Abstract. Based on the results of European Organization for Research and Treatment of Cancer (EORTC) 18991 trial, the US Food and Drug Administration (FDA) approved PEG-interferon α-2b (PEG-IFN) (Sylatron) as adjuvant therapy for high-risk melanoma. The EORTC 18991 trial was an open-label study of resectable stage III melanoma with 1,256 patients who were randomized to observation-alone or to treatment with PEG-IFN for up to 5 years. The median recurrence-free survival of the treatment groups was significantly longer, while overall survival, a secondary endpoint, was not significantly different between the two groups. This review, after a short summary of interferon α-2b trials, critically analyzes the EORTC18991 trial, as well as the subgroup results and future perspectives for this stage of disease.

Currently melanoma represents the fifth most common type of tumor in men and the seventh in women with an annually increasing incidence of 2-5%, especially in the Western world (1). Melanoma affects young individuals in particular and, although new biological drugs are promising in the majority of patients with metastatic disease, therapy remains ineffective (2). When melanoma becomes metastatic, the median survival is about 6 to 12 months and the 5-year survival rate is less than 5% (3, 4). For this reason, early diagnosis remains the primary objective for physicians.

Adjuvant therapy is reserved for patients with American Joint Committee on Cancer (AJCC) stage IIb (primary tumor thickness >4 mm, node-negative) and stage III (any primary tumor, node-positive) melanoma, patients who are at high risk of recurrence after definitive resection (5). The US Food Drug Administration (US FDA) has approved interferon α for adjuvant treatment of melanoma and recently also PEG-interferon α-2b (PEG-IFN) for patients with resected stage III melanoma in 2011 (6).

This review focuses on published articles regarding the adjuvant therapy for high-risk melanoma with interferon α, especially describing the role of PEG-IFN.

Evidence Acquisition

A systematic analysis of the literature was conducted for the period between January 1, 1990 and April 1, 2012, by performing a Mesh search on PUBMED using the words ‘interferon’, ‘PEG-interferon’ and ‘adjuvant therapy’, combined with the Mesh term ‘melanoma’. Consideration for inclusion was given to articles providing data regarding treatment and toxicity results. Selection criteria included articles written in English presenting data from phase II, phase III or phase IV trials. A separate search was conducted on PUBMED to retrieve meta-analyses using the word ‘meta-analysis’. No temporal limit was applied. Abstracts published by the American Society of Clinical Oncology and the European Society of Medical Oncology between 2005 and 2012 were considered, but priority for inclusion was given to peer-reviewed full articles. We finally have included 4 meta-analyses, 2 randomized phase II and 19 phase III studies.

Prognostic Factors

Breslow’s tumor thickness, mitotic rate and ulceration are the most important prognostic factors for patients with localized...
interferons may increase infiltration of CD4+ T-cells into tumor cells, which has been demonstrated, and some studies have suggested that MHC expression, interferons have been theorized to render non-neoplastic host tissues. Through this enhancement of histocompatibility (MHC) antigens in both neoplastic and malignant cells more antigenic. Interferons have also been shown to have a growth-inhibitory effect when added to tumor cells in vitro. An apparent inhibition of angiogenesis with response rates (11). Subsequent analyses showed that HDI down-regulates the MEK/ERK/MAPK, and STAT3, a protein that is involved in cell survival, metastasis, angiogenesis and immune evasion (9).

Recent evidence for indirect immunomodulatory mechanisms of HDI include: increase in tumor infiltrating cells, development of autoantibodies and manifestations of autoimmunity, decrease in circulating T-regulatory cells, modulation of the STAT1/STAT3 balance in tumor cells and host lymphocytes, changes in cytokine concentrations, and normalization of T-cell STAT1 signaling defects in peripheral blood lymphocytes (10).

