# A Phase Ib/II Safety and Efficacy Study of ABC294640 in Patients with Refractory or Relapsed Multiple Myeloma Who Have Previously Been Treated with Proteasome Inhibitors and Immunomodulatory Drugs.

DUKE CANCER INSTITUTE

A National Cancer Institute-designated Comprehensive Cancer Center

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| **Sponsor:** | RedHill Biopharma Ltd., 21 Ha'arba'a St., Tel Aviv 64739, Israel |
| **Funding Source:** | NIH, Apogee Biotechnology Corporation and RedHill Biopharma Ltd. |
| **Protocol Source:** | PI - Duke Cancer Institute |
| **Duke IRB#:** | Pro00062304 |
| **RedHill Study ID** | ABC-103 |
| **IND#** | 124650 |

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|---|---|---|---|
| **Original version:** | September 15, 2014 | **Amended version 8:** | May 17, 2017 |
| **Amended version 1:** | March 19, 2015 | **Amended version 9:** | October 5, 2017 |
| **Amended version 2:** | May 1, 2015 | **Amended version 10:** | April 11, 2018 |
| **Amended version 3:** | May 27, 2015 | **Amended version 11:** | December 4, 2018 |
| **Amended version 4:** | June 15, 2015 | **Amended version 12:** | March 22, 2019 |
| **Amended version 5:** | June 28, 2016 | | |
| **Amended version 6:** | August 19, 2016 | | |
| **Amended version 7:** | October 12, 2016 | | |


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## PROTOCOL SYNOPSIS

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| **TITLE** | **A Phase Ib/II Safety and Efficacy Study of ABC294640 in Patients with Refractory or Relapsed Multiple Myeloma Who have Previously Been Treated with Proteasome Inhibitors and Immunomodulatory Agents** |
| **PHASE** | Ib/II |
| **STUDY RATIONALE** | ABC294640 [3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridin-4-ylmethyl) amide, hydrochloride salt] is an orally available inhibitor of the enzyme sphingosine kinase-2. SK2 is an innovative molecular target for anti-cancer therapy because of its critical role in sphingolipid metabolism, which is known to regulate tumor cell death and proliferation. Our preclinical studies demonstrate that SK2 is overexpressed in multiple myeloma (MM) cell lines and in human MM specimens. Additionally, inhibition of SK2 by RNA interference or treatment with ABC294640 effectively promotes apoptosis in MM cell lines and inhibits the proliferation of primary human myeloma cells. Furthermore, ABC294640 reduces the expression of Mcl-1 and c-Myc by inducing their proteasome degradation. ABC294640 effectively inhibited myeloma tumor growth *in vivo* in mouse xenograft models. Therefore, we hypothesize that ABC294640 will have therapeutic activity in refractory/relapsed MM patients. ABC294640 has recently completed the Phase I safety study and demonstrated an excellent safety profile. In this phase Ib/II study, ABC294640 will be assessed for its safety and efficacy in refractory or relapsed MM patients who are previously treated with proteasome inhibitors and immunomodulatory agents. |
| **STUDY DESIGN** | This is a Phase Ib/II safety and efficacy trial of single agent ABC294640 in refractory or relapsed MM. Cohorts of patients with refractory or relapsed MM who have previously been treated with proteasome inhibitors and immunomodulatory agents will receive increasing doses of oral ABC294640. HIV and hepatitis B and C serology screening will be performed during patient screening. Patients with HIV infection or active hepatitis B or C infection with abnormal liver functions (i.e., LFTs > 2 x upper normal limits) will be excluded. The starting dosage for ABC294640 will be 250 mg BID (known to be safely tolerated as a single-agent), and the ABC294640 dose will be escalated to two additional dose cohorts of 500 and 750 mg BID using Bayesian model average continual reassessment method (BMA-CRM) for dose finding. It is expected that 18 patients will be used to determine the MTD for ABC294640 in refractory or relapsed MM. Pharmacokinetic (PK) assessments of ABC294640 will be conducted on Day 1of Cycle 1. Pharmacodynamics (PD) assessments will be conducted on Day 1 of each cycle. Bone marrow biopsy will be obtained prior to the initiation of ABC294640, at the end of cycle #3 and at the end of cycle #6. In addition to SPEP, UPEP and serum free light chain measurement, correlative studies will be performed to measure SK2 mRNA or enzyme activities, sphingosine metabolites, c-Myc, Mcl-1 and pS6 in CD138+ myeloma cells. |
| **OBJECTIVES – PHASE IB** | **Primary Objectives**<br>• To assess safety and determine the maximum tolerated dose (MTD) of single agent ABC294640 in patients with refractory or relapsed MM who have been previously treated with proteasome inhibitors and immunomodulatory agents.<br>**Secondary Objectives**<br>• To assess the antitumor activity of single agent ABC294640 in patients with refractory or relapsed MM after 3 cycles of treatment.<br>• To determine the pharmacokinetics of ABC294640 following administration of the drug. |

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| | • To describe the effects of ABC294640 on plasma levels of sphingosine 1-phosphate, IL-6, and other cytokines in patients with refractory or relapsed MM.<br>• To assess pharmacodynamic markers (SK2 mRNA level or activity, sphingolipid metabolites, c-Myc, Mcl-1 and pS6) in bone marrow CD138+ myeloma cells. For patients who receive more than 3 cycles of treatment and have a repeated bone marrow biopsy, RNA sequencing and Assay for transposases accessible chromatin sequencing (ATAC seq) will be performed to assess the effects of ABC294640 on global gene and epigenetic expression.<br>• 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 markers and Lyn/Src gene expression in both CD138+ and CD138- cells.<br>• 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 |
| **NUMBER OF PATIENTS (PHASE 1B)** | Up to eighteen patients will be enrolled in the phase IB portion of the trial. |
| **OBJECTIVES – PHASE II** | **Primary Objectives**<br>• Assess overall treatment response rate and overall survival in patients with relapsed or refractory MM treated with single-agent ABC294640.<br>**Secondary Objectives**<br>• To assess the treatment response of ABC294640 in patients with refractory or relapsed MM after 3 cycles of treatment.<br>• 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 ABC294640.<br>• 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.<br>• 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 |
| **NUMBER OF PATIENTS (PHASE II)** | 59 (may include 3 patients treated at MTD in Phase Ib, thus 56 additional patients) |
| **PATIENT ELIGIBILITY CRITERIA** | **Inclusion criteria**<br>1. Patient must have a diagnosis of symptomatic multiple myeloma, who relapses or is refractory after previous treatment with a proteasome inhibitor (bortezomib or carfilzomib) and an immunomodulatory agent (thalidomide, lenalidomide or pamolidomide).<br>2. Have measurable disease as defined by at least one of the following:<br>- Serum monoclonal (M) protein ≥0.5 g/dl by protein electrophoresis<br>- >200 mg of M protein in the urine on 24-hour electrophoresis<br>- Serum immunoglobulin free light chain ≥10 mg/dL AND abnormal serum immunoglobulin kappa to lambda free light chain ratio<br>- Monoclonal bone marrow plasmacytosis ≥30%<br>3. Voluntary signed and dated institutional review board (IRB) approved informed consent form in accordance with regulatory and institutional guidelines.<br>4. Time interval from last systemic chemotherapy (not including low dose |

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| | dexamethasone) more than 2 weeks prior to initiation of ABC294640. Patients receiving high dose dexamethasone defined as 40mg dexamethasone a day for 4 days will need 2 weeks washout prior to initiation of ABC294640<br>5. 18 years of age or older.<br>6. ECOG performance status of 0-2.<br>7. Acceptable liver function:<br>- Bilirubin ≤ 1.5 times upper limit of normal (CTCAE Grade 1 baseline)<br>- AST (SGOT), ALT (SGPT) ≤ 5 x ULN (CTCAE Grade 2 baseline)<br>- Serum creatinine ≤1.5 XULN (CTCAE Grade 1 baseline)<br>8. Acceptable hematologic status (with or without transfusion support):<br>- Absolute neutrophil count ≥1000 cells/$mm^3$,<br>- Platelet count ≥50,000 (plt/$mm^3$),<br>- Hemoglobin ≥9 g/dL.<br>NOTE: If the patient's bone marrow biopsy shows greater than or equal to 50% plasma cells, the platelet count should be ≥30,000 plt/$mm^3$, Hemoglobin ≥8 g/dL and ANC ≥500 cells/$mm^3$ (transfusion support or growth factor support is acceptable).<br>9. Urinalysis: No clinically significant abnormalities.<br>10. PT and PTT ≤ 1.5 X ULN after correction of nutritional deficiencies that may contribute to prolonged PT/PTT.<br>11. As determined by the treating investigator, the patient must have well-controlled blood pressure, defined as systolic blood pressure <150mmHg and/or diastolic blood pressure <100 mmHg for the majority of measurements.<br>**12.** A negative pregnancy test (if female of child bearing potential).<br>**13.** For men and women of child-producing potential, willingness to use effective contraceptive methods during the study. |
| | ***Exclusion criteria***<br>1. Pregnant or nursing women. NOTE: Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; or abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.<br>2. Patients who are currently participating in any other clinical trial of an investigational product.<br>3. Major surgery within 30 days prior to start of treatment<br>4. Treatment with intravenous antibiotics, antivirals, or antifungals within 14 days prior to start of treatment.<br>5. Known human immunodeficiency virus infection<br>6. Active hepatitis B or C infection with abnormal liver functions (i.e., LFTs > 2 x upper normal limits)<br>7. Unstable angina or myocardial infarction within 4 months prior to start of treatment, NYHA Class III or IV heart failure, uncontrolled angina, history of severe coronary artery disease, severe uncontrolled ventricular arrhythmias, sick sinus syndrome, or electrocardiographic evidence of acute ischemia or Grade 3 conduction system abnormalities unless subject has a pacemaker<br>8. Uncontrolled hypertension or uncontrolled diabetes within 14 days prior to start of treatment<br>9. Nonhematologic malignancy within the past 3 years with the exception of a) adequately treated basal cell carcinoma, squamous cell skin cancer, or thyroid cancer; b) carcinoma in situ of the cervix or breast; c) prostate cancer of Gleason Grade 6 or less with stable prostate-specific antigen levels; or d) cancer considered cured by surgical resection or unlikely to impact survival during the duration of the |

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| | study, such as localized transitional cell carcinoma of the bladder<br>10. Subjects with pleural effusions requiring thoracentesis or ascites requiring paracentesis within 14 days prior to initiation of ABC294640<br>11. Any other clinically significant medical or psychiatric disease or condition, or social situation that, in the Investigator's opinion, may interfere with protocol adherence or a subject's ability to give informed consent |
| **TEST ARTICLE ADMINISTRATION** | ABC294640 will be administered orally twice a day (approximately 12 hours apart) continuously for 28 days. A cycle will be defined as 28 days. |
| **DEFINITION OF DOSE LIMITING TOXICITY** | Toxicities 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 ABC294640 during dose escalation in the phase 1 portion of the study.<br><br>**Non hematologic DLT is defined as:**<br>Any Grade ≥ 3 AE, with the following exceptions<br>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.<br><br>**Hematologic DLT is defined as:**<br>≥Grade 4 neutropenia or thrombocytopenia that lasts more than 7 days after the last dose of study drug;<br>≥Grade 3 thrombocytopenia in the presence of ≥grade 3 hemorrhage of any organ/site;<br>Any grade 5 hematologic toxicity |
| **TREATMENT REGIMENS** | The projected ABC294640 doses for the escalation phase are: 250, 500, and 750 mg BID as determined in the single agent trial for ABC294640. The dose will be given under fasting conditions or after a light to moderate meal. Every 28 days as a cycle.<br><br>For phase Ib study, one patient will be enrolled per week per dose. Subsequent patients on each cohort may not be enrolled less than one week after the previous patient. Patients in each dose cohort must complete cycle 1 and undergo toxicity evaluation before the next dose level cohort is opened. The target toxicity probability is set as $\phi_T = 0.33$. We treat a cohort of 3 patients at each dose level. The following dose-finding algorithm will be employed.<br>• Treat the first cohort of patients at the 250 mg BID dose level.<br>• Suppose that the current dose level is $j^0$. Denote the estimated probabilities of toxicity based on the accumulated data to be $\overline{\pi}_1, \overline{\pi}_2, \overline{\pi}_3$ for the 3 dose levels $d_1, d_2, d_3$, respectively. Find the dose level $j^*$ such that $j^* = \text{argmin} \mid \overline{\pi}_j - \phi_T \mid$.<br>Then<br>– if $j^0 > j^*$, de-escalate to dose level $j^0 - 1$;<br>– if $j^0 < j^*$, escalate to dose level $j^0 + 1$;<br>– otherwise, stay at dose level $j^0$ for the next cohort of patients.<br>• Once the maximum sample size is reached, the dose with the toxicity probability closest to $\phi_T$ is declared as the MTD.<br><br>If the lowest dose is still too toxic, as indicated by the fact that the posterior probability |

