

Clinical Perspectives™

Highlights from the Perspectives in Melanoma XII Conference

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ADJUVANT THERAPY

Updated analysis and discussion of current EORTC trials

Alexander M. M. Eggermont, of Erasmus University Medical Center-Daniel den Hoed Cancer Center in Rotterdam, The Netherlands, provided an update of major EORTC trials dealing with adjuvant therapy in melanoma. In particular, he focused his attention on EORTC 18991 and 18952 trials.

EORTC 18991 was a large, randomized controlled phase 3 trial comparing recurrence-free survival (RFS) and other outcomes in patients with resected stage III melanoma who were assigned to either observation or treatment with pegylated interferon (IFN)- α 2b for an intended duration of 5 years.¹ The final results from this trial after a median follow-up of 3.8 years were recently reported in *Lancet* and showed adjuvant pegylated-IFN therapy was associated with a significantly lower RFS rate, but no significant differences for distant metastasis-free survival (DMFS) or overall survival (OS).¹ This is important because it supports the idea that the benefits of adjuvant IFN therapy are durable for RFS.

Moreover, the benefits of adjuvant pegylated-IFN adjuvant therapy in EORTC 18991 appeared to be more pronounced for patients with microscopic nonpalpable (N1) than clinically palpable (N2) disease and those with only 1 positive lymph node versus 2 or more positive lymph nodes.¹ Pegylated-IFN treatment was associated with significantly longer RFS and DMFS in patients with N1 disease (ie, SLN-positive disease) or only 1 positive lymph node, but not those with N2 disease or more than 1 positive lymph node.

Of particular interest, at the *Perspectives in Melanoma* conference (and at the *European Society of Medical Oncology* [ESMO] Congress that occurred a few weeks before), Dr. Eggermont presented the results from a post-hoc analysis after a 5.1-year median follow-up suggesting that the benefits of adjuvant pegylated-IFN adjuvant therapy were particularly pronounced in patients with stage III-N1 disease with *ulcerated* versus nonulcerated primary tumors. As illustrated in **Table 1**, this was true with respect to RFS, DMFS, and OS.²

Table 1. RFS, DMFS, and OS From EORTC 18991 for Patients With Stage III-N1 Stratified by Ulceration

Ulceration	RFS		DMFS		OS	
	Obs	PEG-IFN	Obs	PEG-IFN	Obs	PEG-IFN
Absent (n = 321)						
HR (99% CI)	0.74 (0.46-1.20)		0.88 (0.52-1.50)		1.18 (0.64-2.18)	
P-value	.11		.54		.49	
Present (n = 186)						
No. events	62	53	59	45	44	33
4-year rates	26.8	43.8	30.1	47.4	45.4	65.0
HR (99% CI)	0.69 (0.43-1.12)		0.59 (0.35-0.98)		0.61 (0.34-1.10)	
P-value	.049		.006		.03	

RFS, relapse-free survival; DMFS, distant metastasis-free survival, OS, overall survival; Obs, observation; PEG-IFN, pegylated interferon alfa-2b; HR, hazard ratio; CI, confidence interval.

From Eggermont A. Systemic adjuvant therapy in melanoma: Where are we? Presented at: 33rd Annual ESMO Congress; September 12-16, 2008; Stockholm, Sweden.²

The results from the earlier EORTC 18952 study also support the stage-dependency of the adjuvant IFN effect, Dr. Eggermont noted, and post-hoc analyses further pointed to ulceration as a potentially critical variable impacting sensitivity to this therapy. EORTC 18952 was a randomized controlled study that compared distant metastasis free interval (DMFI) and other endpoints in patients with resected stage IIB or III melanoma.³ Patients were assigned to observation or either 13 or 25 months adjuvant treatment with standard IFN- α 2b (10 MU 5 days/week for 4 weeks followed by either 10 MU tid for 1 year or 5 MU tid for 2 years). The results after a median follow-up of 4.7 years showed significantly longer DMFS, DMFI, and OS with 25-month IFN therapy versus observation for patients with stage IIB disease, but not for those with stage III-N1 or stage III-N2 disease. There was a trend for longer DMFS ($P=.06$), DMFI ($P=.08$), and OS ($P=.11$) with 25-month IFN for patients with stage III-N1 disease. Dr. Eggermont noted that these results are generally consistent and supportive with the findings from EORTC 18991 showing stage dependency of adjuvant IFN therapy.

