An Exploratory Study of Systemic Administration of the Toll-like Receptor-7 Agonist 852A in Patients with Refractory Metastatic Melanoma


Abstract

Purpose: A topical Toll-like receptor 7 (TLR7) agonist induces regression of cutaneous melanocytic neoplasms. We explored antitumor activity of a systemically administered TLR7 agonist, 852A, in patients with metastatic melanoma.

Experimental Design: We undertook a phase II, multicenter, open-label study in patients with chemotherapy-refractory metastatic melanoma. Patients received i.v. 852A, starting at 0.6 mg/m² and increasing to 0.9 mg/m² based on tolerance, thrice per week for 12 weeks. Clinical response was determined by Response Evaluation Criteria in Solid Tumors. Immune effects of 852A were monitored by measuring serum type I IFN and IP-10 together with assessment of immune cell markers in peripheral blood.

Results: Twenty-one patients were enrolled. Thirteen patients completed the initial 12-week treatment cycle, with two discontinuing for adverse events considered to be possibly related to study drug. Four (19%) patients had disease stabilization for >100 days. One patient had a partial remission after two treatment cycles, but progressed during the third. Dose-limiting toxicity was observed in two patients. Serum type I IFN and IP-10 increased in most patients on 852A administration. Serum type I IFN increases were greater after dosing with 852A 0.9 mg/m² than after 0.6 mg/m² (P = 0.009). The maximal increase in IP-10 compared with baseline correlated with the maximal increase in type I IFN (P = 0.003). In the eight patients with immune cell marker data, CD86 expression on monocytes increased significantly post-first dose (P = 0.007).

Conclusion: Intravenous 852A was well tolerated and induced systemic immune activation that eventually resulted in prolonged disease stabilization in some patients with stage IV metastatic melanoma who had failed chemotherapy.

Toll-like receptors (TLR) recognize conserved pathogen-associated molecular patterns (6). Manipulation of the innate immune response through TLR activation may act as a bridge toward enhancement of tumor-specific acquired immunity (7). The small-molecule TLR7 agonist imiquimod induces IFN-α production from plasmacytoid dendritic cells, as well as proinflammatory cytokines from other cells (8). Imiquimod has induced regression in melanoma lesions after topical application.

Melanoma is among the more immunogenic malignancies, and as such, multiple immunotherapeutic treatments have been investigated including IFN-α, interleukin-2, tumor necrosis factor α, adoptive cell transfer, and therapeutic vaccines (1, 2). Overall response rates of 8% to 20% have been reported with IFN-α or interleukin-2 monotherapy in advanced melanoma patients, but overall survival improvement has not been shown (3). In the adjuvant setting, IFN-α is the only drug significantly affecting relapse-free and overall survival (4). Despite preexisting circulating T cells recognizing tumor antigens in some patients and the ability to expand tumor-reactive CTLs with tumor antigen immunization, effective immunotherapy for melanoma remains elusive (5).

Toll-like receptors (TLR) recognize conserved pathogen-associated molecular patterns (6). Manipulation of the innate immune response through TLR activation may act as a bridge toward enhancement of tumor-specific acquired immunity (7). The small-molecule TLR7 agonist imiquimod induces IFN-α production from plasmacytoid dendritic cells, as well as proinflammatory cytokines from other cells (8). Imiquimod has induced regression in melanoma lesions after topical application.
administration (9–11). 852A {N-[4-(4-amino-2-ethyl-1H-imidazo[4,5-c]quinolin-1-yl) butyl]methanesulfonamide, 3M-001} is an analogue severalfold more potent and more selective for TLR7 versus TLR8 activation than imiquimod in a human HEK293 TLR-nuclear factor αB-luciferase cotransfection assay (12). 852A is also more potent in inducing IFN-α production by human peripheral blood mononuclear cells with a median minimum concentration to produce >3-fold increase in cytokines over control of 0.04 μmol/L for 852A versus 1.11 μmol/L for imiquimod (data on file, 3M Pharmaceuticals). 852A activates antigen-presenting cells, up-regulates costimulatory molecule expression, stimulates natural killer cell activity, induces production of IFN-α by plasmacytoid dendritic cells, and promotes plasmacytoid dendritic cell survival (12, 13). Supernatant from 852A-treated human peripheral blood mononuclear cells inhibited Hs294T and A375 melanoma cell proliferation in vitro; the effect was abrogated by plasmacytoid dendritic cell depletion and partially neutralized by preincubation of supernatant with anti–IFN receptor α/β or anti–IFN-α. Although less active in rodents than in humans, 852A delayed the onset of lung colonies in the B16-F10 murine melanoma model. In a phase I dose-finding study in 25 patients with refractory solid organ tumors, i.v. administration of 852A thrice per week was associated with cytokine induction at 0.6 mg/m² whereas 1.2 mg/m² was the maximum tolerated dose (12).

