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IFN-α in the Treatment of Melanoma

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Among the IFNs, IFN-α2 has been the most broadly evaluated clinically. At the molecular level, IFN-α has multiple effects in a variety of malignancies that range from antiangiogenic to potent immunoregulatory, differentiation-inducing, antiproliferative, and proapoptotic effects. A multitude of IFN-α2 regimens that may be classified as low dose, intermediate dose, and high dose have been evaluated as adjuvant therapy in melanoma. A durable impact on both relapse-free and overall survival was seen only with the regimen utilizing high-dose IFN-α2b tested in the Eastern Cooperative Oncology Group and intergroup trials E1684, E1690, and E1694 as adjuvant therapy for high-risk surgically resected melanoma (stage IIB or III). Adjuvant pegylated IFN-α2b has also been evaluated at maximally tolerable doses compared with the observation group in the European Organization for Research and Treatment of Cancer trial 18991 and has shown relapse-free survival benefits in patients with microscopic nodal disease. ***The Journal of Immunology*****, 2012, 189: 3789–3793.**

In their quest to produce a more effective smallpox vaccine, Nagano and Kojima (1) hypothesized that a "viral inhibitory factor" is present in virus-infected tissues that might serve therapeutic goals. They later reported that the isolated virus inhibitory factor lasted 1–4 d (2–4). In parallel, Isaacs and Lindenmann (5) discovered "viral interference" while investigating the underlying mechanisms of this phenomenon, and they have been credited with the first delineation of "interferon." They observed that heat-inactivated influenza virus had growth inhibitory effects on live influenza virus propagation in the chorioallantoic membrane of the chicken egg. This birth of "type I interferon" (6) and the purification of type I IFNs in 1978 (7) led to the rDNA production of IFN-α and IFN-β in the early 1980s as the corresponding genes were cloned (8–10). Today we know that the type I IFN family includes IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω. Additionally, several related molecules such as limitin are known to signal through the IFN-α/β receptor, although utilizing variable downstream signal transduction pathways (11). The cloning of IFN genes showed that IFN-α is encoded by a family of related genes, whereas IFN-β, IFN-ε, IFN-κ, and IFN-ω are each encoded by a single gene (9). Given its distinct structural and functional properties, IFN-γ was designated as type II IFN (11). Type I IFNs signal through the same receptor complex (IFNAR that consists of IFNAR1 and IFNAR2 chains) impacting distinct but related pathways to those affected by IFN-γ, which signals through a distinct cell surface receptor (IFNGR that consists of IFNGR1 and IFNGR2 chains) (12). The most recent addition to the IFN family is IFN-λ (type III IFN), which signals through unique receptors (IFNLR1 and IL-10R2) but shares common intracellular IFN signaling pathways (13, 14). IFN-λ, originally termed IL-29, IL-28A, and IL-28B (also known as IFN-λ1, IFN-λ2, and IFN-λ3, respectively), is also induced during a viral infection similar to type I IFN and is involved in host defense against viruses. IFN-λ is currently being studied for the treatment of hepatitis C infection.

Among the IFNs, IFN-α2 has been the most broadly evaluated clinically. Three commercially available subspecies exist, including IFN-α2a (Roferon-A; Roche Pharmaceuticals), IFN-α2b (Intron A; Merck), and IFN-α2c (Boehringer Ingelheim). At the molecular level, IFN-α has multiple effects in a variety of malignancies that range from antiangiogenic to potent immunoregulatory, differentiation-inducing, antiproliferative, and proapoptotic effects (15). It has significant effects in relationship to promoting tumor immunogenicity and enhancing dendritic cell (DC) response to tumor, DC polarization or maturation, survival, and Ag cross-presentation that lead to antitumor immunity (15–17). IFN-α promotes a Th1 shift in host immunity against tumors, enhancing cell-mediated cytotoxicity, and it has a role in attracting Th1 lymphocyte traffic to the tumor (18–25). Recently, host type I IFNs were found to be critical for the innate immune recognition of a growing tumor in vivo, leading to intratumor accumulation of $CD8\alpha^+$ DCs that promote tumor Ag-specific $CD8^+$ T cell responses (26).