Furthermore, post-hoc analyses of EORTC 18952 presented at the *Perspectives in Melanoma* conference also pointed to ulceration as being critical for benefit from adjuvant IFN. In particular, 13-month or 25-month adjuvant IFN treatments were associated with significantly longer DMFS compared with observation for patients with stage IIB disease and ulcerated primary tumors ($P=.015$), but not for stage IIB patients without ulceration ($P=.81$). Hence, the results from both EORTC 18891 and EORTC 18952 suggest stage dependency (stage IIB or stage III-N1) and ulceration may be useful in identifying patients most likely to benefit from adjuvant IFN therapy.

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STAT signaling and interferon response

Systemic pharmacotherapy of stage IV melanoma is generally ineffective,¹ and only 1 regimen (high-dose interferon [IFN]- α 2b) has been documented to have durable benefit for recurrence-free survival when used as adjuvant therapy in patients with resected stage IIB-III melanoma.² **John M. Kirkwood**, of the University of Pittsburgh Cancer Institute in Pittsburgh, Pennsylvania, discussed data focused on identifying biomarker of melanoma progression and therapeutic response to IFN- α , with particular focus on signal transducer and activator of transcription (STAT) signaling molecules.

Dr. Kirkwood noted that melanoma progression is associated with defects in the host immune response, or “immune paralysis,” and that STAT3 is constitutively activated early in the process. IFN- α therapy leads to STAT3 inactivation³ and, more generally, augments host immune responses, but the question remains whether we can identify biomarkers prognostic for melanoma prognosis or predictive of response to IFN- α . Dr. Kirkwood’s presentation focused on the STAT signaling, both as a biomarker of progression and potential predictor of therapeutic effect of IFN- α .

UPIC 00-008 is clinical trial designed to evaluate clinical and histologic response to neoadjuvant high-dose IFN- α 2b therapy in patients with stage IIIB-C (Tx, N2b or N3, M0) melanoma who subsequently underwent radical regional lymphadenectomy.⁴ An excisional biopsy (sample 1) was performed prior to IFN- α 2b induction therapy (20 MU/m²/d IV, 5 days a week for 4 weeks). Patients then underwent radical regional lymphadenectomy (sample 2) before receiving maintenance therapy (10 MU/m²/d IV, 3 times a week for 48 weeks). The initial report of study results demonstrated that IFN-related tumor regression was associated with modulation of the number of immune cells (CD11c⁺, CD3⁺, and CD83⁺) infiltrating the tumor.⁴ A subsequent report represented further study done in the context off the UPCI 00-008 that were focused on the impact of IFN treatment on STAT1 and STAT3.⁵

This latter report demonstrated that high-dose IFN treatment was associated with downregulation of phosphorylated or active STAT3 (pSTAT3) and total STAT3 in both tumor cells and lymphocytes, and an upregulation in pSTAT1.⁵ As a consequence, IFN treatment produced an increase in the pSTAT1:pSTAT3 ratios in both tumor cells and lymphocytes. Furthermore, higher pSTAT1:pSTAT3 ratios in tumor cells pretreatment were associated with significantly longer overall survival.

Dr. Kirkwood also discussed the results from a recent study comparing gene expression profiles of peripheral blood lymphocytes from melanoma patients and healthy controls, which showed STAT1 phosphorylation was significantly reduced in IFN-stimulated lymphocytes from melanoma patients compared with controls.⁶ The study also demonstrated that IFN signaling was downregulated in lymphocytes from melanoma patients and that prolonged treatment with high-dose (but not low-dose) IFN treatment was able to overcome the impairment in IFN signaling in at least some patients. The authors of the study identified 2 populations of melanoma patients: high- and low-responders to IFN. Finally, Dr. Kirkwood reviewed the results from a study of pSTAT1 and pSTAT3 expression in the biopsied atypical nevi from patients who had received either high- or low-dose IFN- α .⁷ The results from this study indicate that pSTAT3 may be a potential biomarker for melanocytic transformation and progression that is responsive to IFN therapy in a dose-responsive manner.

More recent results from a paper in press that Dr. Kirkwood reviewed suggest that STAT5 may also be a biomarker for melanoma progression that is associated with FOXP3 expression and upregulated in response to high-dose IFN therapy.