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| | that the rate of toxicity for the lowest dose is greater than 0.33 is > 0.9, the trial will be terminated early for safety.<br><br>For phase II study, the patients will be treated with single agent ABC294640 at the MTD determined from the phase IB study until disease progression or intolerable toxicities. |

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| **PRESTUDY ASSESSMENTS** | **Screening Period:**<br>• Informed consent<br>• Eligibility determination<br><br>**Baseline Period (within 30 days of Treatment Day 1)**<br>• Complete medical history<br>• Concomitant medication assessment<br>• Baseline Review of Systems and AE documentation<br>• Focused Physical examination (including vital signs, HEENT, heart/lung/chest/abdomen/skin and neurological assessment)<br>• Vital signs (temperature, blood pressure, pulse rate, and respiratory rate)<br>• ECOG Performance Status<br>• Pregnancy test (for nonsterile women of childbearing potential within 2 days of Treatment Day 1)<br>• 12-lead electrocardiogram<br>• Serum chemistry<br>• Coagulation parameters<br>• CBC with differential<br>• Urinalysis<br>• Bone marrow biopsy and aspirates (should be within 2 weeks of treatment day 1)<br>• SPEP, IFE, UPEP, serum free light chain, LDH, beta-2 microglobulin<br>• Bone skeletal survey |
| **TREATMENT ASSESSMENTS** | Patients will be monitored weekly in Cycle 1, biweekly for Cycles 2-4, and monthly for subsequent cycles. The patient assessments include:<br>• Review of concomitant medications.<br>• Adverse Events monitoring (patient diary, review of systems).<br>• ECOG Performance Status.<br>• Physical exam.<br>• Weight.<br>• Vital signs (temperature, blood pressure, pulse rate, and respiratory rate).<br>• Serum chemistry.<br>• CBC with differential.<br>• Blood sample collection for pharmacodynamics (PD) analysis.<br>• Urinalysis.<br>• Coagulation parameters.<br><br>Myeloma treatment response assessment<br>• SPEP, UPEP, serum free light chain, and beta-2 microglobulin before each cycle.<br>• Bone skeletal survey at screening, then every year or at the end of treatment.<br>• Bone marrow biopsy and aspirates at the last day of cycle #3 and cycle #6 (± 7 days). |
| **PHARMACO-KINETIC ASSESSMENTS** | On Day-1, Cycle-1: a blood sample will be drawn from each subject for PK analysis prior to drug administration. Subsequently, all subjects will receive the morning dose of ABC294640 after which PK sampling will be performed for 8 hours. Patients will take the second dose approximately 12 hours after the initial dose was taken. |

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| **CORRELATIVE STUDY AND PHARMACO-DYMANIC ASSESSMENTS** | **Correlative studies**<br>- plasma PD analysis for sphingosine 1-phosphate will be performed at the beginning of each cycle prior to ABC294640 drug administration and on the PK samples drawn on Cycle 1 Day 1. Plasma IL-6 level will be measured at the beginning of each cycle prior to ABC294640 drug administration for the first 6 cycles. Up to 41 different cytokines will be measured using multiplex cytokine assay.<br>- sphingolipid concentrations (ceramides, sphingosine and sphingosine 1-phosphate will be 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.<br>- expression of sphingolipid signaling genes, SK2 mRNA level or activity, c-Myc, Mcl-1 and pS6 will be measured in bone marrow CD138+ myeloma cells isolated from bone marrow aspirate at the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6 (if sufficient CD138+ myeloma cells can be obtained). For patients who receive more than 3 cycles of treatment and have a repeated bone marrow biopsy, RNA sequencing and Assay for transposases accessible chromatin sequencing (ATAC seq) will be performed to assess the effects of ABC294640 on global gene and epigenetic expression.<br>- 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.<br>- 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 |
| **DATA COLLECTION** | Data will be captured in a secure, web-based, 21 CFR Part 11 compliant system. |
| **INVESTIGATIVE SITES** | This study will be conducted at: Duke University Medical Center, Duke Cancer Institute, Durham, NC 27710. The Phase Ib will be conducted at Duke University Division of Hematologic Malignancies and Cellular Therapy. The Phase II portion may be conducted at multiple sites managed by the Duke Cancer Network. |
| **STATISTICAL ANALYSIS** | The MTD for ABC294640 will be determined using a Bayesian model averaging continual reassessment method, and is the dose at which the estimated probability of toxicity is closest to the target probability 0.33 among all doses. The primary safety analysis will be conducted using descriptive statistics of the incidence of AEs (including DLTs and discontinuations due to AEs). Adverse events will be coded by body system and summary tables with incidence rates of AEs will be generated. Descriptive statistics of AEs will be reported by doses and for subsets of patients with DLTs, patients who discontinue due to AEs, and patients with related AEs. Severity, duration, investigator attributed relationship to treatment, and outcomes of AEs will be reported.<br><br>For the Phase II portion of the study, 59 patients provides 80% power to detect a HR of 0.67 for OS and an increase from 10% to 21% for ORR based on a one-sided $\alpha = 0.025$, controlling for multiple tests.<br><br>The primary analysis will be conducted after all patients are off treatment for at least 30 |

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| | days. A follow-up analysis, which will be an addendum to the primary study report, will be issued once all patients are off treatment for at least 2 years or have died, whichever comes first. |
| **DURATION OF STUDY** | Approximately 5 years (assuming Phase I accrues at Duke Division of Hematologic Malignancies and Cellular Therapy in 12 months, 56 additional Phase II patients accrue in 24 months, with 24 months follow-up after last patient is enrolled).<br>End of study is defined as the time when all subjects have completed the follow-up period. |

## DEFINITION OF TERMS USED

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| $^{o}$C | degrees Centigrade |
| $^{o}$F | degrees Fahrenheit |
| ABC294640 | 3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridin-4-ylmethyl)amide, hydrochloride salt |
| AE | adverse event |
| ALT | alanine aminotransferase |
| ANC | absolute neutrophil count |
| aPTT | activated partial thromboplastin time (also PTT) |
| API | active pharmaceutical ingredient |
| AST | aspartate aminotransferase |
| AUC | area under the drug concentration over time curve |
| BID | twice per day |
| BSA | body surface area |
| BUN | blood urea nitrogen |
| CBC | complete blood count |
| CFR | Code of Federal Regulations |
| CHF | congestive heart failure |
| CI | combination index |
| CPC | Cancer Protocol Committee |
| CR | complete response |
| CrCl | Creatinine Clearance |
| CRF | Case Report Form |
| CSR | Clinical Study Report |
| CT | computer-assisted tomography |
| CTCAE | Common Toxicity Criteria adverse event |
| CTRC | Clinical and Translation Research Center |
| CV | cardiovascular |
| CYP | cytochrome P450 |
| DCI | Duke Cancer Institute |
| DCN | Duke Cancer Network |
| DLT | dose limiting toxicity |
| DMS | N,N-dimethylsphingosine |
| DVT | deep venous thrombosis |
| ECG | electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | electronic Case Report Form |
| EGF | epidermal growth factor |
| FCBP | Females of childbearing potential |
| FDA | Food and Drug Administration |
| FISH | fluorescent in situ hybridization |

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| FLC | free light chain |
| G-CSF | granulocyte colony stimulating factor |
| GCP | Good Clinical Practice |
| GI | gastrointestinal |
| GLP | Good Laboratory Practice |
| GM-CSF | granulocyte macrophage colony stimulating factor |
| h | hour(s) |
| HED | human equivalent dose |
| hERG | human ether-a-go-go related gene |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | human immunodeficiency virus |
| HNSTD | highest nonseverely toxic dose |
| IB | Investigator Brochure |
| ICH | International Conference on Harmonization |
| IC50 | concentration that inhibits by 50% |
| IEC | Independent Ethics Committee |
| IL-1β | interleukin 1-beta |
| IL-6 | interleukin 6 |
| IND | Investigational New Drug (Application) |
| iNOS | inducible nitric oxide synthase |
| IRB | Institutional Review Board |
| IV | intravenous |
| Kg | kilogram(s) |
| LC/MS | liquid chromatography with mass-spectrometry detection |
| LDH | lactate dehydrogenase |
| mg | milligram(s) |
| min | minute(s) |
| mIU | Milli International Units |
| mL | milliliter(s) |
| MM | multiple myeloma |
| $mm^2$ | millimeter(s) squared |
| $mm^3$ | millimeter cubed |
| MR | minimal response |
| MTD | maximum tolerated dose |
| MRI | magnetic resonance imaging |
| NCI | National Cancer Institute |
| NFκB | nuclear factor kappa-B |
| NHL | non-Hodgkin's lymphoma |
| NOAEL | no adverse effect level |
| ORR | overall response rate |
| p | probability factor |
| PBMC | peripheral blood mononuclear cells |
| PD | progressive disease |
| PDGF | platelet-derived growth factor |
| PFS | progression-free survival |
| PI | Principal Investigator |
| PK | pharmacokinetic(s) |
| PO | per os (oral) |
| PR | partial response |
| PSA | prostate-specific antigen |
| PT | prothrombin |
| PTT | prothrombin time |
| QIU | Qualified Investigator Undertaking Form |

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| RBC | red blood cell |
| S1P | sphingosine 1-phosphate |
| SAE | serious adverse event |
| SAP | Statistical Analysis Plan |
| sCR | stringent complete response |
| s.c. | subcutaneous |
| SD | stable disease |
| SEER | Surveillance, Epidemiology, and End Results |
| SEM | standard error of the mean |
| SK | sphingosine kinase |
| SK1 | sphingosine kinase-1 |
| SK2 | sphingosine kinase-2 |
| SPEP | serum protein electrophoresis |
| $STD_{10}$ | severely toxic dose in 10% of animals |
| TLS | Tumor lysis syndrome |
| TNFα | tumor necrosis factor-alpha |
| TTP | time to tumor progression |
| TUNEL | terminal deoxynucleotidyl transferase dUTP nick end labeling |
| ULN | upper limit of normal |
| UPEP | urine protein electrophoresis |
| VEGF | vascular endothelial growth factor |
| VGPR | very good partial response |
| w | week |
| WBC | white blood cells |

# 1. BACKGROUND

## 1.1 MULTIPLE MYELOMA

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].

For decades, low doses of melphalan and prednisone were the cornerstone of MM treatment. However, complete responses under this regimen are rare, and the median time for progression is not higher than 15 months[4,5]. A first significant advance in the management of MM was the upfront use of high doses of melphalan with autologous hematopoietic stem cell transplantation (AHSCT). Such treatment has allowed for improved response rates, progression free survival and, in some trials, prolonged survival in MM[6-9]. High dose melphalan with AHSCT is considered a fundamental therapeutic modality to be explored by younger patients upfront (after response to conventional induction therapy) and/or at the time of disease progression.

The advent of new "biological" agents in treatment regimens for MM has led to marked improvement in the depth and duration of the responses obtained. The immunomodulatory drugs (i.e., thalidomide and lenalidomide), along with the first proteasome inhibitor (i.e., bortezomib), have shown efficacy in the management of both newly diagnosed as well as relapsed and refractory MM patients[1,10-12]. Treatment of MM patients with combination regimens, containing one or more biological agents, followed by autologous HSC transplantation consolidation have resulted in the highest response rates ever reported in the management of newly diagnosed MM patients[13-18].

Despite the use of the "biological" agents and the incoporation of AHSCT, MM remains an incurable disease. Patients may relapse within months after autologous hematopoietic stem cell transplantation. Furthermore, nearly all MM patients will eventually develop resistance to currently available agents. There is an unmet medical need for the development of novel therapeutic agents for this disease. It is particularly important to develop new agents that do not share similar mechanism of action with proteasome inhibitors or immunomodulatory drugs because most of the refractory/relapsed MM patients would have exposed to those agents during their course of treatment.

*Role of bone marrow transplantation in multiple myeloma:* Bone marrow transplant in myeloma is almost always autologous. Allogeneic hematopoietic stem cell transplant is currently only performed under study protocol and is associated with high rates of complications and mortality. Autologous bone marrow transplant is usually employed for patients whose myeloma is in remission after some form of induction chemotherapy, and is not curative. For patients in relapse or being refractory, the value of autologous bone marrow transplant is limited. Our current study focuses on patients with relapse or refractory multiple myeloma. Most of these patients would have already received bone marrow transplantation or they are not candidates for transplant ever due to age or comorbidities. Please note that history of autologous transplant generally does not increase or decrease safety of later lines of therapy including clinical trials, since patients would have full count recovery in about one month after transplant.

