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Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors

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CD40 is a cell-surface molecule that critically regulates immune responses. CP-870,893 is a fully human, CD40-specific agonist monoclonal antibody (mAb) exerting clinical antineoplastic activity. Here, the safety of CP-870,893 combined with carboplatin and paclitaxel was assessed in a Phase I study. Patients with advanced solid tumors received standard doses of paclitaxel and carboplatin on day 1 followed by either 0.1 mg/Kg or 0.2 mg/Kg CP-870,893 on day 3 (Schedule A) or day 8 (Schedule B), repeated every 21 d. The primary objective was to determine safety and maximum-tolerated dose (MTD) of CP-870,893. Secondary objectives included the evaluation of antitumor responses, pharmacokinetics and immune modulation. Thirty-two patients were treated with CP-870,893, 16 patients on each schedule. Two dose-limiting toxicities were observed (grade 3 cytokine release and transient ischemic attack), each at the 0.2 mg/Kg dose level, which was estimated to be the MTD. The most common treatment-related adverse event was fatigue (81%). Of 30 evaluable patients, 6 (20%) exhibited partial responses constituting best responses as defined by RECIST. Following CP-870,893 infusion, the peripheral blood manifested an acute depletion of B cells associated with upregulation of immune co-stimulatory molecules. T-cell numbers did not change significantly from baseline, but transient tumor-specific T-cell responses were observed in a small number of evaluable patients. The CD40 agonist mAb CP-870,893, given on either of two schedules in combination with paclitaxel and carboplatin, was safe for patients affected with advanced solid tumors. Biological and clinical responses were observed, providing a rationale for Phase II studies.

Introduction

CD40-targeting agonist monoclonal antibodies (mAbs) represent an immunomodulatory approach designed to enhance the ability of the immune system to recognize and destroy cancer cells.[1] CD40 is a cell-surface member of the tumor necrosis factor superfamily expressed on antigen presenting cells (APCs) such as dendritic cells, B cells and macrophages.[2] CD40 signaling reportedly activates APCs and trigger tumor-specific T-cell immune responses,[1,3] although CD40-activated macrophages also exert direct tumoricidal functions.[4]

CP-870,893 is a fully human IgG_2 mAb that operates as a potent and selective agonist of CD40.[1,5,6] In preclinical studies, CP-870,893 has been shown to mediate both immune system-dependent and -independent effects on tumor cell survival.[5] In the first-in-human study, promising antitumor activity was observed, especially in patients with melanoma.[6] Pharmacodynamically, the administration of CP-870,893 leads to a transient decrease in peripheral blood B cells and to the upregulation of activation markers on APCs.[6] Because preclinical data suggest a synergy between chemotherapy (which favors the cell death-associated release of tumor-associated antigens) and CD40 agonists (which promotes the activation of APCs),[7] the combination of CP-870,893 and gemcitabine was evaluated in patients with advanced pancreatic carcinoma. In this setting, consistent tumor regressions were observed in about 20% of patients.[4]

Here, we report the results of a Phase I study of CP-870,893 combined with paclitaxel and carboplatin in patients affected by advanced solid tumors. This study was designed to determine the maximum-tolerated dose (MTD) of CP-870,893 given on two different schedules and to assess its clinical activity and potential for immunomodulation in patients. Because it remains unknown how soon after chemotherapy tumor-associated antigens might be released as well as how long their immunogenic potential might persist, the administration of CP-870,893 schedules on either day 3 or day 8 post-chemotherapy was explored.

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Table 1. Patients characteristics*

		Schedule A (n = 16)	Schedule B (n = 16)
Sex	Male	13	10
	Female	3	6
Age	Mean	58.4	53.1
	Range	(25–75)	(32–76)
Race	Caucasian	15	16
	Hispanic	1	0
ECOG PS	0	10	8
	1	6	8
Tumor type	Cholangiocarcioma	0	1
	Colon carcinoma	0	1
	Leimyosarcoma	0	1
	Lung carcinoma	0	1
	Melanoma	15	10
	Ovarian carcinoma	0	1
	Prostate carcinoma	0	1
	Renal carcinoma	1	0
Prior therapy	Systemic therapy	13	13
	Radiation	6	7

*n = 32.

Results

Patient characteristics, toxicity and determination of MTD. Thirty-two patients with advanced solid tumors, 16 on each schedule (i.e., CP-870,893 on day 3 or day 8 post-chemotherapy), were enrolled and treated (**Table 1**). A wide variety of tumor histologies was represented, yet 78% of patients was affected by metastatic melanoma. The majority of patients (81%) were treated with 0.2 mg/Kg CP-870,893. Treatment-related adverse events and laboratory abnormalities are summarized in **Table 2**. There were no dose-limiting toxicities (DLTs) among 3 patients receiving, on either schedule, 0.1 mg/Kg CP-870,893. The first 6 patients treated with 0.2 mg/Kg on either schedule exhibited no DLTs. However, during the expansion phase, there was one DLT for each schedule. On schedule A, a 75 year-old man affected by melanoma developed a grade 3 cytokine release syndrome characterized by chills and rigors on day 3 of cycle 1. On schedule B, a 61 year-old woman affected by leiomyosarcoma developed a transient ischemic attack on day 11 of cycle 1, followed—two days later—by a fatal cerebrovascular accident (CVA). In addition, on schedule A, a 55 year-old man affected by melanoma developed a grade 2 thrombosis and fatal intracranial hemorrhage in the setting of new brain metastases, and, on schedule B, a 64 year-old-woman bearing ovarian cancer developed an optic nerve infarction (grade 2), neither event being considered a DLT. The MTD of CP-870,893 in combination with carboplatin and paclitaxel for both schedules was thus estimated at 0.2 mg/Kg.

