Name of Sponsor/Company: Bristol-Myers Squibb	Individual Study Table Referring to the Dossier	*(For National Authority Use Only)*
Name of Finished Product:		
Name of Active Ingredient:		

SYNOPSIS

Final Clinical Study Report for Study CA186001

TITLE OF STUDY: A Phase I/II, Ascending, Multi-Dose Study of BMS-663513, an Agonistic Anti-CD137 Monoclonal Antibody, Administered Every Three Weeks to Patients with Metastatic or Locally Advanced Solid Malignancies

INVESTIGATORS/STUDY CENTERS: Twelve investigational sites in Canada (1 site), France (5 sites), and the United States (6 sites).

PUBLICATIONS: None

STUDY PERIOD: Study Initiation Date: 09-Dec-2005 **CLINICAL PHASE:** I / II

Study Completion Date: 30-Dec-2008

OBJECTIVES:

Primary Objective

- To assess the safety and tolerability of BMS-663513 in the range of 0.3 mg/kg to 15 mg/kg administered once every 3 weeks to subjects with metastatic or locally advanced solid malignancies in the dose escalation phase of the study. To estimate the reversible Grade 3-4 toxicity rate in the expansion dose cohorts of melanoma, ovarian and renal carcinoma patients at each of the 3 selected doses (1, 3 and 10 mg/kg).

Secondary Objectives

- To assess the PK of BMS-663513
- To assess BMS-663513 dose and biologic effect relationships using:
  - Flow cytometry to quantitate subsets of immune cells (CD3, CD4, CD8, CD19, and CD16/56)
  - Flow cytometry to measure subsets of T cells (activated, memory)
  - qRT-PCR to measure changes in RNA expression in peripheral blood
  - ELISA to measure changes in levels of cytokines and/or other biomolecules
- To screen for anti-tumor activity of single agent BMS-663513
- To assess the effect of BMS-663513 on immune response to non-cancer vaccines (influenza, tetanus, pneumoccocal)
- To assess the immunogenic potential of BMS-663513

- To assess the effect of BMS-663513 on intratumoral immune response pre and post treatment using immunohistochemistry (IHC)
- To obtain fresh and paraffin embedded tumor samples, and blood, to identify potential predictive markers of biological response utilizing ribonucleic acid (RNA) profiling, protein profiling, single nucleotide polymorphism (SNP) analysis, and other techniques

METHODOLOGY: This was an open-label, sequential, ascending, multi-dose Phase I/II study of BMS-663513 in subjects with metastatic or locally advanced solid malignancies. Subjects were to be assigned to each of the 6 sequential cohorts (0.3, 1, 3, 6, 10 and 15 mg/kg). Following completion of the dose escalation phase of the study, an expansion cohort phase was to be conducted to enroll 90 subjects in 3 tumor types (strata) - melanoma (30), ovarian (30) and renal cancer (30) - to receive 1 of 3 doses of BMS-663513. Thirty (30) subjects within each of the 3 tumor types were to be randomized to receive 1, 3 or 10 mg/kg of BMS-663513 in a 1:1:1 ratio (10 subjects at each dose per tumor type) based on a randomization schedule created by the Sponsor.

Continuous evaluation of toxicity events in the expansion cohort was performed. In the event that in 1 arm of the expansion cohort, the number of DLTs was greater than the number of subjects with clinical benefit (ie, objective tumor response or stable disease at Week 12), a reevaluation of the risk/benefit ratio was made by the study Sponsor and investigators for that arm. In the event that any arm of the expansion cohort was determined to have an unfavorable risk/benefit ratio, the treatment arm was closed to new subjects. No additional arms were created to replace a closed cohort.

On Day 1, subjects received BMS-663513 as a 60-minute IV infusion. Following review of Cycle 1 (each 3 week period was considered 1 cycle) clinical and laboratory safety data, and if that dose was deemed safe, the succeeding cohort of 3 subjects received the next higher dose of BMS-663513. Dose escalation proceeded once ≥ 3 subjects had Cycle 1 safety data reviewed. Dose escalation was planned to continue until 15 mg/kg, or one-third or more of the subjects at a particular dose level had a dose limiting toxicity (DLT), whichever occurred first. A DLT was defined as any ≥Grade 3 toxicity that was attributed (i.e., judged by the investigator to be at least possibly related) to BMS-663513. The incidence of DLTs during the first 21-days of treatment was used to define dose escalation and the MTD. If a DLT was reported for 1 of the first 3 subjects in a given dosing cohort, an additional 3 subjects were enrolled to that dose level before further escalation was considered. The maximally tolerated dose (MTD) was defined as the highest dose at which less than one-third of the subjects experienced a DLT.

