	IFN-α2a	S.C. 3MU 3 days a week for 18 mths (A) vs5 yrs (B)	4.3	5 yr DMFS 81.9%(A) vs. 79.7% (B) (NS)	5 yr OS 85.9%(A) vs. 84.9% (B) (NS)	Not available
DeCOG[32]	444	III (TanyN+M0)	IFN-α2a	S.C. 3MU 3 days a week for 24 mths (A) vs. S.C. 3MU 3 days a week for 24 mths + DTIC 850 mg/m^2 every 4–8 weeks for 24	3.9	HR: 0.69 (A) vs. 1.01 (B) vs. 1.0 (C)	HR: 0.62 (A) vs. 0.96 (B) vs. 1.0 (C)	100%

Study Reference	Number of Patients Eligible for Analysis	TNM Stage	Therapy and IFN Sub-species	Dose and Schedule – Treatment Arm	Median Follow-up at Time of Reporting (yrs)	DFS/RFS	OS	% Node positive
				mths (B) vs. observation (C)				

Key: NS – Not significant; S – Significant; HR – Hazard ratio; DFS – Disease free survival; OS – Overall survival

Table 2

Studies of Neo-adjuvant Approachesin Melanoma

Study Reference	Number of Patients Eligible for Analysis	Study Design	Primary Endpoint	Dose and Schedule – Treatment Arm	Responses	Survival	Toxicity
Buzaid AC et al[42]	64	Phase II nonrandomized Study	ORR	3 weekly cycles consisting of: D1-4 I.V. cisplatin 20 mg/m^2 I.V. vinblastine 1.5 mg/m^2 I.V. continuous infusion IL-2 9 MIU/m^2/day D1 only I.V. dacarbazine 800 mg/m^2 D1-5 S.C. IFN-α2a 5 MU/m^2 2-4 cycles prior to surgery. Responders received up to 2 additional cycles.	44% (all PR)	Not reported	Not reported
Gibbs P et al[43]	48	Phase II nonrandomized Study	ORR	3 weekly cycles consisting of: D1-4 I.V. cisplatin 20 mg/m^2 I.V. vinblastine 1.6 mg/m^2 I.V. continuous infusion IL-2 9 MIU/m^2/day D1 only I.V. dacarbazine 800 mg/m^2 D1-5 S.C. IFN-α2a 5 MU/m^2 2 cycles prior to surgery. Responders received up to 2 additional cycles.	38.9% (13 PR, 1 CR)	79% alive, 65% progression-free (median follow-up 2.6 yrs)	Not reported
Shah GD et al[44]	19	Phase II non-randomized study	ORR	TMZ extended dosing cycle 75 mg/m^2/day×6 weeks with 2 weeks off per 8 week cycle	16% (1 PR, 2 CR)	Not reported	Lymphopenia – G3 16% (3/19) Transaminitis G3 16% (3/19)
Moschos SJ et al[45]	20	Phase II non-randomized study	ORR	Standard induction HDI with I.V. 20MU/m^2 5 days a week for 4 weeks Standard maintenance HDI with S.C. 10MU/m^2 3 days a week for 48 weeks Induction prior to surgery. Maintenance following surgery.	55% (8 PR, 3 CR)	90% progression-free (median follow-up 1.5 yrs)	33% dose reductions in 4/20 patients
E3695[45]	395	Phase III randomized study	Primary – OS Secondary – PFS, ORR, duration of response	3 weekly cycles of CVD or BCT CVD: D1-4 I.V. cisplatin 20 mg/m^2 I.V. vinblastine 1.5 mg/m^2 D1 only I.V. dacarbazine 800 mg/m^2 BCT – CVD as above with: D1-4 I.V. continuous infusion IL-2 9 MIU/m^2/day D1-5, 8, 10 and 12 S.C. IFN-α2a 5 MU/m^2	13.8% (CVD) vs. 19.5% (BCT)	OS: 8.7 (CVD) vs. 9.0 (BCT) PFS: 2.9 (CVD) vs. 4.8 (BCT) Duration of response: 9.4 (CVD) vs. 6.1 (BCT)	≥ Grade 3 in 73% (CVD) vs. 95% (BCT)
EORTC 18951[45]	363	Phase III randomized study	Primary – OS	D1-3 I.V. cisplatin 30 mg/m^2 I.V. dacarbazine 250 mg/m^2 D1-5: S.C IFN-α2b 10 MU/m2 D5-10: I.V. IL-2 67.5 MU/m^2 decrescendo schedule	41% (Arm A) vs. 38% (Arm B)	OS: 9.0 (Arm A) vs. 9.0 (Arm B) (NS) PFS: 3.0 (Arm A) vs. 3.96 (Arm B) (NS)	Higher incidence of Grade 3/4 hypotension, fever, lethargy, anorexia and

