STATISTICAL ANALYSIS PLAN

Red Hill Biopharma Ltd.
Tel Aviv, Israel

Protocol ABC-103

A Phase Ib Safety and Efficacy Study of Opaganib in Patients with Refractory or Relapsed Multiple Myeloma Who Have Previously Been Treated with Proteasome Inhibitors and Immunomodulatory Drugs

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SIGNATURES OF APPROVAL

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LIST OF ABBREVIATIONS

AE	adverse event
AHSCT	autologous haematopoietic stem cell transplant
aPTT	activated partial thromboplastin time (also PTT)
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC	area under the drug concentration-time curve
AUC_{0-12}	area under the drug concentration-time curve from 0-12 h
AUC_{0-T}	area under the drug concentration-time curve from 0-time of last measurement
$AUC_{0-T-\infty}$	AUC extrapolated from time 0 to infinity
$AUC_{0/\infty}$	ratio of AUC_{0-T} / $AUC_{0-T-\infty}$
BID	twice per day
BMA-CRM	Bayesian Model Averaging Continual Reassessment Method
BMI	body mass index
BUN	blood urea nitrogen
C_{max}	maximum observed plasma concentration
C_{min}	minximum observed plasma concentration
CR	complete response
CRF	Case Report Form
CTCAE	Common Toxicity Criteria adverse event
DLT	dose limiting toxicity
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EOT	End of treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
h	hour(s)
HBV	hepatitis B virus
HBVsAg	hepatitis B viral antigen
HCV	hepatitis C virus
HEENT	head, ears, eyes, nose and throat
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
IL-6	interleukin 6
IMWG	International Myeloma Working Group
INR	International normalized ratio
IRB	Institutional Review Board
ITT	intention to treat
λ_Z	apparent elimination rate constant
Kg	kilogram(s)
LDH	lactate dehydrogenase
MEDDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
MM	multiple myeloma
MTD	maximum tolerated dose
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NK	natural killer T-cells
ORR	overall response rate

LIST OF ABBREVIATIONS (continued)

PD	progressive disease
PFS	progression-free survival
PK	pharmacokinetic(s)
PR	partial response
P-R	the interval between the P and staat of the QRS on an ECG
PTT	prothrombin time
QRS	a specific interval of peaks and troughs on an ECG
QT	an interval between the Q and T waves on an ECG
QTc	a corrected form of the QT interval
RBC	red blood cell
RR	the interval between successive Q waves on an ECG
S1P	sphingosine 1-phosphate
SAE	serious adverse event
SAP	Statistical Analysis Plan
sCR	stringent complete response
SD	stable disease
SK	sphingosine kinase
SK1	sphingosine kinase-1
SK2	sphingosine kinase-2
SOC	system organ class
SPEP	serum protein electrophoresis
STD	standard deviation
TEAE	treatment-emergent adverse event
T_{LIN}	start of the log-linear elimination phase
T_{half}	terminal elimination half-life
USP/NF	United States pharmacopeia / national formulary
WBC	white blood cells

1.0 INTRODUCTION

This document provides the details of statistical analyses planned for Protocol No. ABC-103. In addition, it discusses the statistical issues relevant to these analyses (e.g., sample data to be used and missing data).

1.1 Background

Multiple Myeloma (MM) is a malignant plasma cell disorder with no standard curative therapy[1]. Symptomatic MM is characterized by a clonal proliferation of plasma cells preceding clinical findings that include bone lesions, fractures, anemia, renal failure and hypercalcemia[2]. MM affects 4.3 per 100,000 individuals yearly[3] and accounts for about 1% of all cancers and 10% of all hematological malignancies in the United States[2].

Sphingosine Kinase (SK) is an innovative molecular target for anti-cancer therapy because of its critical role in sphingolipid metabolism, which is known to regulate cancer cell proliferation and activation. SK is also a critical mediator of the actions of inflammatory cytokines and angiogenic growth factors. Therefore, inhibitors of SK are expected to have utility for the treatment of a variety of hyperproliferative, inflammatory and angiogenic diseases. Sphingomyelin is a building block for cellular membranes and also serves as the precursor for potent lipid messengers that have profound cellular effects. Ceramide is produced by the hydrolysis of sphingomyelin in response to several growth stimulatory and/or inflammatory signals. Ceramide induces apoptosis in tumor cells without disrupting quiescent normal cells. Additionally, ceramide can be further hydrolyzed by the action of ceramidase to produce sphingosine, which is phosphorylated by sphingosine kinases (SK1 and SK2) to produce sphingosine 1-phosphate (S1P). Studies in various cancer cell lines consistently demonstrate that S1P induces proliferation and protects cells from ceramide-induced apoptosis. Therefore, a critical balance, i.e. a ceramide / S1P rheostat, has been hypothesized to determine the fate of tumor cells. In this model, the balance between ceramide and S1P determines whether a tumor cell proliferates or undergoes apoptosis.

Because S1P is the direct mitogenic messenger, inhibition of its production should have antiproliferative effects on tumor cells, making SKs prime targets for inhibition. Research at Apogee has identified structurally novel “drug-like” inhibitors of SK1 and

SK2 [56-62], and opaganib was selected as the first compound in this program for clinical testing.