NUMBER OF SUBJECTS (Planned and Analyzed): The sample size for this study was not based on statistical power considerations. In the dose escalation phase, 3 or 6 subjects were enrolled into each tested drug dose level to determine the maximum tolerated dose (MTD). In the dose expansion phase, 90 subjects were enrolled. This number of subjects (i.e., 30 per tumor type).was chosen based on a type I error of 0.05 and a 90% confidence interval to estimate the reversible Grade 3-4 toxicity rate at one dose level. Detection of 11 drug-related Grade 3-4 reversible toxicity events would provide 95% confidence the true reversible Grade 3-4 toxicity rate was > 22%.

A total of 128 subjects were enrolled and 115 received at least 1 dose of BMS-663513. All 115 subjects who received BMS-663513 and had evaluable tumor imaging results at baseline and post treatment were included in the efficacy data set. All available data from the 115 treated subjects were included in the analysis of safety, PK, PD and the predictive biomarkers.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION: Subjects 18 years of age or older, male or female, who had histologic or cytologic diagnosis of advanced solid malignancy; failed, refused or unable to receive standard treatment were enrolled in the study. Subjects who entered the study during the expansion cohort phase had one of the 3 tumor types (strata) - melanoma (30 subjects), ovarian (30 subjects) or renal cancer (30 subjects).

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, DURATION OF TREATMENT, BATCH NUMBERS: BMS-663513 was administered every 3 weeks as a 60-minute IV infusion. Each 3 week period was considered 1 cycle. The starting dose of BMS-663513 for the initial cohort of 3 subjects was 0.3 mg/kg. Subsequent ascending dose levels were 1 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg and 15 mg/kg, respectively. BMS-663513 for injection was supplied by BMS in individual 10 mL vials each containing 100 mg of the drug substance, BMS-663513-01 (product batch numbers 5F04835, 5F04838, 5F40835, 5FU04835, 5L08603, 6E17537, 7G260261, 7G26061), as a clear, colorless to pale yellow 10 mg/mL solution.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, DURATION OF TREATMENT, BATCH NUMBERS: None.

CRITERIA FOR EVALUATION:

Efficacy: Evaluable or measurable disease response was assessed using a modified form of the WHO criteria. At minimum, tumor measurements by CAT/CT scan, MRI, x-ray or physical exam were obtained during the screening period, at Week 12 and every 6 weeks thereafter.

The efficacy endpoint of objective response rate, defined as the number of complete and partial responders among all treated subjects, was reported by tumor type and dose for treated subjects in the expansion cohort phase, for each of the 3 tumor types (melanoma, ovarian, renal cell)..

Safety: Adverse events (AEs) were evaluated according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 on a continuous basis while the subject was on study and until a minimum of 60 days after the last dose of study drug or until all treatment-related AEs had recovered to baseline or were deemed irreversible. Once a subject had been off treatment due to toxicity, assessments were to be made every 28 days until all study related toxicities resolved to baseline, stabilized, or were deemed irreversible.

Serum, plasma, and urine samples for clinical laboratory evaluations were collected at screening and various post-dose timepoints. In addition, (i) HIV, hepatitis C antibody (anti-HCV), and hepatitis B surface antigen (HBsAg) were obtained during the screening period, (ii) autoimmune panel and endocrine panel were collected during the screening period, before each treatment, and at the 60-day follow-up visit, and (iii) serum CA-125 (ovarian cancer subjects only) was obtained during the screening period ≤14 days prior to Day 1, on Day 1 prior to each cycle, at study discharge and during long-term follow-up. In the event of a CA-125 response, additional CA-125 values were obtained.

The autoimmune panel included erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and antinuclear antibody (ANA) titer and pattern. If ANA was positive (titer ≥1:80), the following tests were performed: anti-DNA antibody, anti-SSA antibody (Ro), anti-SSB antibody (La), anti-phospholipid antibody, and total complement (CH50). The endocrine panel included thyroid stimulating hormone (TSH), cortisol, adrenocorticotropic hormone (ACTH), and free testosterone in male subjects. If TSH was abnormal, then triiodothyronine (T_3), free thyroxine (FT_4), and anti-thyroglobulin antibody were obtained.

A 12-lead ECG was completed, and interpreted/documented by the investigator, during the screening period, before each infusion and at the end of each infusion. Vital signs were assessed at screening, weekly during Cycle 1, prior to each cycle, study discharge, and the 30 and 60-day follow-up visit. In addition, vital signs were assessed at the start of each BMS-663513 infusion, half-way through infusion, at the completion of infusion, 1-hour after the end of infusion or more frequently if clinically indicated.

A negative serum or urine pregnancy test was documented for women of childbearing potential within 72 hours prior to Day 1 of treatment, and was repeated weekly during the 1st cycle and then every 2 weeks until study discharge. ECOG performance status was documented at screening, Day 1, prior to the start of each cycle, at study discharge and the 30 and 60-day follow-up visit.