Mechanisms of Interferon Action

Human interferons are classified as α, β and γ. Interferons function by binding to cell surface receptors, interacting with specific gene sites in both normal and neoplastic cells. They modulate the expression of host natural killer cells, T-cells, monocytes, dendritic cells, and class I and II major histocompatibility (MHC) antigens in both neoplastic and non-neoplastic host tissues. Through this enhancement of MHC expression, interferons have been theorized to render malignant cells more antigenic. Interferons have also been shown to have a growth-inhibitory effect when added to tumor cells in vitro. An apparent inhibition of angiogenesis has been demonstrated, and some studies have suggested that interferons may increase infiltration of CD4+ T-cells into melanoma tumors (9).

The anti-neoplastic mechanisms of interferon (IFN) are not completely known. Pre-clinical and clinical studies suggests that the effects of IFN-α are related to immunomodulatory actions rather than direct cytotoxic activities (10). Moschos et al. tested high-dose interferon (HDI) as neoadjuvant therapy given before lymph node dissection in stage III disease, demonstrating that HDI increased T-lymphocytes and dendritic cells inside the tumor and this directly correlated with response rates (11). Subsequent analyses showed that HDI down-regulates the MEK/ERK/MAPK, and STAT3, a protein that is involved in cell survival, metastasis, angiogenesis and immune evasion (9).

Several clinical trials with interferon in treating high-risk (stage IIB-III) melanoma patients with different doses and duration of treatment (Table I) (12-24). Table I summarizes all published studies and shows that HDI trials were conducted by Eastern Cooperative Oncology Group (ECOG). In these trials, supervised by Kirkwood, interferon therapy was divided into induction and maintenance stages (12-14). The dose of interferon in the induction stage was up to 20x10^6 units on five continuous days per week for 4 weeks. The dosage of interferon in the maintenance stage was 10x10^6 units three times per week for 48 weeks. Interferon was approved as adjuvant therapy for high-risk melanoma in 1996 based on the overall survival (OS) benefit from the ECOG 1684 trial (12).

The serious toxicity of HDI has opened the door to finding an effective treatment with a lower toxicity. Numerous trials, including Austrian, French and English studies, were carried out to evaluate the efficacy of low-dose interferon (LDI) adjuvant therapy for melanoma (15, 16, 18). We found a recurrence-free survival (RFS) benefit in some trials but no OS benefit was gained.

The duration of treatment with interferon in multiple clinical trials was found to widely vary. Hauschild et al. found that extending LDI treatment to 5 years did not improve RFS nor OS of the patients who received LDI treatment for 18 months (25). Although LDI reduced therapy-related toxicity we found no OS improvement, except for the trial by Garbe et al. (21).

In the EORTC 18952 trial, 1,418 patients (stage IIB-III) received intermediate-dose interferon (IDI) for one or two years. The results suggested that IDI treatment for such duration did not produce any RFS or OS benefit (23).

Table I summarizes all published studies and shows that only four clinical trials confirmed an OS improvement with adjuvant interferon: ECOG 1684 study (HDI) (12), the ECOG 1694 (14), the ECOG 2696 (17) and the German...
Dermatologic Cooperative Oncology Group (DeCOG trial) [LDI alone or in combination with dacarbazine was compared to the control] (21).

Although the meta-analysis of Wheatley et al. reported an OS benefit, regardless of the dose and therapeutic regimen, the results are still controversial because this analysis included the ECOG 2696 and ECOG 1694 trials, in which the GM2 vaccine group was used (26). The GM2 vaccine (gangliosides expressed on surface of melanocytic cells with bacillus Calmette-Guerin) induces antibody responses (27) and was compared in a phase III setting in the E1694 and EORTC 18961 with HDI or observation [no treatment], respectively. Both failed to demonstrate any RFS and OS advantage for vaccine therapy and the EORTC 18961 trial