IFN-α was evaluated clinically in a variety of regimens that initially tested nonrecombinant partially purified IFN-α species and, as they became available, recombinant IFN-α species in a variety of malignancies, in addition to its role in the treatment of viral hepatitis and multiple sclerosis. A series of phase I/II studies of the different subspecies of IFN-α in metastatic melanoma have attempted to identify the optimal dose, route, schedule, and duration with an acceptable toxicity profile. The pharmacokinetic properties were shown to de-

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Abbreviations used in this article: CI, confidence interval; DC, dendritic cell; GMK, ganglioside GM2/keyhole limpet hemocyanin; HDI, high-dose IFN-α2b; HR, hazard ratio; OS, overall survival; p_1, stratified log-rank one-sided p value; pIFN-α, pegylated IFN-α2b; RCT, randomized control trial; RFS, relapse-free survival.

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pend on the route of administration, schedule, and formulation (rIFN or polyethylene glycol-bound) (27). Tumor response rates of ~16% were observed, with responses sometimes as late as 6 mo from the initiation of therapy, but with a modest median duration of response of ~4 mo (28). Additionally, tumor burden was noted to correlate with the probability of response, with higher likelihoods of response among patients having lower tumor burden. These observations, as well as data that suggested that the character of the immune response differs in patients with advanced bulky unresectable tumors, supported the potential for a greater impact of IFN-α on postoperative patients with microscopic residual disease at high risk for recurrence and death (which has been termed the "adjuvant" setting of therapy). This hypothesis opened the door for the initial adjuvant evaluations of IFN-α in melanoma.

A multitude of IFN-α2 regimens that may be classified as low dose, intermediate dose, and high dose have been evaluated as adjuvant therapy for disease that may be categorized as intermediate or high risk (corresponding to stage II, III, or IV by the American Joint Committee on Cancer), including deeper primary tumors on the one hand, or with resectable lymph nodes positive for tumor or limited extent of operable distant metastases on the other hand (6). A durable impact on both relapse-free survival (RFS) and overall survival (OS) was seen only with the regimen utilizing the high-dose IFN-α2b (HDI) tested in the Eastern Cooperative Oncology Group and intergroup trials E1684 ($n = 287$), E1690 ($n = 642$), and E1694 ($n = 880$) as adjuvant therapy for high-risk surgically resected melanoma (stage IIB or III) (29–31). These trials and their outcomes are summarized in Table I. The HDI regimen tested in these studies was administered in a 4-wk induction phase given i.v. at 20 million IU/m^2/d for 5 consecutive days out of 7 every week for 4 wk, followed by a maintenance phase given s.c. at 10 million IU/m^2/d every other day three times each week for an additional 48 wk. All three trials have demonstrated significant benefits in relation to RFS, whereas significant benefits in relationship to OS were seen in two of these trials. Trial E1684 demonstrated significant RFS and OS prolongation with HDI versus the observation group (median RFS, 1.72 versus 0.98 y [stratified log-rank one-sided p value (p_1) = 0.0023]; median OS, 3.82 versus 2.78 y [$p_1 = 0.0237$]). In trial E1694, the ganglioside GM2/keyhole limpet hemocyanin (GMK) vaccine was selected as the optimal vaccine control arm at the time (32), and this study reported significant improvements in both RFS (hazard ratio [HR], 1.47; $p_1 = 0.0015$) and OS (HR, 1.52; $p_1 = 0.009$) in favor of HDI. The intent-to-treat analysis of this study showed similar benefits in RFS (HR, 1.49) and OS (HR, 1.38) (31). Of note, the HR is a measure of how often an event of relapse or death occurs in one group compared with how often it occurs in a control group over time. In this case, it is used to measure relapse or survival at any point in time in the HDI group of patients compared with the observation- or vaccine-assigned control group.