At the MTD, the most common treatment-related adverse events were fatigue (81% of patients, but only one patient with grade 3 fatigue), peripheral neuropathy (46%), cytokine release syndrome (42%, one patient with grade 3 toxicity), alopecia (42%), constipation (38%), nausea (38%) and neutropenia (38%, two cases of which were complicated by fever). The rates of these common adverse events were similar across the two schedules, except for grade 3 or 4 neutropenia which was observed at the MTD in only one patient on schedule A (8%), as compared with eight patients on schedule B (62%). The most likely explanation for this observation is that blood draws were performed on days 8, 9 and 10 on schedule B (days corresponding to the expected chemotherapy-induced white blood cell, WBC, nadir) whereas patients on schedule A who had received CP-870,893 on day 3 and not day 8 were were not subjected to follow-up blood draws at the time of chemotherapy-induced nadir.

At the MTD, the second or third infusions of CP-870,893 were delayed in one patient on schedule A (8%) and 5 patients on schedule B (38%), because of neutropenia, thrombocytopenia, or both. The delays on schedule B were more common probably because, as noted above, CP-870,893 was given on day 8 of each cycle, which corresponded to the expected nadir for WBCs and platelets following the administration of chemotherapy.

There were no cases of autoimmune-related colitis, thyroiditis, dermatitis, uveitis, or hypophysitis among the patients enrolled in this study. Two patients, both affected by melanoma, developed new widespread vitiligo during the study. However, neither of them exhibited an objective tumor response.

Tumor response. Six of 30 evaluable patients (20%) had a partial response (PR), as defined by the Response Evaluation Criteria In Solid Tumors (RECIST) (**Fig. 1**). Two of these PRs were on schedule A and four were on schedule B. All PRs were observed with the 0.2 mg/Kg dose. Twelve evaluable patients (40%) had stable disease (SD) and 12 (40%) had progressive disease (PD). There were no complete responses (CRs). Two patients could not be assessed for response: as noted above, one suffered a fatal CVA early during cycle 1, and a second one was taken off study based on brain imaging during cycle 1, revealing lesions that were subsequently determined to represent a benign process that had begun before enrollment. The mean time from the first radiological documentation of a PR to subsequent tumor progression was 75 d, with a range of 50–96 d.

Of the 6 patients who achieved a PR, 3 were affected by metastatic melanoma. Two of these patients had not been previously treated for metastatic melanoma, and the third patient manifested tumor progression in spite of prior immunochemotherapy with cisplatin, dacarbazine, vinblastine, interleukin (IL)-2 and interferonα (IFNα). The fourth patient manifesting a PR was affected by a metastatic, hormone-independent prostate cancer that had progressed despite prior treatment with docetaxel, bevacizumab, prednisone and thalidomide. The fifth of these patients bore a metastatic renal cell carcinoma that had progressed in spite of pelvic radiation therapy combined with 5-fluorouracil and mitomycin. The sixth patient exhibiting a PR was affected a metastatic ovarian cancer that had progressed four years after a previous CR to carboplatin and paclitaxel.

Pharmacokinetics and anti-human antibodies. The pharmacokinetics of CP-870,893 was similar to that demonstrated in previous Phase I studies.[6,8] CP-870,893 was rapidly cleared from the circulation regardless of dosing schedule. At 24 h post-infusion, none of the 6 patients receiving 0.1 mg/Kg and only 8

of 26 patients receiving 0. 2mg/Kg CP-870,893 had detectable levels of CP-870,893 in their circulation. For patients receiving 0.1 mg/Kg CP = 870,893 (n = 5), the mean area under the curve (AUC ± SD) was 2.56 ± 1.39 h-μg/mL, with a coefficient of variation (CV) of 54% and a C_{max} of 0.83 ± 0.36 μg/mL (CV = 44%). For patients receiving 0.2 mg/Kg CP-870,893 (n = 26), the mean AUC ± SD was 8.95 ± 8.48 h-μg/mL (CV = 95%) and the C_{max} was 1.45 ± 0.73) μg/mL (CV = 50%). The levels of human anti-human antibodies for were below the limit of detection in all patients treated with CP-870,893.

Modulation of clinical laboratory parameters. Changes in 11 distinct clinical laboratory variables were assessed (and compared with baseline) at three time points following the infusion of 0.2 mg/Kg CP-870,893 (**Fig. 2**). In this analysis, baseline values were those measured immediately before the first cycle of CP-870,893 infusion. Details on this exploratory statistical analysis are provided in **Table S1**. We observed statistically significant decreases in platelets and leukocytes, notably neutrophils. Decreases in the absolute lymphocyte and monocytes counts at some time points were also observed. Consistent with previous studies,[6] we observed statistically significant elevations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) on both schedules, but there were no hepatic clinical complications. We also observed transient dose-related increases in the levels of D-dimers (a degradation product of cross-linked fibrin), most pronounced on schedule B. D-dimer levels returned to baseline by one week post CP-870,893 infusion and no disseminated intravascular coagulation was observed in any patient. In comparing the changes from baseline in these parameters across schedules (at these times points), we found no statistically significant differences.

Modulation in cytokines. CP-870,893 treatment was associated with a moderate systemic increase in both IL-6 and tumor necrosis factor α (TNFα). For patients belonging to the MTD expansion cohort (n = 7 per schedule), the maximal mean amount of IL-6 measured was 371 pg/mL and 679 pg/mL, on schedule A and B, respectively. The maximal mean amount of TNFα measured was 113 pg/mL and 675 pg/mL on schedule A and B, respectively. Maximum cytokine levels were observed 2 h post infusion.

