Published in final edited form as:

Ann N Y Acad Sci. 2009 December ; 1182: 47–57. doi:10.1111/j.1749-6632.2009.05073.x.

Clinical and Immunologic Basis of Interferon Therapy in Melanoma

Ahmad A. Tarhini and John M. Kirkwood

University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA

Abstract

Interferon $\alpha 2b$ (IFN- $\alpha 2b$) at high dosage is critical to the reversal of signaling defects in T cells of melanoma patients, and to the durable effector (α DC1) polarization of dendritic cells. These immunoregulatory effects appear to be uniquely achieved with levels of IFN- α only attainable *in vivo* using the high-dose regimen of IFN- $\alpha 2b$ (HDI). Three US cooperative group studies have evaluated the benefit of HDI as an adjuvant therapy for high-risk melanoma. All have demonstrated significant and durable reduction in the frequency of relapse, while the first and third trials have demonstrated significant improvements in the fractions of patients surviving compared with observation (E1684) or with a ganglioside vaccine (GMK, E1694). A meta-analysis of 13 randomized trials evaluating adjuvant IFN therapy has now also demonstrated significant benefits for IFN in terms of RFS and OS. Research of IFN- α in melanoma is now focused on identifying prognostic markers of outcome and predictors of therapeutic response.

Keywords

melanoma; interferon- α ; adjuvant; neoadjuvant

Introduction to Melanoma

Survival of melanoma varies widely by stage, from a highly curable disease when detected in early stages, to a disease with dismal prognosis when in advanced inoperable stages.¹ The American Joint Committee on Cancer (AJCC) divides cutaneous melanoma into four stages. Primary tumors confined to the skin without regional lymph node involvement are assigned stages I and II depending on the thickness (depth) of the tumor, and the presence or absence of ulceration of the overlying epithelium, or invasion of the reticular dermis or subcutaneous fat (Clark level IV or V). Stage III comprises a disease with clinical or pathological evidence of regional lymph node involvement, or the presence of in transit or satellite metastases. Stage IV disease is defined by the presence of distant metastasis. Patients with stage I melanoma have an excellent prognosis with surgical treatment alone and a cure rate of more than 85%. The 3–5 years postsurgical relapse rate in patients with stages IIA and IIB is 20–30% and 40–55% respectively. Stage III melanoma patients with regional lymph node involvement have a 5-year relapse rate of 40–80%, while stage IV disease has a dismal prognosis with a median survival of only 6 to 9 months.^{2, 3}

© 2009 New York Academy of Sciences.

Address for correspondence: Ahmad A. Tarhini, University of Pittsburgh, UPMC Cancer Pavilion, 5150 Centre Avenue, 5th floor, Pittsburgh, PA 15232. Voice: 412 648 6507; fax: 412 648 6579. tarhiniaa@upmc.edu.

Conflicts of Interest

John Kirkwood: Schering Plough Speaker's Board.

Immunity in Melanoma and Implications for Adjuvant Immunotherapy

Immunity to melanoma appears to be important for disease control in the adjuvant and advanced disease settings. Spontaneous regression of disease has been reported in patients with melanoma, suggesting a role for host immunity, indirectly supported by the pathological evidence for the presence of lymphoid infiltrates at primary melanoma associated with tumor regression. Host cellular immune responses within melanoma have potential prognostic and predictive significance. T cell infiltrates in primary melanoma have been suggested to be of prognostic significance,⁴ and T cell infiltrates within regional nodal metastases predicts benefit in patients treated with neoadjuvant IFN- α 2b therapy.^{5–7}

The quality of the host immune response has been shown to differ between earlier and more advanced disease settings. While T helper type 1 (Th1)-type CD4⁺ antitumor T-cell function appears to be critical to the induction and maintenance of antitumor cytotoxic T-lymphocyte (CTL) responses *in vivo*, and Th2- or Th3/Tr-type CD4⁺ T-cell responses may subvert Th1-type cell mediated immunity yielding a microenvironment that facilitates disease progression, patients with active melanoma or renal cell carcinoma have been shown to display strong tumor antigen specific Th2-type polarization. On the other hand, normal donors and patients who were disease free following therapy demonstrate either strongly polarized mixed Th1-/Th2-type or str Th1-type responses to the same epitopes.^{8, 9} Therefore, host immune tolerance appears to be an impediment to the therapy of advanced disease, and this may be avoidable in the high-risk setting of operable disease, where the host susceptibility to immunologic interventions may be greater, and where IFN- α 2b has demonstrated its significant impact upon melanoma relapse and survival.

Interferon- α in the Treatment of Melanoma

IFN- α was the first recombinant cytokine to be investigated clinically for the therapy of metastatic melanoma. Initial phase I-II studies yielded overall response rates of about 16% and about one third achieved complete and durable responses. Responses were observed as late as six months from initiation of therapy, and up to one third of the responses were durable. However, the median duration of response was only about four months.^{10–13} The use of IFN- α for adjuvant therapy of patients with melanoma is based on the hypothesis that micrometastatic disease is the source of future relapse, and less established in its induction of host tolerance of tumor. While patients with advanced metastatic melanoma display immunological tolerance, the adjuvant setting may be more susceptible to interventions designed to induce Th1 host-effector mechanisms to eradicate micrometastases.