Table I. Clinical trials of adjuvant interferon (IFN) therapy. Efficacy results.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Eligibility</th>
<th>Time (months, years)</th>
<th>Treatment (arm: patients)</th>
<th>RFS (p-Value)</th>
<th>OS (p-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG 1684 (12)</td>
<td>280</td>
<td>IIB-III</td>
<td>13 m</td>
<td>HDI: 143</td>
<td>0.0023</td>
<td>0.0237</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 137</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG 1690 (13)</td>
<td>608</td>
<td>IIB-III</td>
<td>13 m vs. 24 m</td>
<td>HDI (13 m): 203</td>
<td>0.03</td>
<td>0.744</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LDI (24 m): 203</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 202</td>
<td></td>
<td>0.17</td>
<td>0.672</td>
</tr>
<tr>
<td>ECOG 1694 (14)</td>
<td>774</td>
<td>IIB-III</td>
<td>13 m</td>
<td>HDI: 385 GM2: 389</td>
<td>0.006</td>
<td>0.04</td>
</tr>
<tr>
<td>French CGM (15)</td>
<td>499</td>
<td>II</td>
<td>18 m</td>
<td>LDI: 245 Control: 244</td>
<td>0.035</td>
<td>0.059</td>
</tr>
<tr>
<td>Austrian MMCG (16)</td>
<td>311</td>
<td>II</td>
<td>12 m</td>
<td>LDI: 154</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 157</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG E2696 (17)</td>
<td>107</td>
<td>II-III-IV</td>
<td>-</td>
<td>+ GM2 with and without induction</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AIM HIGH (18)</td>
<td>674</td>
<td>IIB-III</td>
<td>24 m</td>
<td>LDI: 338</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 336</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scottish (19)</td>
<td>94</td>
<td>IIB-III</td>
<td>6 m</td>
<td>LDI: 46</td>
<td>&gt;0.1</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO-16 (20)</td>
<td>426</td>
<td>III</td>
<td>3 y</td>
<td>LDI: 218</td>
<td>0.5</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 208</td>
<td>LDI: 146</td>
<td>0.018</td>
<td>0.0045</td>
</tr>
<tr>
<td>DeCOG (21)</td>
<td>441</td>
<td>III</td>
<td>2 y</td>
<td>LDI+DTIC: 148</td>
<td>0.97</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 147</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeCOG (22)</td>
<td>353</td>
<td>III</td>
<td>13 m vs. 1 m</td>
<td>HDI (13 m): 176</td>
<td>0.90</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HDI (1 m): 177</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EORTC 18952 (23)</td>
<td>1388</td>
<td>IIB-III</td>
<td>25 m vs. 13 m</td>
<td>IDI (25 m): 556</td>
<td>0.17</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IDI (13 m): 553</td>
<td>Control: 279</td>
<td>0.01</td>
<td>0.78</td>
</tr>
<tr>
<td>EORTC 18991 (24)</td>
<td>1256</td>
<td>III</td>
<td>5 y</td>
<td>PEG-IFN: 627</td>
<td>0.01</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control: 629</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Y: Year, DTIC: dacarbazine; HDI: high-dose IFN; IDI: intermediate-dose IFN; LDI: low-dose IFN. PEG-IFN: pegylated interferon; GM2: Gangliosides vaccine. RFS: recurrence-free-survival; OS: overall survival. NS: not significant.

Table II. Grade 3-4 toxicity results for clinical trials of adjuvant interferon therapy.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Low-dose interferon (ref. 15, 18, 22)</th>
<th>Intermediate-dose interferon (ref. 23)</th>
<th>High-dose interferon (ref. 12, 13, 14)</th>
<th>Pegylated interferon (ref. 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>1-6%</td>
<td>13-15%</td>
<td>21-25%</td>
<td>16%</td>
</tr>
<tr>
<td>Liver function test*</td>
<td>2-4%</td>
<td>4-5%</td>
<td>27-29%</td>
<td>11%</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>&lt;1%</td>
<td>19-22%</td>
<td>10-35%</td>
<td>5%</td>
</tr>
<tr>
<td>Headache</td>
<td>0%</td>
<td>6%</td>
<td>10-12%</td>
<td>4%</td>
</tr>
<tr>
<td>Myalgia</td>
<td>3%</td>
<td>8%</td>
<td>15-17%</td>
<td>5%</td>
</tr>
<tr>
<td>Depression</td>
<td>1-4%</td>
<td>10-12%</td>
<td>40%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Ref.: Reference. *Alanine and aspartate aminotransferases, bilirubin/alkaline phosphatase.
was stopped early as evidence suggested that vaccination was ineffective and potentially harmful (28). The authors concluded that vaccination resulted in poorer Distant metastasis-free survival (DMFS) and OS compared to observation (28).