To further explore the antitumor activity, immunologic effects, and safety of 852A, we conducted an open-label study of i.v. administered 852A in patients with metastatic melanoma who relapsed after chemotherapy.

### Patients and Methods

**Patients.** Patients were recruited at six centers (University Hospital, Zürich, Switzerland; Hôpital Ste Marguerite, Marseille, France; Hospital Hotel Dieu, Lyon, France; University Clinic Schleswig-Holstein, Kiel, Germany; University Clinic and Health Center for Skin Diseases, Würzburg, Germany; and Skin Cancer Unit, Mannheim, Germany) in accordance with Good Clinical Practice guidelines and the ethical standards enunciated in the Declaration of Helsinki. The protocol and amendments were reviewed and approved by independent ethics committees and the medicines regulatory agency for each participating country. Each patient gave written informed consent. The study was registered at ClinicalTrials.gov (NCT0018933).

Male or female patients ≥18 years of age were eligible for enrollment if they had advanced melanoma not responding to first-line of chemotherapy; measurable disease by Response Evaluation Criteria in Solid Tumors with cutaneous or s.c. target lesions >10 mm in diameter; Eastern Cooperative Oncology Group performance status ≤2; a life expectancy ≥6 months; WBC count ≥3.0 × 10⁹ cells/L; absolute neutrophil count ≥1.5 × 10⁹ cells/L; platelet count ≥100 × 10⁹/L; hemoglobin >100 g/L; alanine aminotransferase and aspartate aminotransferase ≤2.5 × upper limits of normal; normal total bilirubin; and serum creatinine >1.8 mg/dL or calculated creatinine clearance ≥60 mL/min (14, 15). Patients were excluded if they had immunotherapy for melanoma 6 weeks before first 852A dose (to decrease possible confounding by carry-over immunologic effects); melanoma progression on IFN with/without monochemotherapy (to increase the likelihood of seeing a response in tumors sensitive to IFN-α because 852A mediates its effects mainly through induction of endogenous IFN-α); pregnancy, breast-feeding, significant malignancy, significant cardiac disease including prolonged QT interval, coagulation disorder, active infection or fever, and uncontrolled medical conditions. In addition, patients were excluded if they had therapy within the 4 weeks or required therapy with investigational agents, systemic or high-dose inhaled or topical corticosteroids, immunosuppressive therapy, drugs associated with QT prolongation and/or torsades de pointes, or radiotherapy. Adequate contraception was required if applicable.

**Treatment.** 852A (3M Pharmaceuticals) as a sterile 0.2% solution was administered by i.v. bolus at the clinic. Dosing was initiated at 0.6 mg/m² of 852A thrice per week. Patients tolerating >4 weeks of treatment could escalate to 0.9 mg/m² thrice per week; patients not tolerating treatment could de-escalate to 0.6 mg/m² twice per week. The treatment duration was 12 weeks or until disease progression (treatment cycle 1); patients with at least stable disease were allowed to receive additional 12-week treatment cycles.

