Abstract

Purpose: The precise molecular targets of IFN-α therapy of melanoma are unknown but likely involve signal transducer and activator of transcription (STAT) 1 signal transduction within host immune effector cells. We hypothesized that intermediate and high doses of IFN-α would be equally effective in activating patient immune cells.

Experimental Design: Eleven metastatic melanoma patients who were enrolled in a clinical trial of bevacizumab in combination with escalating doses of IFN-α-2b (5 megaunits/m² and then 10 megaunits/m²) were included in the study. Peripheral blood mononuclear cells (PBMC) were procured from patient blood just before therapy and again 1 h after each dose of IFN-α-2b and analyzed for the presence of phosphorylated STAT1, phosphorylated STAT2, and the induction of IFN-stimulated gene (ISG) transcripts.

Results: Phosphorylated STAT1 was significantly greater at the 5 megaunits/m² dose compared with the 10 megaunits/m² dose of IFN-α-2b (P = 0.02). In contrast, no significant difference in phosphorylated STAT2 was observed at a dose of 5 megaunits/m² compared with 10 megaunits/m² (P = 0.20). There were also no significant differences in the induction of ISGs within PBMCs between the two doses (P > 0.4 for all ISGs). Suppressor of cytokine signaling 1 and 3 (two inhibitors of IFN-α signaling) transcripts were significantly higher among patient PBMCs following the 10 megaunits/m² dose of IFN-α (P < 0.001).

Conclusion: These results suggest that lower doses of IFN-α-2b are as effective as higher doses with respect to the induction of Janus-activated kinase-STAT signal transduction and the transcription of ISGs within immune effector cells.

The IFN-α receptor is widely expressed on both tumor cells and immune effector cells (1, 2). Binding of IFN-α to its receptor activates Janus-activated kinase 1 (JAK1) and tyrosine kinase 2, which in turn phosphorylate tyrosine residues within the cytoplasmic region of the IFN-α receptor. These phosphorylated residues provide docking sites for signal transducer and activator of transcription (STAT) 1 and STAT2, latent cytoplasmic transcription factors that are phosphorylated by the JAKs (3). The prototypical IFN-α signaling reaction results in the formation of IFN-stimulated gene (ISG) factor 3, a DNA-binding complex that consists of STAT1α (or STAT1β) and STAT2, and a chaperon protein known as IFN regulatory factor 9 (4). ISG factor 3 translocates to the nucleus and binds to IFN-stimulated response elements located in the promoter regions of IFN-responsive genes (5). This signaling event induces the expression of several immunoregulatory genes and largely determines the pattern of immune cell activation following exposure to IFN-α (6–8).

High-dose IFN-α is used as an adjuvant in patients who have undergone resection of high-risk lesions (nodal disease or primary tumors with Breslow thickness >4 mm; refs. 9–13). IFN-α is also used to treat individuals with metastatic melanoma and produces clinical responses in ~10% of patients (9, 10, 14). However, it remains difficult to determine the optimal dose of IFN-α for melanoma patients or devise strategies to enhance its antitumor effects because its cellular targets and mechanism of action are largely unknown. Although IFN-α can act directly on melanoma cells to inhibit proliferation and up-regulate the expression of MHC class I antigens, its stimulatory properties on effector cells of the immune system are thought to be most important for its antitumor activity (15–22). In fact, recent data have shown that the occurrence of autoimmune sequelae and the presence of tumor-infiltrating lymphocytes correlate with clinical response in patients receiving IFN-α (23, 24). Our previous analysis of IFN-α–induced JAK-STAT signal transduction in immune cells revealed reduced phosphorylation of these

Cancer Therapy: Clinical

IFN-α-2b–Induced Signal Transduction and Gene Regulation in Patient Peripheral Blood Mononuclear Cells Is Not Enhanced by a Dose Increase from 5 to 10 Megaunits/m²

Jason M. Zimmerer,1,2 Amy M. Lehman,3 Amy S. Ruppert,3 Carl W. Noble,2 Thomas Olencki,4 Michael J. Walker,4 Kari Kendra,4 and William E. Carson III2,4

Abstract Purpose: The precise molecular targets of IFN-α therapy of melanoma are unknown but likely involve signal transducer and activator of transcription (STAT) 1 signal transduction within host immune effector cells. We hypothesized that intermediate and high doses of IFN-α would be equally effective in activating patient immune cells.