The second of the three trials, E1690, was conducted partly before and partly after the U.S. Food and Drug Administration approval of HDI, based on the mature outcome of the E1684 trial. This study demonstrated improvements in RFS (intent-to-treat analysis HR, 1.28; $p_1 = 0.025$) but not OS (30). Surgery guidelines for E1690, unlike E1684, did not require regional removal of lymph nodes. Therefore, patients assigned to observation in E1690 frequently experienced lymph node relapse, and they uniformly were found to have crossed over from the observation-assigned arm to receive HDI at relapse in regional lymph nodes. Retrospective analysis has shown that those who crossed over to HDI ($n = 38$) also demonstrated a large benefit that could have confounded the survival analysis (30).

The progression-free survival and OS analysis of all three trials was updated up to April 2001, and a pooled analysis of the two observation controlled trials (E1684 and E1690) was reported (32). The pooled analysis showed that HDI maintained significant benefits in relapse prevention out to intervals of 20 y. However, this analysis excluded the GMK vaccine-controlled E1694 trial, and because the larger of the two observation-controlled trials (E1690) did not show an OS benefit for HDI, the pooled analysis did not show compelling evidence of OS benefit.

Three large meta-analyses (Table II) of published results or of the individual patient data from cooperative groups have evaluated the survival benefits of adjuvant IFN-α, pooling data from a multitude of randomized control trials (RCTs) testing IFN-α at various dose levels, durations, and routes of administration. The first analysis, which included 12 RCTs, estimated highly significant RFS benefits with IFN compared with the observation group (HR, 0.83; 95% confidence interval [CI], 0.77–0.90; $p = 0.000003$) and OS benefits that

Table I. Summary of the Eastern Cooperative Oncology Group and intergroup trials that tested high dose IFN-α2b (E1684, E1690, E1694) and pIFN-α2b (European Organization for Research and Treatment of Cancer trial 18991) leading to regulatory approval

Cooperative Group/Principal Investigator	Eligibility	n	Treatment Agent: Dosage and Duration	Impact on PFS/OS	Toxicity Attrition Rate (%)
ECOG trial 1684/J.M. Kirkwood	T4, N	287	IFN-α2b: 20 million IU/m^2/d given i.v. for 1 mo, then 10 million IU/m^2 given s.c. three times per week for 11 mo versus observation group	+/+	26
Intergroup trial E1690/J.M. Kirkwood	T4, N	642	IFN-α2b: 20 million IU/m^2/d given i.v. for 1 mo, then 10 million IU/m^2 s.c. three times per week for 11 mo versus 3 million IU/d given s.c. three times per week for 2 y versus observation group	+/−	13
Intergroup trial E1694/J.M. Kirkwood	T4, N	880	IFN-α2b: 20 million IU/m^2/d given i.v. for 1 mo, then 10 million IU/m^2 s.c. three times per week for 11 mo versus GMK vaccine group for 96 wk	+/+	10
EORTC trial 18991/A. Eggermont	Tx, N	1256	pIFN-α: given s.c. at 6 μg/kg/wk (8 wk), then 3 μg/kg/wk (5 y) versus observation group	+/−	37

The toxicity attrition rate indicates the rate of study drug discontinuation owing to toxicity.

ECOG, Eastern Cooperative Oncology Group; EORTC, European Organization for Research and Treatment of Cancer; N, regional lymph node metastasis; PFS, progression-free survival; T4, primary melanomas >4 mm deep; Tx, unknown primary melanoma.

Table II. Three large meta-analyses of published results or the individual patient data from cooperative groups that evaluated the survival benefits of adjuvant IFN-α

	RCT (*n*)	RFS	OS	Comment
Wheatley et al. (33)	12	+	−/+	Did not include trial E1694; ↑ benefit with ↑ IFN dose
Wheatley et al. (34)	13	+: OR, 0.87; 95% CI, 0.81–0.93; $p = 0.00006$	+: OR, 0.9; 95% CI, 0.84–0.97; $p = 0.008$	13% risk reduction in RFS; 10% risk reduction in OS
Mocellin et al. (35)	14	+: HR, 0.82; 95% CI, 0.77–0.87; $p < 0.001$	+: HR. 0.89, 95% CI, 0.83–0.96; $p = 0.002$	18% risk reduction in RFS; 11% risk reduction in OS

OR, Odds ratio.