Pharmacokinetics: Blood samples for the determination of BMS-663513 pharmacokinetics in serum were drawn on Cycle 1 Day 1 (predose, 4 hours and 24 hours after start of infusion), Day 8 and Day 15. Samples were also drawn (i) pre-dose on Cycle 2 Day 1 (Study Day 22) and 24 hours after start of infusion (Study Day 23), (ii) pre-dose on Cycle 3 Day 1 (Study Day 43), 4 hours and 24 hours after start of infusion (Study Day 44), (iii) Cycle 3 Day 8 (Study Day 50), (iv) prior to the start of Cycle 4 (Study Day 64) and subsequent cycles, and (v) at study discharge.

Pharmacodynamics: Protein and mRNA expressions of genes related to anti-tumor immune regulation and potentially BMS-663513 mechanisms of action were assayed. RNA and/or protein were extracted from whole blood collected pretreatment on Day 1, 24-hours post treatment on Day 2, Day 8, pretreatment on Day 22; and from tumor tissue collected pre and post treatment. Additionally, changes in tumor infiltrating lymphocytes and other related proteins were examined in archived, pre and post treatment tumor tissue.

Blood samples for assessment of anti-influenza, anti-pneumococcal and anti-tetanus antibody levels were collected pretreatment on Day 1, and Day 43.

Flow cytometry was used to assess the baseline and serial on treatment percentages of T lymphocyte subsets [including, but not limited to, activated T-cells (CD69 and/or HLA-DR) and memory T-cells (CD45RO and/or CCR7)].

Blood samples for assessment of inflammatory cytokines and/or biomolecules including, but not limited to, neopterin were collected during screening, pretreatment on Day 1, on Day 8, pretreatment on Day 22 (C2D1), Day 29 (C2D8), pretreatment on Day 43 (C3D1) and Day 50 (C3D8).

A single blood sample for SNP analysis in the 4-1BB gene, CTLA-4 gene and other genes that affect the BMS-663513 mechanism of action, was collected pretreatment on Day 1. However, given the low level of response rate in this first-in-human study and the exploratory nature of the analysis, the decision was made not to do the analysis, so an administrative change was made to the protocol and the samples were subsequently discarded and the SNP analysis was not done.

The following samples were banked for future use only from those subjects who signed the "Optional Consent to Bank Blood, Plasma and Tissue for Future Use":

- Remaining/left-over tumor tissue, fresh and/or archive.
- Remaining/left-over blood sample after mRNA and SNP analysis.
- Additional 10 mL blood plasma samples were collected pretreatment on Days 1, 22, 43 and study discharge.

Immunogenicity Testing: Blood for human anti-human antibody (HAHA) testing was drawn pretreatment on Day 1, before each subsequent treatment, and 50 - 60 days after the last dose of BMS-663513. All samples were initially evaluated in a screening assay. Positive samples were subjected to a confirmatory assay, which assessed specificity of antibody binding to BMS-663513.

STATISTICAL CONSIDERATIONS:

Sample Size Considerations: This was a Phase I/II dose escalation study. For the dose escalation phase, the sample size could not be precisely determined because it depended on the observed toxicity. The sample size for this study was not based on statistical power considerations. In the dose escalation phase, 3 or 6 subjects were enrolled into each defined drug dose level to determine the maximum tolerated dose (MTD). In the dose expansion phase, 90 subjects were enrolled. This number of subjects (i.e., 30 per tumor type).was chosen based on a type I error of 0.05 and a 90% confidence interval to estimate the reversible Grade 3-4 toxicity rate at one dose level. Detection of 11 drug-related Grade 3-4 reversible toxicity events would provide 95% confidence the true reversible Grade 3-4 toxicity rate was > 22%.

Efficacy: Objective response rate, defined as the number of complete and partial responders among all treated subjects, was reported by tumor type and dose for treated subjects in the expansion cohort phase, for

each of the 3 tumor types. The two-sided 95% exact confidence interval for the response rate was also calculated. The best overall response for subjects in the dose escalation phase was presented in a listing. Duration of response and progression-free survival (PFS) were analyzed using the Kaplan-Meier method. The median and its two-sided 95% confidence intervals for the duration of response and PFS were reported by tumor type and dose in the expansion cohort phase.

Safety: All recorded AEs were listed and tabulated by system organ class, preferred term and dose. Vital signs and clinical laboratory test results were listed and summarized by dose. Any significant physical examination findings and ECG results were listed. For the expansion cohort phase, safety summaries were also tabulated by tumor type and dose as well as by tumor type pooled across doses.

Pharmacokinetics: PK parameter values of BMS-663513 were derived from serum concentration versus time data. The pharmacokinetic parameters for BMS-663513 (Cycle 1, Day 1 and Cycle 3, Day 1) assessed were: Cmin, Cmax, Tmax, AUC(INF), AUC(0-T), T-HALF, Vss and CL. Individual subject PK parameter values were derived by a non-compartmental method using a validated pharmacokinetic analysis program (Kinetica™ 4.4.1 within the eToolbox [version 2.6.1]).