Experimental Design: Eleven metastatic melanoma patients who were enrolled in a clinical trial of bevacizumab in combination with escalating doses of IFN-α-2b (5 megaunits/m² and then 10 megaunits/m²) were included in the study. Peripheral blood mononuclear cells (PBMC) were procured from patient blood just before therapy and again 1 h after each dose of IFN-α-2b and analyzed for the presence of phosphorylated STAT1, phosphorylated STAT2, and the induction of IFN-stimulated gene (ISG) transcripts.

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proteins at higher doses of IFN-α (25). We therefore hypothesized that intermediate and high doses of IFN-α might be equally effective in activating STAT proteins and inducing the expression of ISGs in patient peripheral blood mononuclear cells (PBMC).

To test this hypothesis, we analyzed JAK-STAT–mediated signal transduction and gene regulation in patients who received escalating doses of IFN-α-2b in the setting of metastatic disease. The phosphorylation of STAT1 and STAT2 was measured by intracellular flow cytometry, and the induction of ISG transcripts was evaluated by real-time PCR and microarray analysis. These studies indicated that the PBMC response to 5 megaunits/m² IFN-α was comparable with that of 10 megaunits/m² in the context of the present trial. Interpretation of these results must be tempered by the observation that the reduced response to the higher dose of IFN-α could be a time-dependent effect rather than a dose-dependent effect.

Materials and Methods

Patient blood samples. Samples were obtained at The Ohio State University following informed consent under an Institutional Review Board–approved protocol (OSU 99H0348). Patients on this National Cancer Institute–sponsored trial received 15 mg/kg bevacizumab (anti–vascular endothelial growth factor monoclonal antibody) every 2 weeks plus high-dose IFN-α-2b thrice weekly via the s.c. route. IFN therapy was initiated at 5 megaunits/m². If this dose was well tolerated for 2 weeks, the patient underwent a dose escalation to 10 megaunits/m². Peripheral blood was obtained from melanoma patients (five females and six males) immediately before and 1 h following the administration of the first dose of IFN-α-2b at 5 or 10 megaunits/m². The characteristics of the patients on this study are listed in Table 1. PBMCs were isolated from blood (8 mL) via centrifugation with Ficoll-Paque Plus (Amersham Pharmacia Biotech) and used immediately in the assays.

Flow cytometric analysis of STAT1 and STAT2. The native and phosphorylated forms of STAT1 (Tyr²¹⁸) and STAT2 (Tyr²⁴⁸) were measured by intracellular flow cytometry as previously described, with modifications (25, 26). Rabbit anti-human primary antibodies (Cell Signaling Technology) were used in combination with a goat anti-rabbit Alexa Fluor 488–conjugated secondary antibody (Molecular Probes).

Real-time PCR. Following Trizol extraction and RNeasy purification, 2 μg of total RNA were reverse transcribed. The resulting cDNA was used as a template to measure gene expression by real-time PCR using predesigned primer/probe sets (Assays-On-Demand, Applied Biosystems) as previously described (27). Human β-actin was used as an internal control in each reaction well (Applied Biosystems). Real-time PCRs were done in triplicate and analyzed using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems).

cRNA preparation and array hybridization. U133 Plus 2.0 Arrays (Affymetrix), which query ~47,000 human transcripts, were used in these analyses. The cRNA was synthesized as suggested by Affymetrix. Briefly, double-stranded cDNA was generated from 8 μg of total RNA using the SuperScript Choice System according to the manufacturer’s instructions (Invitrogen). Biotinylated cRNA was generated by in vitro transcription using the BioArray High Yield RNA Transcript Labeling System (Enzo Life Sciences, Inc.). The cRNA was purified, fragmented, and hybridized to the arrays (28).

Microarray data analysis. Raw data were collected with a confocal laser scanner (Hewlett-Packard) and probe level data were analyzed using dChip software (29). Quantile normalization was done, and only perfect match probes were used in computing the model-based expression indices (MBEI). “Array outliers” identified by dChip at the probe set level were set to missing. The log₂(MBEIs) were then calculated and used for further analysis.

An ANOVA model with random effects to account for repeated measures across patients was used to determine if there was evidence of a linear or nonlinear IFN-α dose effect on gene expression values. These tests were done for 143 preselected probe sets (representing 64 IFN-responsive genes; see Supplementary Table S1) identified based on preliminary studies from our group as well as the previous work of other groups (30, 31). Paired t tests were used to test for a difference in expression between the 5 and 10 megaunits/m² dose levels. Type 1 error was protected by adjusting P values using Holm’s method and two-sided significance levels were set at α = 0.10.