Study Reference	Number of Patients Eligible for Analysis	Study Design	Primary Endpoint	Dose and Schedule – Treatment Arm	Responses	Survival	Toxicity
			Secondary – ORR, PFS, RFS	Arm A: cisplatin, dacarbazine and IFN-α2b vs. Arm B cisplatin, dacarbazine and IFN-α2b and IL-2		RFS: HR 0.97 (BCT vs. chemotherapy) (NS)	diarrhea in Arm B vs. Arm A

Key: NS – Not significant; S – Significant; HR – Hazard ratio; DFS – Disease free survival; OS – Overall survival

Table 3

Phase II/III Studies of CTLA-4 Blockade in Melanoma

Study Reference	Number of Patients Eligible for Analysis	Study Design	Primary Endpoint	Dose and Schedule – Treatment Arm	ORR/OS	PFS (mths)	HR (95% CI)
BMS 008[49]	155	Phase II, open-label, single arm, advanced melanoma	Dose-finding	Ipilimumab – 10mg/kg	47% (1 yr)	N/A	N/A
BMS 022[50]	217	Phase II, randomized, double blind, advanced melanoma	Efficacy of three dose levels of ipilimumab	Ipilimumab – 0.3, 3, 10 mg/kg Induction 0.3/3/10 mg/kg q3weeks for 4 doses Maintenance – 0.3/3/10 mg/kg q6weeks until week 48 then q12weeks afterwards	48% (1yr)	N/A	N/A
BMS 007[51]	115	Phase II, randomized, double blind, advanced melanoma	Rate of Grade 2+ diarrhea	Ipilimumab – 10mg/kg	51% (1 yr)	N/A	N/A
Medarex MDX010–20[10]	676	Phase III, randomized, double blind, advanced melanoma	ORR, subsequently amended to OS	Ipilimumab – 3mg/kg	Ipi alone: 10.1 mths (95% CI 8.0 to 13.8) Ipi + GP-100: 10.0 mths (95% CI 8.5 to 11.5) GP-100 alone: 6.4 mths (95% CI 5.5 to 8.7)	Ipi alone: 2.86 mths (95% CI 2.76 to 3.02) Ipi + GP-100: 2.76 mths (95% CI 2.73 to 2.79) GP-100 alone: 2.76 mths (95% CI 2.73 to 2.83)	Ipi alone (compared to GP-100 alone): 0.66 (95% CI 0.51–0.87) Ipi + GP-100 (compared to GP-100 alone): 0.68 (95% CI 0.55–0.85)
BMS 024[11]	502	Phase III, randomized, double blind, advanced melanoma	OS	Ipilimumab + DTIC: Induction 10mg/kg + DTIC (850 mg/m^2) q3weeks for 4 doses Maintenance10mg/kg + DTIC (850 mg/m^2) q12weeks	Ipi+DTIC: 47.3% (1yr), 28.5% (2yr), 20.8% (3yr). DTIC alone: 36.3% (1yr), 17.9% (2yr), 12.2% (3yr).	Ipi+DTIC:2.8 DTIC alone: 2.6	Ipi+DTIC: OS 0.72 PFS 0.76
Sarnaik AA et al[52]	75	Phase II, single-arm, open-label, resected high-risk melanoma	40% rate of tolerable irAE	HLA A*0201 positive: Ipi3 or 10 mg/kg q8weekly for 12 months + multipeptide (MART-1/gp100/ tyrosinase) vaccine HLA A*0201 negative: IPI 10 mg/ kg q8weekly for 12 months	Not reached (29.5 mths follow up)	Resected stage IV: 40.5 mths Resected stage IIIc - not reached (29.5 mths follow up)	Not reached (29.5 mths follow up)
Margolin K et al[53]	72	Phase II, single-arm, open-label, advanced melanoma with brain metastases 2 cohorts: Cohort A (asymptomatic; no steroids) and; Cohort B	DCR	Ipilimumab: Induction 10mg/kg q3weeks for 4 doses Maintenance 10mg/ kgq12weeks	Cohort A: 18% Cohort B: 10%	N/A	N/A

Study Reference	Number of Patients Eligible for Analysis	Study Design	Primary Endpoint	Dose and Schedule – Treatment Arm	ORR/OS	PFS (mths)	HR (95% CI)
		(symptomatic, on stable dose of steroids)					
NIBIT-M1[54]	86	Phase II, single-arm, open-label, advanced melanoma	Immune-response DCR using irRC	Ipilimumab + fotemustine: Ipilimumab – Induction 10mg/kg q3weeks for 4 doses Maintenance 10mg/kg + DTIC (850 mg/m^2) q12weeks Fotemustine – 100 mg/m^2 qweekly for 3 weeks then q3weekly	irORR - 29.1% OS at 1 yr – 51.8% (median OS not reached)	Median irPFS – 5.3 mths	Not reported

Key: DCR – disease control rate; ORR – overall response rate; OS – overall survival; PFS – progression free survival; irRC – immune related response criteria; irAE – immune related adverse events; HR – hazard ratio; N/A – not applicable