Multiple IFN- α 2b regimens, that may be categorized as high-dose, intermediate-dose, or low-dose regimens, have been evaluated as adjuvant therapy for intermediate/high-risk (T3–4, lymph node positive) surgically resected melanoma (Table 1). The only randomized controlled trials that have shown durable relapse-free survival (RFS) and overall survival (OS) impact have utilized the high-dose IFN- α 2b regimen (HDI).^{14–16} A meta-analysis of 12 randomized trials of adjuvant IFN- α 2b for high-risk melanoma confirmed a highly significant reduction in the odds of recurrence in patients treated with IFN compared to observation. The analysis also demonstrated evidence of increased benefit with increasing IFN dose and a trend for improved benefit with increasing total dose. This meta-analysis did not find a statistically significant OS benefit for IFN- α 2b, although a larger individual patient data meta-analysis of 13 randomized trials showed a significant though small impact of IFN upon OS.^{17, 18} In this latter meta-analysis, there was statistically significant benefit for interferon (IFN) for both relapse-free survival (RFS) (OR = 0.87, CI = 0.81–0.93, P = 0.00006) and overall survival (OS) (0.9, 0.84–0.97, P = 0.008). This survival advantage translates into an absolute benefit of about 3% (CI 1%–5%) at 5 years. This analysis did not, however, clarify whether there is an optimal (high, intermediate or low) dose of IFN.¹⁸

Adjuvant High-dose IFN- α 2b (HDI) for High-risk Resected Melanoma

Since 1984, three national cooperative group studies have evaluated the benefit of high-dose IFN (HDI) as adjuvant therapy for resectable high-risk cutaneous melanoma. These included patients with regional lymph node metastases (T_{1-4} , N_1 , M_0) and primary localized deep melanomas (T_4 , N_0 , M_0) that have a 5-year postsurgical relapse rate of more than 40–50%.

The first and third of these studies both demonstrated significant survival prolongation, compared to observation (E1684; The median RFS was 1.72 years in the high-dose interferon α -02b (HDI) arm versus 0.98 year in the Obs arm [stratified log-rank one-sided P value (P_1) = 0.0023], and the median OS was 3.82 versus 2.78 years (P_1 = 0.0237), respectively)¹⁴ and compared to a vaccine (GMK) that was selected as the optimal vaccine candidate at the time (E1694). The results of this trial were reported in 2001 based on a final analysis in June 2000, with a median follow-up interval of 16 months. Among eligible patients in this trial, HDI provided a statistically significant RFS benefit (HR = 1.47; P_1 = 0.0015) and OS benefit (HR = 1.52; P_1 = 0.009) compared with GMK. A similar benefit was observed in the intent-to-treat analysis of RFS (HR = 1.49) and OS (HR = 1.38).¹⁹

The second trial, E1690, conducted in part before and in part after the approval of HDI, was associated with systematic crossover of patients from the observation-assigned arm to treatment at nodal relapse with HDI. This trial showed differences in terms of RFS but not OS. In the intent-to-treat analysis of RFS, treatment with HDI was associated with a statistically significant benefit compared with Obs (HR = 1.28; P_1 = 0.025). In contrast, LDI was not associated with a significant RFS benefit compared with Obs. Neither HDI nor LDI regimens had any apparent impact on OS compared with Obs in this trial. However, a retrospective analysis of salvage therapy demonstrated the occurrence of a disproportionate crossover of patients from the Obs arm to HDI therapy postprotocol in those patients who developed regional recurrence (stage IIB patients in this trial were not required to undergo lymphadenectomy), which may have confounded the survival analysis.¹⁵

The analysis of each of the foregoing studies has been conducted at the closure of each study^{14, 15, 19} and has been updated in a pooled analysis of survival and relapse-free outcomes to April 2001.²⁰ The pooled analysis has firmly demonstrated that melanoma relapse has been prevented by IFN to intervals that now approach 20 years, and yet this analysis has not yielded compelling evidence of an impact upon OS despite the positive survival results of two randomized US Cooperative Group and Intergroup studies (E1684 and E1694). This may not be surprising, given that the larger of the two observation-controlled trials included in the pooled analysis (E1690) did not show an OS benefit for HDI. As discussed previously, the confounding of the OS analysis of E1690 by the routine crossover to HDI of all but one of 37 patients assigned to observation who had nodal relapse, associated with an unusually prolonged postrelapse survival of those patients in the observation arm treated with HDI, may have been responsible for this outcome variability. Patients treated with HDI in E1694 have not been included in the pooled analysis because the comparator in that trial was the GMK vaccine and not observation as was the case in E1684 and E1690.