Statistical analysis. For Fig. 1, differences in phosphorylated STAT1 (P-STAT1) and phosphorylated STAT2 (P-STAT2) levels between doses were assessed using a random-effects ANOVA model to allow for correlations between replicate observations from the same patient. For Figs. 2 and 3, respectively, the STAT activation efficiencies (determined by calculating the ratio of activated versus native forms of STAT1 and STAT2) and fold changes were log transformed and statistical comparisons between treatments were done using random-effects ANOVA. Treatment differences and 95% confidence intervals were estimated using a two-sided z = 0.05 level of significance. All analyses were done using Statistical Analysis System v9.1.3 (SAS Institute, Inc.).

Results

Native and activated forms of STAT1 and STAT2 in PBMCs following escalation of IFN-α-2b dose from 5 to 10 megaunits/m². We hypothesized that an intermediate dose of IFN-α would

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>Metastatic disease site</th>
<th>Prior treatments</th>
<th>Major toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>F</td>
<td>Liver, lung</td>
<td>Radiation, surgical resection</td>
<td>Grade 3 fatigue</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>F</td>
<td>Colon, lung</td>
<td>Interleukin-2, radiation</td>
<td>Grade 3 weight loss</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>F</td>
<td>Liver</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>M</td>
<td>Liver, lymph node</td>
<td>Radiation, recurrent lesion resected, liver chemobilization</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>M</td>
<td>Right scapula, bladder</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>M</td>
<td>Lung, lymph nodes</td>
<td>IFN-α (20 megaunits/m²)</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>F</td>
<td>Liver, sacrum, lymph nodes</td>
<td>Recurrent lesion resected, IFN-α (20 megaunits/m²)</td>
<td>Severe psychological effects, grade 3 fatigue, grade 3 diarrhea</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>M</td>
<td>Auricular/parotid mass</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>74</td>
<td>M</td>
<td>Chest wall, lymph node</td>
<td>—</td>
<td>Grade 3 neutropenia</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>M</td>
<td>Lung</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>69</td>
<td>F</td>
<td>Right groin, lymph node</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
be as effective as a higher dose for the activation of immune cells. To test this hypothesis, PBMCs from 11 metastatic melanoma patients were obtained immediately before treatment, 1 h following the s.c. administration of 5 megaunits/m² IFN-α-2b, and 1 h following the administration of 10 megaunits/m² IFN-α-2b 2 weeks later. Ten of 11 patients received 6 injections of IFN-α-2b at a dose of 5 megaunits/m² before dose escalation (patient no. 10 received 12 injections of 5 megaunits/m² over 4 weeks). Following isolation from blood, PBMCs were immediately analyzed for the native and activated forms of STAT1 (activated residue Tyr701) and STAT2 (activated residue Tyr690) by flow cytometry. Flow cytometric data were derived from at least 10,000 events gated on the lymphocyte populations as determined by light scatter properties.

Within individual patients, overall levels of unphosphorylated STAT1 did not seem to diminish greatly following administration of IFN-α at 10 megaunits/m² except for patient no. 3 (Fig. 1B). Activation of STAT2 (P-STAT2) was not significantly different between a dose of 5 megaunits/m² and a subsequent dose of 10 megaunits/m² (P = 0.20; Fig. 1C). Within individual patients, overall levels of unphosphorylated STAT2 did not seem to diminish greatly following administration of IFN-α at 10 megaunits/m², except for patient no. 5 (Fig. 1D). Because of the trial design, it is not known whether the reduced IFN responsiveness (at the level of STAT1 phosphorylation) would have been observed following continued administration of the lower dose of IFN-α (i.e., the drop in P-STAT1 may have been a time-dependent effect rather than a dose-dependent effect).