HDI was approved by the US-FDA in 1996 and later in 2004 by the European Medicine Agency (EMA) as adjuvant therapy, but no international consensus has yet been reached on the best schedule for maximal risk-benefit ratio. One reason for this may be attributed to the different approaches, taken by the different specialists who treat patients with melanoma i.e. dermatologists vs. medical oncologists (dermatologists prescribe LDI and IDI and medical oncologists HDI).

PEG-Interferon Therapy (PEG-IFN): EORTC 18991 Trial

As previously stated, interferon α-2b is the most studied agent for adjuvant treatment of patients at high risk of recurrence after radical surgery for melanoma (5, 6).

However, HDI regimens are associated with high toxicity and need to be given three to five times per week (14-17).

The conjugation of polyethylene glycol (PEG) to therapeutically useful proteins is widely used to reduce clearance and increase systemic exposure while preserving biological activity. PEG-IFN is a type of recombinant human IFN α-2b, chemically modified by addition of a 12-kDa mono-methoxy-PEG. This molecule has a sustained absorption and prolonged half-life and is more effective compared with the non-pegylated form in patients with hepatitis C (29-31). Pegylation of interferon α-2b has been shown to maintain maximum exposure to interferon α with less frequent injections than with unpegylated IFN. In fact PEG-IFN can be administered once a week, compared to the standard three to five times per week (29, 30).

PEG-IFN has also shown promise in the treatment of various solid tumors (32). Given this information, investigators started studying high-dose PEG-IFN for treatment of melanoma in the late 1990s.

The FDA approved PEG-IFN (Sylatron) for adjuvant treatment of lymph node-positive melanoma in 2011. Approval of FDA was based on the results of the phase III EORTC 18991 randomized trial.

In this trial, Eggermont et al. evaluated the efficacy and toxicity of PEG-IFN versus observation-alone in 1256 patients with resected AJCC stage III melanoma (24). Patients were stratified by disease stage (N1: microscopic non palpable nodal involvement, including those staged with sentinel node biopsy and N2: clinically palpable lymph nodes), Breslow thickness, number of involved lymph nodes, ulceration of the primary melanoma, sex and centre. The PEG-IFN schedule included an induction dose (6 μg/kg subcutaneously/weekly for eight weeks) followed by a maintenance dose (once weekly injections at 3 μg/kg for five years). The median length of therapy with PEG-IFN was 12 months. After 4 years, 22.5% of the patients remained in the treatment group. 31% of the 608 patients who were treated with PEG-IFN discontinued therapy due to side effects: fatigue (25%), anorexia (15%), depression (16%), liver function tests (13%), headache (12%), nausea (12%), pyrexia (11%).

At a median of 3.8 years of follow-up, the primary end-point of the study, RFS was increased by 18% (HR=0.82; p=0.1) in patients treated with PEG IFN.

Median RFS was significantly longer in the PEG-IFN group compared to the patients under observation alone (34.8 vs. 25.6 months).

DMFS was longer in the PEG-IFN than in the observation population although the difference was not statistically significant. OS, a secondary endpoint, was not significantly different between the two groups.

The most commonly observed side-effects were fatigue, anorexia, nausea, myalgia, and asthenia. Other adverse effects were myalgia, anorexia and nausea. A total of 525 deaths were reported during the trial (262 in the PEG-IFN group and 263 in the observation group). The most frequent causes of death were infection, malignant disease, and cardiovascular disease.

Considering the adverse effects of adjuvant treatment with PEG-IFN, Bottomley et al. examined the health–related quality of life (HRQOL) and symptoms including HRQOL as a secondary endpoint in the EORTC 18991 trial (33). The EORTC Quality of Life Questionnaire C30 (QLQ-C30) was used to evaluate HRQOL. At baseline, both groups were comparable using the global HRQOL scale.