Immune pharmacodynamics. To assess the pharmacodynamics of CP-870,893 given at the MTD of 0.2 mg/Kg, flow cytometric analysis of peripheral blood B cells was performed before and at three time points after the first infusion cycle. Consistent with previous studies,[6] the infusion of CP-870,893 caused a rapid decline in both the absolute count and percentage of circulating $CD19^+$ B cells (**Fig. 3A**). This effect was consistent across schedules and dose levels. Nadirs for both absolute numbers and percentages were lower than 20% of baseline and remained lower than 40% of baseline for at least 48 h post- infusion. Changes in peripheral B cells were statistically significant at all time points after infusion (**Table S2**). CP-870,893 infusion also resulted in a rapid increase in the expression of the co-stimulatory molecule CD86 on B cells, to levels 3–5 times higher than baseline. This was a statistically significant change for all three time points in schedule B, but only at 48 h in schedule A. A trend toward an increased expression of the MHC molecule HLA-DR after infusion was observed, but this did not achieve statistical significance

Table 2. Adverse events or laboratory abnormalities*

Adverse event	Any grade (n)	Grade 3 (n)	Grade 4 (n)	Grade 5 (n)
Schedule A				
CP- 870,893 0.1 mg/Kg (n = 3)				
Alopecia	2	0	0	0
Anemia	2	0	0	0
Arthralgia	2	0	0	0
Back pain	2	0	0	0
Chest pain	2	0	0	0
Constipation	2	0	0	0
Cytokine release syndrome	2	0	0	0
Fatigue	3	0	0	0
Leukopenia	1	1	0	0
Lymphopenia	2	2	0	0
Nausea	2	0	0	0
Neutropenia	2	1	1	0
Thrombocytopenia	1	1	0	0
CP- 870,893 0.2 mg/Kg (n = 13)				
ALT increase	1	1	0	0
Alopecia	8	0	0	0
Arrhythmia	2	2	0	0
Anemia	2	0	0	0
Arthralgia	4	0	0	0
Constipation	3	0	0	0
Cytokine release syndrome	5	1#	0	0
Fatigue	11	0	0	0
Febrile neutropenia	1	0	1	0
Gastresophageal reflux	3	0	0	0
Hematuria	2	1	0	0
Hemorrhage, intracranial	1	0	0	1
Hypogeusia	2	0	0	0
Mental status changes	1	1	0	0
Myalgia	1	1	0	0
Nausea	4	0	0	0
Neuropathy, peripheral	4	0	0	0
Pyrexia	3	0	0	0
Schedule B				
CP-870,893 0.1 mg/Kg (n = 3)				
Alopecia	2	0	0	0
Decreased appetite	2	0	0	0
Diarrhea	3	0	0	0
Fatigue	2	0	0	0
Headache	2	0	0	0

*events listed if present in > 10% of patients for toxicities grade 1 and 2, or if n ≥ 1 for grade 3–5 toxicity; #dose limiting toxicity.

Table 2. Adverse events or laboratory abnormalities* (continued)

Adverse event	Any grade (n)	Grade 3 (n)	Grade 4 (n)	Grade 5 (n)
Neutropenia	1	0	1	0
Thrombocytopenia	2	0	0	0
Upper respiratory tract infection	2	0	0	0
CP- 870,893 0.2 mg/Kg (n = 13)				
Alopecia	3	0	0	0
Anemia	3	2	0	0
Arthralgia	5	1	0	0
Back pain	2	1	0	0
Chills	2	0	0	0
Cerebrovascular accident	1	0	0	1#
Constipation	7	0	0	0
Cytokine release syndrome	6	0	0	0
Decreased appetite	6	0	0	0
Diarrhea	5	0	0	0
Dizziness	2	0	0	0
Drug hypersensitivity	1	1	0	0
Fatigue	10	1	0	0
Febrile neutropenia	1	1	0	0
Headache	3	0	0	0
Hypersensitivity	2	1	0	0
Hypogeusia	2	0	0	0
Hypomagnesemia	1	1	0	0
Lymphedema	2	0	0	0
Lymphopenia	1	1	0	0
Myalgia	2	0	0	0
Nausea	6	0	0	0
Neuropathy, peripheral	8	0	0	0
Neutropenia	8	1	6	0
Pruritus	2	0	0	0
Pyrexia	5	0	0	0
Stomatitis	2	0	0	0
Thrombocytopenia	5	0	0	0
Upper respiratory tract infection	2	0	0	0
Vomiting	5	0	0	0

*events listed if present in > 10% of patients for toxicities grade 1 and 2, or if n ≥ 1 for grade 3–5 toxicity; #dose limiting toxicity.

in either arm. Expression of the adhesion molecule CD54 was found to increase on both schedules, but this change was statistically significant only for schedule A at 48 h. In comparing the two schedules for 6-, 24- and 48-h changes in these parameters from baseline, we found no statistically significant differences.

Modulation of T-cell subsets. The phenotype of patient-derived T cells was analyzed using blood samples obtained at baseline and at the end of study for patients treated at the University of Pennsylvania (**Fig. 3B**). There was no statistically significant change in the mean percentage or absolute count of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, or $CD3^+CD8^+$ T cells at the end of study relative to baseline, nor was there any statistically significant change in the subsets as a function of dose or schedule. For regulatory T cells (Tregs, $CD3^+CD4^+FOXP3^+$), some patients demonstrated a large increase in the percentage or absolute numbers, but a similar number of patients demonstrated a marked Treg decrease. Overall, there was no statistically significant change in the percentage or absolute count of Tregs at the end of study relative to baseline. For both, the percentage and absolute counts of ICOS-expressing $CD4^+$ and $CD8^+$ T cells was on average higher upon CP-870,893 infusion than at baseline (including > 2-fold elevations in a few patients on schedule A), but these changes were not statistically significant.