An interim analysis of a phase III trial (EORTC 18961) of adjuvant GMK vaccination versus observation after resection of the primary in AJCC stage II (T_{3-4} , N_0 , M_0) melanoma patients was recently reported.²¹ For the primary endpoint, RFS, the criteria for stopping for futility were met. For OS, the results suggested a detrimental effect of the vaccine, although this trial is so early in its follow-up that it is difficult to interpret this finding given that no adverse effect was seen in terms of the generally more sensitive endpoints of RFS, and distant metastasis-free survival. The investigators concluded that GMK vaccination is ineffective and might even be detrimental in the stage II melanoma

adjuvant setting. These data have led some investigators to question the evidence for a benefit of HDI upon OS in high-risk melanoma patients studied in E1694. On the other hand, there continues to be a wide agreement upon the RFS benefit of HDI supported by all three randomized trials (E1684, E1690, E1694) as well as the data from multiple lower-dose IFN trials summarized by Wheatley and Ives.²² As discussed earlier, this individual patient data meta-analysis of 13 randomized trials showed a significant though small impact of IFN upon OS (HR = 0.9 with an absolute 5-year increment of 3%). In addition, no other agent has ever been demonstrated to provide similar relapse-free or survival benefits for this patient population. Therefore, HDI continues to be the current standard and the only option available for these patients outside of a clinical trial.

The Role of Dose, Route, and Duration of IFN- α Therapy in Melanoma

Less intensive (less toxic) regimens tested in the melanoma adjuvant setting have not previously ever demonstrated durable effects upon relapse or death as has been observed with HDI. These include very low-dose IFN (1 MU SC QOD) as tested in EORTC 18871 (AJCC TNM melanoma stage T3–4, N1)²³ and low-dose IFN (3 MU SC TIW) as tested in WHO Trial 16 (N1–2),²⁴ ECOG 1690 (T4, N1),¹⁵ UKCCR AIM-High trial (T4, N1),²⁵ and the Scottish trial.²⁶ These also include intermediate-dose IFN (given subcutaneously) regimens tested in EORTC 18952 (T4, N1–2)²⁷ and EORTC 18961 (TxN1–2).²¹

All trials of IFN- α with durable RFS and OS impact utilized an intravenous (i.v.) induction phase given at 20MU/m² five days a week for four weeks ($C_{\max} > 10,000$ u/ml). Based on this experience, a US intergroup trial, E1697 was designed to evaluate the impact of a 4-week course of high dose IFN- α 2b similar to the induction phase of the HDI regimen tested in E1684/E1690/E1694 trials. Eligibility for this trial includes patients with resected melanoma in the following categories: (1) T_{2b} N₀ (2) T_{3a–b} N₀ (3) T_{4a–b} N₀ (4) T_{1–4} N_{1a}, 2a (microscopic), and the control arm is observation. Out of the 1420 patients planned for accrual (to demonstrate a 7% improvement in RFS) to this study, more than 950 have been accrued as of April 2009. Other groups are also testing the role of the I.V. induction phase of HDI, including the Italian Melanoma Intergroup (HDI Induction given every 2 months for a total of four courses; 80 doses, $n = 300$) and DeCOG (HDI Induction given every 4 months for one year; 60 doses, $n = 800$).

The Hellenic Oncology Group conducted a randomized, phase 3 trial to evaluate intravenous induction therapy with IFN- α 2b for 4 weeks as compared with the same regimen followed by 11 months of adjuvant IFN- α 2b therapy. This trial used a modified high-dose adjuvant IFN regimen derived from the E1684/1690/1694 HDI regimen. The study design proposed that the one-month treatment would be considered at least as good as the one-year regimen treatment, if the relapse rate at 3 years from study entry is at most 15% higher in the former arm. A relapse rate of 60% was assumed in both treatment arms. A sample size of 152 (182 enrolled) patients per treatment arm was planned. The trial concluded that at the 5% level of significance the 3-year relapse rate of the one month group was not 15% higher than the relapse rate of the one-year group. There were also no significant differences in OS, disease-free survival (DFS) or severe toxicities between the arms. The issues with this study are related to the adoption of a nonstandard IFN induction dose regimen for one month (15 Mu/M² compared to the 20 Mu/M² HDI dosing; $n = 182$) and a nonstandard 3/4 IFN maintenance regimen for the balance of one year (delivering 10 Mu/dose rather than 10 Mu/M² as specified in the FDA-approved HDI dosage regimen; $n = 182$). This study arm of one month would have termed as noninferior, a treatment with 15% lower 3-year RFS/OS. Beyond this, in the absence of an observation control it is not certain what level of activity was achieved by either arm. By comparison, a US cooperative group proposal to test equivalence of one month and one year (with a 5% threshold) was designed and abandoned when it became apparent that it would have required 3000 patients in 1991.