Ratio of activated to total STAT1 and STAT2 in PBMCs following escalation of IFN-α-2b dose from 5 to 10 megaunits/m². To determine the efficiency of STAT activation in PBMCs following IFN-α administration, the ratio of activated STAT protein to the total level of the STAT protein was evaluated. This
measure is an artificial means of quantifying the ability of the cell to phosphorylate the available STAT protein within the cytoplasm. However, it does not imply a greater activation of STAT1 because the functional effect of a STAT protein is mediated by the binding of its phosphorylated form to specific DNA elements. The estimated ratio of P-STAT1 to total STAT1 at the 10 megaunits/m² dose of IFN-α was only 40% of the ratio at the 5 megaunits/m² dose (95% confidence interval, 21.6-67.5%; \( P = 0.002 \); Fig. 2A). Similarly, the ratio of P-STAT2 to total STAT2 at the 10 megaunits/m² dose of IFN-α was only 58% of the ratio at the 5 megaunits/m² dose (95% confidence interval, 32.6-94.8%; \( P = 0.003 \), Fig. 2B). These results indicate that the efficiency of STAT1 and STAT2 activation in response to exogenous IFN-α was significantly higher at the 5 megaunits/m² compared with the 10 megaunits/m² dose.

ISG expression following IFN-α-2b administration. To characterize the induction of ISGs following IFN-α-2b administration at the two doses, patient PBMCs were analyzed for the induction of specific ISGs by real-time PCR. As with the activation of STAT1 and STAT2, the expression of several well-characterized ISGs (OAS3, GIP2, IFIT1, and IFIT2) was not significantly enhanced following the increase in IFN-α-2b dose from 5 to 10 megaunits/m² (\( P > 0.4 \) for all genes; Fig. 3A-D).

Suppressor of cytokine signaling gene expression following IFN-α-2b administration. Investigators have identified a family of proteins known as suppressors of cytokine signaling (SOCS) that negatively regulate JAK-STAT signal transduction (32). Our group has shown that SOCS1 and SOCS3 mediate potent inhibitory effects on IFN-α–stimulated signal transduction, gene regulation, and antitumor activity in a murine melanoma model (22, 33–36). However, the effect of exogenous IFN-α on SOCS expression in resting immune cells has yet to be defined in the context of cancer immunotherapy. For both SOCS1 and SOCS3, expression was induced to a greater degree following administration of IFN-α at 10 megaunits/m² compared with the 5 megaunits/m² dose (\( P < 0.001 \) for both SOCS1 and SOCS3; Fig. 3E and F). These data suggest that IFN-α may induce the transcription of genes that negatively regulate the IFN-α response.

Gene regulation in PBMCs from metastatic melanoma patients receiving increasing doses of IFN-α. PBMCs from four melanoma patients undergoing immunotherapy with escalating doses of IFN-α-2b were evaluated by microarray analysis (patient nos. 2, 5, 6, and 7 from above). Sixty-four known IFN-regulated genes represented by 143 probe sets were analyzed (Supplementary Table S1; refs. 30, 31). Of these, 34 genes showed a dose response, with a significant difference in expression after treatment with IFN-α at 10 megaunits/m² compared with pretreatment expression (adjusted \( P < 0.10 \); Supplementary Table S1). Only 1 of the 34 genes, LGA33BP (endothelial adhesion molecule), was induced (or suppressed) to a greater extent by IFN-α at 10 megaunits/m² compared with 5 megaunits/m² (1.46- and 0.99-fold inductions, respectively; adjusted \( P = 0.10 \); Table 2, group 1; ref. 37). Of the remaining 33 genes, 13 were similarly up-regulated or down-regulated by IFN-α at the 5 and 10 megaunits/m² dose levels. More specifically, a test for a nonlinear dose response showed that these 13 genes were more similarly up-regulated or down-regulated than expected by the two doses of IFN-α (adjusted \( P < 0.10 \); Table 2, group 2). These results were validated by real-time PCR for several genes and corresponded well with the microarray results in that the increase in levels of IFIT44, OAS1, and MX1 transcript were similar following both doses of IFN-α. Likewise, LGA33BP transcript was induced to a greater extent by the higher dose of IFN-α (Fig. 4A-D).

Discussion

The present study showed that IFN-α–induced signal transduction and gene transcription in patient PBMCs in response to IFN-α at 5 megaunits/m² was not enhanced by an increase to a dose of 10 megaunits/m². Specifically, levels of P-STAT1 and P-STAT2 were not induced to a greater degree with the higher dose of IFN-α, despite higher cellular levels of STAT1 and STAT2. The higher levels of total STAT1 and STAT2 after the 10 megaunits/m² dose probably reflect the fact that STAT1 and STAT2 are IFN-inducible genes that can be up-regulated during the course of treatment with IFN-α. Thus, the higher levels of total STAT1 and STAT2 present in PBMCs at the time of the 10 megaunits/m² dose may reflect the fact that there was more of each of these proteins in cells before this dose, as a result of their previous IFN exposure. Interestingly, two important
inhibitors of IFN-α signaling, SOCS1 and SOCS3, were induced more effectively by higher doses of IFN-α.