**Clinical assessments.** Tumor burden was assessed at pre-study, at the end of each treatment cycle, and at the end of study by physical examination and imaging. Adverse event and concomitant medication information was collected at each visit. Physical examination, vital sign measurements, routine clinical laboratory tests, 12-lead electrocardiograms, and Eastern Cooperative Oncology Group status determination were obtained at pre-study, at least weekly during treatment cycle 1, every 2 weeks during additional treatment cycles, and at the end of study. Cardiovascular hormone was measured at pre-study, every 4 weeks during treatment, and at the end of study. Transthoracic echocardiograms, added after the start of the study, were done at pre-study, at the end of each treatment cycle, and at the end of study. Clinical response was determined by Response Evaluation Criteria in Solid Tumors based on tumor evaluation done at baseline and on completion of the treatment at week 13 or earlier using physical examination of palpable lesions and imaging [computed tomography (CT), positron emission tomography (PET), and/or magnetic resonance imaging scans]. Study conduct and source data were monitored by sponsor personnel for accuracy. Clinical laboratories were accredited. A single cardiologist interpreted all electrocardiograms. Data were double data entered with verification. All queries were resolved and subject evaluability was determined before database lock.

**Immune monitoring.** Immunologic monitoring included serum cytokine measurement from blood obtained at pre-dose and 6 and 24 h post-dose for dose 1 of weeks 1, 6, and 12. Type 1 IFN was measured by a bioassay with a lower limit of quantitation of 1 IU/mL (16). IP-10 was measured by an enzyme-linked immunosorbent assay (R&D Systems) with a lower limit of quantitation of 40 pg/mL. For patients enrolled at the Zürich study center, blood was taken at pre-dose and 24 h post-dose for dose 1 of weeks 1, 6, and 12 to assess activation markers on monocytes (CD80 and CD86) and plasmacytoid dendritic cells (CD80, CD86, and CCR7), as well as to measure peripheral plasmacytoid dendritic cell and myeloid dendritic cell frequency by fluorescence-activated cell sorting. In addition, pre-dose plasmacytoid dendritic cell and myeloid dendritic cell frequency was measured for dose 1 of weeks 2 and 4. Within 60 min after blood draw, antibodies were added to a test tube as recommended by the manufacturer (antibodies and control isotypes listed in Supplementary Table S1) followed by addition of 100 μL of whole blood. After incubation with OptiLyz e No-Wash lysing solution (Beckman Coulter Int.), erythrocytes were lysed and stained cell populations fixed. For activation marker expression, plasmacytoid dendritic cells and monocytes were gated from whole blood peripheral blood mononuclear cells using forward scatter/side scatter followed by gating on CD123^hi/CD34^- or CD123^hi/HLADR^ (for plasmacytoid dendritic cells) and by gating on CD14^-CD19^ (for monocytes). Mean fluorescence intensity was measured by flow cytometry.
was determined for each parameter for the selected population. The frequencies of CD123high/HLA-DR+/lin 1- (for plasmacytoid dendritic cells) and CD11c+/HLA-DR+/lin 1- (for myeloid dendritic cells) in peripheral blood mononuclear cells were used to determine the frequencies of the respective dendritic cell populations in peripheral blood.

Statistical powering. This was a two-stage open-label study using a Simon's minimax design (17). The null hypothesis (H0) assumed a true response rate $V_{10\%}$ versus $V_{30\%}$ for the alternative hypothesis. In stage I, for 15 to 18 evaluable patients, >2 patients with a partial response or complete response were required to proceed to stage II. With a total enrollment of 19 to 28 evaluable patients, there was 80% ($\beta = 0.20$) power to detect a response rate $\geq 30\%$ ($x = 0.05$). With this design, the average expected H0 sample size was 19.6 with an average probability of early study stoppage of 0.38.

Data analysis. The primary end point was overall patient tumor status by Response Evaluation Criteria in Solid Tumors. The sum of the longest diameter for all target lesions for a patient was calculated and used as the reference. All patients who received >1 dose were included in the safety assessment; adverse events were coded using the Medical Dictionary for Regulatory Activities. Descriptive clinical analyses were done using SAS version 9 (SAS Institute, Inc.). Group changes in serum type I IFN, IP-10, and cell activation markers were summarized over time. Natural log-transformed type I IFN and IP-10 values were used to calculate the maximal observed positive change. Statistical analyses were done using SPSS version 12 (SPSS) and Excel 2003 (Microsoft Corp.).