A significant lowering of global HRQOL was recorded for patients treated with PEG-IFN compared with the group under observation alone (p<0.0004) and this difference was also clinically significant at 3 months (lower by 11.6 points; 99% CI, 8.2 to 15.0 point reduction) and 2 years (reduction of 10.5 points; 99% CI, 6.6 to 14.4 points).

Considering the EORTC QLQ-C30 functioning scales, the difference between the two groups of patients was the strongest for social functioning. Social functioning of patients receiving therapy with PEG-IFN was compromised for a long period during therapy, as compared to patients under the observation alone. The role functioning of the patients treated with PEG-IFN was also significantly worse at 3 months after baseline if compared with the observation group. This significant difference was transient and limited to one year’s duration.

The group of patients treated with PEG-IFN reported a statistically greater frequency of headaches, fever, muscles/stiffness and pain, and suffered significantly more from appetite loss, fatigue and dyspnea compared with patients under observation alone.
In conclusion, this study showed that although adjuvant treatment with PEG-IFN for resected-stage III melanoma patients offers a significant sustained amelioration in RFS, it has a negative effect on global HRQOL and on various symptoms.

An important message from the EORTC 18991 study is the effect of PEG-IFN in patients with microscopical disease involvement (N1). Responses to therapy were more pronounced in patients with earlier stage III melanoma than in those with later stage disease. In patients with N1 disease, the effects of PEG-IFN began quite early and were maintained throughout the follow-up period. In terms of RFS at 4 years an advantage of 12% with PEG-IFN among the patients with microscopic nodal disease in stage III melanoma was found (24).

At ASCO 2011, the results of EORTC 18991 with a longer follow-up were presented. At 7.6 years follow-up, PEG-IFN had a significant and sustained benefit for RFS but not for DMFS or OS.

Patients with palpable node-positive disease (stage III N2) failed all endpoints while these with sentinel node-positive (stage III N1) experienced greater benefit in terms of RFS, with a trend for better DMFS and OS. Patients with ulcerated primary sentinel node-positive (stage III N1) melanoma significantly benefit on RFS (HR=0.72), DMFS (HR=0.65) and OS (HR=0.59) (34).

Recently Eggermont et al. presented a meta-analysis of two large adjuvant randomized trials of interferon/PEG-IFN therapy (35). A total of 2,644 patients were randomized in the two trials: the EORTC 18991 trial compared adjuvant PEG-IFN treatment to observation in 1,256 stage III melanoma patients, while the EORTC 18952 trial compared IDI versus observation in 1,388 patients with stage IIb/III melanoma. These trials stratified patients by ulceration and sentinel node staging (stage IIb/III-N1: microscopic involvement or stage III-N2: macroscopic nodal disease) and permitted analysis of the potential interaction between ulceration and therapy, as well as tumor load and treatment (35).

Overall, patients comparison of IFN/PEG-IFN versus observation yielded hazard ratios (HR) that were significant for RFS (HR=0.85) and DMFS (HR=0.89), but not for OS (HR=0.94). In the ulceration group the impact of treatment was greater compared to non-ulceration patients for RFS (p=0.02), DMFS (p<0.001) and OS (p<0.001). In stage IIb/III-N1 patients with ulceration of primary melanoma, all estimated HR values were <0.7; RFS:HR=0.69 (p=0.003), DMFS:HR=0.59 (p<0.0001) and OS:HR=0.58 (p<0.0001). The efficacy of therapy was lower in stage III-N2 patients with ulceration and absent in patients without ulceration. The results of Eggermont et al. showed that only the stage IIb/III-N1 melanoma group and patients with ulceration of the primary melanoma benefited significantly from IFN/PEG-IFN therapy.

Patients with stage IIb/III-N1 and ulceration benefited greatly (HRs=0.56-0.69) with regard to RFS, DMFS and OS. Stage III-N2 patients had no significant benefit, not even in case of ulceration of the primary cutaneous tumor. This meta-analysis demonstrated that ulceration and stage are strong prognostic factors that are also predictive of the efficacy of adjuvant treatment with IFN/PEG-IFN, but only in a small subgroup of melanoma patients at high risk for relapse. These observations explain why IFN trials have shown marginal effects. This meta-analysis confirmed the data of Wheatley et al. regarding IFN sensitivity and ulceration but the authors did not include the results of the EORTC 18991 trial (26).