It is important to note the order in which the two doses of IFN-α were administered. If IFN-α were first administered at a dose of 10 megaunits/m² followed later by the 5 megaunits/m² dose, it is possible that superior results would have been obtained for the higher dosage of IFN-α. However, the dose escalation schema used in this study is more likely to be tolerated by patients and is therefore of greater clinical utility. Because of the trial design, it is not known whether the reduced
IFN responsiveness (at the level of STAT1 phosphorylation) would have been observed following continued administration of the lower dose of IFN-α (i.e., the drop in P-STAT1 was a time-dependent effect rather than a dose-dependent effect). That being stated, we currently have data from two patients that received the same 10 megaunits/m² dose of IFN-α over a time period of 6 weeks. P-STAT1 levels were induced to a similar degree following each dose of cytokine in both patients (data not shown). Additionally, we evaluated a patient who underwent dose escalation (from 5 to 10 megaunits/m²) and then required a dose reduction back to 5 megaunits/m². This patient exhibited lower levels of P-STAT1 following the dose escalation. However, STAT1 activation returned to previous levels following the next dose reduction (data not shown). These data are not conclusive, but these results suggest that the observed drop in the generation of P-STAT1 following the IFN-α dose escalations may not have been a time-dependent event. The use of IFN-α in patients with metastatic disease (as opposed to patients with no evidence of disease) and the concurrent administration of bevacizumab may also have influenced our results.

Although melanoma cells routinely express functional IFN-α receptors and exhibit reduced proliferation and/or increased apoptosis in response to this cytokine in vitro (33, 38–41), we have found that tumor expression of STAT proteins did not correlate with the effectiveness of adjuvant IFN-α (21). This prompted us to investigate if IFN-α was acting primarily as an immune activator. Using STAT1-deficient mice and STAT1-deficient melanoma tumor cells, our group showed that the antitumor effects of IFN-α are dependent on STAT1 signaling within immune cells and not the cancer cell (42, 43). In lieu of a panel of ISGs that directly correlates with the antitumor effects of IFN-α, we have used the activation of JAK-STAT signaling intermediates as a marker of the ability of IFN-α to stimulate immune effector cells. We acknowledge that this approach relies on several assumptions (e.g., that activated STAT1 and STAT2 are the prime mediators of the antitumor activity of IFN and that induction of P-STAT1 correlates well with the induction of key ISGs); however, we believe that this technique is suitable for monitoring the effects of IFN-α on the immune system.

Our analyses showed that IFN-α–induced intracellular signaling and gene regulation within patient PBMCs was higher for the initial lower dose of IFN-α (5 megaunits/m²). However, a few patients deviated from this pattern with higher STAT activation and/or ISG transcription at 10 megaunits/m² IFN-α. Our previous work showed that maximal activation of STAT proteins may occur at different IFN-α doses in different patients (25). Furthermore, it has previously been shown by microarray analysis that IFN-responsive genes exhibit the greatest degree of

Table 2. IFN-α-2b dose response

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene</th>
<th>Function</th>
<th>Fold change (5 megaunits/m² vs pretreatment)</th>
<th>Fold change (10 megaunits/m² vs pretreatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP)</td>
<td>Cell adhesion, scavenger receptor</td>
<td>0.99</td>
<td>1.46</td>
</tr>
<tr>
<td>2</td>
<td>2',5'-Oligoadenylate synthetase 1 (OAS1)*</td>
<td>Antiviral activity, nucleic acid metabolism</td>
<td>4.16</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>2',5'-Oligoadenylate synthetase 2 (OAS2)*</td>
<td>Antiviral activity, nucleic acid metabolism</td>
<td>3.05</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>Chemokine (C-X-C motif) ligand 10 (CXCL10)</td>
<td>Chemotaxis, monocyte stimulation</td>
<td>22.13</td>
<td>32.82</td>
</tr>
<tr>
<td></td>
<td>Interleukin 1 (ICAM1) IFN-induced protein 44 (IFI44)</td>
<td>Cellular adhesion</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>IFN-induced protein with tetratricopeptide repeats 1 (IFIT1)</td>
<td>Invasive growth, antiviral response</td>
<td>12.91</td>
<td>14.80</td>
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<tr>
<td></td>
<td>IFN-induced protein with tetratricopeptide repeats 2 (IFIT2)*</td>
<td>Immune response</td>
<td>24.08</td>
<td>27.48</td>
</tr>
<tr>
<td></td>
<td>IFN-induced protein with tetratricopeptide repeats 3 (IFIT3)</td>
<td>Immune response</td>
<td>15.40</td>
<td>14.60</td>
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<td>IFN-induced protein with tetratricopeptide repeats 5 (IFIT5)*</td>
<td>Immune response</td>
<td>12.34</td>
<td>16.22</td>
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<tr>
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<td>Interleukin-1 β (IL1B)*</td>
<td>Immune response</td>
<td>5.96</td>
<td>7.06</td>
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<td>Myxovirus (influenza virus) resistance 1 (MX1)</td>
<td>Anti-viral activity</td>
<td>3.16</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>Promyelocytic leukemia (PML)</td>
<td>Transcription factor, cell growth</td>
<td>1.78</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>V-jun sarcoma virus 17 oncogene homologue (JUN)*</td>
<td>Regulation of transcription</td>
<td>0.10</td>
<td>0.18</td>
</tr>
</tbody>
</table>