A second major question that has not been clearly answered is whether prolonged adjuvant therapy may offer improved results. A Dermatologic Cooperative Oncology Group (DeCOG) study of lower-dose IFN- α 2a treatment at 3 MU 3x/wk s.c. for 5 years versus 18 months showed no difference in RFS or OS.²⁸

The EORTC 18952 trial tested two intermediate dosages of IFN (IDI) administered over 2 years versus one year and showed nonsignificant differences in favor of the 2-year regimen.²⁹ The Nordic IFN- α trial comparing an identical regimen for 2 years versus one year of IDI showed nonsignificant differences in favor of the one-year regimen. Most recently, the EORTC trial 18991 testing Peg-IFN- α has shown neither improved OS nor DMFS overall, although RFS benefits overall have been noted on analysis for regulatory review, and these appear to be confined to the subset of patients without gross nodal disease (termed N1 by the EORTC). The RFS difference of 16% at 5 years was significant in the N1 group with microscopic nodal disease. Unfortunately, this trial designed to deliver 5 years of therapy has shown a median treatment interval of only one year, so the question of whether longer therapy with this regimen achieves more significant antitumor effects can not be answered at this time. The hypothesis that was being tested, that prolonged lower-dose therapy might achieve anti-angiogenic effects has not yet been analyzed.

The meta-analysis of individual patient data that was undertaken by Ives, Wheatley and the leaders of the cooperative group trials of IFN through 2007 initially suggested that the presence of primary tumor ulceration predicted increased susceptibility to IFN therapeutic effects.³⁰ A more recent analysis of the adjuvant trials EORTC18952 and EORTC18991 has assessed the predictive value of ulceration in relation to the therapeutic impact of IFN- α in terms of RFS, DMFS and OS, overall, and according to stage: IIB and III (N1 microscopic nodal, N2 macroscopic nodal disease). Among 2,644 patients randomized into these studies less than one-third (849) had ulcerated primaries, and 1,336 nonulcerated primaries, while for 459 the ulceration status was unknown. In the group of patients with ulcerated primary melanomas, the impact of IFN was noted to be greater than in the nonulcerated group for RFS (test for interaction: $P = 0.02$), DMFS ($P < 0.001$), and OS ($P < 0.001$). The greatest effects of therapy were noted in patients with ulceration and stages IIB/III-N1. Based on this retrospective analysis, the EORTC have planned the EORTC 18081 trial, which will compare the benefit of Peg-IFN- α 2b versus observation in patients with ulcerated primaries and Breslow depth of more than 1mm (node negative). It is noteworthy that unlike US cooperative groups, the EORTC does not require central pathology review for EORTC melanoma trials.³¹

Neoadjuvant HDI Treatment of Potentially Resectable Local-Regional Metastases of Cutaneous Melanoma

Patients with clinically palpable regional lymph node metastases (AJCC stage IIIB-C; T_{any}, N_{1b}, 2b, 2c, 3) carry a risk of relapse and death that approaches 70% at 5 years.^{1, 32, 33}

In a pilot study, neoadjuvant HDI was investigated in this group of patients, who underwent surgical biopsy at study entry and then received standard intravenous HDI (20 million units/m², 5 days per week) for 4 weeks followed by complete lymphadenectomy. This was followed by standard maintenance sub-cutaneous HDI (10 million units/m² three times per week) for 48 weeks. Biopsy samples were obtained before and after intravenous HDI and subjected to immunohistochemical (IHC) analysis as well as routine pathologic study. Twenty patients were enrolled, and biopsy samples were informative for 17. Eleven patients (55%) demonstrated objective clinical response, and three patients (15%) had complete pathologic response. At a median follow-up of 18.5 months (range, 7–50 months) 10 patients had no evidence of recurrent disease.³⁴ In the context of this neoadjuvant study, HDI was found to upregulate pSTAT1, whereas it downregulates pSTAT3 and total *STAT3*

levels in both tumor cells and lymphocytes. Higher pSTAT1/pSTAT3 ratios in tumor cells pretreatment were associated with longer OS ($P = 0.032$). The pSTAT1/pSTAT3 ratios were augmented by HDI both in melanoma cells ($P = 0.005$) and in lymphocytes ($P = 0.022$). Of the immunologic mediators and markers tested, *TAP2* was augmented by HDI (but not *TAP1* and MHC class I/II).³⁵ In addition, HDI was found to regulate MAPK signaling differentially in melanoma tumor cells and host lymphoid cells. HDI was found to downregulate pSTAT3 ($P = 0.008$) and phospho-MEK1/2 ($P = 0.008$) levels significantly in tumor cells. Phospho-ERK1/2 was downregulated by HDI in tumor cells ($P = 0.015$), but not in lymphoid cells. HDI downregulated *EGFR* ($P = 0.013$), but pSTAT3 activation appeared not to be associated with *EGFR* expression and the MEK/ERK MAPK pathway, indicating that *STAT3* activation is independent of the EGFR/MEK/ERK signaling pathway.³⁶ Clinical responders had significantly greater increases in endotumoral CD11c⁺ and CD3⁺ cells and significantly greater decreases in endotumoral CD83⁺ cells compared with nonresponders.⁷