**IFN Autoimmunity**

Several studies (E1690, E1694, EORTC 18952, EORTC 18991) have demonstrated that IFN treatment benefit is limited to the population of patients that may be identified by pathological appearance of ulceration and/or microscopic node-positive disease, and also by the ability to develop autoimmunity.

Autoimmune manifestations are a peculiarity of IFN therapy in hematologic malignancies and in chronic viral hepatitis. Therapy with PEG-IFN for chronic hepatitis C has been associated with autoimmune-related toxicities such as autoimmune thyroiditis, type 1 diabetes mellitus, systemic lupus erythematosus and celiac disease (36, 37). The appearance of autoimmunity in melanoma has been considered as a good prognostic factor of response to adjuvant treatment for several years.

Gogas et al. noted that patients who developed autoimmunity had better DFS and OS for up to 46 months; indeed patients who generated autoimmune manifestations showed reductions in relapse (13% vs. 73%) and overall mortality (4% vs. 54%), compared to those who did not develop autoantibodies (38).

Bouwhuis et al. evaluated the prognostic significance of autoantibodies in the EORTC 18991 trial. Seroconversion (the appearance of autoantibodies in initially autoantibody-negative patients) occurred in 76 (35%) out of 220 patients with prevalence of antinuclear antibodies, and was associated with an improved outcome according to the Cox model which considers the occurrence of autoantibodies as a time-independent co-variate (HR=0.56; p=0.1).

When the effects of seroconversion were studied in two groups of patients (PEG-IFN group and observation alone), similar results were found, indicating that seroconversion is not a predictive serological marker for adjuvant therapy with PEG-IFN. However no significance test was used.

In conclusion, appearance of autoimmune antibodies is neither a prognostic nor a predictive factor to evaluate efficacy of treatment with PEG-IFN in stage III melanoma patients. No
significant treatment differences on RFS were observed between the two groups of patients \( (p=0.63) \), probably because a larger portion of patients were in IIIN2 stage. The number of positive lymph nodes remained an independent strong prognostic factor throughout all analyses (39).

**Circulating Melanoma Cells (CMC)**

Some interesting previous studies evaluated the role of the prognostic significance of the circulation of melanoma cells in the peripheral blood using reverse transcriptase polymerase chain reaction (RT-PCR) in stage I, II, III melanoma patients (40-41).

In a study based on resected stage IIB, IIIA, IIIB and IV melanoma patients enrolled in a vaccination trial, Reynolds et al. revealed a statistically significant correlation between the presence of circulating melanoma cells and RFS. The risk of recurrence in positive patients was 2.6-times higher than in negative patients (40).

Scoggins et al. confirmed time-dependent tyrosinase RT-PCR as a significant prognostic variable for DMFS in stage I, II and III melanoma patients with an HR of 5.5 (41).

Palmieri et al. analyzed a consecutive series of 200 melanoma patients with stage of disease ranging from I to IV in order to evaluate the prognostic role of circulating melanoma cells (CMCs) detected by semiquantitative RT-PCR. The authors concluded that the presence of CMCs was predictive of prognosis in the univariate analysis, but did not provide any additional prognostic information about the stage of disease in multivariable models (42).

Recently Fusi et al. conducted a multicentre study of serial RT-PCR analysis of the two melanoma markers (Mart-1/Melan-A and tyrosinase) in order to assess the prognostic and predictive role of RT-PCR assessment of circulating melanoma cells for DMTS (43).

Among the 299 stage-III melanoma patients enrolled in EORTC 18991, 109 (36.5%) had at least one positive sample, either at the time of randomization \( (N=17) \), or subsequently \( (N=92) \). When comparing these patients to the negative group no marked differences were observed, with the exception of a higher percentage of patients with microscopic nodal involvement (62.4% vs. 52.6%) in the positive group. Analysis of baseline blood samples revealed no differences in terms of DMFS between the two groups of patients at a given time point. Then, RT-PCR results had no prognostic impact on subsequent DMFS. The Cox time-dependent model estimated the hazard ratio for the latest RT-PCR-positive versus-negative to be 2.23 (95% CI: 1.40-3.55; \( p<0.001 \)). It indicated a significant risk of developing metastasis/death in RT-PCR-positive patients compared to negative patients. Patients were also divided in three categories: strongly-positive, moderately-positive and negative for Mart-1/Melan-A and tyrosinase.