were less significant (HR, 0.93; 95% CI, 0.85–1.02; $p = 0.1$). Furthermore, it suggested increased benefit of IFN-α with increasing the dose (33). The second and larger individual patient data meta-analysis evaluated 13 RCTs and demonstrated significant benefits for IFN-α in reducing the risk of recurrence (HR, 0.87; 95% CI, 0.81–0.93; $p < 0.0001$) and the risk of death (HR, 0.90; 95% CI, 0.84–0.97; $p = 0.008$) versus observation or vaccination groups (34). The latest meta-analysis included 14 RCTs and estimated significant reductions in the risk of recurrence (HR, 0.82; 95% CI, 0.77–0.87; $p < 0.001$) and death (HR, 0.89; 95% CI, 0.83–0.96; $p = 0.002$) for adjuvant therapy with IFN-α (35). The toxicity profile of IFN-α includes a wide range of adverse events affecting numerous organ systems. However, through the development and adoption of practical recommendations for patient education as well as patient assessment and monitoring, the HDI regimen has been shown to be appropriately managed by experienced medical oncologists and to be administered through completion of 1 y in 90% of patients who do not relapse (31).

Adjuvant pegylated IFN-α2b (pIFN-α) has also been evaluated at maximally tolerable doses compared with observation groups in European Organization for Research and Treatment of Cancer trial 18991 ($n = 1256$) to determine whether prolonged weekly administration up to 5 y in patients with stage III melanoma might improve the benefit/toxicity ratio (Table I). This study hypothesized an antiangiogenic effect of IFN-α (36). The treatment regimen consisted of an 8-wk induction phase mirroring trial E1684, in which pIFN-α is given at higher dosage (6 μg/kg/wk) and maintenance follows at lower doses titrated against symptoms (3, 2, or 1 μg/kg/wk) for up to 5 y. The first report in 2007 at 3.8 y median follow-up demonstrated significant improvement in RFS in favor of pIFN-α compared with observation groups (median, 34.8 versus 25.5 mo; HR, 0.82; $p = 0.011$). No overall significant benefits were shown in either distant metastasis-free survival or OS. Subgroup analysis showed no significant benefits in the group of patients with gross nodal disease in any end point, whereas pIFN-α significantly improved RFS and distant metastasis-free survival, but not OS, in patients with microscopic nodal involvement. The median duration of therapy on trial was a little more than 1 y, and only 23% of patients continued into the fourth or fifth year as originally planned, and therefore the main hypothesis that prolonged treatment duration is needed to achieve survival benefits could not be adequately assessed. pIFN-α was approved by the U.S. Food and Drug Administration for the adjuvant treatment of stage III melanoma in March 2011. Updated data were presented at the 2011 American Society of Clinical Oncology Annual Meeting for a median follow-up of 7.6 y (36), which showed continued significant benefit in overall RFS but with reduction in magnitude (HR, 0.87; 95% CI, 0.76–1.00; $p = 0.05$).

Attempts to identify a subset of patients likely to benefit from adjuvant treatment with IFN-α have failed to discover clinical or demographic features of true therapeutic predictive value. Other studies evaluating methylthioadenosine phosphorylase tumor expression (37), serum S100 levels (38), and HLA class I and II Ags (39) have correlated these with prognosis but need further study to test their predictive value. Analysis of the mechanism of IFN-α has been undertaken using more efficient study designs, in which translational studies of tumor tissue biopsies are taken at initial evaluation and before therapy, followed by treatment given for a month or 6 wk, followed by definitive surgery (complete lymph node resection). This approach, known as neoadjuvant therapy in multiple other solid tumors ranging from breast cancer to esophageal cancer, has allowed evaluation of the role of a multitude of biological activities in relationship to therapeutic activity. Our studies of IFN-α have shown significant effects of IFN-α upon STAT signaling, as well as clinical activity associated with a profound influx of DCs and T cells to the tumor (25). This suggests that the STAT3 inhibition and the concurrent induction of STAT1 in the lymph nodes of patients correspond to the potent reversal of T cell signaling defects in up to a third of patients with melanoma studied by Lee and colleagues (40, 41) using Phosflow approaches. More interesting, this is consistent with the data that suggest that a type I IFN signature is associated with an effector immune response to cancer, specifically in murine and human melanoma (26). Ongoing melanoma adjuvant trials are testing the next generation of immunotherapeutics that target immune regulatory checkpoints, and these may build on this foundation of understanding that has emerged regarding the immunomodulatory role of IFNs in human cancer. Specifically, the CTLA4 blocking Ab ipilimumab that has recently demonstrated significant survival benefits in the treatment of advanced metastatic melanoma offers new options to enhance our immunotherapy of cancer in general (42, 43). In melanoma, the European Organization for Research and Treatment of Cancer 18071 trial has compared ipilimumab versus placebo in stage III melanoma and has recently completed accrual, and the ongoing U.S. intergroup E1609 trial is testing ipilimumab versus standard HDI in stages IIIB, IIIC, and IV (M1a, M1b). Adjuvant vaccination with the MAGE-A3 vaccine is also being tested in patients with tumor expression of the MAGE-A3 Ag. This phase III vaccine study employs a potent new TLR9 agonist (CpG) as a vaccine adjuvant and evaluates a predictive gene signature that may further refine our approach to this therapy (44, 45). Parallel