## 1.2 SPHINGOLIPIDS, SPHINGOSINE KINASES AND CANCER

Sphingolipids are an extremely diverse group of water insoluble molecules that include ceramides, sphingoid bases, ceramide phosphates and sphingoid base phosphates. In addition to supporting the structure and fluidity of the lipid bilayer, sphingolipid metabolites function as second messengers and

hormones, and regulate cytokine-mediated cell signaling[19,20]. Sphingolipids are involved in a wide range of biological and pathological events including inflammation, cell proliferation, apoptosis, angiogenesis, and transformation (reviewed in[21-26]). More recently, sphingolipid metabolism is being increasingly recognized as a key pathway in tumor cell survival and in cancer biology[27-34].

Among sphingolipid metabolites, ceramide, sphingosine and sphingosine-1-phosphate (S1P) are the key players for their biophysiological functions. Ceramide can be produced via hydrolyzation of sphingomyelin in response to stimuli such as cytokines and growth factors. Ceramide is further hydrolyzed to sphingosine. Sphingosine is then rapidly phosphorylated by sphingosine kinases (SK) to S1P. Ceramide and sphingosine are pro-apoptotic, inducing apoptosis in tumor cells without disrupting quiescent normal cells[35-38]. In contrast, S1P is mitogenic and anti-apoptotic. A critical balance, i.e. a ceramide:S1P rheostat, is hypothesized to determine the fate of the cell[28,39,40].

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.

### *1.2.1 Sphingolipid metabolism in cancer*

The mechanisms and effects of the interconversion of sphingolipids have been the subjects of a growing body of scientific investigation. Sphingomyelin is a building block for cellular membranes and also serves as the precursor for potent lipid messengers that have profound cellular effects. As indicated in Figure 1, 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. Inhibition of SK activity causes the accumulation of ceramides along with the depletion of S1P, thereby driving cancer cells into apoptosis. Many studies have shown that a variety of proliferative factors rapidly elevate SK activity. Signaling via the Ras-MAP kinase pathway is dependent on SK activity, as are angiogenic processes such as cell motility, mitogenesis in smooth muscle cells and endothelial cell differentiation.

In another aspect of tumor biology, sphingolipids regulate the sensitivities of cancer cells to anticancer drugs and ionizing radiation. For example, ceramides enhance apoptosis in response to paclitaxel and etoposide. Therefore, disruption of metabolism of ceramides to S1P is a new method to enhance drug and radiation sensitivity in cancer cells, including those that are resistant to traditional cytotoxic drugs such as multiple myeloma (MM).

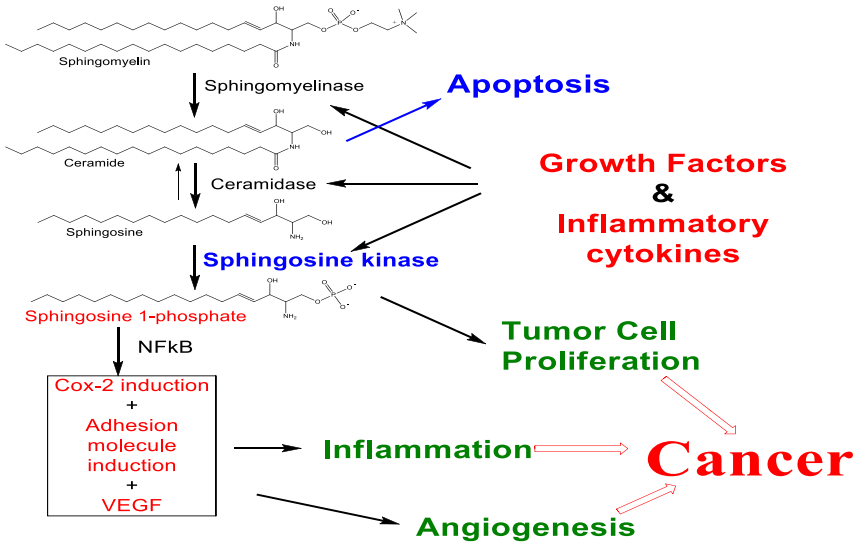


**Figure 1.** ***A simplified model of sphingolipid metabolism.*** Growth stimulatory signals increase the activity of ceramidase and SK driving the production of S1P, which promotes cell proliferation, angiogenesis and inflammation; while depleting the cells of ceramide, thereby inhibiting apoptosis.

### 1.2.2 *Differential roles of SK1 and SK2*

Sphingosine kinase has 2 isoforms: SK1 and SK2. The relative roles of SK1 and SK2 in tumor biology have been of great interest to many investigators, and were a central issue in the selection of ABC294640 as a clinical agent. Space limitations preclude full discussion of this issue, but many studies have suggested different biological roles of the two SK isozymes[33,41-45]. Most studies have focused on SK1 because it is the predominant isozyme in most cells, and is upregulated in many cancers. The latter response is likely due to hypoxia because SK1 is HIF-regulated [46-49], making it difficult to discern whether overexpression is required to drive tumor growth or is a consequence of tumor growth. Target validation studies using cDNA transfection or RNA interference are inconsistent in ascribing dominance to either isozyme. For example, overexpression of SK1 has been shown to be oncogenic [50,51], while transfection with SK2 was originally reported to inhibit cell growth and to induce apoptosis [52]. However, these effects of SK2 are not dependent on its catalytic activity, suggesting that the antiproliferative effects may be mediated by its BH3 domain [52]. Consistent with this are the observations that physiological levels of SK2 do not inhibit DNA synthesis [53]. Similarly, EGF is reported to induce the expression of SK1 but not SK2 in MCF-7 cells [54]; however, it has also been reported that EGF activates SK2 in MDA-MB-453 cells [55]. Thus additional work is needed to refine our knowledge about SK1 vs. SK2 biology.

### 1.2.3 *Development of SK inhibitors*

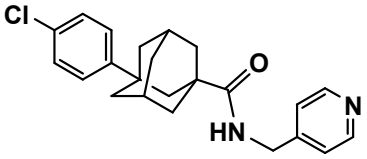


**Figure 2. ABC294640**

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 ABC294640 was selected as the first compound in this program for clinical testing. Much of the pharmacology of ABC294640 [61,62], as well as its antitumor [61,63-69] and anti-inflammatory [58,59,70-72] activities have been published. Space limitations preclude full discussion of the properties and *in vivo* activities of ABC294640, but key points include: $K_i$ for SK2 = 9 µM, competitive with respect to sphingosine; No inhibition of SK1 or panel of protein kinases; Depletes S1P and elevates ceramide in tumor cells; Plasma concentrations can reach >200 µM without toxicity; Good pharmacokinetics, oral bioavailability and biodistribution; No hematologic or major organ toxicity; Effective in multiple cancer models; Pharmacodynamic marker (plasma S1P) for *in vivo* and clinical testing; Therapeutically effective in multiple animal models of inflammation including:

Crohn's Disease, ulcerative colitis, rheumatoid arthritis, osteoarthritis and ischemia / reperfusion injury; cGMP synthesis and formulation (gelatin caps) completed; cGLP toxicology studies in rats and dogs completed; IND application approved by FDA; Single-agent phase I clinical trial in patients with advanced solid tumors completed as discussed below; and Excellent correlation between mouse and dog PK parameters with human data from the clinical trial.

### 1.3 Summary of ABC294640 Phase I Clinical Data in Solid Tumors

***1.3.1. Study design.*** The first-in-human phase I clinical trial of ABC294640 in patients with advanced solid tumors has completed enrollment at the Hollings Cancer Center at MUSC (ClinicalTrials.gov ID NCT01488513). ABC294640 was administered orally on a continuous schedule, with 28 days constituting a Cycle. Tumors were reimaged every two Cycles (8 weeks), and patients were allowed to continue receiving the drug if there is no disease progression by RECIST criteria. Primary endpoints for the trial were: identification of the Maximum Tolerated Dose (MTD); determination of the Dose Limiting Toxicities (DLTs); and evaluation of safety. Secondary endpoints were: determination of PK (Day 1 and Day 28 of Cycle 1); evaluation of PD effects; and assessment of antitumor activity.

***1.3.2. Safety data.*** Twenty-two patients were enrolled, as outlined in Table 1. Three patients (one at 250 mg bid, and two at 500 mg bid) were unable to complete Cycle 1 due to complications from their disease, and were replaced. The first patient on study at 250 mg qd developed grade 4 hyperglycemia in the setting of rapidly progressing pancreas cancer; however, no other patients experienced possibly drug-related hyperglycemia. Among the four patients enrolled at 750 mg bid, one ovarian cancer patient had dose-limiting grade 3 nausea and vomiting, and two patients were unable to complete Cycle 1 due to diverse possibly drug-related toxicities. The 750 mg bid dose level was declared not tolerable, and the 500 mg dose level was expanded. One patient at the expanded 500 mg dose level was dose reduced at Cycle 3 due to grade 1 AEs. The other five patients at 500 mg bid tolerated the drug well, and the 500 mg bid dose level was established as the recommended phase II dose. Across all dose levels, the most common drug-related toxicities were nausea, fatigue, vomiting, and diarrhea. In addition, 2 patients experienced psychiatric disorders including agitation/anxiety, mood changes, and/or hallucinations; and 5 patients experienced grade 1-2 nervous system disorders, including dizziness, dysarthria, dysgeusia, dysesthesia, memory loss, muscle spasms, paraesthesias, somnolence, and/or spasticity that resolved upon discontinuation of ABC294640.

| Patient Study ID | Dose Level | Primary Histologic Diagnosis | Number of Treatment Cycles | DLT |
|---|---|---|---|---|
| | 250 mg qd | Pancreatic adenocarcinoma | 1 | Grade 4 hyperglycemia |
| 2 | 250 mg qd | Cholangiocarcinoma | 19 | None |
| 3 | 250 mg qd | Rectal adenocarcinoma | 3 | None |
| 4 | 250 mg qd | Colon adenocarcinoma | 3 | None |
| 5 | 250 mg qd | Carcinoma of unknown primary | 5 | None |
| 6 | 250 mg qd | Urothelial carcinoma | 13 | None |
| 7 | 250 mg bid | Colon adenocarcinoma | 3 | None |
| 8 | 250 mg bid | Rectal adenocarcinoma | 1 | None |
| 9 | 250 mg bid | Colon adenocarcinoma | 4 | None |
| 10 | 250 mg bid | Hepatocellular carcinoma | 4 | None |
| 11 | 500 mg bid | Lung carcinoma | 3 | None |
| 12 | 500 mg bid | Bile duct cancer | 5 | None |
| 13 | 500 mg bid | Pancreatic adenocarcinoma | 1 | None |
| 14 | 500 mg bid | Pancreatic adenocarcinoma | 2 | None |
| 15 | 750 mg bid | Ovarian cancer | 1 | Grade 3 nausea/vomiting |
| 16 | 750 mg bid | Pancreatic adenocarcinoma | 1 | None |
| 17 | 500 mg bid | Bladder cancer | 3 | None |
| 18 | 750 mg bid | Colon adenocarcinoma | 1 | None |
| 19 | 500 mg bid | Cholangiocarcinoma | 4 | None |
| 20 | None | Lung carcinoma | None | None |
| 21 | 750 mg bid | Urothelial carcinoma | 2 | None |
| 22 | 500 mg bid | Adrenal cortical carcinoma | 1 | None |

***1.3.3. Efficacy data.*** Of the 21 ABC294640-treated patients, 11 (52%) completed at least 2 drug cycles and 7 (64%) of those had Stable Disease at that time. The number of treatment cycles is also indicated in Table 1. Subjects included a patient with advanced HCC who lived for 10 months after

completing 4 cycles, a patient with recurrent metastatic bladder cancer who received 12 cycles and is alive more than 2 years after starting the trial, and a patient with advanced cholangiocarcinoma who received 18 cycles (PFS for 72 weeks).

***1.3.4. Pharmacokinetic data.*** PK profiling was conducted on Days 1 and 28 of Cycle 1. The data from patients treated with 500 mg of ABC294640 are shown in Figure 3. After oral dosing, plasma concentrations of ABC294640 typically peak at 1-2 hours, and then decline with a half-time of clearance of ~4 hours. The peak plasma concentrations ($C_{max}$) and AUCs within a cohort were generally similar. Notably, the $C_{max}$ and AUC values for most patients were not significantly different between Day 1 and Day 28, indicating a lack of metabolic adaptation to the drug. Importantly, $C_{max}$ levels of ABC294640 in patients receiving 500 mg of the drug were in the range expected to have therapeutic activity. Specifically, antitumor activity in mouse models occurs at ABC294640 doses of 25-50 mg/kg [73], which results in plasma $C_{max}$ levels of 9-20 µM. Patients receiving a 500 mg dose of ABC294640 had $C_{max}$ levels averaging 16.4 µM (Figure 3), with 9 of 12 profiles exceeding the threshold of 9 µM. Patients given 250 mg of ABC294640 exceeded the $C_{max}$ threshold approximately 50% of the time, while all patients receiving 750 mg of ABC294640 reached this level. Because the $t_{1/2}$s are equal in mice and humans, the drug exposure in patients at 500 mg and higher dose levels is expected to be sufficient to inhibit SK2 in the tumors.