Melanoma-specific T-cell immune responses. Among the 9 melanoma patients treated at the University of Pennsylvania, two patients, each manifesting SD as a best response, were found to be HLA-$A2^+$. Peripheral blood $CD8^+$ T cells from these patients were then analyzed with peptide-HLA-A2 tetramers specific for a panel of viral and melanoma-associated epitopes. For one patient (schedule B, 0.1 mg/Kg CP-870,893, 3 cycles), we found evidence for the induction of T cells specific for MART-1 at the end of cycle 1, which were no longer detectable at the end of cycle 2 (**Fig. 4A**). Influenza-specific T cells, however, were evident at baseline and throughout the study in this patient. For another patient (schedule B, 0.2 mg/Kg CP-870,893, 3 cycles), an induction of MART-1 specific T cells was also observed, but only at the end of cycle 3 (**Fig. 4B**). There was evidence for the induction of T cells specific for gp100 at the end of cycle 1, but these were no longer detectable at the end of cycles 2 or 3 (**Fig. 4B**). Cytomegalovirus (CMV)-specific T cells were observed at baseline and throughout the study in this patient.

Discussion

The purpose of this study was to determine the safety of CP-870,893, a fully human, CD40-targeting agonist mAb, administered intravenously in combination with paclitaxel and carboplatin to patients with advanced solid tumors. Patients were treated on two schedules in an attempt to optimize the timing between chemotherapy and immunotherapy. CP-870,893 in combination with chemotherapy was well-tolerated, and 6 out of 30 evaluable patients (20%) had PRs as best responses. Consistent with its proposed mechanism of action, CP-870,893 exhibited immunomodulatory effects, notably an acute depletion and activation of peripheral B cells, and (at least in a small set of patients) the induction of melanoma-specific T-cell responses. Chemotherapy did not prevent these immunostimulatory effects. No major differences were detected in toxicity, tumor response, or immune pharmacodynamics between the two dosing schedules tested in this study.

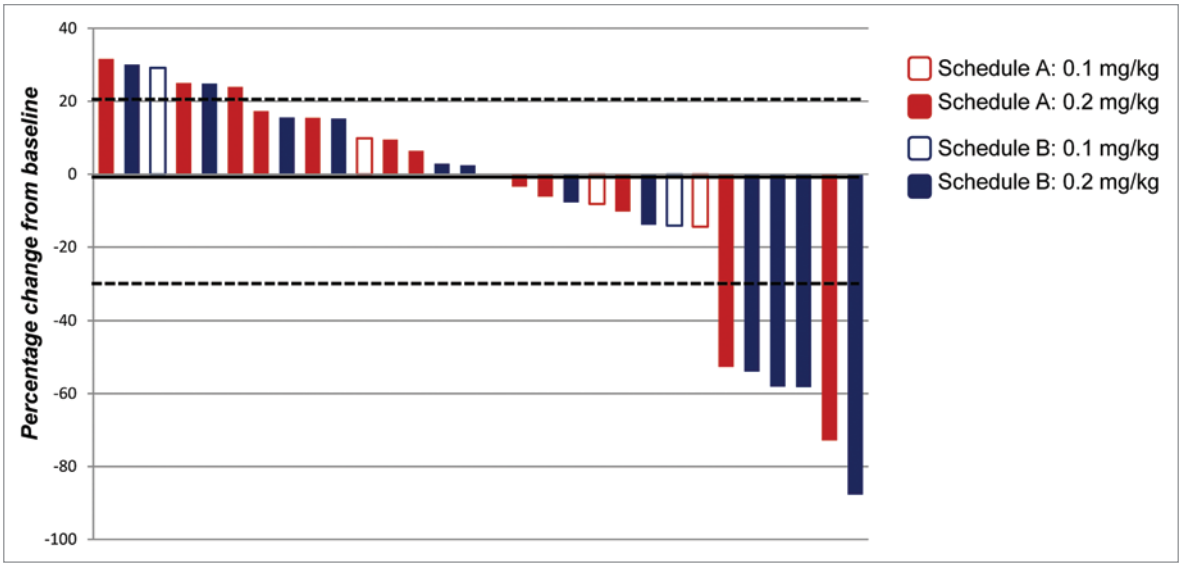

Figure 1. Best overall percentage change from baseline in target tumor lesion measurements. Percentage changes are shown as waterfall plots for 30 evaluable patients treated with CP-870,893 in combination with carboplatin and paclitaxel with two dose levels on two schedules. Data do not reflect new lesions.

The rate of objective clinical responses was encouraging, and—interestingly—all were observed at the MTD. This said, it is difficult to separate the effect of CP-870,893 on tumor responses from that of chemotherapy, because the combination of carboplatin and paclitaxel are known to exert antitumor activity against the neoplasms represented in our cohort. For example, in two recent studies enrolling patients with advanced melanoma (a Phase II study with 61 patients[9] and a Phase III study with 135 patients on the carboplatin/paclitaxel/placebo arm[10]), objective tumor response rates to carboplatin and paclitaxel (given every 21 d) ranged from 4.9% to 11%. A previous smaller study of 15 patients documented an objective PR rate of 20%.[11] In our study, some patients may have been affected by lesions that were sensitive to carboplatin and paclitaxel (such as the patients bearing ovarian cancer or melanoma). However, in other cases, patients exhibited PRs on study in spite of tumor progression after previous treatment with chemotherapy, including docetaxel or cisplatin. The tumor of the renal cell carcinoma patient who responded on study was unlikely to be highly sensitive to either carboplatin or paclitaxel, given this histology, suggesting that CP-870,893 contributed to tumor regression.