neoadjuvant trials testing ipilimumab or ipilimumab/IFN-α combinations are either complete or ongoing. Preliminary data have demonstrated significant downregulation of the host immune suppressive elements by ipilimumab that may contribute to the clinical benefits observed with this agent (46).

The potent immune impact of IFN-α can clearly be suppressed by tumor tolerogenic mechanisms, explaining the limited clinical activity of IFN-α as monotherapy in metastatic melanoma. Combinations with immune regulatory checkpoint blocking mAbs (e.g., targeting CTLA4, programmed death-1) may alter this balance, downregulating counterregulatory mechanisms and possibly releasing inhibitory influences on activated CD4 and CD8 effector cells. This is supported by the promising results of the phase II trial that tested the combination of HDI and tremelimumab in metastatic melanoma, paving the way for the ongoing Eastern Cooperative Oncology Group trial E3611 testing the combination of HDI and ipilimumab in the same population (47). Trials testing other combinations in melanoma such as the combination with BRAF inhibitors are in development.

Conclusions

IFN-α has potent immune modulating and antitumor impacts on melanoma where a significant likelihood of curing this disease was first seen with IFN-α at high dosage in the adjuvant treatment of high-risk surgically resected disease targeting residual micrometastases (29, 31). As adjuvant therapy for melanoma, the HDI regimen is unique in its impact on both RFS and OS, as demonstrated in the Eastern Cooperative Oncology Group and intergroup trials E1684, E1690, and E1694. The newly U.S. Food and Drug Administration-approved pIFN-α has RFS benefits that appear to be confined to the microscopic nodal disease population and no overall impact upon OS, and therefore it may be applicable for patients who cannot undertake conventional HDI.