Summary statistics were tabulated for the PK parameters (listed above) by dose and study cycle (Cycle 1 and Cycle 3). Geometric means and coefficients of variation were presented for Cmax, Cmin, AUC(INF), AUC(0-T) and CL. Medians and ranges were presented for Tmax. Means and standard deviations were provided for the other PK parameters. To assess attainment of steady state geometric mean Cmin versus study day was provided by dose level.

To assess the dependency on dose, scatter plots of Cmax, AUC(0-T), and AUC(INF) versus dose were provided for Cycle 1. Furthermore, the power model described by Gouch et al:

$$\text{PK Parameter} = A * \text{Dose}^{\beta}$$

was used to assess whether Cmax, AUC(0-T), and AUC(INF) were proportional to dose (i.e., whether the slope (β) could be assumed to be no different than 1). An estimate of β and a 90% CI for each parameter during Cycle 1 was obtained by simple linear regression of the natural log of the PK parameter on the natural log of dose:

$$E[\text{Log(PK Parameter)}|\text{Dose}] = A + \beta * \log(\text{Dose}).$$

A β equal to 1 would indicate perfect dose proportionality.

Pharmacodynamics and Immune Response Analyses: Summary statistics were tabulated by dose and time for the following PD and immune response parameters: flow cytometry outcomes, serum cytokines and/or other biomolecules, mRNA outcomes, and responses to influenza, tetanus and 23-valent pneumococcal vaccines and their changes from baseline. Frequency distributions for responses to vaccines were also summarized by dose. Plots of mean percent changes in the markers were presented versus Day/Time by dose. In order to explore possible associations between the PD or immune response parameters and exposure, scatter plots of changes (from baseline) in the outcomes versus BMS-663513 concentration (or AUC) were provided.

Other Exploratory Research Analyses: This study was not statistically powered for exploratory analyses and probabilities were not adjusted for the multiplicity of tests conducted. Additional post hoc exploratory assessments were expected and could be performed. These data could be combined with other trials of BMS-663513 to identify potential biomarkers that are predictors of objective tumor response and/or immune response and DLTs.

Subjects were classified as a Responder or Non-Responder for the purpose of evaluating potential predictors of response to the treatment with BMS-663513 using modified WHO criteria and objective tumor response. The best overall response was used for each subject. The Responder group included those

subjects with a CR, PR, and those with SD lasting ≥ 4 months. The Non-Responder group included all treated subjects that were not classified as Responders.

Demographic factors such as race/ethnicity, age, and gender were examined to determine whether stratification or adjustments were to be made within the subsequent statistical analyses and, if so, the appropriate stratification or adjustment to be made. The relationships among SNP frequencies, protein or mRNA expressions or changes in expressions and immune response, objective tumor response and DLT were summarized using descriptive tabulations of frequency distributions, means, standard deviations or scatter plots.

Flow cytometry data were analyzed by paired t-tests and linear or nonlinear regression models as appropriate.

If sufficient tumor samples were obtained, differences in gene expression between pre-treatment and post-treatment tumor samples were assessed using paired t-test. Repeated-measures ANOVA were used to study the effect of dose on changes in gene expression. Genes assessed included, but were not limited to, IFN-γ, Bcl-xl, Granzyme B, Cyclin D2, Perforin 1, TNF-α, IL-2, IL-12 β, FOXP3, IL-12 α, NOS2, IDO, Arginase I, Arginase II, and B7-H1.

To identify additional genes either induced or suppressed by the anti-CD137 antibody (BMS-663513), gene expression profiling could be done on selected tumor samples using Affymetrix GeneChip™. Paired t-tests were performed to detect differentially expressed genes and rank them by significance. The top 200 ranked genes with the P-value < 0.05 were listed. Logistic regression was used to identify markers that could potentially predict BMS-663513 response based on the pretreatment tumor samples. Other methods such as chi square tests, analysis of variance, generalized linear models, nonparametric tree-based models, principal components analyses for data dimension reduction or clustering algorithms, was also explored.

The statistical analyses for blood samples were handled similarly to that for the tumor samples.

SUMMARY OF RESULTS:

Disposition and Baseline/Demographic Characteristics: All subjects were off-treatment and no subject was continuing in the follow-up phase (Table 1).

Demographic characteristics are presented in Table 2. All 115 subjects had received prior chemotherapy for their disease with 45.2% of subjects having ≥ 3 regimens of prior chemotherapy. One hundred thirteen (113) subjects (98.3%) had undergone prior surgery, and 36 subjects (31.3%) had received prior radiotherapy. The tumor types reported at the baseline disease diagnosis were melanoma (55 subjects; 48%), kidney (27 subjects; 23%), ovary (28 subjects; 24%), prostate (1 subject; 1%), and other (4 subjects; 3%).