Results

Patients

The first subject was enrolled on 19 May 2005 and the last subject completed the study on 12 October 2006. The analysis

### Table 1. Baseline patient characteristics and response during study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y); sex; race</th>
<th>Primary tumor type; AJCC stage (ECOG score)</th>
<th>Target tumors (n); LD sum (cm)</th>
<th>Nontarget involvement</th>
<th>Prior therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-61</td>
<td>62; female; White</td>
<td>Unknown; IV M1a (1)</td>
<td>1; 2.6</td>
<td>Not applicable</td>
<td>Dacarbazine, peg-IFNα, thalidomide</td>
</tr>
<tr>
<td>11-62</td>
<td>43; female; White</td>
<td>Nodular; IV M1a (2)</td>
<td>4; 25</td>
<td>Not applicable</td>
<td>Dacarbazine, carboplatin, vinblastin, radiation Temozolomide</td>
</tr>
<tr>
<td>11-63</td>
<td>64; male; White</td>
<td>Unknown; IV M1c (1)</td>
<td>4; 20.7</td>
<td>Lymph node, bone, pleura, mesentery</td>
<td></td>
</tr>
<tr>
<td>11-64</td>
<td>31; female; White</td>
<td>Nodular; IV M1b (0)</td>
<td>1; 3.5</td>
<td>Lung, skin</td>
<td>Adeno-IL-2, dacarbazine, thalidomide</td>
</tr>
<tr>
<td>11-65</td>
<td>52; female; White</td>
<td>Unknown; IV M1c (0)</td>
<td>2; 9.9</td>
<td>Kidney, bladder</td>
<td>Adeno-IL-2, thalidomide, dacarbazine</td>
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<tr>
<td>11-66</td>
<td>73; male; White</td>
<td>Nodular; IV M1b (0)</td>
<td>1; 3.8</td>
<td>Lung</td>
<td>Dacarbazine, IFNα</td>
</tr>
<tr>
<td>11-67</td>
<td>62; male; White</td>
<td>Acral lentiginous; IV M1 (0)</td>
<td>7; 32</td>
<td>Lymph node, adrenal, kidney, spleen, pancreas</td>
<td></td>
</tr>
<tr>
<td>11-68</td>
<td>44; male; White</td>
<td>Superficial spreading; IV M1b (0)</td>
<td>1; 2.1</td>
<td>Lymph node, lung, kidney</td>
<td></td>
</tr>
<tr>
<td>21-01</td>
<td>77; female; White</td>
<td>Nodular; IV M1c (1)</td>
<td>2; 6.4</td>
<td>Lung</td>
<td>Peg-IFNα, dacarbazine, radiation</td>
</tr>
<tr>
<td>21-03</td>
<td>54; male; White</td>
<td>Unclassified; IV M1c (1)</td>
<td>4; 20.7</td>
<td>Lymph node, lung, liver, skin, pancreas, spleen</td>
<td></td>
</tr>
<tr>
<td>31-37</td>
<td>56; female; White</td>
<td>Unknown; IV M1a (0)</td>
<td>5; 8.5</td>
<td>Lymph node</td>
<td>IFNα, dacarbazine</td>
</tr>
<tr>
<td>31-38</td>
<td>63; male; White</td>
<td>Acral lentiginous; IV M1 (0)</td>
<td>4; 12.8</td>
<td>Lymph node, lung</td>
<td>Dacarbazine, fotemustine</td>
</tr>
<tr>
<td>31-39</td>
<td>69; male; White</td>
<td>Nodular; IV M1c (1)</td>
<td>3; 6.3</td>
<td>Lung</td>
<td>IFNα, dacarbazine</td>
</tr>
<tr>
<td>31-40</td>
<td>77; male; White</td>
<td>Superficial spreading; IV M1b (1)</td>
<td>2; 6.3</td>
<td>Lymph node, lung, skin</td>
<td></td>
</tr>
<tr>
<td>32-43</td>
<td>29; male; White</td>
<td>Unclassified; IV M1c (0)</td>
<td>1; 5.2</td>
<td>Bone, skin</td>
<td>Dacarbazine</td>
</tr>
<tr>
<td>32-44</td>
<td>76; male; White</td>
<td>Unknown; IV M1b (0)</td>
<td>5; 20.7</td>
<td>Lymph node, lung, liver</td>
<td></td>
</tr>
<tr>
<td>32-45</td>
<td>64; male; White</td>
<td>Unclassified; IV M1c (0)</td>
<td>1; 3.2</td>
<td>Lymph node, lung, liver, skin</td>
<td></td>
</tr>
<tr>
<td>32-46</td>
<td>60; male; White</td>
<td>Unclassified; IV M1c (0)</td>
<td>1; 2.6</td>
<td>Lung, liver</td>
<td>Fotemustine, imatinib</td>
</tr>
<tr>
<td>32-47</td>
<td>68; male; White</td>
<td>Lentigo maligna; IV M1a (0)</td>
<td>1; 2.1</td>
<td>Lymph node, lung</td>
<td>Dacarbazine</td>
</tr>
<tr>
<td>33-49</td>
<td>48; female; White</td>
<td>Superficial spreading; IV M1a (1)</td>
<td>2; 7.8</td>
<td>Lymph node, lung, liver, skin</td>
<td></td>
</tr>
<tr>
<td>33-50</td>
<td>72; male; White</td>
<td>Unknown; IV M1b (1)</td>
<td>1; 2.5</td>
<td>Lymph node, thoracic</td>
<td>Treosulfan, gemcitabine, dacarbazine</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC, American Joint Commission on Cancer; ECOG, Eastern cooperative oncology group; LD, longest diameter; DC, discontinued; C, cycle; W, week; EOS, end of study; SD, stable disease; PR, partial response; PD, progressive disease; IL-2, interleukin 2; AE, adverse event.
database was finalized on 05 January 2007. Of 31 patients screened, 21 were enrolled into stage I; there were no enrollments for stage II (for explanation see below). The patients were all White, mostly male (62%), and had a mean age of 59 ± 14 years (Table 1). Nineteen percent of patients were American Joint Committee on Cancer stage IV M1a, 24% IV M1b, and 57% IV M1c. Most patients had 1 (43%), 2 (19%), or 4 (19%) target lesions (range, 1-7). Mean sum longest diameter was 9.7 ± 8.7 cm (range, 2.1-31). Lung (67%) and lymph nodes (57%) were the most common nontarget sites. Thirteen patients completed treatment cycle 1; five withdrew for adverse events and three for lack of therapeutic effect. Three patients had one additional treatment cycle and a fourth had two additional cycles.