Disclosures

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References

1. Nagano, Y., and Y. Kojima. 1954. [Immunizing property of vaccinia virus inactivated by ultraviolets rays]. *C. R. Seances Soc. Biol. Fil.* 148: 1700–1702.
2. Ozato, K., K. Uno, and Y. Iwakura. 2007. Another road to interferon: Yasuichi Nagano's journey. *J. Interferon Cytokine Res.* 27: 349–352.
3. Nagano, Y., and Y. Kojima. 1958. [Inhibition of vaccinia infection by a liquid factor in tissues infected by homologous virus]. *C. R. Seances Soc. Biol. Fil.* 152: 1627–1629.
4. Watanabe, Y. 2004. Fifty years of interference. *Nat. Immunol.* 5: 1193.
5. Isaacs, A., and J. Lindenmann. 1957. Virus interference. I. The interferon. *Proc. R. Soc. Lond. B Biol. Sci.* 147: 258–267.
6. Tarhini, A. A., and J. M. Kirkwood. 2009. Clinical and immunologic basis of interferon therapy in melanoma. *Ann. N. Y. Acad. Sci.* 1182: 47–57.
7. Pestka, S. 2007. The interferons: 50 years after their discovery, there is much more to learn. *J. Biol. Chem.* 282: 20047–20051.
8. Weissenbach, J., Y. Chernajovsky, M. Zeevi, L. Shulman, H. Soreq, U. Nir, D. Wallach, M. Perricaudet, P. Tiollais, and M. Revel. 1980. Two interferon mRNAs in human fibroblasts: in vitro translation and *Escherichia coli* cloning studies. *Proc. Natl. Acad. Sci. USA* 77: 7152–7156.
9. Nagata, S., N. Mantei, and C. Weissmann. 1980. The structure of one of the eight or more distinct chromosomal genes for human interferon-alpha. *Nature* 287: 401–408.
10. Taniguchi, T., Y. Fujii-Kuriyama, and M. Muramatsu. 1980. Molecular cloning of human interferon cDNA. *Proc. Natl. Acad. Sci. USA* 77: 4003–4006.
11. Kawamoto, S., K. Oritani, E. Asakura, J. Ishikawa, M. Koyama, K. Miyano, M. Iwamoto, S. Yasuda, H. Nakakubo, F. Hirayama, et al. 2004. A new interferon, limitin, displays equivalent immunomodulatory and antitumor activities without myelosuppressive properties as compared with interferon-α. *Exp. Hematol.* 32: 797–805.
12. Gray, P. W., and D. V. Goeddel. 1982. Structure of the human immune interferon gene. *Nature* 298: 859–863.
13. Kotenko, S. V., G. Gallagher, V. V. Baurin, A. Lewis-Antes, M. Shen, N. K. Shah, J. A. Langer, F. Sheikh, H. Dickensheets, and R. P. Donnelly. 2003. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol.* 4: 69–77.
14. Sheppard, P., W. Kindsvogel, W. Xu, K. Henderson, S. Schlutsmeyer, T. E. Whitmore, R. Kuestner, U. Garrigues, C. Birks, J. Roraback, et al. 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol.* 4: 63–68.
15. Kirkwood, J. M., T. Richards, H. M. Zarour, J. Sosman, M. Ernstoff, T. L. Whiteside, J. Ibrahim, R. Blum, S. Wieand, and R. Mascari. 2002. Immunomodulatory effects of high-dose and low-dose interferon α2b in patients with high-risk resected melanoma: the E2690 laboratory corollary of intergroup adjuvant trial E1690. *Cancer* 95: 1101–1112.
16. Wang, W., H. D. Edington, U. N. Rao, D. M. Jukic, S. R. Land, S. Ferrone, and J. M. Kirkwood. 2007. Modulation of signal transducers and activators of transcription 1 and 3 signaling in melanoma by high-dose IFNα2b. *Clin. Cancer Res.* 13: 1523–1531.
17. Paquette, R. L., N. C. Hsu, S. M. Kiertscher, A. N. Park, L. Tran, M. D. Roth, and J. A. Glaspy. 1998. Interferon-α and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells. *J. Leukoc. Biol.* 64: 358–367.