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Update on DeCOG interferon trials

Axel Hauschild, of the University of Kiel, in Kiel, Germany, provided a brief overview of recent or ongoing Dermatologic Cooperative Oncology Group (DeCOG) trials of adjuvant treatment in post-excision melanoma patients with intermediate or high-risk of recurrence. A number of DeCOG trials were designed to evaluate low-dose interferon (IFN)- α adjuvant therapy. In 2003, Dr. Hauschild and colleagues reported the results from a trial comparing low-dose IFN α plus low-dose Interleukin-2 (IL-2) with observation in 223 melanoma patients, showing no differences between treatment groups for recurrence-free survival (RFS) or overall survival (OS).¹

Another DeCOG trial compared low-dose IFN- α 2a (3 MU sc, 3 times a week, for 2 years), low-dose IFN- α 2a plus dacarbazine (DTIC), and observation in 444 patients who received a completion lymph node dissection for documented regional lymph node involvement.² Low-dose IFN- α 2a treatment, but not the combination regimen, was associated with significant improvement in disease-free survival (DFS) and overall survival (OS) compared with observation.

A third trial compared low-dose interferon- α 2a with or without an intermediate-dose induction phase (10 MU/m²) in 657 melanoma patients with ≥ 1.5 -mm thick primary tumors, and found no improved outcomes with the induction regimen. The final results of the trial will be published shortly.³ Similarly, results from a phase 3 trial comparing 18 months versus 60 months adjuvant therapy with low-dose IFN- α 2a in 850 melanoma patients with ≥ 1.5 mm tumor thickness showed no differences between the regimens for RFS, distant metastasis-free survival, or OS.⁴

Interim results are expected sometime in 2009 from a study comparing adjuvant pegylated IFN- α 2a with conventional low-dose IFN- α 2a in 880 patients with stage IIA/IIIB melanoma. Pegylated IFN- α 2a was subcutaneously administered once a week at a dose of 180 μ g, compared with 3 MU 3 times per week for conventional low-dose IFN- α 2a.

Another interesting and ongoing trial is comparing adjuvant “high-dose pulsed IFN- α 2b treatment” with 3 induction cycles (4 weeks each) every 4 months (dose: 20 MU/m²) with conventional high-dose IFN- α 2b in patients with stage IIIA/IIIC melanoma. At the time of the meeting, 540 patients had been randomized into the trial, and initial results indicated significantly better quality of life with the high-dosed pulsed regimen.

Lastly, Dr. Hauschild described the design of a very recently initiated DeCOG “hypothesis-building” trial for future adjuvant treatment regimens. The study is designed to examine pegylated IFN- α 2b plus sorafenib in 55 patients with stage IV melanoma and serum lactate dehydrogenase (LDH) $< 2\times$ the upper limit of normal. Pegylated IFN- α 2b is administered subcutaneously as 3 μ g/kg once a week, and sorafenib orally as 400 mg, twice daily, for 2 cycles of 4 weeks before re-evaluation. The primary endpoint for the trial is to determine disease control rate (clinical response, partial response, stable disease) after 8 weeks of treatment. Full recruitment will be reached in early 2009 and results from the trial are eagerly awaited.

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New biomarkers or prognostic factors in adjuvant therapy trial design

Henrik Schmidt, of Aarhus University in Aarhus, Denmark, discussed possible new prognostic biomarkers in adjuvant therapy, with a focus on serum YKL-40 levels. YKL-40 is a protein expressed by various immune cells (such as macrophages and neutrophils) but also by solid tumor cells. High serum YKL-40 level has been suggested to be a prognostic biomarker of short survival in various cancers.¹ The exact function or mechanism by which YKL-40 promotes cancer progression is unclear. Dr. Schmidt described the results from 3 studies examining the potential role of serum YKL-40 level as a prognostic biomarker in patients with melanoma.

One study demonstrated significantly higher serum YKL-40 levels in patients with stage IV melanoma than healthy controls ($P < .001$), and a multivariate Cox analysis identified serum YKL-40 as a significant independent predictor of survival (HR, 1.9; 95% CI, 1.2-2.8; $P = .004$), along with serum LDH level (HR, 1.9; 95% CI, 1.2-2.9; $P = .004$).² The risk of early death was quadrupled in patients with elevated levels of both YKL-40 and LDH. Another study examined serum YKL-40 as a possible prognostic factor for survival in patients with stage I ($n = 162$) or stage II ($n = 72$) melanoma.³ After a median follow-up of 66 months, there were 41 relapses and 39 deaths, and a time-dependent multivariate Cox analysis showed serum YKL-40 was an independent prognostic factor for recurrence-free survival (HR, 1.6; 95% CI, 1.1-2.5; $P = .03$) and overall survival (HR, 1.8; 95% CI, 1.2-2.6; $P = .002$). In addition serum YKL-40 level at the time of diagnosis (normal or elevated) was an independent prognostic factor for overall survival (HR, 3.6; 95% CI, 1.7-7.7; $P = .001$).