**Tumor response**

During the study, 71% (15 of 21) of patients progressed, 19% (4 of 21) had disease stabilization, and 10% (2 of 21) were not evaluable (Table 1). Patient 61, who had two additional treatment cycles, had a partial response after the second cycle (Fig. 1), but progressed after the third cycle. Four patients had stable disease for >100 days (275, 197, >105, and >185 days, where “>” indicates stable disease at last study visit).

**Safety**

Mean cumulative treatment cycle 1 exposure was 19.28 ± 8.53 mg/m² (1.80-28.28). During the study no patient died. Nine (43%) patients had 17 serious adverse events, of which 11 (2 adverse events of nausea and 1 each of vomiting, fatigue, chills, pyrexia, dizziness, muscle weakness, muscle tightness, pain in extremity, and anemia) were considered possibly/probably related to study drug. Five (24%) patients discontinued for adverse events, of which two had adverse events (grade 3 fatigue and grade 2 dizziness and nausea) considered possibly related to study drug. Two (10%) patients experienced dose-limiting toxicities such as grade 3 thrombocytopenia and grade 3 cholestasis. Eight (38%) patients had at least one adverse event of maximal intensity of severe, whereas 12 (57%) had adverse events of maximal intensity of
moderate. The adverse events reported by the most patients by preferred terms were pyrexia (67%), fatigue (62%), nausea (52%), chills (48%), myalgia (29%), pain in extremity (29%), and headache (29%), most of which are reminiscent of “flu-like” syndrome described after treatment with other TLR7 agonists and IFN-α (refs. 12, 18–20; Table 2). No inflammatory reaction of tumor lesions in the skin possibly or probably related to study drug was observed. Severe adverse events reported by >10% of patients were anemia, nausea, and pain. For laboratory tests, there was tendency toward a decrease in hemoglobin, hematocrit, RBC count, and serum phosphorus (data not shown); three patients had adverse events of anemia.