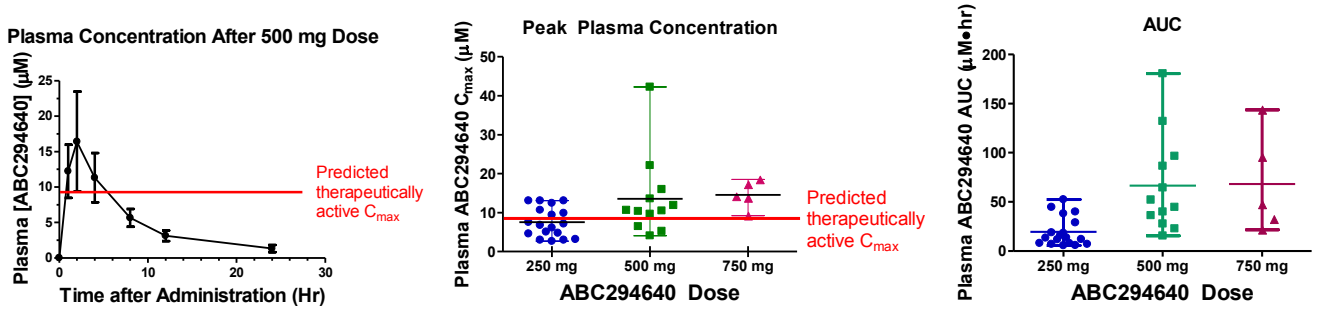


Figure 3. **Pharmacokinetics of ABC294640 in humans. Left Panel.** Plasma was isolated from patients given 500 mg of ABC294640 on either Day 1 or Day 28 of Cycle 1 and levels of ABC294640 were quantified using a GLP LC/MS method. Values represent the mean ± SEM for 7 data sets. Antitumor activity in mouse models occurs at ABC294640 doses ≥25 mg/kg, which provides plasma $C_{max}$ levels of 9 µM. The red line indicates the $C_{max}$ predicted to have antitumor activity based on the mouse tumor models. **Middle Panel**. $C_{max}$ values are shown for patient samples collected on either Day 1 or Day 28 (there is no change in $C_{max}$ or AUC in this period). The red line indicates the Cmax predicted to have antitumor activity based on the mouse tumor models. **Right Panel.** The AUCs for ABC294640 at each dose level are indicated.

***1.3.5. Pharmacodynamic data.*** The most direct PD biomarker for inhibition of SK2 by ABC294640 is S1P in the target tissue. However, tumor samples were not obtained in the phase I clinical trial, so we measured S1P in plasma as a biomarker for inhibition of SK2. ABC294640 treatment caused rapid decreases in plasma S1P levels (Figure 4). In general, plasma S1P reached a minimum at 12 hr after ABC294640 treatment and recovered to baseline by 24 hr. This is consistent with the PK of the drug and supports the BID dosing schedule. The maximum S1P decreases for the 250, 500 and 750 mg cohorts were 51±6% (n=10), 46±9% (n=5) and 54±14% (n=2), respectively, indicating that ABC294640 exposure attained with the 250 mg dose are sufficient to optimally inhibit S1P generation in the patients.

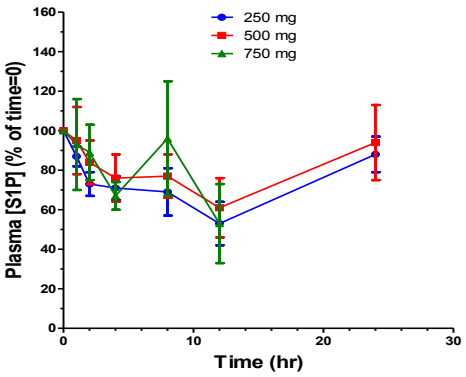


**Figure 4. Effect of ABC294640 treatment on plasma S1P levels.** S1P concentrations were normalized to the Time 0 levels for individual patients at each dose of ABC294640. S1P levels in patients receiving 250, 500 mg and 750 mg of ABC294640 are indicated in blue, red and green, respectively.

### 1.4 Rationale for a clinical trial evaluating ABC294640 in patients with MM

MM is characterized by the accumulation of malignant plasma cells that are defective in apoptotic cell death [74,75]. Sphingolipid metabolism is being increasingly recognized as a key pathway in regulating myeloma cell survival, migration and adhesion, and responses to treatment. For instance, S1P up-regulates Mcl-1 expression in myeloma cells and protects myeloma cells from apoptosis[76]. Additionally, S1P promotes myeloma cell adhesion and migration to bone marrow[77]. Consistent with the importance of S1P in myeloma pathogenesis, targeting S1P receptors using FTY720 induces apoptosis and overcomes drug resistance[78]. Furthermore, sphingolipid metabolites (i.e., ceramide) play a critical role in thalidomide-induced antiangiogenic effects [79]. Importantly, SK mediates the survival and proliferation signal pathway induced by IL-6, the most critical cytokine in myeloma pathogenesis[80].
*There is an unmet medical need for the development of novel therapeutic agents for MM. It is particularly important to develop new agents that do not share similar mechanism of action with proteasome inhibitors or immunomodulatory drugs because most of the refractory/relapsed MM patients would have exposed to those agents during their course of treatment.*

### 1.5 Pre-clinical efficacy of ABC294640 for Multiple Myeloma

We have published data indicating significant single-agent activity for ABC294640 (at clinically achievable plasma concentrations) in an established xenograft model for myeloma, including treatment for established tumors[81]. The data are summarized below.

#### 1.5.1 Sphingosine Kinase 2 is over-expressed in myeloma cells.

To determine the potential utility of targeting SKs for the treatment of MM, we measured the gene expression levels of SK1 and SK2 in myeloma cells. We measured SK1 and SK2 mRNA expression in a publicly available myeloma microarray dataset, in myeloma cell lines, and in freshly isolated human bone marrow $CD138^+$ myeloma cells (**Fig. 5**). We downloaded the GSE6477 Affymetrix microarray dataset originated by Mayo Clinic.[82,83] This dataset contained microarray mRNA gene profile on purified plasma cells isolated from normal control subjects (n=15) or newly diagnosed MM patients (n=73). We generated the Robust Multi-array Average (RMA)- normalized gene expression data for SK1 and SK2 and compared their expression levels between normal subjects and newly diagnosed MM patients. As shown in **Fig. 5A**, SK2 expression was increased in MM patients compared to normal subjects (p=0.046), whereas there was no significant difference in SK1 expression level in plasma cells between MM patients and normal subjects.

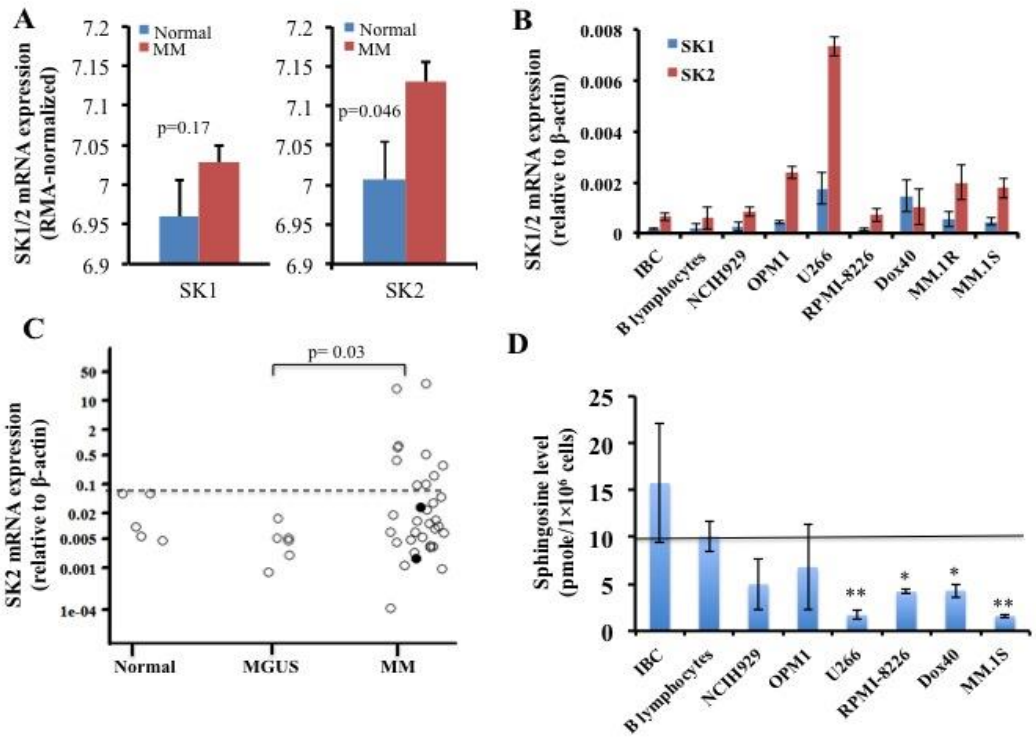


**Figure 5: SK2 was over-expressed in myeloma cells. A: SK1 and SK2 gene expression in GSE6477 Affymetrix microarray dataset.** Publicly available Affymetrix microarray data set GSE6477 was downloaded and the RMA normalized gene expression data were generated. The SK1 and SK2 expression level between plasma cells from normal subjects (blue bar; n=15) and purified CD138$^+$ cells from newly diagnosed MM (red bar; n=73) were compared. **B: SK1 and SK2 expression in myeloma cell lines and B cell lines.** RNA was extracted from two B cell lines (EBV-Immortalized B cells (IBC) and ATCC® CCL-156 B lymphocytes) and seven MM cell lines (NCI-H929, OPM1, U266, RPMI-8226, RPMI-8226-Dox40, MM.1R, and MM.1S) and RT-PCR was performed for SK1 and SK2. Relative SK1 and SK2 mRNA expression with respect to β-Actin was shown (Mean± SEM of 3 separate sets of experiments). **C: SK2 expression in primary human bone marrow CD138$^+$ cells.** Primary human CD138$^+$ cells were isolated using CD138 enrichment kit from the BM aspirates of normal subjects (n=5), MGUS patients (n=6) and myeloma patients (n=34). SK2 gene expression was normalized against β-Actin control (Each dot represented one individual patient and the two solid circles represented amyloidosis patients). **D: Sphingosine level in myeloma cell lines and B cell lines.** Sphingosine was measured by HPLC in freshly prepared EBV-Immortalized B cells, ATCC B lymphocytes, and 6 MM cell lines (NCI-H929, OPM1, U266, RPMI-8226, RPMI-8226-Dox40, and MM.1S). Data represented sphingosine concentration (pmole/1×10$^6$ cells) (Mean± SEM of one of 4 separate sets of experiments) (*: $p<0.05$; **: $p<0.01$).

SK1 and SK2 mRNA expression levels in two B cell lines (EBV-immortalized B cell line and ATCC B lymphocyte cell line) and seven myeloma cell lines (NCI-H929, OPM1, U266, RPMI-8226, RPMI-8226-Dox40, MM.1R and MM.1S) were measured by quantitative RT-PCR. As shown in **Fig. 5B**, the mRNA expression level of SK2 was higher than that of SK1 in all tested myeloma cell lines except RPMI-8226-Dox40 cells. Furthermore, the mRNA expression level of SK2 was higher in all seven myeloma cell lines than that in the two B cell lines.

We also determined the SK1 and SK2 mRNA gene expression in freshly isolated primary human bone marrow (BM) CD138$^+$ MM specimens. CD138$^+$ plasma cells were isolated from the BM aspirates of normal controls, MGUS (monoclonal gammopathy of undetermined significance) patients, or MM patients including amyloidosis patients. No difference in SK1 mRNA expression was observed between these three populations of patients (data not shown). Interestingly, SK2 gene expression was increased in the CD138$^+$ cells in 10 out of 34 (29%) MM patients (**Fig. 5C**). We performed additional subset analyses to determine whether SK2 mRNA expression correlated with myeloma disease stage, cytogenetic profile, M protein level or BM plasma cell number. No correlation was observed with these subset analyses (data not shown).