18. Parlato, S., S. M. Santini, C. Lapenta, T. Di Pucchio, M. Logozzi, M. Spada, A. M. Giammarioli, W. Malorni, S. Fais, and F. Belardelli. 2001. Expression of CCR-7, MIP-3β, and Th-1 chemokines in type I IFN-induced monocyte-derived dendritic cells: importance for the rapid acquisition of potent migratory and functional activities. *Blood* 98: 3022–3029.
19. Brinkmann, V., T. Geiger, S. Alkan, and C. H. Heusser. 1993. Interferon α increases the frequency of interferon γ-producing human $CD4^+$ T cells. *J. Exp. Med.* 178: 1655–1663.
20. Wenner, C. A., M. L. Güler, S. E. Macatonia, A. O'Garra, and K. M. Murphy. 1996. Roles of IFN-γ and IFN-α in IL-12-induced T helper cell-1 development. *J. Immunol.* 156: 1442–1447.
21. Rogge, L., L. Barberis-Maino, M. Biffi, N. Passini, D. H. Presky, U. Gubler, and F. Sinigaglia. 1997. Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J. Exp. Med.* 185: 825–831.
22. Palmer, K. J., M. Harries, M. E. Gore, and M. K. Collins. 2000. Interferon-alpha (IFN-α) stimulates anti-melanoma cytotoxic T lymphocyte (CTL) generation in mixed lymphocyte tumour cultures (MLTC). *Clin. Exp. Immunol.* 119: 412–418.
23. Yurkovetsky, Z. R., J. M. Kirkwood, H. D. Edington, A. M. Marrangoni, L. Velikokhatnaya, M. T. Winans, E. Gorelik, and A. E. Lokshin. 2007. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-α2b. *Clin. Cancer Res.* 13: 2422–2428.
24. Tough, D. F. 2004. Type I interferon as a link between innate and adaptive immunity through dendritic cell stimulation. *Leuk. Lymphoma* 45: 257–264.
25. Moschos, S. J., H. D. Edington, S. R. Land, U. N. Rao, D. Jukic, J. Shipe-Spotloe, and J. M. Kirkwood. 2006. 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.* 24: 3164–3171.
26. Fuertes, M. B., A. K. Kacha, J. Kline, S. R. Woo, D. M. Kranz, K. M. Murphy, and T. F. Gajewski. 2011. Host type I IFN signals are required for antitumor $CD8^+$ T cell responses through $CD8\alpha^+$ dendritic cells. *J. Exp. Med.* 208: 2005–2016.
27. Kirkwood, J. M., M. S. Ernstoff, C. A. Davis, M. Reiss, R. Ferraresi, and S. A. Rudnick. 1985. Comparison of intramuscular and intravenous recombinant α-2 interferon in melanoma and other cancers. *Ann. Intern. Med.* 103: 32–36.
28. Creagan, E. T., D. L. Ahmann, S. Frytak, H. J. Long, and L. M. Itri. 1986. Recombinant leukocyte A interferon (rIFN-αA) in the treatment of disseminated malignant melanoma. Analysis of complete and long-term responding patients. *Cancer* 58: 2576–2578.
29. Kirkwood, J. M., M. H. Strawderman, M. S. Ernstoff, T. J. Smith, E. C. Borden, and R. H. Blum. 1996. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J. Clin. Oncol.* 14: 7–17.
30. Kirkwood, J. M., J. G. Ibrahim, V. K. Sondak, J. Richards, L. E. Flaherty, M. S. Ernstoff, T. J. Smith, U. Rao, M. Steele, and R. H. Blum. 2000. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J. Clin. Oncol.* 18: 2444–2458.
31. Kirkwood, J. M., J. G. Ibrahim, J. A. Sosman, V. K. Sondak, S. S. Agarwala, M. S. Ernstoff, and U. Rao. 2001. 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.* 19: 2370–2380.
32. Kirkwood, J. M., J. Manola, J. Ibrahim, V. Sondak, M. S. Ernstoff, and U. Rao; Eastern Cooperative Oncology Group. 2004. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. *Clin. Cancer Res.* 10: 1670–1677.
33. Wheatley, K., N. Ives, B. Hancock, M. Gore, A. Eggermont, and S. Suciu. 2003. Does adjuvant interferon-α for high-risk melanoma provide a worthwhile benefit? A meta-analysis of the randomised trials. *Cancer Treat. Rev.* 29: 241–252.