The pharmacodynamic changes observed in this study, including B-cell activation, were similar to those previously observed with CP-870,893 in the absence of chemotherapy.[6] Thus, it seems unlikely that chemotherapy is incompatible with CD40-mediated immune activation. Importantly, we did not observe systemic T-cell depletion in this study as we did in a prior study when CP-870,893 was administered weekly to a similar patient population.[8] In that study, it was hypothesized that a hyperacute T-cell activation as a result of frequent CP-870,893 dosing would lead to T-cell apoptosis and depletion.[8] Here, the administration of CP-870,893 every three weeks in combination with chemotherapy failed to significantly affect T-cell subsets and, in fact, the induction of tumor-specific T-cell responses was observed. In ongoing studies, therefore, we are dosing CP-870,893 (combined with other agents) no more frequently than every three weeks (http://www.clinicaltrials.gov; NCT01103635 and NCT01456585).

In summary, CP-879,893 in combination with chemotherapy can be given safely to patients affected by solid tumors and this regimen achieves tumor responses without compromising the immunomodulatory effects of the antibody. For combination with carboplatin and paclitaxel, the recommended dose of CP-870,893 is 0.2 mg/Kg. There were no major detectable differences in toxicity, response rate, or immune system-related pharmacodynamic parameters between the two dosing schedules tested. However, because grade 3 or 4 neutropenia was more commonly observed on schedule B at the time of CP-870,893 dosing, schedule A appears to be preferable for future clinical trials. Overall, further studies to optimize the potential synergy between CD40-targeted immunotherapy and chemotherapy are warranted.

Patients and Methods

Patients. Thirty-two patients with advanced solid malignancies, for which paclitaxel and carboplatin were the appropriate therapy, were enrolled between November 2007 and July 2009 at three clinical centers in the United States (Abramson Cancer Center at the University of Pennsylvania, South Texas Accelerated Research Therapeutics and The Angeles Clinic and Research Institute). Signed informed consent was required; the consent form and protocol were approved by each institution's review board. The study was conducted in accordance with the Helsinki Declaration of 1975. Primary objectives were to assess the safety and tolerability of CP-870,893 in two different schedules and to determine the MTD of CP-870,893 when given

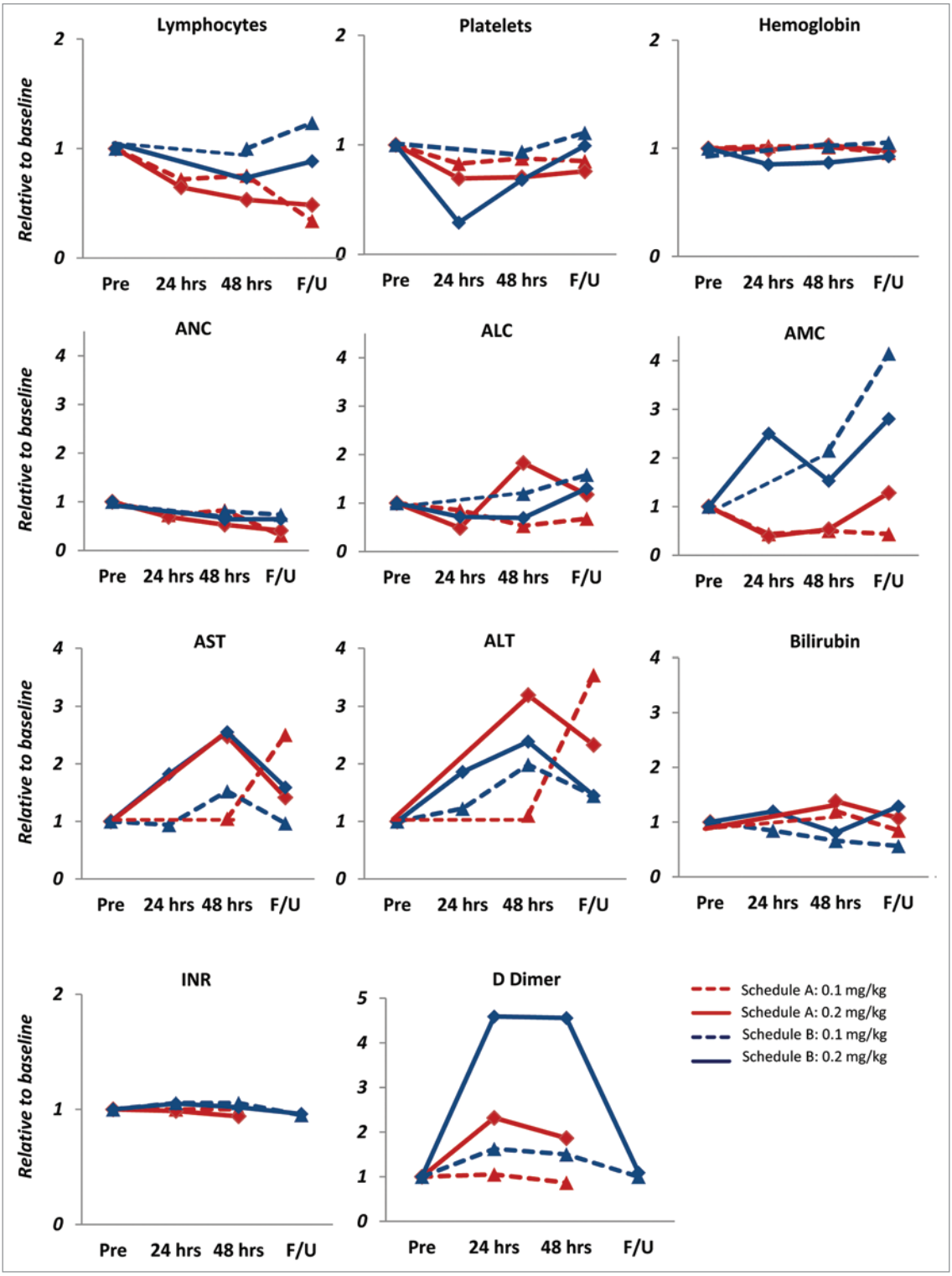

Figure 2. Changes relative to baseline of hematological, chemical and coagulation parameters after the first infusion of CP-870,893. Data are grouped for all patients on a given schedule and dose level and reported as mean values. Schedule A: 0.1 mg/Kg (dashed red) and 0.2 mg/Kg (solid red); schedule B: 0.1 mg/Kg (dashed blue) and 0.2 mg/Kg (solid blue). Follow up (F/U) is at day 8 (5 d after infusion) for schedule A and day 15 (7 d after infusion) for schedule B. Detailed statistical analyses are provided in **Table S1**. ALC, absolute lymphocyte count; AMC, absolute monocyte count ANC, absolute neutrophil count.