SKs catalyze the phosphorylation of sphingosine to S1P and sphingosine derives from ceramide. To determine if the over-expression of SK2 in myeloma cells affects the levels of ceramides, sphingosine and S1P, mass spectrometry measurement of 14 different ceramides, sphingosine and S1P was performed in two B cell lines and six MM cell lines. The levels of ceramides and S1P varied highly among MM cell lines and between B cell lines and MM cell lines (data not shown). Interestingly, sphingosine level was lower in MM cells than that in B cells and the difference was statistically significant for 4 out of 6 MM cell lines we tested (**Fig. 5D)**. The decrease in the level of sphingosine in MM is consistent with increased sphingosine kinase gene expression.

### 1.5.2. SK2-specific shRNA inhibits myeloma proliferation and induces Caspase 3-mediated cell death.

To determine the roles of SK2 in MM cell survival and proliferation, we used specific shRNA to knockdown SK2 expression in MM cells. Lentiviral vector expressing SK2-specific shRNA or control shRNA was constructed and used to transduce MM cell lines. Both SK2-specific shRNA and control shRNA effectively transduced MM cell lines as demonstrated by high level of DsRFP expression (**Fig. 6A**). SK2-specific shRNA decreased SK2 mRNA expression by ~80% (**Fig. 6B**). SK2-specific shRNA effectively inhibited myeloma cell proliferation as measured by MTT assay (**Fig. 6C**). To further determine the effect of SK2 on myeloma cell proliferation, we transduced OPM1 myeloma cells with SK2-specific shRNA or control shRNA. We then labeled the cells with CellTrace Violet Cell Proliferation dye and measured dye fluorescence intensity 7 days later (**Fig. 6D**). With cell division, the dye is diluted and the fluorescence intensity is reduced. As shown in **Fig. 6D**, compared to control shRNA, SK2-specific RNA inhibited myeloma cell proliferation and division. Additionally, we found that SK2-specific shRNA activated Caspase 3 (**Fig. 6E**). These data suggested that SK2 plays an important role in both cell proliferation and survival of myeloma cells, and thus provides a therapeutic target for the treatment of MM.

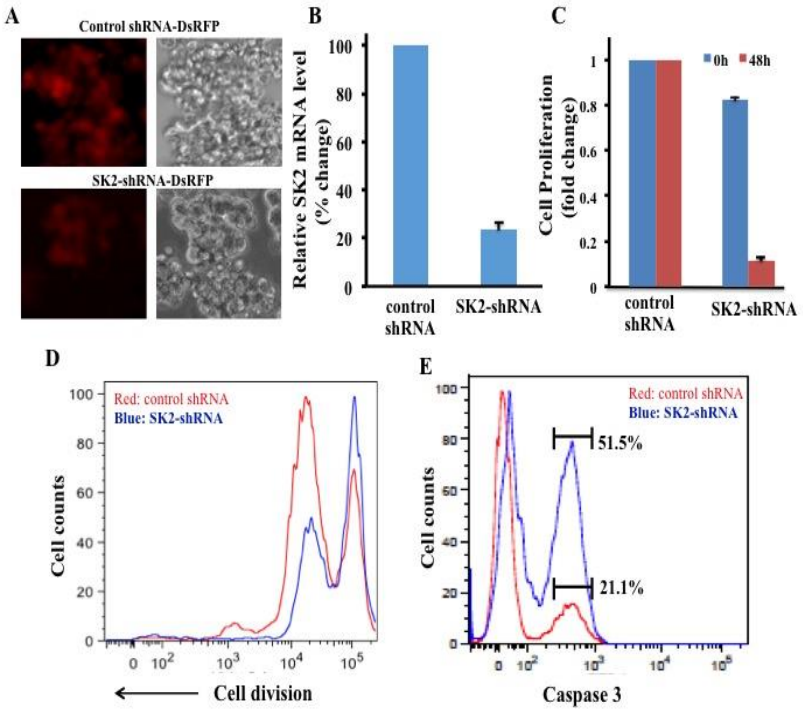


**Figure 6: SK2-specific shRNA inhibited cell proliferation and induced Caspase 3 activation in myeloma cells.** OPM1 cells were transduced with lentiviruses expressing SK2-shRNA-DsRFP or control shRNA-DsRFP for 4h. The cells were then washed and grew in regular culture medium for additional 48h. **A: Fluorescent microscopy image showing DsRFP expression. B: Expression of SK2 mRNA in SK2-shRNA- or control shRNA- transduced OPM1 cells. C: Cell proliferation by MTT assay.** Cell proliferation was measured using MTT assay at 0h and 48h following transduction. **D: Cell proliferation by flow cytometry.** OPM1 cells transduced with SK2-shRNA or control shRNA were stained with CellTrace Violet Cell Proliferation dye and allowed to proliferate for 7 days. The dye fluorescence intensity was measured by flow cytometry. **E: Activation of Caspase 3.** OPM1 cells were transduced with SK2-shRNA viruses or control shRNA viruses. Forty-eight hrs later, the cells were stained with Fixable Live/Dead cell dye, then fixed and permeabilized, and stained with Caspase-3 antibody. Caspase 3 intensity was gated on live cell population. Data were representative of 4 separate experiments.

### 1.5.3. SK2-specific inhibitor (ABC294640) inhibits myeloma growth in vitro.

We next tested the effectiveness of the SK2-selective inhibitor (ABC294640) in killing myeloma cells *in vitro*. ABC294640 is the most advanced, non-lipid-based oral SK2 inhibitor and shows no inhibition for SK1 or panel of protein kinases.[61] ABC294640 is currently undergoing single agent phase I/II clinical trial at our institute for solid tumors. We treated 6 different MM cell lines with various concentrations of ABC294640 and found that ABC294640 inhibited myeloma cell growth, including steroid resistant MM.1R cells (**Fig. 7A**). Among the 6 MM cell lines we tested, OPM1 appears to be the most sensitive cell line to ABC294640 treatment whereas U266 is relatively resistant. ABC294640 inhibited myeloma cell growth as early as 16 hours after exposure (**Fig. 7B**).

To determine if ABC294640 induces cytotoxic or cytostatic effects on MM cells, we cultured MM cells with 30μM of ABC294640 and then quantified live cell numbers over time. As shown in **Fig. 7C**, ABC294640 exhibited cytotoxic effects on the majority (7 out of 8) of MM cell lines we tested, except for U266 cells. We further found that ABC294640 treatment induced apoptotic cell death in myeloma cells as demonstrated by Annexin V staining (**Fig. 7D**), Caspase 3 activation (**Fig. 7E**), Caspase 9 activation, and PARP cleavage (**Fig. 7F**). ABC294640 induced growth arrest, but no apoptosis in U266 cells (data not shown).

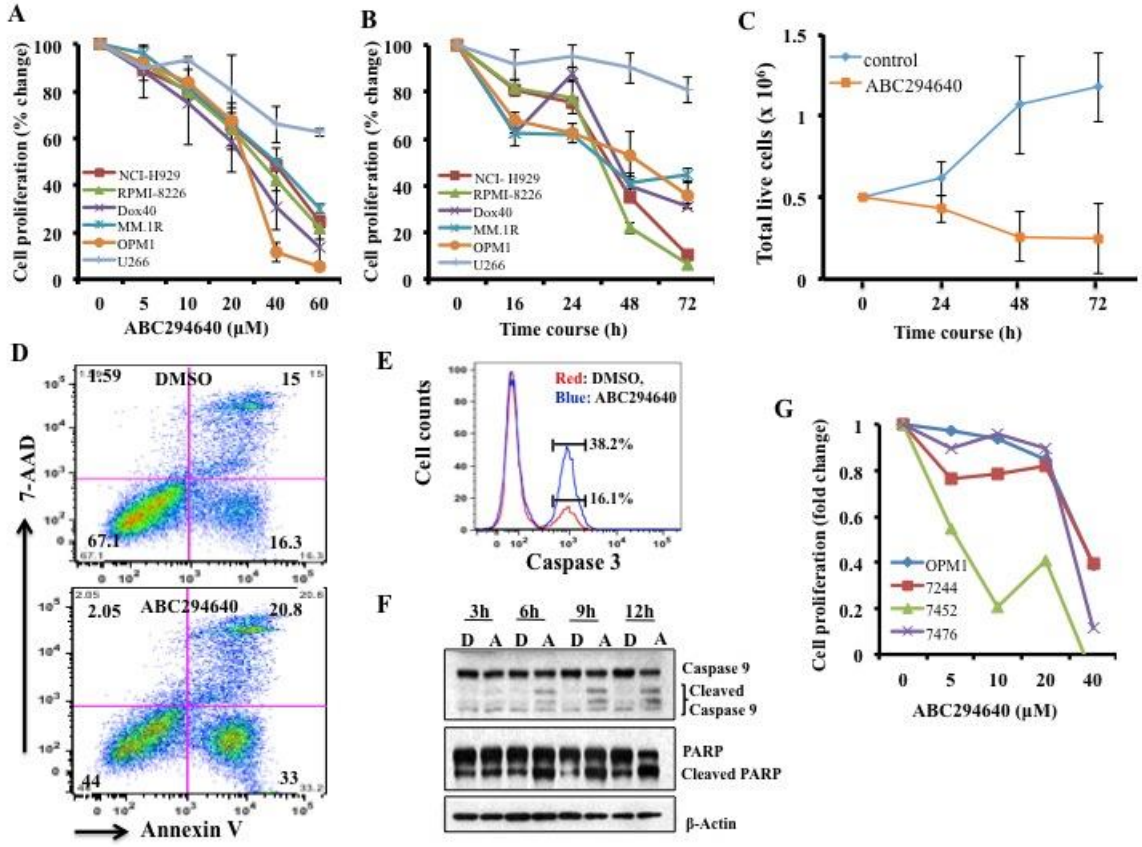

**Figure 7: ABC294640 inhibited cell proliferation and induced apoptosis in MM cells. A: Dose-dependent inhibition of cell proliferation by ABC294640.** Six different MM cell lines were treated with various concentrations of ABC294640 for 48h and cell proliferation was measured by MTT assay (Mean± SEM of one of 3 separate sets of experiments). **B: Time course of proliferation inhibition by ABC294640.** Six different MM cells lines were treated with 30μM of ABC294640 or DMSO for various durations. Cell proliferation was analyzed by MTT assay at the time-points indicated (Mean± SEM of one of 3 separate sets of experiments). **C: Cytotoxic effects of ABC294640 on MM cells.** OPM1 cells were treated with 30μM of ABC294640 or DMSO and total live cells were quantified at the time-points indicated (Mean± SEM of 3 separate sets of experiments). **D: Increased Annexin V$^+$ cells by ABC294640.** OPM1 cells were treated with 30μM of ABC294640 or DMSO for 16h and cells were stained with Annexin V and 7-AAD and analyzed by flow cytometry. Data shown were representative of 3 separate sets of experiments. **E: Caspase 3 activation.** OPM1 cells were treated with 30μM of ABC294640 or DMSO for 16h and cells were then fixed, permeabilized, and stained Caspase3 antibody. **F: Caspase 9 activation and PAPR cleavage.** OPM1 cells were treated with 30μM of ABC294640 (indicated as A) or DMSO control (indicated as D) for 3h, 6h, 9h and 12h and analyzed for PARP and cleaved PARP, full length Caspase-9 and cleaved Caspase -9 by western blot. β-actin was used as loading control (data were representative of 3 separate sets of experiments). **G: Inhibition of primary human CD138$^+$ myeloma cells by ABC294640.** Primary human CD138$^+$ cells were freshly isolated using CD138 enrichment kit from the BM aspirates of myeloma patients and cultured in triplicate at $1\times10^4$ cells in 100 μl of RPMI1640 medium supplemented with 2 mM Glutamax and 10% fetal calf serum containing DMSO control or various concentrations of ABC294640 for 24h at $37^0$C under 5% CO2. Cell proliferation was then measured by MTT assay. OPM1 cells were similarly treated for comparison (n=3; ID#: 7244, 7452 and 7476).

We also tested the anti-tumor effects of ABC294640 on primary human CD138$^+$ myeloma cells. Human CD138$^+$ myeloma cells were freshly isolated from myeloma patient's BM aspirate and treated with various concentrations of ABC294640. Cell proliferation was then measured by MTT assay. ABC294640 inhibited primary human myeloma cells with the same efficacy as with the most sensitive myeloma cell lines (OPM1) (**Fig. 7G**), demonstrating the potential clinical utility of ABC294640 in the treatment of MM.

The effects of SK1 inhibitor, Myriocin (a natural product inhibitor of serine palmitoyltransferase), and FTY720 (a S1P receptor antagonist) on myeloma cell proliferation were investigated (**Figure 8**). Consistent with previous reports by others,[78] FTY720 inhibited myeloma cell growth (**Figure 8A**). No inhibitory effects of Myriocin or SK1 inhibitor on myeloma cells were observed (**Figure 8B** and **8C**). These data again support the important role of SK2 in myeloma cell proliferation and survival.