Inhibition of SK2 by treatment with opaganib effectively promotes apoptosis in MM cell lines and inhibits the proliferation of primary human myeloma cells. Furthermore, opaganib reduces the expression of Mcl-1 and c-Myc by inducing their proteasome degradation. opaganib effectively inhibited myeloma tumor growth *in vivo* in mouse xenograft models. Therefore, Red Hill and Apogee hypothesize that opaganib will have therapeutic activity in refractory/relapsed MM patients. In this phase Ib/II study, opaganib will be assessed for its safety and efficacy in refractory or relapsed MM patients who are previously treated with proteasome inhibitors and immunomodulatory agents.

2.0 OBJECTIVES

2.1 Primary Objectives

The primary objective of the phase 1b component of the clinical trial is to determine the maximum tolerated dose (MTD) and evaluate the safety of single agent opaganib in patients with relapsed or refractory multiple myeloma who have previously been treated with a proteasome inhibitor and an immunomodulatory agent.

2.2 Secondary Objectives

- To assess the antitumor activity of single agent opaganib in patients with refractory or relapsed MM after 3 cycles of treatment the treatment
- To describe the effects of opaganib on plasma levels of sphingosine 1-phosphate and IL-6 in patients with refractory or relapsed MM.
- To determine if pharmacodynamic markers (SK2 mRNA or activity, sphingolipid metabolites, c-Myc, Mcl-1 and pS6) in bone marrow CD138+ myeloma cells predict tumor response to the treatment with opaganib.
- To perform correlative studies including serum cell-free PINK1/PARK2 (mitophagy markers), cell free DNA for next generation sequencing (for signal pathway analyses), serum bone destruction/formation markers (TRAP, Osteocalcin), and bone gene markers and Lyn/Src gene expression in both CD138+ and CD138- cells.
- To evaluate immunologic function and immunologic panels (NK, NK T, T, B cells and myeloid derived suppressive cells (MDSCs) and the immune co-signalling molecules) in bone marrow environment.
- To determine the pharmacokinetics of opaganib following administration of a single dose on day 1 of cycle 1.

3.0 STUDY DESIGN

The study employed the Bayesian model average continual reassessment method (BMA-CRM) for dose escalation to determine the maximum tolerated dose of opaganib.

After signing informed consent, patients completed the evaluation process to determine eligibility. Once a patient was determined to be eligible for the study, he/she was enrolled in the dose escalation Phase Ib study testing 3 opaganib doses (250 mg, 500 mg, and 750 mg BID). A cycle of treatment was defined as 28 days. A single patient was enrolled weekly in a given dose group. Subsequent patients on each cohort were required enroll no sooner than one week after the previous patient. Patients in each dose cohort were required to complete cycle 1 and undergo toxicity evaluation before the next dose level cohort was opened. Up to 18 patients were used for phase 1b study.

A cohort of 3 patients was treated at each dose level. Individual patients continued to receive therapy at the same dose of opaganib provided they do not experience unacceptable toxicity or clinical disease progression. Per Protocol, study drug withheld from patients who experienced Grade 3 or 4 hematologic toxicity. If Grade 3 or 4 toxicity continued after 4 weeks, the patient was removed from the study. Each patient was to have an End of Treatment assessment scheduled 4-6 weeks after their last dose of study drug.

The Schedule of Assessments for both phases of the trial is presented in Table 1.

Table 1. Clinical and Laboratory Evaluation Schedule

Activity	Screening Phase [b]	Cycle 1 [a]				Cycle 2				Cycle 3				Cycle 4				Subsequent cycles	End of Treatment[u]	Follow-up[p]
		W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4	W 1	W 2	W 3	W 4			
Informed Consent [c]	X																			
Serum Pregnancy Test [d]	X																			
Study Registration [e]	X																			
History and Physical Exam[t]	X[s]	X[q]	X	X	X	X		X		X		X		X		X		X	X	X
ECOG Performance Status	X[s]	X[q]	X	X	X	X		X		X		X		X		X		X	X	
Vital Signs	X	X	X	X	X	X		X		X		X		X		X		X	X	
Assessment of Concomitant Medications	X[s]	X	X	X	X	X		X		X		X		X		X		X	X	
Serum chemistry [f]	X[r,s]	X[q]	X	X	X	X		X		X		X		X		X		X	X	X
PT/INR and aPTT [g]	X[s]	X[q]	X	X	X	X				X				X				X		
Complete blood count with differential [h]	X[s]	X[q]	X	X	X	X		X		X		X		X		X		X	X	X
Screening HIV, HBV and HCV [i]	X																			
12 Lead ECG [J]	X																			
Echocardiogram [k]	X																			
Urinalysis	X[s]	X[q]				X				X				X				X	X	
SPEP (serum protein electrophoresis)	X[s]	X[q]				X				X				X				X	X	
Serum free light chain	X[s]	X[q]				X				X				X				X	X	
Urine monoclonal protein	X[s]	X[q]				X				X				X				X	X	
IFE (immunofixation electrophoresis)	X[s]	X[q]				X				X				X				X	X	
Immunoglobulin profile	X[s]	X[q]				X				X				X				X	X	
Beta-2 microglobulin	X[s]	X[q]				X				X				X				X	X	
Metastatic bone survey [l]	X																	X		
Administration of ABC296460		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse Events Assessment [m]		X	X	X	X	X		X		X		X		X		X		X	X	
Review Patient Diary and Pill Count		X	X	X	X	X		X		X		X		X		X		X	X	
Plasma PK [n]		X																		
Plasma PD [v]		X				X				X				X				X[v]		
Bone marrow biopsy [o]	X												X					X[o]		