Table 2. Adverse events reported by ≥10% of patients (preferred term)

<table>
<thead>
<tr>
<th>System organ class</th>
<th>Preferred term</th>
<th>n (%)</th>
<th>total patients = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>Anemia</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>Abdominal pain</td>
<td>4 (19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abdominal pain upper</td>
<td>5 (24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constipation</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td>11 (52)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vomiting</td>
<td>5 (24)</td>
<td></td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>Chills</td>
<td>10 (48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>13 (62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malaise</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pain</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyrexia</td>
<td>14 (67)</td>
<td></td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>Nasopharyngitis</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>Anorexia</td>
<td>5 (24)</td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>Arthralgia</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myalgia</td>
<td>6 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pain in extremity</td>
<td>6 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shoulder pain</td>
<td>4 (19)</td>
<td></td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>Dizziness</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>Insomnia</td>
<td>5 (24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sleep disorder</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>Respiratory, thoracic, and mediastinal</td>
<td>Cough</td>
<td>4 (19)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Clinical response to B52A in patient 61 visualized by PET-CT. Baseline PET-CT evaluation of the infracarinal lymph node metastasis (A; fused axial PET-CT image showing pathologically increased [18F]fluorodeoxyglucose (FDG) uptake of the metastasis in bottom left corner). PET-CT evaluation after two treatment cycles shows decrease both in size and in fluorodeoxyglucose activity of the infracarinal lymph node metastasis (B; fused axial PET-CT image in bottom left corner).
(Table 2). No clinically meaningful qualitative changes in electrocardiogram measurements were noted and none of the quantitative changes were considered related to 852A. The distribution of absolute Bazett QTc (in milliseconds) was comparable when patients were dosed at 0.6 or 0.9 mg/m². The majority in both groups had a Bazett QTc between 391 and 420 ms; one patient had an increase of >60 ms on one occasion. One patient had an echocardiogram consistent with a cardiomyopathy; no baseline study evaluation was available but similar abnormalities were noted on echocardiography before the study.

Immunologic markers

Serum cytokines. Thirteen patients had cytokine data through week 12 and five patients through week 6 of treatment cycle 1. Serum type I IFN increased post-dose for week 1 (P = 0.025; Fig. 2A) and for IP-10 for weeks 1 and 6 (P = 0.035 and 0.001, respectively; Fig. 2B). Increases were greater at 6 h than at 24 h for both parameters. In the 13 patients who dosed at 0.9 mg/m² thrice per week and had cytokine measurements, the maximal increase for type I IFN post-dose was greater after the 0.9 mg/m² dose than the 0.6 mg/m² dose (mean, 3.24 ± 2.32 versus 1.04 ± 1.06; P = 0.009, paired t test). IP-10 levels similarly increased but not significantly (mean, 1.67 ± 1.27 versus 0.99 ± 0.91; P = 0.110). For the four patients who remained at 0.6 mg/m² and had cytokine data after the initial dose, the mean maximal increases for type I IFN and IP-10 were 1.45 ± 1.66 and 0.92 ± 0.65 after the first dose versus 1.86 ± 2.17 and 0.46 ± 0.33, respectively, after a subsequent 0.6 mg/m² dose. The maximal increase in IP-10 compared with baseline seemed to correlate with the maximal increase in type 1 IFN (Pearson’s correlation coefficient r = 0.705, P = 0.003). No differences were observed in the maximal cytokine increase between patients who had progressive disease throughout the study (n = 15) versus those who were stable disease or partial response for at least one treatment cycle (n = 4; data not shown).