NOTE: Fold changes for a subset of genes in patient PBMCs are similar after administration of IFN-α-2b at the 5 and 10 megaunits/m² doses. All genes were significantly different in expression following 10 megaunits/m² compared with pretreatment (Holm’s adjusted P < 0.10). Group 1: significantly higher gene expression at 10 megaunits/m² compared with 5 megaunits/m² (Holm’s adjusted P < 0.10). Group 2: test for a nonlinear dose response was significant (Holm’s adjusted P < 0.10; i.e., expression was similarly up-regulated or down-regulated by IFN-α at the 5 and 10 megaunits/m² doses).

*Significant gene up-regulation was observed in multiple probe sets. The displayed changes in expression are the average (geometric mean).
interindividual variation in gene expression within unstimulated PBMCs of healthy donors (44). The authors of these studies acknowledged that this might be attributable to subclinical or recent infection in some donors; however, the existence of similar variability for ISG transcripts was later documented in other studies (45). The existence of interpatient variation in the response to IFN-$\alpha$ suggests that a careful evaluation of the immune response to IFN-$\alpha$ is necessary to identify what might be termed an optimal dose.

IFN-$\alpha$-2b is the only Food and Drug Administration–approved adjuvant therapy for melanoma patients who have undergone successful surgery for high-risk lesions. There is no alternative therapy that has shown efficacy in randomized trials. Therefore, IFN-$\alpha$ will continue as the standard of care for the foreseeable future. It is well documented that a year of adjuvant high-dose IFN-$\alpha$ at 10 megaunits/m$^2$ leads to a significant increase in relapse-free survival for melanoma patients. Importantly, trials of very low-dose IFN-$\alpha$ (i.e., 1 megaunit/m$^2$) have not shown significant efficacy in the adjuvant setting (46). Thus, if it were not for the attendant side effects of high-dose IFN-$\alpha$, it is likely that this treatment regimen would be widely used in high-risk patients. The results of the present study suggest that administration of IFN-$\alpha$ at doses below 10 megaunits/m$^2$ may still produce significant immune stimulation. We have shown that increasing doses of IFN-$\alpha$ did not enhance signal transduction and gene stimulation in melanoma patient immune cells. These results suggest that peak activation of immune cells by IFN-$\alpha$ may occur at doses below 10 megaunits/m$^2$. Individualized optimization of IFN-$\alpha$ dosing in the setting of melanoma is currently under investigation.

Fig. 4. Real-time PCR validation of microarray data from PBMCs of patients receiving escalating doses of IFN-$\alpha$. The expression of representative genes was validated by real-time PCR analysis of IFI44, OAS1, MX1 (expression values at 5 and 10 megaunits/m$^2$ were more similar than expected), and LGALS3BP (expression was significantly higher at 10 megaunits/m$^2$ than at 5 megaunits/m$^2$). Data were expressed as the mean fold increase relative to baseline levels (pretreatment). All real-time PCR data were normalized to the level of $\beta$-actin mRNA (housekeeping gene). Columns, mean of triplicate wells; bars, SD.

References


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Enhanced by a Dose Increase from 5 to 10 Megaunits/m²

IFN-α-2b–Induced Signal Transduction and Gene Regulation in Patient Peripheral Blood Mononuclear Cells Is Not Enhanced by a Dose Increase from 5 to 10 Megaunits/m²


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