Table 1. Clinical and Laboratory Evaluation Schedule (continued)

a) Cycle = 28 days. For cycle 1 to 4, ± 3 days for all visits except cycle 1 day 1. For cycle 5 and beyond, ± 7 days for all visits.
b) Screening Evaluations must be completed within 30 calendar days prior to Treatment Day 1, except for bone marrow biopsy, which should be performed within 2 weeks prior to Treatment Day 1.
c) Informed Consent must be signed within 30 days before Treatment Day 1, and will be updated and re-signed by Investigator and Subjects, as needed.
d) Pregnancy test (for WOCBP) must be performed within 48 hours of first administration of study drug on Treatment Day 1.
e) Investigator must confirm that patient will not have taken chemotherapy for at least 14 days prior to Treatment Day 1.
f) Serum Chemistries will include: sodium, chloride, carbon dioxide, potassium, calcium, phosphorous, magnesium, BUN, creatinine, blood sugar (random), albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and bilirubin (total, direct).
g) INR and aPTT will be performed at screening, every week during cycle 1 and then the first week of each following cycle.
h) Complete blood count will include hemoglobin, hematocrit, total leukocyte count including differential, platelets.
i) Patients undergo HIV and hepatitis screening (HBVsAg, anti-HBs, anti-HBc; anti-HCV qualitative).
j) ECGs will be obtained during screening.
k) Performed at baseline and end of treatment, and if patient demonstrates signs or symptoms of myocardial degeneration.
l) Metastatic bone survey: at the screening, then every year or at the end of treatment.
m) Performed according to CTCAE version 4.
n) Plasma for PK analysis opaganib alone will be collected Cycle 1, Day 1 immediately before dosing and at approximately 1, 2, 4, and 8, hours after dosing and will be sent to Dr. Kang's lab and/or Apogee Biotechnology Corporation for analysis.
o) Bone marrow biopsy must be obtained prior to initiation of treatment with opaganib (within 2 weeks prior to the treatment), at the end of cycle 3 (± 7 days) and at the end of cycle 6 (± 7 days). For patients who discontinue therapy for any reason before completing 3 cycles, bone marrow biopsy will be obtained at the time of discontinuation or as soon as feasible thereafter. After cycle 6, the bone marrow biopsy will be performed at the discretion of the treating physician.
p) If the patient was removed from treatment for toxicity, the patient should be followed with weekly clinic visit and laboratory studies as appropriate, at Duke Cancer Center or the patient's local MD, until the AE has resolved to baseline.
Phase II patients should be followed every 3 months for survival, for 2 years from the time of their End of Treatment visit.
q) If Screening was performed within 1 week prior to Cycle 1, Day 1, these procedures/labs do not need to be repeated. If urine monoclonal protein was performed within 14 days of Cycle 1, Day 1, it will not need to be repeated.
r) Includes LDH at screening.s) Screening procedures/labs which can be assessed prior to drug administration may be completed on Cycle 1, Day 1, if necessary.
t) Physical exam at screening, and day 1 of each cycle will be a focused exam including vital signs, HEENT, heart/lung/chest/abdomen/skin and neurological assessment. Physical exam at all other time points will be symptom-directed.
u) EOT should occur within 4-6 weeks after the last dose of study drug.
v) PD samples will be collected on Cycle 1, Day 1 prior to study drug administration and at approximately 1, 2, 4, and 8, hours after dosing along with PK samples and prior to opaganib drug administration on D1 of each subsequent cycle through C6.

3.1 Sample Size Determination

The BMA-CRM was used for dose escalation to determine the MTD from among 3 candidate dose levels of opaganib. No more than one patient was enrolled per week, per dose. Subsequent patients in each cohort were enrolled 7 or more days after the previous patient. Patients in each dose cohort were required to complete cycle 1 and undergo toxicity evaluation before the next dose cohort could be initiated. Up to 18 patients were to be enrolled into this phase 1b study. The patients at the MTD in Phase 1b were to continue on to the phase 2 portion of the trial. However, due to slow accrual, a decision was made to terminate the trial without advancing to phase 2.

4.0 STUDY PROCEDURES

4.1 Pharmacokinetic Sampling

All PK sampling was done on Day 1 of Cycle 1 immediately before their morning dose. Blood samples for PK assessment were drawn from each subject at 1, 2, 4, and 8 hours after the morning dose. All subjects received an afternoon dose approximately 12 hours after the C1D1 morning dose.