Cellular markers. Eight patients had adequate data on markers of cell activation. There was an increase in monocyte CD86 expression 24 h post-dose at week 1 (P = 0.007, paired t test) that persisted to a lesser extent at weeks 6 and 12 (P = 0.108 and P = 0.064, respectively; Fig. 3). In weeks 1 and 6, serum IFN-α increases correlated with CD86 expression on monocytes measured at 24 h post-dose (Pearson’s correlation coefficient r = 0.734, P = 0.038 and r = 0.840, P = 0.036, respectively). No consistent pattern was observed for monocyte CD80 expression or CD80, CD86, and CCR7 expression on plasmacytoid dendritic cells. During treatment cycle 1, most patients had decreases in pre-dose peripheral plasmacytoid dendritic cell (six of seven) and myeloid dendritic cell frequency (five of seven; Fig. 4A and B, respectively). Irrespective of the changes in plasmacytoid dendritic cell frequency, over the treatment duration, individual patients
tended toward increased pre-dose expression of CD80, CD86, and CCR7 on plasmacytoid dendritic cells (data not shown). No such changes were evident for monocytes.

Discussion

In this study of systematic 852A administration in patients with advanced melanoma refractory to first-line chemotherapy, although no patient achieved a complete response, one experienced a nonsustained partial response and four had overall stable disease lasting ≥100 days. The limited efficacy observed with 852A monotherapy was not necessarily surprising, given the advanced disease of the enrolled population.

The observed adverse events attributable to study drug were consistent with those previously observed with systemic administration of 852A, imiquimod, resiquimod, as well as with exogenous recombinant IFN-α, and seemed to reflect cytokine-mediated effects rather than direct toxicity (12, 18–20). The dose levels selected for evaluation in this study were lower than the 1.2 mg/m² dose considered to be the maximum tolerated dose in a phase I study (12). Although ∼1/3 of patients had adverse events of severe intensity, and dosemultiplying toxicities occurred at 0.6 and 0.9 mg/m², the overall safety profile was deemed tolerable, given the underlying disease. Although limited by the patient number and historical comparison, the gastrointestinal adverse events observed in this study seemed to be less frequent and/or less severe than observed in clinical studies of oral administration of imiquimod or resiquimod (19, 20). The intestinal tract contains a large number of immune cells, including dendritic cells, and TLR7 expression has been reported on hepatocytes (21, 22). Oral administration may result in high localized cytokine induction, affecting gastrointestinal tolerance, but may also spill over into the systemic circulation despite low serum drug levels.

Prolongation of action potential duration in hERG potassium ion channels has been observed in vitro at 3,000 ng/mL of 852A, along with QTc increases in dogs at 2.5 mg/kg (data on file, 3M Pharmaceuticals). However, no trends in electrocardiogram QTc measurements of concern were observed in this study. In addition, mean serum 852A concentrations at 5 min post-infusion ranged from 10 to 100 ng/mL after doses of 0.15 to 2.0 mg/m² in the phase I study, well below the concentration where in vitro effects were observed (12).

By activating TLR7 on plasmacytoid dendritic cells, 852A was shown to induce IFN-α production in vitro as well as in vivo (12). Similar to other TLR7 agonists, 852A also enhances production of IP-10 (CXCL10), which is in turn inducible by IFN-α and dependent on IFN-α receptor (12, 23, 24). In addition, IP-10 has been shown to promote the induction of T helper 1 cytokines (e.g., IFN-γ; refs. 25, 26), which are necessary for an efficient antitumor response (27). IP-10 represents therefore a downstream marker of presence of IFN-α as well as an indicator of T helper 1 responses. 852A pharmacologic activity was thus evidenced by increases in serum type I IFN and IP-10 in the majority of patients. Inducibility of IP-10 by IFN-α was supported by the correlation between the two. Preclinical studies suggest that 852A can also activate monocytes, likely through IFN-α because monocytes do not express TLR7 (28). Expression of monocyte activation markers in most of the patients (that had cell marker analyses done) was increased on treatment with 852A and correlated with type I IFN activity, confirming this observation. Disappointingly, changes in expression of activation markers as well as frequency of other immune cell populations in peripheral blood that were analyzed in this study (plasmacytoid dendritic cells, myeloid dendritic cells, and monocytes; data for B cells not shown) failed to reveal any pattern of association with the study drug.