Improved Survival and Relapse-free Interval Associated with Autoimmunity Induced by HDI Therapy for High-risk Melanoma

Recent studies of immunotherapy for melanoma including high-dose IL-2,³⁷ anti-CTLA4 antibody^{38–40} have suggested a correlation of antitumor effects and autoimmune phenomena like thyroiditis, hypophysitis, enteritis, hepatitis, and dermatitis. More recently, patients who have shown a strong correlation of prolonged RFS and OS after treatment with the modified adjuvant IFN regimen (Hellenic) related to the E1684/1690/1694 HDI regimen have demonstrated a strong correlation with autoimmune phenomena and/or the appearance of auto-antibodies in the serum.⁴¹ Autoantibodies were detected in 52 (26%) of the group of 200 patients tested. Clinical manifestations of autoimmunity were observed among 15 (7%) of patients including vitiligo-like depigmentation in 11 (5%). A total of 113 patients have relapsed and 82 have died. The median time to progression (TTP) was 27.6 months and the median survival was 58.6 months. The median TTP for the patients who did not develop clinical or serological evidence of autoimmunity was 15.9 months while it has not been reached for the 52 patients who developed autoimmunity (106 vs 7; $P < 0.0001$). The median survival was 37.5 months for those who were negative and has not been reached for the other group (80 vs. 2 $P < 0.001$). In multivariate analysis the presence of autoimmunity was an independent favorable prognostic marker.

We evaluated the E2696 and E1694 trials to better understand the prognostic value of autoimmunity induced by HDI. In E2696, patients with resectable high risk melanoma were randomized to GM2-KLH/QS-1 (GMK) vaccine plus concurrent HDI, GMK plus sequential HDI, or GMK alone. In E1694, patients were randomized to either GMK or HDI. Sera from 103 patients in E2696 and 691 patients in E1694 banked at baseline and up to three additional time points were tested by ELISA for the development of five autoantibodies. In E2696, autoantibodies were induced in 17 subjects (25%; $n = 69$) receiving HDI and GMK versus 2 (6%; $n = 34$) receiving GMK without HDI (2 P -value = 0.029). Of 691 patients in E1694, 67 subjects (19.3%; $n = 347$) who received IFN developed autoantibodies versus only 15 (4.4%; $n = 344$) in the vaccine control group (2 P -value < 0.001). In the HDI arms, almost all induced autoantibodies were detected at 12 weeks after initiation of therapy. A one-year landmark analysis of E1694 resected stage III patients, showed survival advantage associated with HDI-induced autoimmunity that approaches statistical significance (HR = 1.54; $P = 0.072$) adjusting for treatment.^{42, 43}

These observations support the hypothesis that prevention of melanoma relapse and mortality with IFN is associated with immunomodulation that may increase resistance to melanoma. IFN- α 2b induction of autoimmunity may provide a useful surrogate biomarker of adjuvant therapeutic benefit. Studies of autoimmunity and its genetic determinants may help identify patients most likely to benefit from HDI and other newer immunotherapies

associated with autoimmunity, such as the anti-CTLA-4 blocking antibodies ipilimumab and tremelimumab.

Baseline Proinflammatory Cytokine Levels Predict Relapse-free Survival Benefit with HDI

The detection of serum biomarkers that are either prognostic of clinical outcome, or predictive of response to IFN- α 2b has been pursued using high-throughput xMAP® multiplex immunobead assay technology (Luminex Corp.). This technology was utilized to simultaneously measure the levels of 29 cytokines, chemokines, angiogenic and growth factors, as well as soluble receptors in the sera of 179 patients with high-risk melanoma who have participated in the E1694, and 378 healthy age and gender-matched controls. These banked sera that have been tested were prospectively collected in the course of the intergroup E1694 trial. The 179 melanoma patients were chosen at random from the two trial arms according to disease status (whether the subject had relapsed at <1 year, between one and 3 years, or more than 5 years). Of those samples tested, 93 were derived from patients who received GMK vaccination and 86 were derived from patients treated with HDI. The clinical data from the E1694 trial were then mature to a median of 4.6 years of follow-up.

The results demonstrated that serum concentrations of IL-1 α , IL-1 β , IL-6, IL-8, IL-12p40, IL-13, G-CSF, MCP-1, MIP-1 α , MIP-1 β , IFN- α , TNF- α , EGF, VEGF, and TNFR2 are significantly higher among patients with resected high-risk melanoma, when compared to healthy controls. Serum levels of immunosuppressive, angiogenic/growth stimulatory factors (VEGF, EGF, HGF) were decreased by IFN- α 2b therapy significantly while levels of anti-angiogenic IP-10 and IFN- α were elevated post treatment. Comparing patients according to relapse outcome, the pretreatment levels of pro-inflammatory cytokines IL-1 β , IL-1 α , IL-6, TNF- α , and chemokines MIP-1 α , and MIP-1 β were significantly higher in sera of patients with longer RFS of greater than 5 years, compared with patients who experienced shorter RFS of less than one year.