4.2 Pharmacodynamic Sampling

Pharmacodynamic assays were employed to verify the hypothesized effects of opaganib on S1P in the plasma and in bone marrow as well as on expression of c-Myc and Mcl-1 oncogenes on myeloma cells. A blood sample was drawn from each subject on Day-1 of Cycle-1. A bone marrow biopsy also was obtained from each subject within 14 days prior to initiation of opaganib treatment. Biopsies were also to be obtained at the end of cycle 3 (± 7 days) and at the end of cycle 6 (± 7 days) and/or at the time of discontinuation or shortly thereafter. However, since only one subject had a post-treatment marrow biopsy, there is not sufficient data to compare pre- vs. post-treatment PD profiles. Therefore, bone marrow PD endpoint summaries will be limited to the baseline assessments.

The key plasma and bone marrow endpoints for pharmacodynamic and mechanistic studies are summarized in Table 2.

Table 2. Pharmacodynamic Endpoint Assays

PD endpoint	Sample Source	Sampling Times
S1P levels	Peripheral blood plasma	In the PK draws and at the beginning of each cycle prior to opaganib drug administration (up to cycle#6)
IL-6 level	Peripheral blood plasma	At the beginning of each cycle prior to opaganib drug administration (up to cycle#6)
Cell free PINK1/PARk2 expression, Serum bone formation/destruction markers	Peripheral blood plasma	At the beginning of each cycle prior to opaganib drug administration (up to cycle#6)
Cell free DNA for next generation sequencing	Peripheral blood plasma	At the beginning of each cycle prior to opaganib drug administration (up to cycle#6)
Ceramide, sphingosine, and S1P levels	Bone marrow supernatant	At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6
SK mRNA or activity in CD138+ cells	Bone marrow CD138+ cells	At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6
Protein level of c-Myc, Mcl-1 and pS6 on CD138+ cells)	Bone marrow CD138+ cells	At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6
Gene expression (Lyn/Src, Osteoclast/osteoblast genes, mitophagy markers)	Bone marrow CD138+ cells and CD138- cells	At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6
Immunologic function and immunologic panel (immune cells and co-signaling molecules)	Bone marrow aspirate	At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6

4.3 Response Assessment

Treatment response assessment and full myeloma restaging were to be performed at the end of Cycles 3 and 6 (+/- 7 days) and consisted of serum protein electrophoresis, serum and urine immunofixation, 24h urine protein electrophoresis, serum free light chains, beta-2 microglobulin, bone marrow aspiration and biopsy, complete blood counts and metabolic panel. Disease response were categorized according to the International Myeloma Working Group (IMWG) uniform response criteria (Durie et al. 2006; Appendix A).

4.4 Blinding and Randomization

This is an open-label Phase 1b study. Subjects were enrolled sequentially to the next scheduled dose cohort in the dose-escalation phase. No randomization was performed.

5.0 ANALYSIS POPULATIONS

5.1 Intent-to-Treat Population

The efficacy analysis will be performed using the intent to treat (ITT) population that is composed of all patients enrolled and who have received at least one dose of study medication.

5.2 Safety Population

The safety population comprises all subjects receiving any amount of study drug. In safety analyses, subjects will be grouped according to treatment actually received.

5.3 Pharmacokinetic Population

The PK population is defined as all subjects who provided evaluable opaganib plasma concentration data on day 1 of cycle 1. These patients should have at least one quantifiable plasma concentration, should not have violated any major entry criterion likely to confound the PK analysis, and should not have deviated significantly from the protocol between enrollment and successful study completion.

5.4 Protocol Deviations

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the sponsor and the IRB and agreed to by the investigator. A major deviation occurs when there is nonadherence to the protocol by the subject or investigator that jeopardizes the scientific soundness of the study or the rights, safety, or welfare of patients. Major deviations can include any deviation from the investigational plan to protect the life or physical well-being of a patient, collection of data outside the allowable sampling or assessment times, missing or otherwise invalid baseline data, nonadherence to inclusion or exclusion criteria, enrollment of the subject without prior sponsor approval, nonadherence to FDA regulations or ICH GCP guidelines, or concurrent use of prohibited medications and will lead to the subject being withdrawn from the study.

5.5 Subject Disposition

Subject disposition will be summarized and tabulated for ITT, PK and Safety populations. The summaries will include the number and percentage of subjects that were evaluable for safety (i.e., received at least 75% of the planned initial 28-day cycle of therapy and 30 days' follow-up after the last dose unless they discontinued or received less due to toxicity). Each patient's reason for study discontinuation will be listed and tabulated.

6.0 GENERAL STATISTICAL ANALYSIS SPECIFICATIONS

6.1 Descriptive and Summary Statistics

Summary tables of data will be provided by dose and (where appropriate) by cycle, showing the number of subjects with non-missing data (n), mean, standard deviation (STD), median, minimum and maximum for continuous variables, and counts and percentages for categorical variables. For categorical measurements collected by cycle within dose cohort, the denominator of percentages will be the number of subjects with a nonmissing value within each cohort.

All statistical analyses will be performed and data displays will be created using SAS®, Version 9.4. eCRFs will be used to capture study results and data.