Table 1: Subject Disposition

	Number of Subjects (%)
No. of Subjects Enrolled	128
No. of Subjects Treated	115
No. of Subjects Never Treated	13
No. of Subjects Discontinued	
Disease progression	81 (70.4)
Deterioration w/o progression	13 (11.3)
Study drug toxicity	9 (7.8)
Withdrawal of consent	4 (3.5)
Other	4 (3.5)
Unrelated AEs	3 (2.6)
Death*	2 (1.7)
Subject request	2 (1.7)
Physician decision	1 (0.9)

*Subjects [redacted]. Eight other subjects died within 60 days of last dose ([redacted])

Table 2: Demographic Characteristics

	Number of Subjects (%) N=115
Age (yrs), median	61.0
Age Categorization n (%)	
< 65	75 (65.2)
≥ 65	40 (34.8)
Gender n (%)	
Male	61 (53.0)
Female	54 (47.0)
Race n (%)	
White	112 (97.4)
Black/African American	1 (0.9)
Asian	1 (0.9)
Other	1 (0.9)
Ethnicity n (%)	
Hispanic / Latino	1 (0.9)
Not Hispanic / Latino	89 (77.4)
Not Reported	25 (21.7)

Efficacy Results: Four subjects (3%), all with melanoma as primary tumor, met the criteria for PR; none of the subjects met the criteria for CR. Stable disease was recorded as the best clinical response for a total of 25 subjects for a rate of 22%.

Safety Results:

DLT/MTD: A DLT was defined as any ≥Grade 3 toxicity that was attributed (i.e., judged by the investigator to be at least possibly related) to BMS-663513. The incidence of DLTs during the first 21-days of treatment was used to define dose escalation and the MTD. These criteria were met in 2 subjects: (i) Grade 3 neutropenia in subject CA186001-4-2 (0.3 mg/kg), and (ii) Grade 4 neutropenia in subject CA186001-3-6 (15 mg/kg). Dose escalation proceeded to the maximum planned dose of 15 mg/kg. The MTD in this study was not formally reached, as 15 mg/kg was the predefined maximum dose level.

Adverse Events: Across all dose cohorts, AEs were reported in 114 of 115 subjects (99.1%) treated with BMS-663513. The most frequently reported AEs (>20% of subjects) were (i) fatigue (54 subjects, 47.0%), (ii) increased alanine aminotransferase (40 subjects, 34.8%), (iii) increased aspartate aminotransferase (40 subjects, 34.8%), (iv) decreased appetite (36 subjects, 31.3%), (v) nausea (32 subjects, 27.8%), (vi) rash (32 subjects, 27.8%), (vii) diarrhea (27 subjects, 23.5%), (viii) headache (27 subjects, 23.5%), (ix) pyrexia (25 subjects, 21.7%), (x) constipation (23 subjects, 20.0%), and (xi) pruritis (23 subjects, 20.0%). The majority of AEs that were reported after administration of BMS-663513 were Grade 1 to 2 in severity. However, a majority of subjects (67 of 114) reported at least one AE that was Grade 3 to 5 in severity.

A total of 44 deaths were reported during this study: 10 deaths were reported during the study or within 60 days of the last dose of study medication. The reasons for these 10 deaths were disease progression (6 subjects), perforated duodenal ulcer (1 subject), sepsis due to bowel perforation due to metastatic ovarian cancer (1 subject), hypotension (1 subject), or unknown (1 subject).

A total of 37 subjects (32.2%) reported SAEs (including laboratory values reported as SAEs) during study participation. The most frequently reported SAEs (≥2% of subjects) were (i) malignant neoplasm progression (6 subjects, 5.2%), (ii) increased alanine aminotransferase (4 subjects, 3.5%), (iii) increased aspartate aminotransferase (4 subjects, 3.5%), (iv) thrombocytopenia (4 subjects, 3.5%), (v) dyspnea (3 subjects, 2.6%), (vi) febrile neutropenia (3 subjects, 2.6%), (vii) neutropenia (3 subjects, 2.6%), and (viii) pyrexia (3 subjects, 2.6%). A total of 17 subjects discontinued study treatment due to AEs. Study therapy was discontinued due to 1 or more of the following AEs: abnormal hepatic function, ALT increased, AST increased, blood alkaline phosphatase increased, febrile neutropenia, neutropenia, leukopenia, thrombocytopenia, leukaemia, hypercalcemia, lower abdominal pain, gastrointestinal obstruction, subileus, cholestasis, musculoskeletal pain, pain in extremity, bone pain, fatigue.