Conclusion

IFN- α 2b at high dosage has demonstrated durable adjuvant therapeutic impact upon melanoma patients that are at high risk for recurrence and death after surgical resection, with either deep primary melanoma alone, or nodal involvement by microscopic or macroscopic tumor. An immunological mechanism of action has been suggested for the therapeutic actions of IFN- α 2b and the reversal of signaling defects in T cells of melanoma patients and to the polarization of dendritic cells. The induction of autoimmunity is a marker of altering immunologic tolerance and may be utilized as a post-treatment correlate of therapeutic benefit. A baseline proinflammatory serum cytokine profile (IL-1 α , β ; MIP-1 α , β ; IL-6, TNF- α) pre-therapy is predictive of benefit from HDI at 5 years. The restoration of T cell STAT1 signaling has also been advanced as a predictive tool for the selection of patients for therapy. In addition, biological features of the primary tumor such as ulceration have been advanced as potential tools for the selection of patients with a greater likelihood of benefit from lower-dose regimens of therapy, although the role of ulceration of the primary has not proven to be of use in relation to the FDA-approved regimen of HDI. These will be tested prospectively over the next 5 years. Ultimately, genetic factors that may distinguish patients who are susceptible to the immunoregulatory effects of IFNs and other immunotherapies are being sought in relation to current US intergroup trial E1697 that may be informative in the next few years.

References

1. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J. Clin. Oncol.* 2001; 19:3635–3648. [PubMed: 11504745]
2. Manola J, Atkins M, Ibrahim J, et al. Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials. *J. Clin. Oncol.* 2000; 18:3782–3793. [PubMed: 11078491]
3. Kirkwood JM, Agarwala SS. Systemic cytotoxic and biologic therapy of melanoma. In: DeVita, VT.; Hellman, S.; Rosenberg, SA., editors. *Cancer: Principles and Practice of Oncology*. Lippincott; Philadelphia, PA: 1993. p. 1-16.
4. Clemente CG, Mihm MC Jr, Bufalino R, et al. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer.* 1996; 77:1303–1310. [PubMed: 8608507]
5. Hakansson A, Gustafsson B, Krysanter L, et al. Tumour-infiltrating lymphocytes in metastatic malignant melanoma and response to interferon alpha treatment. *Br. J. Cancer.* 1996; 74:670–676. [PubMed: 8845294]
6. Mihm MC, Clemente CG, Cascinelli N. Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response. *Lab. Invest.* 1996; 74:43–47. [PubMed: 8569196]
7. Moschos SJ, Edington HD, Land SR, et al. Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. *J. Clin. Oncol.* 2006; 24:3164–3171. [PubMed: 16809739]
8. Tatsumi T, Kierstead LS, Ranieri E, et al. Disease-associated bias in T helper type 1 (Th1)/Th2 CD4(+) T cell responses against MAGE-6 in HLA-DRB10401(+) patients with renal cell carcinoma or melanoma. *J. Exp. Med.* 2002; 196:619–628. [PubMed: 12208877]
9. Tatsumi T, Herrem CJ, Olson WC, et al. Disease stage variation in CD4⁺ and CD8⁺ T-cell reactivity to the receptor tyrosine kinase EphA2 in patients with renal cell carcinoma. *Cancer Res.* 2003; 63:4481–4489. [PubMed: 12907621]
10. Creagan ET, Ahmann DL, Frytak S, et al. Phase II trials of recombinant leukocyte A interferon in disseminated malignant melanoma: results in 96 patients. *Cancer Treat. Rep.* 1986; 70:619–624. [PubMed: 3518925]
11. Creagan ET, Ahmann DL, Frytak S, et al. Recombinant leukocyte A interferon (rIFN-alpha A) in the treatment of disseminated malignant melanoma. Analysis of complete and long-term responding patients. *Cancer.* 1986; 58:2576–2578. [PubMed: 3779607]
12. Creagan ET, Kovach JS, Long HJ, et al. Phase I study of recombinant leukocyte A human interferon combined with BCNU in selected patients with advanced cancer. *J. Clin. Oncol.* 1986; 4:408–413. [PubMed: 3950678]
13. Creagan ET, Schutt AJ, Long HJ, et al. Phase II study: the combination DTIC, BCNU, actinomycin D, and vincristine in disseminated malignant melanoma. *Med. Pediatr. Oncol.* 1986; 14:86–87. [PubMed: 3713642]
14. Kirkwood JM, Strawderman MH, Ernstoff MS, et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J. Clin. Oncol.* 1996; 14:7–17. [PubMed: 8558223]
15. Kirkwood JM, Ibrahim JG, Sondak VK, et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J. Clin. Oncol.* 2000; 18:2444–2458. [PubMed: 10856105]
16. Kirkwood JM, Ibrahim J, Lawson DH, et al. High-dose interferon alfa-2b does not diminish antibody response to GM2 vaccination in patients with resected melanoma: results of the Multicenter Eastern Cooperative Oncology Group Phase II Trial E2696. *J. Clin. Oncol.* 2001; 19:1430–1436. [PubMed: 11230488]
17. Wheatley K, Ives N, Hancock B, et al. Does adjuvant interferon-alpha for high-risk melanoma provide a worthwhile benefit? A meta-analysis of the randomised trials. *Cancer Treat. Rev.* 2003; 29:241–252. [PubMed: 12927565]