Percentages will be rounded to one decimal place, except 0% and 100% will be displayed without any decimal places. Minima and maxima will be rounded to the precision of the original value; means and medians will be rounded to one decimal place greater than the precision of the original value; STDs will be rounded to two decimal places greater than the precision of the original value.

A subject's baseline value will be defined as the latest non-missing value prior to taking opaganib on day 1 of cycle 1.

Subject data listings will be provided for variables that are that are summarized in tables or figures.

6.2 Endpoints

6.2.1 Safety Endpoints

- The primary endpoint in this study is the maximum tolerated dose (MTD) of opaganib. The MTD is the dose at which the estimated toxicity probability based on all 18 patients is closest to the targeted probability 0.33 among all doses.
- Adverse events (AEs) and serious adverse events (SAEs) that are considered related to study drug, all events of death, and any study specific issue of concern will be monitored and reported.

- Dose limiting toxicities (DLTs) will be graded according to the NCI CTCAE version 4.0.3 criteria. DLTs will be defined as any of the following events that are at least (possibly, probably, or definitely) attributable to Opaganib during dose escalation:

  Non hematologic DLT is defined as:
  Any Grade ≥ 3 AE, with the following exceptions: Symptomatic adverse events such as nausea, vomiting and diarrhea will not be considered dose limiting if they can be reduced to less than grade 3 within 72 hours with standard supportive measures such as antiemetics and antidiarrheals.

  Hematologic DLT is defined as:
  ≥Grade 4 neutropenia or thrombocytopenia that lasts more than 7 days after the last dose of study drug; ≥Grade 3 thrombocytopenia in the presence of ≥grade 3 hemorrhage of any organ/site; Any grade 5 hematologic toxicity

- Standard clinical labs including hematology, serum chemistries, coagulation tests and urinalysis endpoints will be collected in each cycle according to the schedule of clinical and lab evaluations (Table 1).

- Vital signs (pulse, respiration rate, blood pressure, oral temperature, body weight) will be recorded and physical exams of body systems, organs and tissues will be conducted throughout the trial (Table 1).

6.2.2 Efficacy Endpoints

- Treatment response will be determined using IMWG criteria after 3 cycles and 6 cycles of treatment (Appendix A). Frequencies and rates of Stringent complete response, complete response, partial response, stable disease, and progressive disease will be reported.

- Myeloma markers that form the basis for the IWMG criteria will include: serum M-Protein (g/dl), serum free light chain κ (mg/dl), serum free light chain λ (mg/dl), serum free light chain κ/λ ratio, urine M-Protein (mg/dl). These endpoints will be summarized in spaghetti plots of each subject's assessed values (and/or corresponding change from baseline values) over time from baseline to EOT.

6.2.3 PD and Correlative Endpoints

Pharmacodynamic (PD) endpoints will be computed from data obtained from blood samples collected immediately before dosing and at 1, 2, 4, and 8 hours after dosing on day 1 of cycles 1-6. Sufficient data were obtained for the following PD endpoints to support descriptive analyses:

- Plasma sphingosine 1-phosphate (performed using the PK samples and at the beginning of each cycle prior to opaganib drug administration)
- Plasma IL-6 performed at the beginning of each cycles for the first 6 cycles
- Ceramides, sphingosine and sphingosine 1-phosphate concentrations (assessed on the bone marrow aspirate supernatants at the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6)
- Immunologic function and immunologic panel endpoints including: NK, NK T, T, B cells and myeloid derived suppressive cells (MDSCs) and the immune co-signaling molecules in the bone marrow environment.

6.2.4 PK Endpoints

At minimum, the following PK endpoints will be computed from data obtained from blood samples collected immediately before dosing and at 1, 2, 4, and 8 hours after dosing on days1 of cycle 1.

- C_{max} = Maximum observed plasma concentration
- T_{max} =Time of maximum observed plasma concentration; if it occurs at more than one time point, T_{max} is defined as the first time point with this value
- AUC_{0-8} = Cumulative area under the plasma concentration time curve calculated from 0 to 12 hours using the linear trapezoidal method

If the terminal elimination phase is reached by the eighth hour post-dose, additional PK parameters will be estimated and reported.

6.3 Specifications for Displays

Displays (including headers) will be produced by using the Courier New 8-point font. All displays are intended to be printed as landscape on 8.5- × 11-in paper. The top and bottom margins will be 0.50 in, and the left and right margins will be 0.75 in. Further details related to these specifications are provided in Section 6.1

6.4 Methods for Handling Dropouts and Missing Data

All data will be summarized as observed. No imputation will be made for missing data, with the exception that partially missing dates will be imputed to the least favorable date, where necessary. For example, adverse events (AEs) with partially missing dates that occur in the same month as the first dose of study drug that was administered will be considered treatment emergent.

For the analysis of tumor response, patients lost to follow-up prior to the 12-week evaluation will be considered failures.

In the case where concentrations for PK analyses cannot be determined due to bioanalytical or clinical reasons, these values are to be set to missing for the statistical and pharmacokinetic analysis. Concentrations below limit of quantitation (coded BLQ) will be treated as zeroes for all analyses.