Laboratory Data: For hematology parameters, Grade 3 values were reported for absolute neutrophil count (4 subjects, 3.5%), hemoglobin (5 subjects, 4.3%), platelet count (3 subject, 2.6%), and leukocytes (5 subjects, 4.3%). Grade 4 values were reported for absolute neutrophil count (6 subjects, 5.2%), platelet count (3 subject, 2.6%), and leukocytes (2 subjects, 1.7%). No Grade 4 values were reported for hemoglobin.

For chemistry parameters, Grade 3 values were reported for ALT (14 subjects, 12.2%), AST (13 subjects, 11.3%), alkaline phosphatase (6 subjects, 5.2%), bilirubin (3 subjects, 2.6%), and albumin (1 subject, 0.9%). Grade 4 values were reported for ALT (8 subjects, 7.0%), AST (6 subjects, 5.2%), and bilirubin (2 subjects, 1.7%). No Grade 4 values were reported for alkaline phosphatase and albumin.

Grade 3 values were reported for hyponatremia (5 subjects, 4.3%), creatinine (3 subjects, 2.6%) and hypokalemia (1 subject, 0.9%). No Grade 4 values were reported for hyponatremia, creatinine or hypokalemia. No Grade 3 or Grade 4 values were reported for hyperkalemia or hypernatremia.

Grade 3 values were reported for inorganic phosphorus (2 subjects, 1.7%), hypocalcemia (1 subject, 0.9%), and hyperglycemia in fasting (1 subject, 0.9%). Grade 4 values were reported hypocalcemia (2 subjects, 1.7%) but not for inorganic phosphorus or hyperglycemia. No Grade 3 or Grade 4 values were reported for hypercalcemia, hypoglycemia in fasting, hypo- or hypermagnesemia.

Analysis of on-study hepatoxicity data showed that AST was either normal (38.3%) or between >ULN to ≤3xULN (37.4%) in a majority of subjects. The worst value was >3xULN to ≤5xULN in 9 subjects (7.8%), >5xULN to ≤8xULN in 6 subjects (5.2%), and >8xULN in 13 subjects (11.3%). Similarly, ALT was either normal (40.0%) or between >ULN to ≤3xULN (32.2%) in a majority of subjects. The worst value was >3xULN to ≤5xULN in 10 subjects (8.7%), >5xULN to ≤8xULN in 7 subjects (6.1%), and >8xULN in 15 subjects (13.0%). Analysis of on-study hepatoxicity data showed that total bilirubin was ≤2xULN in 108 of 115 subjects (93.9%), and >2xULN in 7 of 115 subjects (6.1%).

Drug-related concurrent elevations in ALT or AST (>3 X ULN) and total bilirubin (>2 X ULN) (Hy's law) are considered to be indicators of severe drug-induced liver injury (DILI). Of the 115 subjects treated in this study, 6 subjects experienced this level of concurrent ALT/AST and total bilirubin elevations, 3 of which were considered by the investigator to be at least possibly related to BMS-663513 (one each at 1 mg/kg, 3 mg/kg, and 6 mg/kg, respectively). Given there were no Hy's law events considered at least possibly related to BMS-663513 at the 10 mg/kg and 15 mg/kg dose levels, there does not seem to be a clear dose relationship for the occurrence of these Hy's law events associated with DILI.

Pharmacokinetic Results: Analysis of the PK data suggest the following key findings: (i) the assays were precise and accurate for the determination of BMS-663513 concentrations in serum, (ii) BMS-663513 Cmax and AUC increased in proportion to dose when administered at dose levels of 0.3-15 mg/kg, (iii)

across the dose groups and study days 1 and 43, mean serum terminal half-lives ranged from 135 to 289 hours (5.6-12.0 days), (iv) both CL and Vss showed no apparent dose dependency over the 0.3-15 mg/kg dose range, and (v) steady-state appeared to occur between Cycles 2-4.

Pharmacodynamic Results: For mRNA expression derived from whole blood, significant ($p < 0.05$) changes in expression over time were observed for 15 of the 21 target genes with available data. Significant ($p < 0.05$) differences in expression among dose groups were observed for 2 of the 21 target genes, CD8A and MS4A1.

Genes with greatest increase in mean expression following treatment with BMS-663513 included a group of IFN-inducible genes (OAS2, RSAD2, MX1, CXCL10, and GBP1). Dose levels of 0.3, 1 and 3 mg/kg resulted in a ≥2-fold increase in the mean gene expression for RSAD2 (viperin), OAS2, and MX1 on Day 8; at later times the mean expression levels of these genes was in general maintained at higher levels than baseline but lower than on Day 8. Doses of 6, 10, and 15 mg/kg did not elicit changes for OAS2 and MX1, while ≥2-fold increases in expression were detected for RSAD2 8 days after the second dose of BMS-663513. CXCL10 and GBP1 mean levels of expression increased by Day 8 in subjects dosed with BMS-663513 at 0.3, 1, 3, 10 and 15 mg/kg. Conversely, subjects that received a dose of 6 mg/kg showed a maximum increase (≥2-fold) for CXCL10 and GBP1 8 days after the second dose. It is not clear whether the differences in patterns of expression for IFN-inducible genes at various dose levels reflect a dose effect. The small study size and imbalance in prognostic variables between dose cohorts makes interpretation of the data difficult.