18. Wheatley, K.; Ives, N.; Eggermont, A.; Kirkwood, JM. Interferon-alfa as adjuvant therapy for melanoma: an individual patient data meta-analysis of randomised trials. ASCO Annual Meeting; Chicago. 2007.
19. Kirkwood JM, Ibrahim JG, Sosman JA, et al. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. *J. Clin. Oncol.* 2001; 19:2370–2380. [PubMed: 11331315]
20. Kirkwood JM, Manola J, Ibrahim J, et al. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. *Clin. Cancer Res.* 2004; 10:1670–1677. [PubMed: 15014018]
21. Eggermont, A.; Suci, S.; Ruka, W. EORTC 18961: Post-operative adjuvant ganglioside GM2-KLH21 vaccination treatment vs observation in stage II (T3-T4N0M0) melanoma: 2nd interim analysis led to an early disclosure of the results.. *J. Clin. Oncol*; ASCO Annual Meeting; Chicago. (May 20 suppl; abstr 9004), 2008; 2008.
22. Wheatley, K.; Ives, N.; Eggermont, A.; et al. International Malignant Melanoma Collaborative Group. Interferon- α as adjuvant therapy for melanoma: An individual patient data meta-analysis of randomised trials.. In: *Oncology, JoC, editor. Journal of Clinical Oncology: ASCO Annual Meeting; Chicago.* 2007.
23. Kleeberg UR, Suci S, Bocker EB, et al. Final results of the EORTC 18871/DKG 80-1 randomised phase III trial. rIFN- α 2b versus rIFN- γ versus ISCADOR M versus observation after surgery in melanoma patients with either high-risk primary (thickness >3 mm) or regional lymph node metastasis. *Eur. J. Cancer.* 2004; 40:390–402. [PubMed: 14746858]
24. Cascinelli N, Belli F, MacKie RM, et al. Effect of long-term adjuvant therapy with interferon α -2a in patients with regional node metastases from cutaneous melanoma: a randomised trial. *Lancet.* 2001; 358:866–869. [PubMed: 11567700]
25. Hancock BW, Wheatley K, Harris S, et al. Adjuvant interferon in high-risk melanoma: the AIM HIGH Study–United Kingdom Coordinating Committee on Cancer Research randomized study of adjuvant low-dose extended-duration interferon α -2a in high-risk resected malignant melanoma. *J. Clin. Oncol.* 2004; 22:53–61. [PubMed: 14665609]
26. Cameron DA, Cornbleet MC, Mackie RM, et al. Adjuvant interferon α 2b in high risk melanoma – the Scottish study. *Br. J. Cancer.* 2001; 84:1146–1149. [PubMed: 11379605]
27. Eggermont AM, Suci S, MacKie R, et al. Post-surgery adjuvant therapy with intermediate doses of interferon α 2b versus observation in patients with stage IIB/III melanoma (EORTC 18952): randomised controlled trial. *Lancet.* 2005; 366:1189–1196. [PubMed: 16198768]
28. Hauschild, A.; Volkenandt, M.; Tilgen, W., et al. Efficacy of interferon α 2a in 18 versus 60 months of treatment in patients with primary melanoma of 1.5 mm tumor thickness: A randomized phase III DeCOG trial. *American Society of Clinical Oncology; Chicago, IL:* 2008. p. 15S
29. Eggermont AM, Suci S, Santinami M, et al. Adjuvant therapy with pegylated interferon α -2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. *Lancet.* 2008; 372:117–126. [PubMed: 18620949]
30. Ives, NJ.; Stowe, RL.; Lorigan, P.; Wheatley, K. Biochemotherapy versus chemotherapy for metastatic malignant melanoma: A meta-analysis of the randomised trials.. ASCO Annual Meeting; 2007.
31. Eggermont, M.; Suci, S.; Testori, A., et al. Ulceration of primary melanoma and responsiveness to adjuvant interferon therapy: Analysis of the adjuvant trials EORTC18952 and EORTC18991 in 2,644 patients.. ASCO Annual Meeting; Orlando, FL. 2009.
32. Balch CM, Soong SJ, Murad TM, et al. A multifactorial analysis of melanoma: III. Prognostic factors in melanoma patients with lymph node metastases (stage II). *Ann. Surg.* 1981; 193:377–388. [PubMed: 7212800]
33. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J. Clin. Oncol.* 2001; 19:3622–3634. [PubMed: 11504744]