6.4 Interim Analysis

There are no interim analyses planned for this study.

7.0 DEMOGRAPHIC AND BASELINE ASSESSMENTS

This section will summarize data that were collected prior to subjects receiving their first dose of opaganib.

7.1 Demographic and Baseline Characteristics

The continuous demographic variables; age (years), baseline height (cm), baseline weight (kg), baseline body mass index (BMI) and body surface area (BSA), will be summarized using descriptive statistics; while sex, race, and ethnicity will be provided using frequency tabulations. Age will be calculated as the integer part of (Informed Consent Date - Birth Date + 1)/365.25. BMI will be calculated as kg/m2 where kg is a person's weight in kilograms and m2 is their height in meters squared.

7.2 Electrocardiogram

Screening assessments of 12-lead electrocardiogram parameters including RR, P-R interval, QRS, QT, QTc and whether or not it was normal, will be recorded for each subject at the screening visit.

7.3 Echocardiogram

Clinical significance, ejection fraction (%) and whether or not the Screening echocardiogram (and end of study assessments if done) was normal, will be recorded for each subject.

7.4 Medical History/Prior Therapies/Prior Therapies line of treatment

Prior myeloma therapies will be listed by opaganib dose. Category (e.g., Hematopoietic stem cell transplant, agent, proteasome inhibitors, etc.), start and stop dates and whether or not the therapy is ongoing and IMWG response category (i.e., sCR, CR,…Clincal relapse) will be included in the listings.

8.0 EVALUATION OF TREATMENT EXPOSURE AND COMPLIANCE

8.1 Treatments

Opaganib capsules contain 250 mg of the milled active pharmaceutical ingredient (API) opaganib along with the excipients microcrystalline cellulose, USP/NF (Avicel® PH102, FMC) (FMC biopolymer) and colloidal silicon dioxide, NF (Cab-O-Sil® M5P). Opaganib is a white to off-white powder and for clinical use it is encapsulated in white opaque hard gelatin capsules. The study drug will be administered approximately every 12 hours, under fasting conditions or after a light to moderate meal for 28 days according to the dose escalation and de-escalation guidelines outlined in Section 4 of the protocol.

8.2 Exposure to Study Treatment

Depending on the dose group to which they were assigned, each subject will receive one, two or three 250 mg capsules of opaganib, twice a day, on each day of each 28-day cycle. Because subjects will be self-administering their medications, it is possible the subject may not always take all of 2, 4 or 6 of their capsules on a given day and/or may miss one or more days of dosing (e.g., because of severe nausea). To address these issues, three measures of patient exposure to AV-101 (total amount received during the trial, average total daily exposure (mg), planned total daily exposure (mg)) and 2 measures of compliance (relative daily exposure, % of scheduled doses taken) will be computed over each subject's duration of dosing.

Subjects will record the number of capsules they took in a daily dosing diary that will be returned to the site at the end of the cycle. Accurate records will be kept at the sites regarding when and how much test article is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. For a given subject, in a given cycle, the maximum total dose received is 250 mg × total number of capsules taken during the 28 day cycle. The average daily dose is then computed as:

$$\text{Avg. actual daily exposure (mg)} = \frac{Total\ over\ all\ days\ a\ dose\ (mg) was\ to\ be\ taken}{number\ days\ patient\ took\ at\ least\ 1\ capsule}$$

8.3 Compliance to Planned Exposure and Dosing Schedule

Given the estimated exposure measures, two components of compliance during a given stage of the study can then be computed as:

$$\text{Relative daily exposure} = \frac{avg\ daily\ exposure\ \ (mg)}{expected\ daily\ exposure\ (mg)} \times 100,$$

where expected daily exposure = 500 mg or 1000 mg or 1500 mg, depending on the dose cohort.

$$\%\ \text{Compliance} = \frac{total\ number\ days\ over\ all\ cycles\ that\ patient\ was\ dosed}{number\ scheduled\ dosing\ days} \times 100,$$

where number of scheduled dosing days = number of days the subject is in the study and taking study medication.

Exposure and Compliance will be summarized by dose cohort for all subjects who received at least one dose of opaganib.

9.0 STATISTICAL METHODS

SAS® (SAS Institute, Inc.) version 9.4 will be used for all statistical analyses described in this document with the exception of the BMA-CRM.

Inferential analyses reported in this study generally will include statistics such as 2-sided 95% confidence intervals (CI), and p-values. Unless stated otherwise, all statistical tests will be 2-sided hypothesis tests, performed at the 0.05 level of significance.

All data will be included in subject data listings for all subjects.

A final, statistical report that will include both efficacy and safety evaluations will be generated upon completion of the trial. The final report will be distributed to the Trial Steering Committee.

9.1 Primary Endpoint Analysis

Per Protocol, a Bayesian model averaging continual reassessment method (BMA-CRM)[11-13] was applied for dose escalation. Based on the BMA-CRM results, the MTD was determined to be 750 mg.

9.2 Secondary Analyses

The secondary analyses were intended to assess tumor response to opaganib and to explore mechanistic relationships among tumor markers, gene expression and clinical laboratory endpoints that may mediate tumor response to opaganib dosage.