Immunoglobulin-related genes (IGG134431M, IGKC and IGL@) showed a different pattern of expression compared with the interferon-inducible genes. The mean expression value for this group of genes increased by Day 8 and it was followed by at least a 2-fold decrease in mean gene expression at doses of 1, 3, 6, 10, and 15 mg/kg. Subjects that received BMS-663513 at 0.3 mg/kg also showed a decrease in expression for these genes without the apparent increase on Day 8. In addition, the mean expression levels for MS4A1 (CD20) decreased after the second dose of BMS-663513 across dose levels with a change of ≥ 2-fold at 0.3, 1, 6 and 15 mg/kg; at 10 mg/kg a modest (<2-fold) decrease in CD20 expression level was observed.

Global changes in mRNA expression levels from whole blood samples were assessed using the HG-U133A_2 HT GeneChipTM microarray system (Affymetrix). Out of 22215 probe sets tested, 3421 had a normalized log2 expression value of at least 4 for at least one sample and a maximum value of at least 2 for the ratio comparing mean expression for 2 dose groups at post-treatment time points. Significant (q-value <0.05) dose effects on expression were observed for 333 of these 3421 probe sets, representing 293 unique genes. Out of the 22215 probe sets tested, 1695 had a normalized log2 expression value of at least 4 for at least one sample and a maximum value of at least 2 for the ratio comparing mean expression at 2 time points within a dose group. Significant (q-value < 0.05) changes in expression over time were observed for 9 of these 1695 probe sets, representing 7 unique genes. Several of these genes (RPL36, RPL22, DDX17, ST20) are associated with tumor cell proliferation, therefore, changes in expression for these genes may reflect tumor growth. IFI27 belongs to the group of interferon-inducible genes; changes in expression for this gene might be related to immune modulation by BMS-663513.

Serum Antibody: Eleven (11) antibody results, including AH1N1, AH3N2, IFZB, PNP14, PNP19, PNP2, PNP23, PNP6, PNP8, PNP9, and TETAB were obtained on Day 1 (C1D1) and Day 43 (C3D1) from some subjects in this study. It is not clear if BMS-663513 affected antibody levels due to lack of a control group where BMS-663513 was not administered. However, among subjects administered with BMS-663513, there were a proportion of subjects with increased antibody level (with positive log of fold change), and dose level or Cmin does not seem to affect the fold change of antibody level for almost all antibody tests.

Serum Cytokines: Three (3) cytokine tests, IL6, INFα, and TNFα, were obtained on Day 1 (C1D1), Day 2 (C1D2), Day 8 (C1D8), and Day 22 (C2D1) from some subjects in this study. Another cytokine, neopterin (NEOP), was obtained on Day 1 (C1D1), Day 2 (C1D2), Day 8 (C1D8), Day 22 (C2D1), Day 29 (C2D8), Day 43 (C3D1), and Day 50 (C3D8). The box plots showed that at least 75% of available subjects had an

increase of NEOP from baseline at all visit days except on Day 29 of 10 mg/kg cohort, where slightly more than 50% of subjects experienced a NEOP increase. Particularly, NEOP change from baseline tends to follow a similar pattern for subjects on 1 mg/kg and 3 mg/kg BMS-663513, that is, a relatively large increase on Day 8 followed by a relatively smaller increase on Day 22, and then relatively same level of increase on Day 1 as on days 29, 43 and 50. For subjects on 10 mg/kg BMS-663513, the increase at Day 8 tends to be smaller than for the subjects on the other two dose levels.

Immunophenotyping of Peripheral Blood: Flow cytometry data were obtained on Day 1 (C1D1), Day 8 (C1D8), Day 22 (C2D1), Day 29 (C2D8), Day 43 (C3D1), and Day 50 (C3D8). An exploratory analysis was performed to investigate the relationship between on-treatment activated CD4 T cells (T4ACT_P) and dose at each visit with a linear mixed model. The model included baseline values of activated CD4 T cells, visit day as categorical variable, the first dose, and interaction between visit day and the first dose. An unstructured correlation was specified to the within-subject measurements. The model showed that the inclusion of the first dose and its interaction with visit day did not improve significantly over the reduced model that included baseline and visit day only. The same model was also used for on-treatment activated T CD8 T cells (T8ACT_P) to explore its relationship with the first dose. Similarly, the first dose and its interaction with visit day do not significantly improve over the model without them.