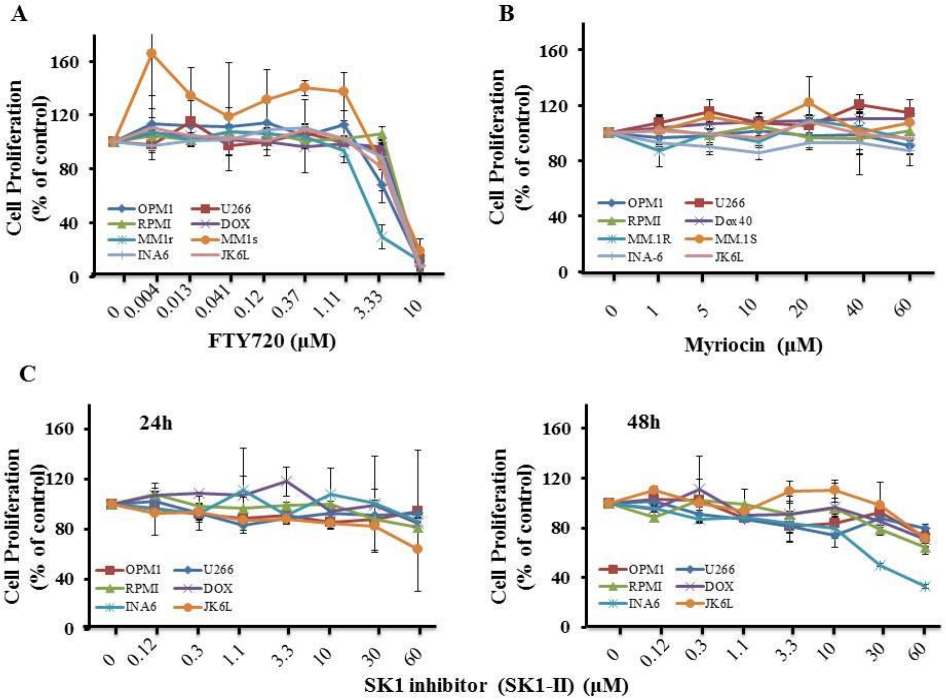


**Figure 8: Effects of FTY720, Myriocin and SK1-II on myeloma cell proliferation.** MM cell lines were treated with various concentrations of FTY720 (**A**), Myriocin (**B**) for 48hr or SK1I (**C**) for 24hr or 48hr, and cell proliferation was measured by MTT assay.

### 1.5.4. *ABC294640 up-regulates Noxa expression and promotes proteasome degradation of Mcl-1.*

We performed extensive mechanistic studies to understand the pathways through which SK2 inhibition induces myeloma cell death. Extensive studies have demonstrated an essential role of Mcl-1in the survival of human myeloma cells.[84,85] Mcl-1 is over-expressed in more than half of newly diagnosed MM patients and in ~81% of relapsed MM patients.[86] Furthermore, recent studies suggested that SK1 plays a key role in Mcl-1 expression induced by IL-6.[76] Therefore, we examined if ABC294640 could affect the expression of Mcl-1 in MM. As shown in **Fig. 9A**, ABC294640 treatment led to down-regulation of Mcl-1 protein expression in MM cells, with the largest reduction occuring in OPM1 cells, which is also the most sensitive cell line for inhibition of proliferation by ABC294640. ABC294640 caused minimal changes in Mcl-1 expression in U266 cells.

Protein expression is controlled by the rate of biosynthesis and degradation. We thus determined if ABC294640 affected Mcl-1 gene transcription. MM cell lines were treated with ABC294640 for 16 hr and Mcl-1 mRNA was quantified by RT-PCR. Additionally, OPM1 cells were stably transduced with SK2-specific shRNA or control shRNA and Mcl-1 mRNA was measured. Inhibition of SK2 by either ABC294640 or shRNA did not affect Mcl-1 gene transcription (**data not shown**), suggesting that ABC294640 down-regulated Mcl-1 expression at the post-transcription level. We next tested if ABC294640 would increase the rate of Mcl-1 degradation. To this end, we treated OPM1 cells with DMSO or ABC294640 for 3 hrs, and then added cycloheximide to inhibit new protein synthesis. Mcl-1 protein levels were measured by immunoblot every hour for 4 hrs. As shown in **Fig. 9B**, ABC294640 treatment significantly increased Mcl-1 degradation.

Proteasome degradation plays an important role in regulating protein stability. We thus tested if ABC294640 treatment promoted Mcl-1 degradation in a proteasome-dependent manner. OPM1 cells were treated with ABC294640 alone, proteasome inhibitor (either MG132 or bortezomib) alone or in combination of ABC294640 with proteasome inhibitor. MG132 and bortezomib partially but reproducibly protected Mcl-1 from degradation induced by ABC294640 treatment (**Fig. 9C**). These data suggested that ABC294640 at least in part increased Mcl-1 proteasome degradation.

Noxa is a pro-apoptotic, Bcl-2 homolog (BH) 3-only member of the Bcl-2 family. Treatment with Bortezomib or arsenic trioxide induced up-regulation of Noxa while down-regulating Mcl-1.[87,88] These studies and others[89] suggested that Noxa and Mcl-1 form a complex and the Noxa/Mcl-1 ratio plays an important role in regulating apoptosis. We thus examined the effects of ABC294640 on Noxa expression. ABC294640 treatment increased Noxa protein expression levels by 50% to 2 fold (**Fig. 9D**). Additionally, ABC294640 up-regulated Noxa mRNA expression in all eight MM cell lines we tested by at least 5 fold (**Fig. 9E**). These data suggested that ABC294640 treatment shifted the Noxa/Mcl-1 apoptosis rheostat towards favoring cell death.

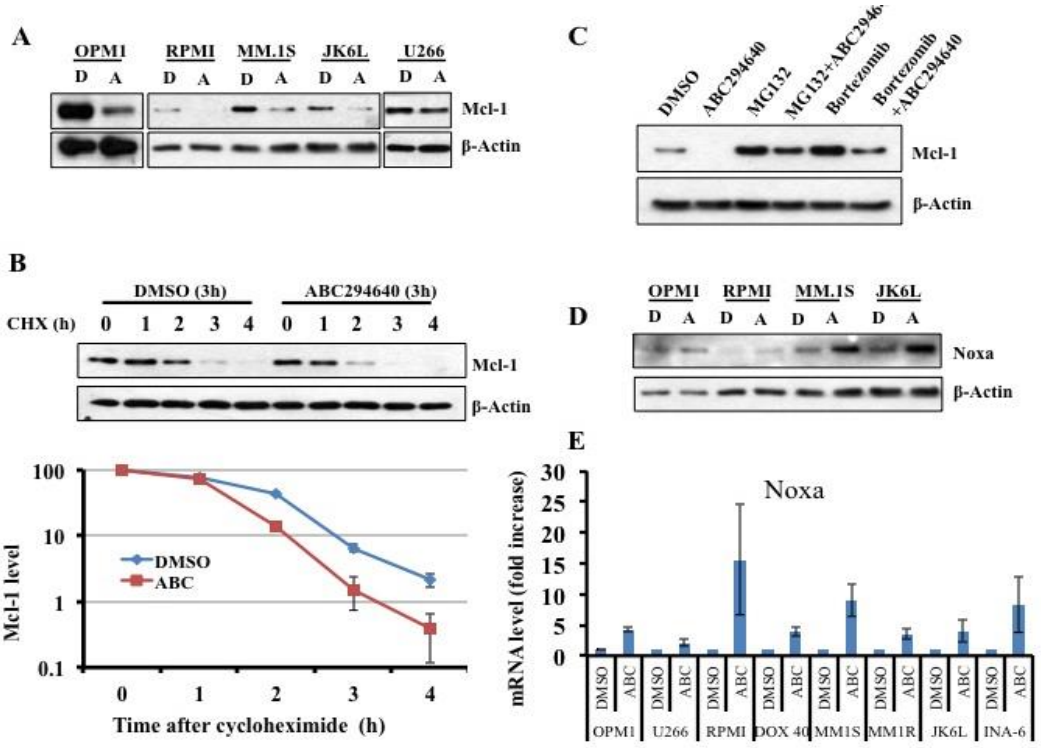


**Figure 9: ABC294640 enhanced Mcl-1 proteasome degradation and increased Noxa expression. A: ABC294640 down-regulated Mcl-1 protein expression.** MM cells (OPM1, RPMI-8226, MM.1S, JK6L, and U266) were treated with 30μM of ABC294640 (A) or DMSO (D) for 16 hr and whole cell lysates were prepared and analyzed for Mcl-1 expression by Western blot. β-actin was used as the loading control. Data were representative of 3 separate sets of experiments. **B: A294640 increased Mcl-1 protein degradation.** OPM1 cells were treated with 30μM of ABC294640 or DMSO for 3 hr and then cycloheximide (100mg/ml) was added. Cells were collected at each hour after cycloheximide treatment and whole cell lysate was prepared and analyzed for Mcl-1 expression by Western blot. β-actin was used as the loading control. Graph below represented the quantification of western blots. Western blots were quantified using ImageJ. Data were representative of 2 separate sets of experiments. **C: Proteasome inhibitor (MG132 and bortezomib) prevented the degradation of Mcl-1 by ABC294640.** OPM1 and JK6L cells were treated with DMSO control buffer, proteasome inhibitor MG132 (1μM) or Bortezomib (50nM) for 1 h, followed by treatment with DMSO or 30 μM of ABC294640 for additional 6h. Whole cell lysate was prepared and analyzed for Mcl-1 expression by Western blot. Data were representative of 2 separate sets of experiments. **D: ABC294640 increased Noxa protein expression.** OPM1, RPMI8226, MM.1S, and JK6L were treated with 30μM of ABC294640 (A) or DMSO (D) for 16 hr and whole cell lysates were prepared and analyzed for Noxa expression by Western blot. **E: ABC294640 induced Noxa gene expression.** Eight MM cell lines were treated with 30μM of ABC294640 (A) or DMSO (D) for 16 hr and RNA was isolated and analyzed for Noxa gene expression by RT-PCR. Gene expression was normalized against β-actin internal control. Graphs represented the fold change of Noxa mRNA in ABC294640- treated MM cells lines compared to DMSO- treated cells. Data shown in the figure were representative of at least 2 separate sets of experiments.

### 1.5.5. *ABC294640 down-regulates pS6 and promotes proteasome degradation of c-Myc.*

MM originates from post-germinal-center B cells and c-Myc is a class oncogene dysregulated in post-germinal center malignancies including MM. Using specific FISH probes, translocation involving Myc was found in 19 of 20 MM cell lines and approximately 50% of advanced primary MM tumors[90]. c-Myc plays a critical role in the progression from MGUS to MM [91,92]. The progression of MGUS to myeloma is associated with several-fold increase in MYC RNA expression [93]. Overexpression of MYC leads to the development of myeloma phenotypes in the Vk*MYC mouse model [94] and synergizes with IL-6 in plasma cell tumor formation in mice[95]. Furthermore, targeting MYC using short hairpin RNA or a selective small molecule inhibitor of MYC-Max heterodimerization (10058-F4) induces myeloma cell death [96]. These data suggest that myeloma cells are addicted to c-MYC activity, and that c-MYC is indispensable in myeloma development [93]. Furthermore, constitutive activation of PI3K/AKT/mTOR pathway is a common event in MM pathogenesis and contributes to MM proliferation and survival.[97] Our unpublished data indicated that the downstream of mTOR pathway, i.e., pS6, was highly up-

regulated in nearly all myeloma patients' bone marrow samples. Given the importance of c-Myc and pS6 in MM cell survival, we examined the effects of ABC294640 on the expression of c-Myc and pS6 in MM cells. ABC294640 treatment significantly down-regulated the expression of c-Myc and pS6 (**Fig. 10A**). U266 has no detectable c-Myc expression by Western blot analysis. We performed additional studies similar to those described with Mcl-1 to understand the mechanisms through which ABC294640 down-regulated c-Myc expression. We found that ABC294640 or SK2-specific shRNA did not affect the rate of c-Myc gene transcription (**data not shown**). Cycloheximide study suggested that ABC294640 increased c-Myc protein degradation (**Fig. 10B**). MG132 protected c-Myc from ABC294640-induced c-Myc degradation, suggesting that ABC294640 enhances proteasome degradation of c-Myc (**Fig. 10C**).