Dr. Schmidt also discussed results from the Nordic adjuvant interferon (IFN) trial, which examined the prognostic significance of serum YKL-40 level and other factors in patients with stage I-III melanoma treated with observation or 1 or 2 years high-dose IFN- $\alpha 2b$ regimen. Baseline serum YKL-40 level was a significant independent prognostic factor for short survival in the control group (HR, 1.8; 95% CI, 1.2-2.7; $P = .008$), but not in the treatment arms. However, patients in the 2-year IFN treatment arm with a high baseline YKL-40 level had significantly better survival compared to the control arm (HR, 0.5; 95% CI, 0.3-0.9; $P = .02$), and there was a trend towards better survival for patients in the 1-year IFN treatment arm with high baseline YKL-40 level as well (HR, 0.7; 95% CI, 0.4-1.1; $P = .15$). Low baseline YKL-40 level was not associated with survival differences among the groups. Furthermore, changes in serum YKL-40 levels during the study were not related to recurrence-free survival or overall survival. Further studies are needed to clarify the role of YKL-40 in disease progression and its possible regulation upon IFN treatment.

Dr. Schmidt also discussed results from a recent trial of 227 stage I/II melanoma patients suggesting that tumor-associated macrophages in primary tumors may be prognostic factors for overall survival.⁴ More specifically, dense CD68+ or CD163+ cell infiltration in primary tumors was associated with poor survival.

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HLA, CTLA-4 polymorphisms and autoimmunity

Helen Gogas, of the University of Athens in Athens, Greece, discussed 2 studies exploring the relationship between human leukocyte antigen (HLA) profiles or cytotoxic T-lymphocyte antigen 4 (CTLA-4) polymorphisms, respectively, and outcomes in melanoma patients receiving adjuvant interferon (IFN)- α therapy. The reason for the study is that HLA and CTLA-4 genes have been linked with autoimmunity,¹⁻³ and a prior study by Gogas and coworkers suggested autoimmunity is an independent prognostic marker for improved recurrence-free survival (RFS) and overall survival (OS) in stage IIB-III melanoma patients treated with high-dose adjuvant IFN- α .⁴ Only a subset of melanoma patients at high risk for recurrence responds to high-dose adjuvant IFN- α , a toxic and costly therapy, so predictors of response would be invaluable.

The purpose of the first study reviewed by Dr. Gogas was to evaluate the impact of HLA class I and class II expression on the outcome of high-risk melanoma patients receiving high-dose adjuvant IFN- α . This was a substudy of the prospective randomized phase 3 study 13A98 of the Hellenic Cooperative Oncology Group. A total of 284 stage IIB, IIC, or III melanoma patients and 246 healthy controls were included in the study. Peripheral blood was obtained prior to initiation of adjuvant IFN- α therapy. DNA was used for determination of *HLA-A*, *HLA-B*, *HLA-Cw*, *HLA-DRB1*, and *HLA-DQB1* genotypes. No significant differences were seen in the HLA profiles of melanoma patients and healthy controls, or in patients with evidence of recurrence and those with recurrent disease. In addition, none of the HLA class I and II alleles showed significant positive or negative association with treatment outcome, with the exception of Cw*06. HLA-Cw*06-positive patients had significantly better median RFS ($P=.002$) and median overall survival (.017). Approximately 30% of patients with HLA-Cw*06 exhibited signs of autoimmunity upon treatment with adjuvant IFN.

The second study reviewed by Dr. Gogas evaluated the relationship between *CTLA-4* polymorphisms and treatment outcome in high-risk melanoma patients receiving adjuvant IFN- α . Since multiple polymorphisms within *CTLA-4* have previously been found to be associated with autoimmune disease, Dr. Gogas and coworkers genotyped DNA for 49 A/G, 60 C/T, 318 C/T, JO27, JO30, and JO31 using polymerase chain reaction and pyrosequencing technology. No polymorphism of *CTLA-4* defined by the single-nucleotide polymorphisms studied was significantly correlated with improved RFS or overall survival upon treatment with adjuvant IFN.

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