in combination with paclitaxel and carboplatin. Secondary objectives included measuring the pharmacokinetic and pharmacodynamic behavior of CP-870,893 as well as its antitumor activity and effects on immune cell compartments. Patients were at least 18 years of age with an ECOG performance status of 0 or 1. Patients with brain metastases (unless treated and clinically

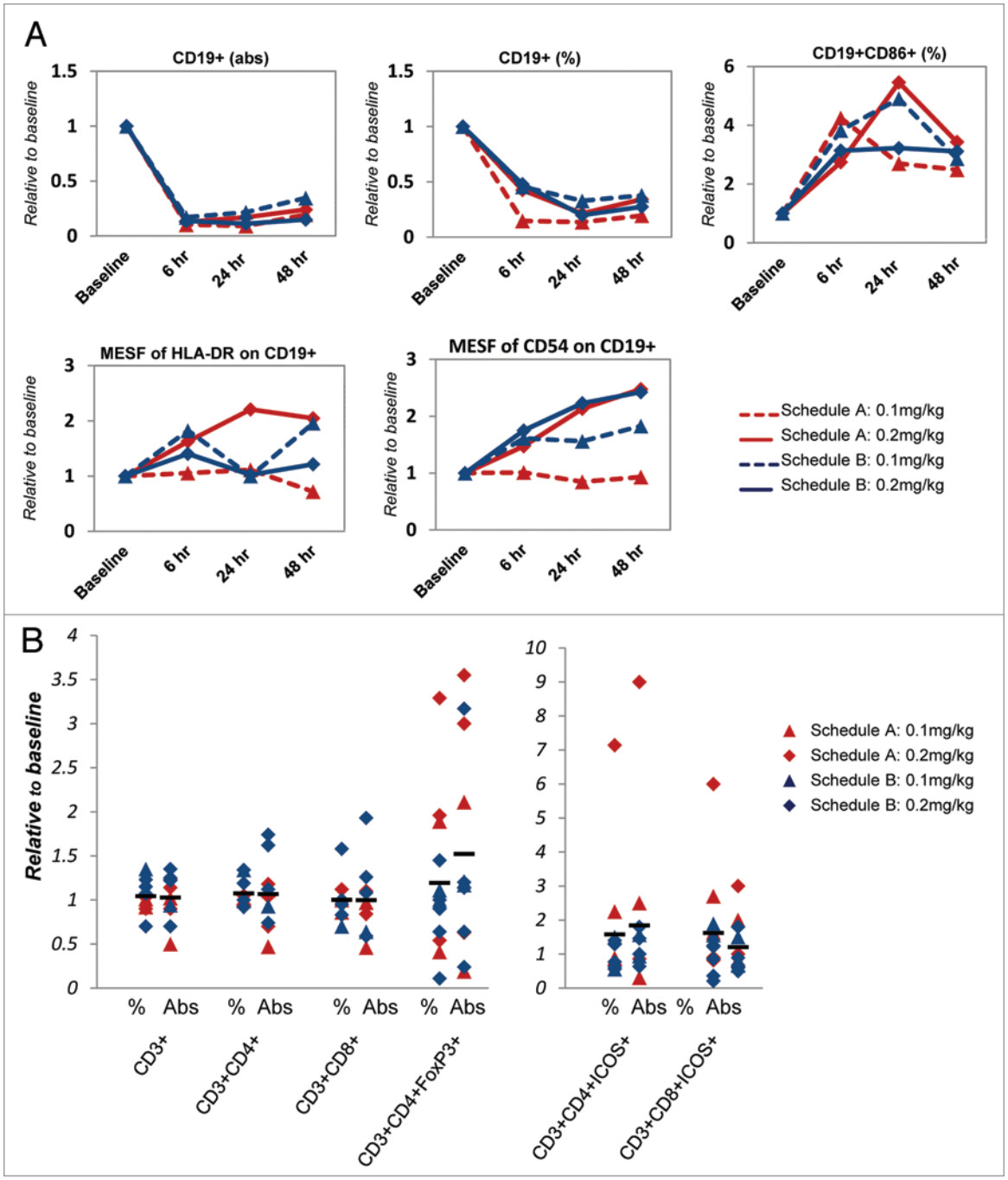

Figure 3. Change relative to baseline of B-cell and T-cell immune parameters after the first infusion of CP-870,893. (**A**) B-cell parameters including absolute count and percentage of peripheral CD19⁺ B cells, percentages of CD86⁺ B cells among total CD19⁺ B cells and molecules of equivalent soluble fluorophore (MESF) of HLA-DR and CD54 on CD19⁺ B cells. Data are grouped for all patients on a given schedule and dose level and reported as mean values. Schedule A: 0.1 mg/Kg (dashed red) and 0.2 mg/Kg (solid red); schedule B: 0.1 mg/Kg (dashed blue) and 0.2 mg/Kg (solid blue). (**B**) Change in T-cell subsets at end of study relative to baseline shown as both percentage and absolute counts (Abs). The black bar represents mean value. Detailed statistical analyses are reported in **Table S2**.

stable), autoimmune disorders, coagulopathies or major co-morbidities or who were pregnant or lactating were excluded from this study. There were no exclusion criteria regarding prior cancer therapies.