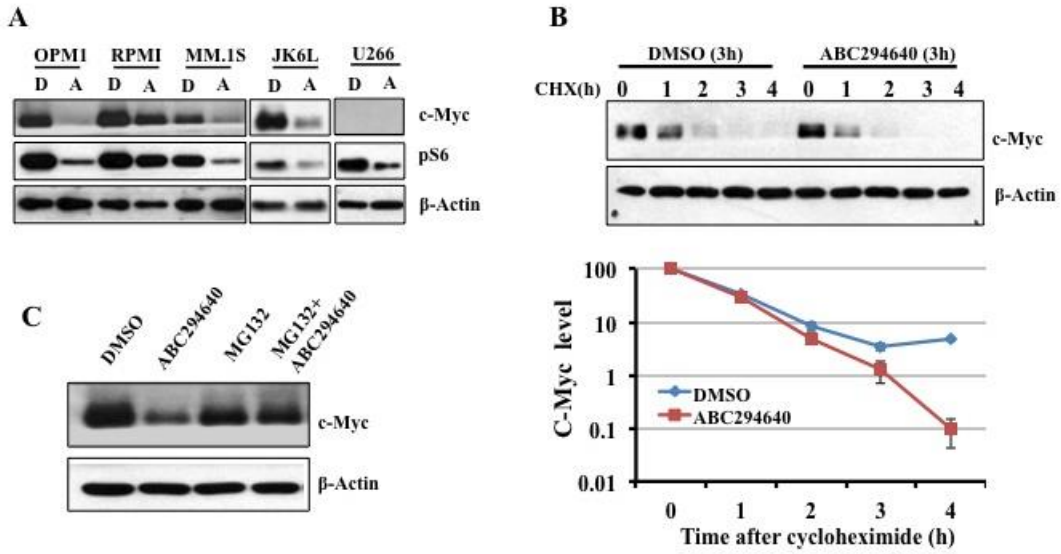


**Figure 10: ABC294640 enhanced c-Myc proteasome degradation. A: ABC294640 down-regulated c-Myc and pS6 protein expression.** MM cells (OPM1, RPMI-8226, MM.1S, JK6L, and U266) were treated with 30μM of ABC294640 (A) or DMSO (D) for 16 hr and whole cell lysates were prepared and analyzed for c-Myc and pS6 expression by Western blot. β-actin was used as the loading control. **B: A294640 increased c-Myc protein degradation.** OPM1 cells were treated with 30μM of ABC294640 or DMSO for 3 hr and then cyclohexamide (100mg/ml) was added. Cells were collected at each hour after cyclohexamide treatment and whole cell lysate was prepared and analyzed for c-Myc expression by Western blot. β-actin was used as the loading control. Graph below represented the quantification of western blots using ImageJ. **C: Proteasome inhibitor (MG132) prevented the degradation of c-Myc by ABC294640.** OPM1 and JK6L cells were treated with DMSO control buffer or MG132 (1μM) for 1 h, followed by treatment with DMSO or 30 μM of ABC294640 for additional 6h. Whole cell lysate was prepared and analyzed for c-Myc expression by Western blot. Data shown in the figure were representative of at least 2 separate sets of experiments.

### 1.5.6. *ABC294640 synergizes with Bcl-2 inhibitor in the killing of myeloma cells.*

We reasoned that combined chemotherapy incorporating drugs with different mechanisms of action is likely to be more effective in killing myeloma cells and could overcome drug resistance. To this end, we examined the anti-myeloma activity of ABC294640 in combination with other agents. We were particularly interested in combining ABC294640 with Bcl-2 inhibitor for the treatment of MM, because ABC294640 per se did not affect Bcl-2 expression (**Fig. 11A**). We treated MM cell lines with various concentrations of ABT-737 (a Bcl-2 inhibitor) in combination with 15μM of ABC294640. Cell proliferation was then measured by MTT assay. Combining ABC294640 with ABT-737 led to greater inhibition of MM cell proliferation (**Fig. 11B**). Additional ABC294640 concentrations were tested with ABT-737 and the combination index (CI) value was calculated. Fa-CI plot analysis demonstrated synergism between ABC294640 and ABT-737 in inhibiting myeloma cell proliferation (**Fig. 11C**).

### 1.5.7. *ABC294640 induces myeloma cell apoptosis in the presence of bone marrow stromal cells.*

BM stromal cells support the growth and survival of myeloma cells and confer them with drug resistance. We sought to test if ABC294640 could still effectively kill myeloma cells in the presence of BM stromal cells. We co-cultured e-GFP expressing myeloma cells with HS5 BM stromal cells. We then added ABC294640 to the co-culture system and measured Annexin $V^+$ cells gated on the $eGFP^+$

myeloma cells. ABC294640 did not induce apoptosis of HS5 BM stromal cells (**Fig. 11D**). Interestingly, as shown in **Fig. 11D,** the percentage of Annexin V$^+$ myeloma cells following ABC294640 treatment was quite similar in OPM1 cells alone and in OPM1 cells co-cultured with HS5 BM stromal cells. These data suggested that ABC294640 could induce myeloma cell apoptosis even in the presence of BM stromal cells.

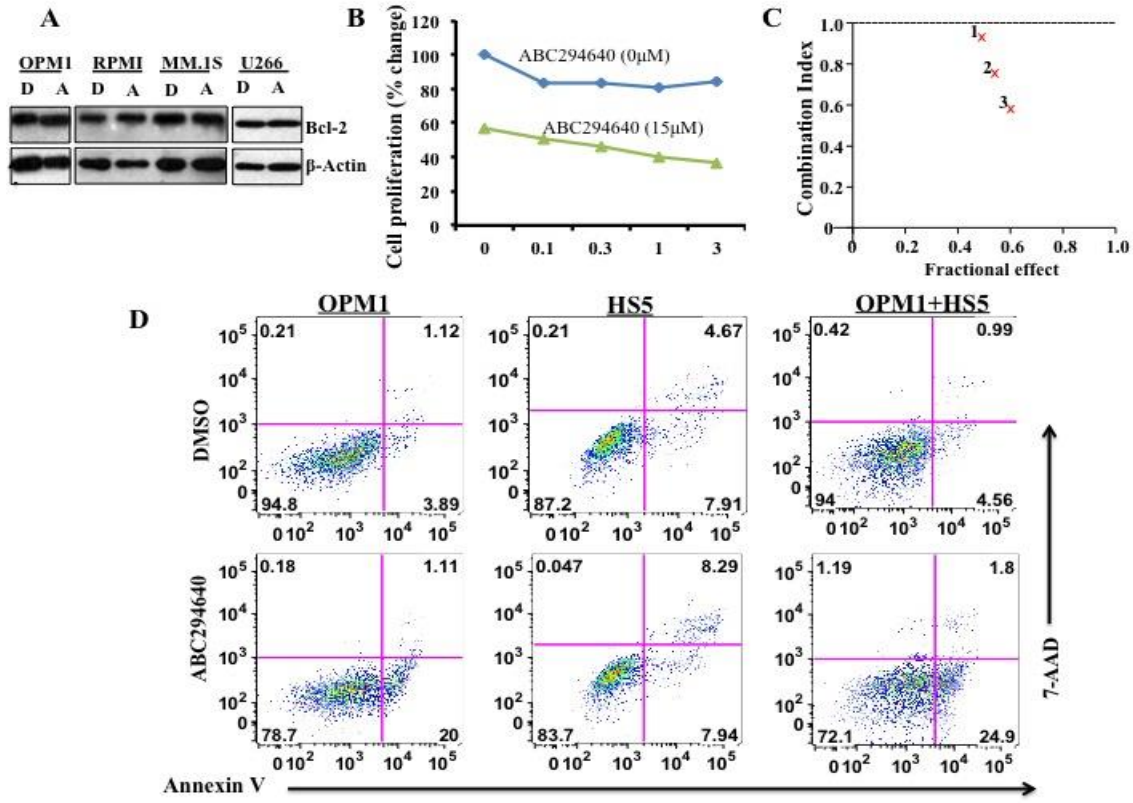


**Figure 11: ABC294640 acted synergistically with Bcl-2 inhibitor in inhibiting myeloma cell growth and induced myeloma cell apoptosis in the presence of bone marrow stromal cells. A. ABC294640 did not affect Bcl-2 expression.** MM cells (OPM1, RPMI-8226, MM.1S, and U266) were treated with 30μM of ABC294640 (A) or DMSO (D) for 16 hr and whole cell lysates were prepared and analyzed for Bcl-2 expression by Western blot. **B: Combination of ABC294640 and ABT-737 led to enhanced inhibition of cell proliferation.** OPM1 cells were treated with various concentrations of ABT-737 in the absence or presence of ABC294640 (15 μM) for 48 hr and cell proliferation was measured by MTT assay. **C: FA-CI plots showing the synergistic effect of ABC294640 and ABT-737.** Fa-CI plots for OPM1 cells revealed a synergistic inhibitory effect for ABC294640 15μM and ABT-737 at 0.1μM (indicated as 1), 0.3μM (indicated as 2) and 1μM (indicated as 3). In the Fa-CI plot, the dashed line [combination index (CI) =1] indicates an additive reaction between the two substances. Values below this dashed line imply synergism. **D: ABC294640 induced myeloma apoptosis in the presence of bone marrow stromal cells.** GFP expressing OPM1 cells were cultured on the monolayer of HS5 BM stromal cells and were treated for 8h with 30μM of ABC294640 or DMSO. The cells were stained with Annexin V and 7-AAD and Annexin V$^+$ apoptotic cells were gated on GFP positive OPM1 cells.

### 1.5.8. *ABC294640 suppresses MM tumor growth in mouse xenograft models.*

The *in vivo* anti-myeloma activity of ABC294640 was assessed using mouse xenograft models. We transduced MM.1S myeloma cells with lentiviral vector expressing luciferase and generated MM.1S cell line stably expressing luciferase. We performed two series of *in vivo* experiments. In the first series of experiments, the luciferase expressing MM.1S cells were injected via tail vein (**Fig. 12A**) or subcutaneously (**Fig. 12B**) into sublethally irradiated NOD/SCID IL-2γ (NSG) mice. Two days after the tumor injection the mice were treated with ABC294640 (50mg/kg daily i.p.) or vehicle control buffer for 30 days. In our second series of experiments, MM.1S cells were injected subcutaneously into irradiated (2.5 Gy) NSG mice. ABC294640 treatment was started 14 days later when bioluminescence imaging showed tumor engraftment, and continued daily for ~1 month (**Fig. 12C**). Tumor growth was monitored by bioluminescence imaging at the time points indicated. As shown in **Fig. 12A-12C**, ABC294640 effectively inhibited myeloma growth *in vivo.*