Among 109 RT-PCR-positive patients, 51 were strongly positive and in this sub-group, using a Cox time-dependent covariates model, the risk of metastasis/death was 3.15-times \( (p<0.001) \) higher that in negative patients, and was 1.45-times higher in those who were moderately-positive versus the negative group.

These results remained unchanged after adjustments for nodal status, number of positive nodes and sex. In conclusion, results of this study showed that the presence of circulating melanoma cells detected by tyrosinase and Mart-1/Melan-A RT-PCR is a moderate time-dependent prognostic factor for subsequent development of distant metastasis in melanoma patients with stage III, but did not reveal significant differences in terms of DMTS. For this reason, RT-PCR analysis should not be used to select subgroup of patients for adjuvant therapy with PEG-IFN (43).

**Considerations and Perspectives**

The EORTC 18991 study investigated the efficacy and the importance of prolonged durations of therapy with PEG-IFN versus observation in patients with stage III cutaneous melanoma. Taking into account the results of this trial some considerations can be made.

Primarily, this study does not permit conclusions regarding the impact of longer durations of therapy with PEG-IFN, because median length of treatment was 14.9 months, and after 4 years about 23% of patients remained in the treatment group.

In addition, 31% out of 608 patients discontinued therapy due to toxicity. Grade 3 and grade 4 adverse effects occurred in 45% of patients (fatigue 16%, aminotransferase elevations 11%). Depression occurred in 59% of patients treated and was severe or life-threatening in 7%. It might be interesting to evaluate different schedules of PEG-IFN in terms of low dose and duration of treatment, but only randomized trials could clarify the real effect of this agent.

Recently Grob et al. presented an abstract of EADO phase III trial. The authors evaluated low-dose of PEG-IFN versus low-dose of IFN α-2b in 896 patients with stage IIA-IIIB melanoma without clinically detectable nodal disease. Patients were randomized to 36 months of PEG-IFN (100 mcg subcutaneously once weekly), or 18 months of LDI (3 MU subcutaneously thrice weekly).

These results were characterized by the high rate of drop-out (72% before the end of study), secondary to severe toxicity in the PEG-IFN group (44.6% versus 26.6%). RFS, OS and DMFS results were similar in both groups (44).

Concerning the correct duration of treatment, the results of the ongoing EORTC 18081 trial (Table III) may give a better answer.

Based on a subgroup analysis of the EORTC 18991 trial, Eggermont et al. concluded that patients with microscopic nodal metastasis (N1) and ulcerated primary melanoma
represented the groups who benefited the most from treatment with PEG-IFN, in terms of RFS, OS and DMFS, concluding that the effects of adjuvant therapy with PEG-IFN are confined to these subgroups.

However, it may be incorrect to compare results of retrospective and subgroup analyses, and the conclusions drawn from these comparisons cannot be considered as a guide for therapy choice in a large melanoma population. We are currently waiting for the results of the ongoing EORTC 18081 trial to make one more step towards the management of the disease.

The exciting survival advantage gained in the metastatic melanoma setting, with anticytotoxic T-lymphocyte antigen-4 (anti-CTLA-4, ipilimumab) (45) has raised hope that this therapy might be beneficial in the adjuvant setting. Multiple trials are investigating the role of ipilimumab in the adjuvant setting: in specific, the results of ECOG 1609 (ipilimumab versus HDI in high-risk stage III MM), and EORTC 18071 (ipilimumab versus placebo in high-risk stage III melanoma) trials are widely expected (table III).

Vemurafenib, a BRAF inhibitor, has demonstrated an improvement in overall and progression-free survival in patients with previously untreated melanoma with the BRAF V600E mutation (46).