Study design and procedures. This was a Phase I, open-label, multicenter, standard 3+3 dose-escalation study of two doses of CP-870,893 (0.1 mg/Kg and 0.2 mg/Kg) in combination with carboplatin and paclitaxel. Pfizer was the IND sponsor of the trial (http://www.clinicaltrials.gov; NCT00607048). Carboplatin was given at a dose of AUC 6 and paclitaxel at 175 mg/m^2. Patients were assigned to one of two schedules, A or B. All patients received carboplatin and paclitaxel on day 1. Those

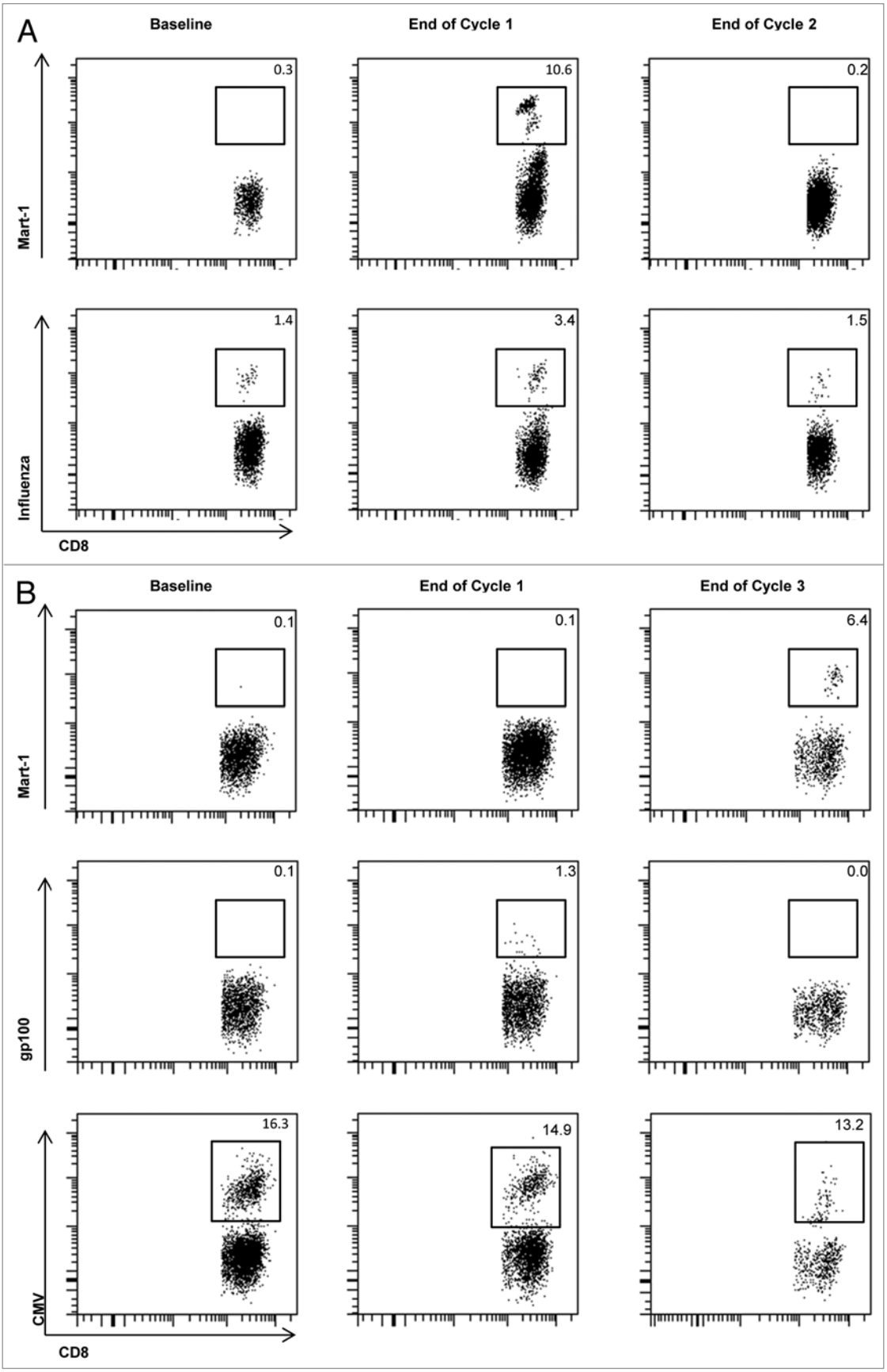

Figure 4. For figure legend, see page e23033-9.

Figure 4 (See previous page). Tetramer-based assessments of T-cell immune responses after therapy. **(A and B)** Cytoflurometric analyses of two HLA-A2$^+$ patients with malignant melanoma on schedule B, 0.1 mg/Kg CP-870,893 (**A**), and schedule B, 0.2 mg/Kg CP-870,893 (**B**). The percentages of tetramer-positive CD8$^+$ T cells are shown for each gate.

on schedule A then received CP-870,893 on day 3 while those on schedule B received CP-870,893 on day 8. Cycles of therapy were repeated every 21 d until tumor progression or the development of DLTs. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. For each schedule, 3 to 6 patients were enrolled at each dose level and the MTD of CP-870,893 was defined as the highest dose level at which < 2 of 6 patients experienced DLT during cycle 1. A minimum of 6 additional patients were then enrolled at the MTD of each schedule.