34. Wheatley, K., N. Ives, A. Eggermont, J. Kirkwood, N. Cascinelli, S. N. Markovic, B. Hancock, S. Lee, and S. Suciu; International Malignant Melanoma Collaborative Group. 2007. Interferon-α as adjuvant therapy for melanoma: an individual patient data meta-analysis of randomised trials. *J. Clin. Oncol.* 25(Suppl.): 8526 (Abstr.).
35. Mocellin, S., S. Pasquali, C. R. Rossi, and D. Nitti. 2010. Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. *J. Natl. Cancer Inst.* 102: 493–501.
36. Eggermont, A. M, S. Suciu, M. Santinami, W. Kruit, A. Testori, J. Marsden, C. J. A. Punt, M. E. Gore, R. MacKie, R. Dummer, et al.; EORTC Melanoma Group. 2011. EORTC 18991 phase III trial: long-term adjuvant pegylated interferon-α2b (PEG-IFN) versus observation in resected stage III melanoma: long-term results at 7.6-years follow-up. *J. Clin. Oncol.* 29(Suppl.): 8506 (Abstr.).
37. Meyer, S., P. J. Wild, T. Vogt, F. Bataille, C. Ehret, S. Gantner, M. Landthaler, M. Klinkhammer-Schalke, F. Hofstaedter, and A. K. Bosserhoff. 2010. Methylthioadenosine phosphorylase represents a predictive marker for response to adjuvant interferon therapy in patients with malignant melanoma. *Exp. Dermatol.* 19: e251–e257.
38. Tarhini, A. A., J. Stuckert, S. Lee, C. Sander, and J. M. Kirkwood. 2009. Prognostic significance of serum S100B protein in high-risk surgically resected melanoma patients participating in Intergroup Trial ECOG 1694. *J. Clin. Oncol.* 27: 38–44.
39. Gogas, H., J. M. Kirkwood, C. S. Falk, U. Dafni, V. K. Sondak, D. Tsoutsos, A. Stratigos, C. Markopoulos, D. Pectasides, and M. Spyropoulou-Vlachou. 2010. Correlation of molecular human leukocyte antigen typing and outcome in high-risk melanoma patients receiving adjuvant interferon. *Cancer* 116: 4326–4333.
40. Critchley-Thorne, R. J., N. Yan, S. Nacu, J. Weber, S. P. Holmes, and P. P. Lee. 2007. Down-regulation of the interferon signaling pathway in T lymphocytes from patients with metastatic melanoma. *PLoS Med.* 4: e176.
41. Simons, D. L., G. Lee, J. M. Kirkwood, and P. P. Lee. 2011. Interferon signaling patterns in peripheral blood lymphocytes may predict clinical outcome after high-dose interferon therapy in melanoma patients. *J. Transl. Med.* 9: 52.
42. Hodi, F. S., S. J. O'Day, D. F. McDermott, R. W. Weber, J. A. Sosman, J. B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J. C. Hassel, et al. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363: 711–723.
43. Robert, C., L. Thomas, I. Bondarenko, S. O'Day, J. W. M D, C. Garbe, C. Lebbe, J. F. Baurain, A. Testori, J. J. Grob, et al. 2011. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* 364: 2517–2526.
44. Kruit, W. H, S. Suciu, B. Dreno, V. Chiarion-Sileni, L. Mortier, C. Robert, M. Maio, V. G. Brichard, F. Lehmann, and U. Keilholz. 2008. Immunization with recombinant MAGE-A3 protein combined with adjuvant systems AS15 or AS02B in patients with unresectable and progressive metastatic cutaneous melanoma: a randomized open-label phase II study of the EORTC Melanoma Group (16032–18031). *J. Clin. Oncol.* 26(May 20 Suppl.): 9065 (Abstr.).
45. Louahed, J., O. Gruselle, S. Gaulis, T. Coche, A. M. Eggermont, W. Kruit, B. Dreno, V. Chiarion Sileni, F. Lehmann, and V. G. Brichard. 2008. Expression of defined genes identified by pretreatment tumor profiling: Association with clinical responses to the GSK MAGE-A3 immunotherapeutic in metastatic melanoma patients (EORTC 16032–18031). *J. Clin. Oncol.* 26(May 20 Suppl.): 9045 (Abstr.).
46. Tarhini, A. A., H. Edington, L. H. Butterfield, M. Sinha, S. J. Moschos, H. Tawbi, Y. Shuai, J. Shipe-Spotloe, M. Simonetta, C. Milburn, et al. 2011. Neoadjuvant ipilimumab in patients with stage IIIB/C melanoma: immunogenicity and biomarker analysis. *J. Clin. Oncol.* 29(Suppl.): 8536 (Abstr.).
47. Tarhini, A. A., J. Cherian, S. J. Moschos, H. A. Tawbi, Y. Shuai, W. E. Gooding, C. Sander, and J. M. Kirkwood. 2012. Safety and efficacy of combination immunotherapy with interferon alfa-2b and tremelimumab in patients with stage IV melanoma. *J. Clin. Oncol.* 30: 322–328.