9.2.1 Efficacy Analysis

The objective response rate (ORR) of subjects receiving opaganib treatment will be calculated as the proportion of subjects who achieved a partial response (PR) or better by the end of their last response assessment in the trial. Tumor response will be further investigated descriptively through waterfall plots of subjects' percent change from baseline M-protein measure (e.g., κ/λ light chain ratio) associated with subjects' best IMWG categorical response (e.g., CR), by subjects' opaganib dose group.

9.2.2 PD and Correlative Analyses

All pharmacodynamic endpoints will be listed for every subject. Spaghetti plots of selected PD endpoint (lipids and cytokines) values and/or change from baseline over time since C1D1 will be graphed for those PD parameters that were measured at baseline and on at least one occasion post-C1D1. Baseline data for all parameters will be summarized (n, mean, std. dev. and range), by dose group, in a table. Spearman linear rank correlations between baseline S1P or IL-6 and the total time each subject was dosed with opaganib (ie, total duration) will be computed. Scatterplots of S1P vs. total duration and of IL-6 vs total duration will be made with subject dose indicated by symbol type and symbol color. Coordinate points for the 3 doses will be plotted within the same scatter plot for visual comparison. Dose-specific values of the correlations will be printed in an open corner of the Graphs.

9.3 PK Analysis

PK endpoints will be computed from data obtained from blood samples collected immediately before dosing and at 1, 2, 4, and 8 hours after dosing on days1 of cycle 1. Pharmacokinetic analyses will be generated using validated pharmacokinetic software (i.e. Phoenix® WinNonlin® version 6.3 or higher, Phoenix® Connect™ version 1.3.1 or higher).

Below limit of quantitation concentrations (coded BLQ) were to be treated as zero for all analyses. All reported sampling time deviations will be taken into consideration for evaluation of PK parameters. In the case where concentrations could not be determined due to bioanalytical or clinical reasons, these values will be set to missing for the statistical and pharmacokinetic analysis.

The main plasma absorption and disposition parameters will be estimated using a non-compartmental approach with a log-linear terminal phase assumption. The trapezoidal rule was to be used to estimate the area under the curve (linear trapezoidal linear interpolation) and the terminal phase will be estimated by maximizing the coefficient of determination estimated from the log-linear regression model. However, disposition parameters were not to be estimated for individual concentration-time profiles where the terminal log-linear phase could not be reliably characterized.

Descriptive statistics will be calculated for plasma concentrations at each individual time point and for all PK parameters. The individual plasma concentration/time profiles will be presented using the actual sampling times whereas the mean plasma concentration/time profiles will be presented using the theoretical sampling times.

9.4 Safety Analysis

The safety population will consist of all subjects who received any dose of study drug. The main analyses will be frequency of DLTs and SAEs including mortality.

9.4.1 Adverse Events

An AE is any untoward medical occurrence in a study subject administered a study drug, whether or not considered drug related. This can be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, without any judgment of causality

For the purposes of this study, the period of observation for AEs extends from the time the subject is administered study drug (i.e., randomization) until the end-of-treatment (EOT) procedures are completed. All AEs that are noted prior to study drug administration are considered to be medical history and are recorded as such.

For the purposes of this study, the period of observation for SAEs extends from the point of giving informed consent until the end of the study. If the investigator detects an SAE in a study subject after the end of the period of observation and considers the event possibly related to prior study treatment, he or she should contact the sponsor to determine how the SAE should be documented and reported.

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable.

AEs with a missing start date or time that leads to ambiguity in whether or not the AE is treatment-emergent will be considered treatment-emergent. For AEs ongoing at the end of the study/early termination visit, the duration of an AE will be calculated by imputing the stop date to the termination/early discontinuation date.

Denominators for AE summary tables should be number of subjects receiving treatment in the corresponding dose group.

A treatment emergent AE (TEAE) is defined as any event not present prior to exposure to any of the three study medications or any event already present that worsens in either intensity or frequency following exposure to any of the study drugs. Unless otherwise stated, all AEs referenced in this SAP may be regarded as TEAEs.

TEAE analyses will consider the frequency of TEAEs and discontinuations due to TEAEs. TEAEs will be summarized by presenting, for each treatment group, the number and percentage of subjects having at least one AE, having an AE in each body system and preferred term, by severity and relatedness to study medication. The frequencies and incidences of AEs occurring in subjects in the drug and placebo control groups will be summarized within treatment group by the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC). The frequencies and incidences of TEAEs and discontinuations due to TEAEs occurring in subjects in the AV-101 and placebo control groups will be summarized within treatment group.

If a given subject had more than one AE mapped to the same preferred term, then that subject will be counted only once within that preferred term.

When reporting TEAEs by maximum toxicity grade (1-5), if a given subject had more than one AE mapped to the same preferred term, then that AE will be counted once according to the maximal level of toxicity.

When reporting TEAEs by relationship to study treatment, if a given subject had more than one AE mapped to the same preferred term, then that AE will be counted once according to the highest level of relatedness (Related, Probably, Possibly, Unrelated).