34. Kirkwood JM, Ernstoff MS, Davis CA, et al. Comparison of intramuscular and intravenous recombinant alpha-2 interferon in melanoma and other cancers. *Ann. Intern. Med.* 1985; 103:32–36. [PubMed: 4003987]
35. Wang W, Edington HD, Rao UN, et al. Modulation of signal transducers and activators of transcription 1 and 3 signaling in melanoma by high-dose IFNalpha2b. *Clin. Cancer Res.* 2007; 13:1523–1531. [PubMed: 17332298]
36. Wang W, Edington HD, Rao UN, et al. Effects of high-dose IFNalpha2b on regional lymph node metastases of human melanoma: modulation of STAT5, FOXP3, and IL-17. *Clin. Cancer Res.* 2008; 14:8314–8320. [PubMed: 19088050]
37. Liu K, Rosenberg SA. Interleukin-2-independent proliferation of human melanoma-reactive T lymphocytes transduced with an exogenous IL-2 gene is stimulation dependent. *J. Immunother.* 2003; 26:190–201. [PubMed: 12806273]
38. Ribas A. Phase I trial of monthly doses of the human anti-CTLA4 monoclonal antibody CP-675, 206 in patients with advanced melanoma. *Proc. ASCO.* 2005:716.
39. Phan GQ. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. USA.* 2003; 100:8372–8377. [PubMed: 12826605]
40. Ribas A, Camacho LH, Lopez-Berestein G, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J. Clin. Oncol.* 2005; 23:8968–8977. [PubMed: 16204013]
41. Gogas H, Ioannovich J, Dafni U, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N. Engl. J. Med.* 2006; 354:709–718. [PubMed: 16481638]
42. Tarhini, AA.; Stuckert, J.; Lee, S., et al. Prognostic significance of serial serum S100 protein levels in high-risk surgically resected melanoma in ECOG phase II trial E2696, AACR. Los Angeles: 2007.
43. Stuckert, J.; Tarhini, AA.; Lee, S., et al. Interferon alfa-induced autoimmunity in patients with high-risk melanoma participating in ECOG trial E2696, AACR. Los Angeles: 2007.

TABLE 1

Published Clinical Trials of Adjuvant IFN- α for Intermediate/High-risk (T3-4, Lymph Node Positive) Surgically Resected Melanoma

Cooperative group/PI	Eligibility	n	Treatment agent/dosage/duration	Impact on	
				DFS	OS
NCCCTG 837052 Creagan	T3-4, N1	262	IFN- α 2a 20 MU/m2/D IM TIW $\times$ 3 mos	-	-
ECOG 1684 Kirkwood	T4, N1	287	IFN- α 2b 20 MU/m2/D IV $\times$ 1 mo 10MU/m2 SC TIWfor 11 mos	+	+
E1690 Intergroup Kirkwood	T4, N1	642	IFN- α 2b 20 MU/m2/D IV $\times$ 1 mo 10 MU/m2 SC TIW $\times$ 11 mos vs 3 MU/D SC TIW $\times$ 2 yrs	+	-
WHO #16 Cascinelli	N1-2	444	IFN- α 2a 3 MU/D SC TIW $\times$ 3 yrs	-	-
EORTC 18871 Kleeberg	T3-4, N1	830	IFN- α 2b 1 MU/D SC QOD $\times$ 1 yr vs IFN α 0.2 mg/D SC QOD $\times$ 1 yr	-	-
E1694 Intergroup Kirkwood	T4, N1	880	IFN- α 2b 20 MU/m2/D IV $\times$ 1 mo 10 MU/m2 SC TIW $\times$ 11 mos vs GMK vaccine $\times$ 96 wks	+	+
ECOG 2696 Kirkwood	T4, N1, M1	107	GMK + IFN or ->IFN vs GMK	+	-
UKCCR Aim-High Hancock	T4, N1	674	IFN- α 2a 3 MU/D SC QOD $\times$ 2 yrs	-	-
EORTC 18952 Eggermont	T4, N1-2	1488	IFN- α 2b 10MU/d then 10 MU TIW $\times$ 1 or 5 MU TIW $\times$ 2 years	+/-	-
EORTC 18991 Eggermont	T $\times$ N1-2	1256	Peg-IFN- α 2b SC 6 μ g/kg/week (8 weeks) then 3 μ g/kg/week (5 years) vs Observation	+	-
French Grob	T2-T4 (1.5 mm), N0	489	IFN- α 2a 3MUSCTIW $\times$ 18 mo vs Obs	$\pm$	-
Austrian Pehamberger	T2-T4 (1.5 mm), N0	311	IFN- α 2a 3MUSCQD $\times$ 3 wks then TIW $\times$ 1 yr vs Obs	$\pm$	-