Whether vemurafenib will play a role in the adjuvant therapy of melanoma remains to be investigated in a phase III adjuvant trial and in the neoadjuvant setting over the next years.

**Conclusion**

PEG-IFN has improved median recurrence-free survival in the EORTC 18991 trial and it has been approved as adjuvant therapy even without an overall survival improvement.

The recent results, presented at ASCO 2011, confirmed that ulcerated primary sentinel node-positive melanoma patients (stage III N1) significantly benefit in terms of RFS, DMFS and OS.

We hope that new agents will lead to novel, more rational, combined-modality regimens that will improve the overall survival benefits of adjuvant treatment. The identification of clinicopathological, molecular and immunological markers can be very important in personalized interferon treatment and in deciding how many patients benefit from adjuvant therapy as we have seen in other cancers.

**Conflicts of Interest**

Paolo A. Ascierto is consultant from Merck Sharp & Dohme and had an advisory role for Bristol Myers Squibb, Merck Sharp & Dohme, Roche-Genentech, Glaxo Smith-Kline, Amgen, Celgene, Medimmune, Novartis; moreover received honoraria from Bristol Myers Squibb, Merck Sharp & Dohme, Roche-Genentech. Ester Simeone received honoraria from Bristol Myers Squibb, Giuseppe Di Lorenzo received honoraria from Pfizer, Novartis, Sanofi, Johnson and Johnson Medical, Teva. All other Authors have no conflicts of interest.

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**Table III. Ongoing phase III clinical trials in adjuvant melanoma.**

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>Interventions</th>
<th>Primary end-point</th>
<th>Estimated completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC 18081</td>
<td>Adjuvant PEG-IFN α-2b 3 μg/kg weekly for 2 years vs. observation in ulcerated MM</td>
<td>RFS</td>
<td>2020</td>
</tr>
<tr>
<td>NCT01502696</td>
<td></td>
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</tr>
<tr>
<td>EORTC 18071</td>
<td>Ipilimumab vs. placebo after complete resection of high risk Stage III MM</td>
<td>RFS</td>
<td>2014</td>
</tr>
<tr>
<td>NCT00636168</td>
<td>High dose ipilimumab vs. low-dose ipilimumab (LIP) vs. high-dose recombinant interferon α-2b for resected high-risk MM</td>
<td>RFS</td>
<td>2015</td>
</tr>
<tr>
<td>EORTC 18071</td>
<td>Adjuvant ganglioside GM2-KLH/QS-21 stage II, vs. observation</td>
<td>DFS</td>
<td>Not reported</td>
</tr>
<tr>
<td>NCT00005052</td>
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<tr>
<td>MD Anderson</td>
<td>Interferon α-2b vs. biochemotherapy using cisplatin plus vinblastine plus DTIC plus interferon plus IL-2 in MM with regional lymph node metastases</td>
<td>OS</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT00002882</td>
<td></td>
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<tr>
<td>GlaxoSmithKline</td>
<td>GSK 2132231A antigen-specific cancer immunotherapeutic vs. placebo</td>
<td>DFS</td>
<td>2016</td>
</tr>
<tr>
<td>NCT00796445</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nordic Adjuvant IFN MM</td>
<td>Interferon α-2b for 1 year vs. 2 years in patients with high-risk MM</td>
<td>OS</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT01259934</td>
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<td></td>
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<tr>
<td>Nantes University Hospital</td>
<td>TIL (Tumor infiltrating lymphocytes) and IL2 vs. abstention in MM with only 1 invaded lymphnode after lymphnodes excision</td>
<td>RFS</td>
<td>Completed</td>
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<tr>
<td>NCT00200577</td>
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<td></td>
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</tr>
</tbody>
</table>

RFS: Recurrence-free survival; DFS: disease-free survival; OS: overall survival. DTIC: Dacarbazine; MM: melanoma; IL2: interleukine 2.
References


7. Balch CM, Gershenwald JE, Soong SJ

8. Thompson JF, Soong SJ, Balch CM


15. Kirkwood JM, Ibrahim JG, Gershenwald JE, Soong SJ


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