The following summaries (tables) of AEs and TEAEs will be provided by number (percentage) of subjects for each treatment group; where treatment group is defined by the treatment (opaganib dose cohort) the subject was randomized to at the time the AE was first recorded:

- All AEs - Overview
- All TEAEs by MedDRA SOC and by preferred term

- All TEAEs by relationship to study treatment (Related, Probably, Possibly, Unrelated) by MedDRA SOC and by preferred term.
- All TEAEs by maximum toxicity (Grades 1-5) by MedDRA SOC and by preferred term.

The following listings of AE occurrences will be provided:

- All AEs by MedDRA SOC and by preferred term
- All SAEs by MedDRA SOC and by preferred term
- All DLTs
- All AEs resulting in study discontinuation by MedDRA SOC and by preferred term
- All AEs leading to death by MedDRA SOC and by preferred term

9.4.2 Vital Signs

Vital signs obtained during the course of the study will be presented by patient in listings. Clinically significant abnormalities in vital signs were recorded as adverse events and will be included in adverse event summaries and listings.

9.4.3 ECOG Scores

Spaghetti plots of subjects' ECOG scores will be made, by dose group, over the time from baseline to EOT.

9.4.4 Electrocardiograms

Screening Electrocardiogram parameters of all subjects who were randomized to study drug will be included in listings. Clinically significant abnormalities in screening ECGs will be included in medical history.

9.4.5 Echocardiograms

Screening (and EOT, if collected) echocardiogram parameters of all subjects who were randomized to study drug will be summarized by dose cohort and tabulated.

9.4.6 Clinical Laboratory Results

Values of all clinical chemistry, hematology and urinalysis parameters will be listed by subject and assessment date. Spaghetti plots will be made, showing individual subject profiles by dose cohort, over time from baseline to EOT, for the following clinical selected laboratory parameters:

Endpoint Category	Endpoint
Hematology	Hemoglobin (g/dl)
	Platelets ($\times 10^9$/L)
	WBC($\times 10^9$/L)
	Neutrophils($\times 10^9$/L)
	Lymphocytes($\times 10^9$/L)
	Monocytes($\times 10^9$/L)
	Eosinophils($\times 10^9$/L)
	Basophils($\times 10^9$/L)
	%circulating plasma cells
Clinical Chemistry	Urea nitrogen (mg/dl)
	Creatinine (mg/dl)
	Serum Glucose (mg/dl)
	Calcium (mg/dl)
	AST (U/L)
	ALT (U/L)
	Total bilirubin (mg/dl)
	Albumin(g/dl)
	Total Protein(g/dl)
	LDH(U/L)
	Uric acid(mg/dl)
	Alkaline Phosphatase(U/L)
	β2-microglobulin(mg/dl)

9.4.7 Prior and Concomitant Medications

Prior and concomitant medications will be listed by WHO Drug preferred name within dose group.

10.0 REFERENCES

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11.0 APPENDIX A. IMWG UNIFORM MULTIPLE MYELOMA RESPONSE CRITERIA

Response category	Criteria
Stringent complete response (sCR)	CR as defined below plus • Normal FLC ratio • Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
Complete response (CR)	• Negative immunofixation of the serum and urine and • ≤ 5% plasma cells in bone marrow and • Disappearance of any soft tissue plasmacytomas.
Very good partial response (VGPR)	• Serum and urine M-protein detectable by immunofixation but not on electrophoresis or • 90% or greater reduction in serum M-protein plus urine M-protein level <100 mg per 24 hour.
Partial response (PR)	≥ 50% reduction of serum M-protein and reduction in 24-hour urinary M-protein by ≥ 90% or to < 200 mg per 24 hour. • If the serum and urine M-protein are unmeasurable, a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required In place of the M-protein criteria • If serum and urine M-protein are unmeasurable, and serum free light assay is also unmeasurable, ≥ 50% reduction in bone marrow plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30% • In addition to the above listed criteria, if a plasmacytoma is present at baseline, ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
Progressive Disease	Progressive Disease requires any one or more of the following: Increase of ≥25% from baseline in • Serum M-component and/or (the absolute increase must be ≥0.5 g/dl) • Urine M-component and/or (the absolute increase must be ≥200 mg/24hour • Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels. The absolute increase must be >10 mg/dl • Bone marrow plasma cell percentage: the absolute % must be ≥1-% • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas • Development of hypercalcemia (corrected serum calcium > 11.5 mg/dl or 2.65 mmol/l) that can be attributed solely to the plasma cell proliferative disorder

Response category	Criteria
Clinical relapse	Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features). It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice 1. Development of new soft tissue plasmacytomas or bone lesions 2. Definite increase in the size of existing plasmacytmas or bone lesions. A definte increase is defined as a 50% (and at least 1cm) increase as measured serially by the sum of the products of the corr-diameters of the measurable lesion. 3. Hypercalcemia (> 11.5 mg/dl) [2.65 mmol/l] 4. Decrease in hemoglobin of ≥ 2g/dl [1.25 mmol/l] 5. Rise in serum creatinine by 2 mg/dl or more [177 micro mol/l or more]