There was tendency for the maximal increase in serum type I IFN to be greater after the 0.9 mg/m² dose as compared with after the initial or a subsequent 0.6 mg/m². This may due to increased 852A dose as well as to repeated dosing; augmentation of effect has been observed after multiple topical dosing as
compared with single dosing with resiquimod, a related mixed TLR7/TLR8 analogue (29). One possibility for the lack of observed efficacy, despite evidence of pharmacologic activity, is that the activation signal induced by systemic 852A was too generalized and decoupled from tumor antigen, or that this signal just insufficient. It is conceivable that the increase in predefined expression of activation markers on plasmacytoid dendritic cells over the treatment, which was observed in some patients, may be the consequence of this generalized over-activation.

Combining 852A with the depletion of regulatory T cells may be an approach to enhance efficacy, as has been tried with other immunotherapeutic modalities (30, 31). Nevertheless, the major concern for lymphoablative chemotherapy, denileukin-diftitox, or anti-CD25 antibody is still their specificity in eliminating regulatory T cells (e.g., CD25 is also expressed on activated CD4+ and CD8+ T cells, driving their expansion and survival), which requires additional investigations to clarify their effects on antitumor immunity (32). An alternative strategy would be to block the molecules that mediate immune suppression, such as CTLA-4, which has shown promising objective response in melanoma when coadministered with peptide vaccine (33). TLR activation seems to induce proliferation of regulatory T cells thereby enhancing their immunosuppressive activity. In addition to other TLRs, regulatory T cells express TLR8 and adoptive transfer of TLR8 agonist--treated regulatory T cells was surprisingly shown to enhance antitumor activity in animals (34–36). Unfortunately, because TLR8 is nonfunctional in rodent species except under special conditions, exploring the individual contributions of TLR7 versus TLR8 in animal tumor models may be difficult because TLR7, TLR7/TLR8, and TLR8 agonists behave similarly (37).

Alternative dosing schedules might prove to be more effective, if the dosing interval is too short to allow adequate plasmacytoid dendritic cell recovery; TLR-induced plasmacytoid dendritic cell maturation to a non–IFN-producing phenotype or counterregulatory mechanisms may result in functional plasmacytoid dendritic cell “exhaustion” (38–40). Combining 852A with other therapeutic modalities may also enhance efficacy. Imiquimod combined with cyclophosphamide was more effective than either alone in the murine MC-26 model (41). Interleukin-2 combined with topical imiquimod cleared cutaneous melanoma metastases refractory to imiquimod alone (42). CpG, a TLR9 agonist that also induces plasmacytoid dendritic cell IFN-α production, is being investigated in combination with chemotherapy for metastatic melanoma. Combining topical imiquimod with photodynamic therapy induced regression not only of treated cutaneous melanoma metastasis but also of a distant metastasis (43). It can be postulated that the physical destruction of the cells makes more tumor antigens available, allowing imiquimod to more effectively enhance tumor-specific cell-mediated immunity. Thus, administration of 852A after radiation therapy of deep solid tumors and its use as an adjuvant during immunization with selected tumor antigens represent the alternatives to be explored. Combining 852A with other therapies, of course, may also result in enhance toxicity, especially with agents or modalities that are causing flu-like syndrome, have hematologic toxicities, or cause inflammation.

In conclusion, whereas objective clinical responses were not observed, disease stabilization, adequate tolerance, and systemic immune activation were observed with systemic administration of 852A in patients with advanced metastatic melanoma. Future studies of 852A in melanoma will provide more information on approaches (combination or adjunctive) warranting highest efficacy.

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References


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