Patients were monitored for adverse events throughout the study and at least once more 21 d after the final dose of CP-870,893. DLT was defined for CP-870,893-related adverse events during cycle 1 as grade 4 neutropenia persisting for greater than 7 days, grade 3 or 4 febrile neutropenia, platelets ≤ 25,000/mm^3, non-hematologic grade 3 or greater adverse event despite optimal supportive care, grade 3 or greater cytokine release syndrome, failure to recover to ≤ grade 1 toxicity after delaying the initiation of the next cycle for a maximum of two weeks, inability to give CP-870,893 on either day 3 or 8 of cycle 1 due to absolute neutrophil counts < 1,000 mm^3 or platelet counts < 80,000 mm^3 or clinically meaningful non-hematologic toxicity ≥ grade 2. Disease-appropriate imaging was performed at baseline and at the completion of each cycle. Tumor response was assessed by the RECIST version 1.0. Blood samples were drawn prior to infusion and on cycle days 8, 9, 10 and 15 for blood chemistries, coagulation profiles and organ function tests. Samples for cytofluorometric analysis were collected prior to and 6, 24 and 48 h after CP-870,893 infusion. Serum was also obtained at various times before and after infusion for pharmacokinetics, cytokine and auto-antibody analyses. The auto-antibody panel included antinuclear, antineutrophilcytoplasmic, antimicrosomal, antithyroglobulin, antiliver-kidney microsomal, anti-islet cell, anti-Ro anti-La and antiphospholipid antibodies.

Pharmacokinetic and pharmacodynamic assessments. Serum samples were used to determine half life, AUC and C_{max} of CP-870,893 as previously described.[6] The pharmacodynamics of CP-870,893 was assessed by cytofluorometry of peripheral blood CD19$^+$ B cells using good laboratory practices to measure the expression of CD86, CD54 and molecules of equivalent soluble fluorophore (MESF) of HLA-DR, as previously described.[6]

Human peripheral blood and lymphocyte isolation. Peripheral blood mononuclear cells (PBMCs) were obtained only from patients treated at the Abramson Cancer Center of the University of Pennsylvania after signed, informed consent for an additional protocol allowing phlebotomy of patients enrolled on the treatment study. The protocol was approved by the Institutional Review Board at the University of Pennsylvania. Absolute lymphocyte count was obtained from a complete blood count and differential as measured by an accredited clinical lab. PBMCs were obtained by Ficoll centrifugation (Amersham Pharmacia Biotech) and viably frozen at -150°C until use.

Flow cytometry. Flow cytometry was performed on PBMCs in PBS with 5% heat-inactivated fetal calf serum using a FACSCanto cytometer and FACSDiva (BD Biosciences) software. Fluorochrome-conjugated mAb used were as follows: PerCP-, PE-Cy7-, or APC-Cy7-CD3 clone SK7; PerCP-CD4 clone SK3, APC-H7- or APC-CD4 clone RPA-T4, PerCP- or APC-H7-CD8 clone SK1; PE-CD56 clone NCAM16.2 (BD Biosciences); biotinylated anti-human ICOS clone ISA-3 (eBioscience); and Alexa Fluor 488-anti-FoxP3 clone 259D (Biolegend). PE-streptavidin was from BD Biosciences. Soluble HLA-A2 tetramers conjugated to PE were purchased from Beckman Coulter Immunomics.

In vitro peptide stimulation. Thawed PBMCs (10^6/well) were incubated with autologous irradiated (32Gy) PBMCs (10^6/well) in the presence of 1 μg/mL peptide (New England Peptide) and 2.5 μg/mL β2-microglobulin in complete culture medium supplemented with 10 ng/mL IL-7 (Sigma) in 24-well tissue culture plates. The HLA-A2-binding peptides used were: pp65 from CMV (NLVPMVATV), M1 from influenza virus (GILGFVFTL), MART-1 (ELAGIGILTV), gp100 (IMDQVPFSV), tyrosinase (YMDGTMSQV), PRAME (VLDGLDVLL) and MAGE-3 (FLWGPPALV). After 24 h and again on day 5, 20 IU7mL IL-2 (Chiron Corp) was added and cells analyzed on day 8 by flow cytometry with peptide-HLA-A2 tetramers, as previously described.[12]

Statistical methods. A standard 3 + 3 dose escalation design was followed. At any dose level, escalation ceased for > 1 DLT in 3 or 6 patients. At the MTD, a minimum of 6 additional patients were enrolled. All statistical testing was exploratory. Linear mixed effects models were used to analyze trends over time in clinical laboratory and pharmacodynamic variables using the xtmixed command in STATA v. 12. An unstructured covariance structure was assumed. Comparable to repeated measures ANOVA, these models defined time as a class variable. Prior to modeling, probability plots were employed to assess the normality of variables. Natural log transformation was subsequently applied to all variables. Comparisons of changes in clinical laboratory and pharmacodynamic variables between the two arms were performed by analysis of covariance (ANCOVA), which adjusts for each patient's baseline value. Due to the many variables examined, the Bonferroni-Holm step-down method was employed to control for multiple comparisons and determine statistical significance.[13] All analyses were performed in STATA v. Twelve (StataCorp). For T-cell analysis GraphPad Prism (GraphPad Software) was used to perform paired Student's t-tests with p values < 0.05 considered as statistically significant. The clinical data from the trial resides at Pfizer. Correlative science studies were done by the University of Pennsylvania, using data from the Pfizer clinical

database and, in addition, some data collected and stored at University of Pennsylvania.

Disclosure of Potential Conflicts of Interest

M.N. Shaik is an employee of Pfizer Corp. and Dongguang Li is a former employee; Anthony W. Tolcher reports receiving honoraria from Pfizer; Robert H. Vonderheide, Anthony W. Tolcher and Omid Hamid have received research funding from Pfizer.

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Supplemental Materials

Supplemental Materials may be found here: www.landesbioscience.com/journals/oncoimmunology/article/23033

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