Another linear mixed model was used to model the frequency of activated CD4 T cells (T4ACT_P) change from baseline by baseline values, and visit day as categorical variable. The same model was used to estimate the frequency of activated CD8 T cells (T8ACT_P) change from baseline. In general, both activated CD4 T cells change from baseline and activated CD8 T cells change from baseline showed a similar pattern over 3 cycles of dosing period, that is, a relatively larger increase on Day 8 followed by modest changes over time. Particularly for activated CD8 T cells, its value tended to fall to about the baseline value during Cycle 3 (days 43 and 50).

Correlations between all pairs of flow cytometry data were explored. Two pairs, naive CD4 T cells (T4NAI_P) and effector memory CD4 T cells (T4MEM_P), naive CD8 T cells (T8NAI_P) and effector memory CD8 T cells (T8EM_P), showed stronger correlation than all the other possible pairs.

Other Results:

Predictive Biomarkers: The results of the predictive biomarker analyses suggest that there were no correlations between the IHC markers and clinical response in this small sample set.

Tumor biopsies were collected from 14 different subjects enrolled in this study. Pre- and post-treatment biopsy samples were collected for 11 of the 14 subjects and pre-treatment samples only were collected for 3 of the 14 subjects. This breakdown is equivalent to 25 different tissue samples. QualTek stained serial sections of 22 of the 25 clinical samples with each of the following biomarkers: CD4, CD8, Interferon Gamma (IFNg), Perforin, Granzyme B, IDO, CD137, and FOXP3. QualTek also stained serial sections of the remaining 3 clinical samples with 4 or 5 of the biomarkers. Each biomarker labels a subset of lymphoid cells by IHC. The subject biopsy samples were scored for infiltration of positive lymphoid cells into areas of tumor for each biomarker. The tumor types tested included melanoma, renal carcinoma, and ovarian carcinoma. No evidence of tumor (NET) was present in sections of subject [redacted] stained with Granzyme B and INFg. As such, scoring results were not possible for these antibodies in this tissue. No infiltration score is provided for staining of subject [redacted] with CD4 because no evidence of tumor was present in this section.

Clinical responses were observed in the following subjects with IHC data:

- Pre-screen only: [redacted]
- Pre- and Post screen: [redacted]

Immunogenicity: The overall immunogenicity rate observed was 11%. An immunogenicity rate of 32% was observed for the 1 mg/kg dose cohort. Due to the number of subjects in dose cohorts at and above 3 mg/kg with trough concentrations above the drug tolerance of the assay, the immunogenicity rates at these doses may be underestimated. Although lower and undetectable rates of immunogenicity were observed at higher dose cohorts (3-15 mg/kg), an inverse relationship between immunogenicity and dose cannot be confirmed in the absence of more complete sampling analysis after withdrawal and clearance of BMS-663513.

CONCLUSIONS:

- No MTD for BMS-663513 was achieved using the maximum protocol-specified dose (15 mg/kg every 3 weeks) defined by the frequency of DLTs following a single treatment.
- The most frequent and clinically important toxicity (hepatotoxicity) can occur after any treatment cycle, but was observed to occur within the first 3 to 4 cycles of treatment in the majority of cases.
- The most common and clinically important BMS-663513-related toxicities were hepatotoxicity, neutropenia, thrombocytopenia, rash, pruritus, and pyrexia.
- The frequency and severity of rash, diarrhea, thrombocytopenia, and neutropenia were not related to the BMS-663513 dose. In contrast, AST/ALT elevations were similar across dose cohorts from 1 mg/kg to 15 mg/kg, but were diminished in rate and severity at the 0.3 mg/kg dose.
- Only subjects with melanoma attained responses. There was a trend for improved anti-tumor activity at the 1 mg/kg dose relative to the antitumor activity at doses above 1 mg/kg.
- The data from this study confirmed that following intravenous administration over 60 minutes, once every three weeks, BMS-663513 has linear PK in humans; Cmax and AUC increased in proportion to dose when administered at dose levels of 0.3 mg/kg to 15 mg/kg.
- Both CL and Vss showed no apparent dose dependency over the 0.3-15 mg/kg dose range
- Steady-state levels of BMS-663513 in serum are reached within 2-4 cycles following intravenous administration over 60 minutes, once every three weeks.
- BMS-663513 at all doses tested produced immunomodulatory effects. BMS-663513 dosed at 1, 3 and 10 mg/kg increased the frequency of activated CD4 and CD8 T cells and levels of interferon-inducible genes and neopterin, confirming the costimulatory effects of CD137 agonism on T and other cells of the immune system. In addition, reduced expression of immunoglobulins and B cell genes were observed suggesting that the effect of BMS-663513 in B cell function may be inhibitory. The magnitude of the changes was more pronounced after 8 days following the first dose than after the second or third dose.

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