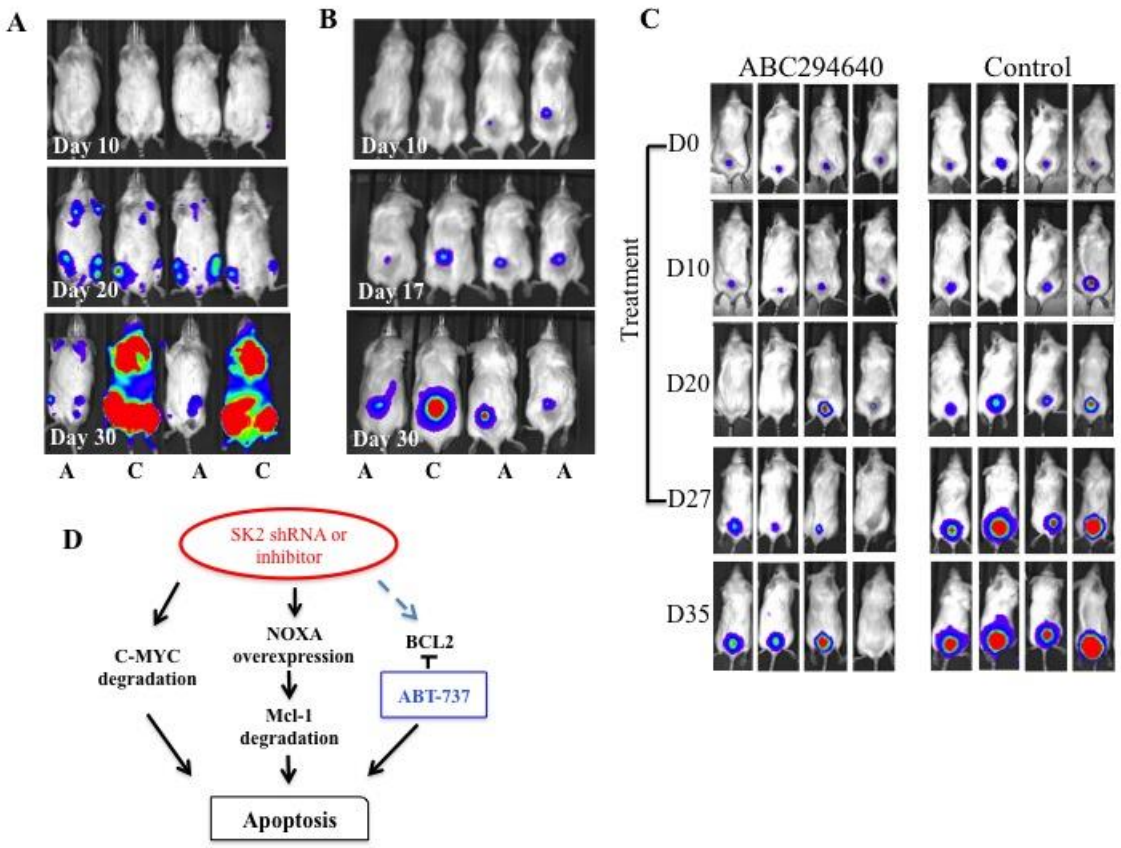


**Figure 12: ABC294640 suppressed myeloma growth *in vivo* in mouse xenograft models. A: ABC294640 inhibited myeloma growth *in vivo* in intravenously administrated mouse xenograft model.** NSG mice were sublethally irradiated (2.5 Gy) and injected via tail vein $0.5x10^6$ MM.1S myeloma cells stably expressing leuciferase. Two days later, the mice were divided randomly into 2 groups and treated either with ABC 294640 50mg/kg i.p. (A) or control vehicle (C; PBS+ 0.3% Tween-80) for 30 days. Every 10 days the mice were imaged using Perkin Elmer Ivis 200 imager and Live Image software. **B: ABC294640 inhibited myeloma growth *in vivo* in subcutaneously inoculated mouse xenograft model.** NSG mice were sublethally irradiated (2.5 Gy) and injected subcutaneously with $0.5x10^6$ MM.1S myeloma cells stably expressing leuciferase. Two days later, the mice were treated either with ABC294640 50mg/kg i.p. (Mice indicated as "A") or control vehicle (Mouse "C") for 30 days. Tumor growth was monitored by bioluminescence imaging. **C: ABC294640 inhibited myeloma growth *in vivo*.** NSG mice were sublethally irradiated (2.5 Gy) and injected subcutaneously with $0.5x10^6$ MM.1S myeloma cells stably expressing leuciferase. Two weeks later, the mice were imaged using bioluminescence imaging and showed tumor engraftment (D0). The mice were then treated with ABC294640 50mg/kg i.p. or control daily for 27 days (from D0 to D27). Tumor growth was monitored by bioluminescence imaging at time-points indicated (up to one week after the discontinuation of injection, i.e., D35). **D: Schematic diagram of the mechanisms of action of ABC294640 in MM cells.**

*Our pre-clinical data justify continued development of ABC294640 as a therapy for patients with myeloma.*

### *1.5.9. The roles of SK2 in the regulation of mitophagy and in bone marrow microenvironment – our most recent studies.*

Mitophagy is the autophagy process of mitochondria. We recently found that suppressed mitophagy (i.e., reduced level of PINK1 and PARK2 expression) correlated with myeloma pathogenesis and was associated with worse survival in patients with multiple myeloma. Furthermore, we found that SK2 plays a critical role in the regulation of mitophagy in multiple myeloma. More interestingly, when we injected VK*MYC myeloma cells into SK2 knockout mice or wildtype mice. SK2 knockout recipient mice did not develop myeloma whereas wildtype recipient mice did, suggesting a critical role of SK2 in the regulation of bone marrow microenvironment (such as osteoclasts, osteoblasts, macrophages or bone matrix). More recently, we found that SK2 knockout mice that did not develop myeloma had increased number of NK cells, NK T cells and B cells and reduced number of myeloid derived suppressive cells, compared to wildtype littermates that developed myeloma. These data implicated a role of SK2 in the regulation of anti-myeloma immunity.

Thus, it would be important to perform correlative studies to understand the effects of ABC294640 on mitophagy, bone formation/destruction markers and on Lyn/Src gene expression.

## 2. STUDY DESIGN

This is a two-part study comprised of a Phase I Dose Escalation and Expansion portion and a Phase II Efficacy at MTD portion.

Dexamethasone was commonly used in myeloma treatment. After debating the effects of combining ABC294640 with dexamethasone in the study, it was decided to exclude the combination of dexamethasone for several considerations: 1) All the patients would have been treated with dexamethasone before and are likely resistant to dexamethasone when they relapse or become refractory. Thus, including dexamethasone offers little benefits while adding toxicities and side effects; 2) The effects of combining ABC294640 with dexamethasone are unknown and they could antagonize each other. In addition, dexamethasone could affect the mechanism of ABC294640; 3) Many agents such as Carfilzomib, Elotuzumab and pomalidomide were tested as a single agent without the combination of dexamethasone.

### 2.1 Study description

The study will be conducted in two parts, an initial Phase Ib dose-finding study with dose escalation of the study drug ABC294640. The projected ABC294640 doses for the escalation phase are: 250, 500, and 750 mg BID as determined in the single agent trial for ABC294640. The dose will be given under fasting conditions or after a light to moderate meal.

### 2.2 Phase 1B

**Primary Objectives**

- To assess safety and determine the maximum tolerated dose (MTD) of single agent ABC294640 in patients with refractory or relapsed MM who have been previously treated with proteasome inhibitors and immunomodulatory agents.

**Secondary Objectives**

- To assess the antitumor activity of single agent ABC294640 in patients with refractory or relapsed MM after 3 cycles of treatment.
- To determine the pharmacokinetics of ABC294640 following administration of the single drug.
- To describe the effects of ABC294640 on plasma levels of sphingosine 1-phosphate, IL-6 and other

cytokines in patients with refractory or relapsed MM.

- To assess pharmacodynamic markers (SK2 mRNA level or activity, sphingolipid metabolites, c-Myc, Mcl-1 and pS6) in bone marrow CD138+ myeloma cells that may predict tumor response to ABC294640. For patients who receive more than 3 cycles of treatment and have a repeated bone marrow biopsy, RNA sequencing and Assay for transposases accessible chromatin sequencing (ATAC seq) will be performed to assess the effects of ABC294640 on global gene and epigenetic expression.
- 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.

## 2.3 Phase 1B design

After signing informed consent, patients will complete the evaluation process to determine eligibility. Once a patient is determined to be eligible for the study, he/she will be enrolled in the dose escalation Phase Ib study testing 3 ABC294640 doses (250 mg, 500 mg, and 750 mg BID). A cycle of treatment is defined as 28 days. One patient will be enrolled per week per dose. Subsequent patients on each cohort may not be enrolled less than one week after the previous patient. Patients in each dose cohort must complete Cycle 1 and undergo toxicity evaluation before the next dose level cohort is opened.

We use the Bayesian model averaging continual reassessment method (BMA-CRM)[98-100] for dose finding. In BMA-CRM, multiple CRM models are used, each equipped with a different skeleton and different skeletons represent different prior guesses of the toxicity profile of the drug. A discrete prior probability is assigned to each CRM model. The estimate of the toxicity probability at each dose level is the weighted average of the estimated probabilities at that dose level from the multiple CRM models, where the weight is the estimated probability of each model. BMA-CRM adaptively assigns a larger weight to a model of a better fit, and thus ensures the estimated probabilities to be always close to the best estimates among all the candidate models.

Let $d_1, d_2, d_3$ be ABC294640 dose levels 250 mg BID, 500 mg BID and 750 mg BID, respectively. Let $M_k$ be the CRM probability model associated with the $k$th skeleton $(p_{k1}, p_{k2}, p_{k3})$, for $1 \le k \le 3$. Under model $M_k$, the toxicity probability at dose level $j$ is assumed to be $\pi_{kj}(\alpha_k) = p_{kj}^{e^{\alpha_k}}, \quad 1 \le j \le 3,$ where the $\alpha_k$'s are unknown parameters. Prior data suggest that the toxicity probability should fall in the intervals (0, 0.1), (0.25, 0.35), and (0.4, 0.6), for the 3 doses $d_1, d_2, d_3$, respectively. Therefore, we use 3 skeletons, with specified probabilities $(p_{k1}, p_{k2}, p_{k3})$ as (0.03, 0.25, 0.4), (0.06, 0.3, 0.5), and (0.09, 0.35, 0.6.), for $k = 1, 2, 3,$ respectively. The prior distribution of $\alpha_k$ is taken as $N(0, 2^2)$, for $1 \le k \le 4$. Assume equal prior probabilities for the 3 models. Suppose that up to the current stage of the trial, $y_j$ patients have experienced toxicity among $n_j$ patients treated at dose level $j$, for $1 \le j \le 3$. Given observed data, use the method described previously[98-100] to obtain the BMA-CRM estimate of the toxicity probability at dose level $j$, which is denoted by $\overline{\pi}_j$, for $1 \le j \le 3$.

The target toxicity probability is set as $\phi_T = 0.33$. We treat a cohort of 3 patients at each dose level.

Individual patients may continue to receive therapy at the same dose of ABC294640 provided they do not experience unacceptable toxicity or clinical disease progression. No intrapatient dose escalation is

allowed.

Patients will continue on therapy until the development of intolerable toxicity, withdrawal of patient consent or other event as outlined in patient discontinuation section 8.2. Patients may remain on study until there is unequivocal evidence of clinical progression or the development of intolerable toxicities. Patients will have an End of Treatment visit 4-6 weeks after last dose of study drug.

### 2.4 Definition of dose-limiting toxicities

Toxicities will be graded according to the NCI CTCAE version 4.0.3 criteria. Dose-limiting toxicities (DLTs) will be defined as any of the following events that are at least (possibly, probably, or definitely) attributable to ABC294640 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

### 2.5 Phase II

**Primary Objectives**
- Assess overall treatment response rate and OS in patients with relapsed or refractory MM treated with single-agent ABC294640.

**Secondary Objectives**
- To assess the antitumor activity of ABC294640 in patients with refractory or relapsed MM after 3 cycles of treatment.
- 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 ABC294640.
- 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

The Phase II portion of the study will commence once the MTD of ABC294640 has been identified, which will be the Phase II dose.

## 3. STUDY PATIENTS

Patients with relapsed or refractory multiple myeloma who have previously been treated proteasome

inhibitors or immunomodulatory agents are eligible for this trial, regardless of the patients' stem cell transplant status. Both patients who have had a transplant and patients who did not receive a transplant are eligible for the study. Potential study patients will be screened according to the Eligibility Criteria. Study-specific evaluations may be performed only after written informed consent has been obtained.

## 3.1 Eligibility criteria

### Inclusion criteria

1. Patient must have a diagnosis of symptomatic multiple myeloma, who relapses or is refractory after previous treatment with a proteasome inhibitor (bortezomib or carfilzomib) and an immunomodulatory agent (thalidomide, lenalidomide or pomalidomide).

2. Have measurable disease as defined by at least one of the following:
   - Serum monoclonal (M) protein ≥0.5g/dl by protein electrophoresis
   - >200 mg of M protein in the urine on 24-hour electrophoresis
   - Serum immunoglobulin free light chain ≥10 mg/dL AND abnormal serum immunoglobulin kappa to lambda free light chain ratio
   - Monoclonal bone marrow plasmacytosis ≥30%

3. Voluntary signed and dated institutional review board (IRB) approved informed consent form in accordance with regulatory and institutional guidelines.

4. Time interval from last systemic chemotherapy (not including low dose dexamethasone) more than 2 weeks prior to initiation of ABC294640. Patients receiving high dose dexamethasone defined as 40mg dexamethasone a day for 4 days will need 2 weeks washout prior to initiation of ABC294640.

5. 18 years of age or older.

6. ECOG performance status of 0-2.

7. Acceptable liver function:
   - Bilirubin ≤ 1.5 times upper limit of normal (CTCAE Grade 1 baseline)
   - AST (SGOT), ALT (SGPT) ≤ 5 x ULN (CTCAE Grade 2 baseline)
   - Serum creatinine ≤1.5 XULN (CTCAE Grade 1 baseline)

8. Acceptable hematologic status (with or without transfusion support):
   - Absolute neutrophil count ≥1000 cells/$mm^3$,
   - Platelet count ≥50,000 (plt/$mm^3$),
   - Hemoglobin ≥9 g/dL.

NOTE: If the patient's bone marrow biopsy shows greater than or equal to 50% plasma cells, the platelet count should be ≥30,000 plt/$mm^3$, Hemoglobin ≥8 g/dL and ANC ≥500 cells/$mm^3$ (transfusion support or growth factor support is acceptable).

9. Urinalysis: No clinically significant abnormalities.

10. PT and PTT ≤ 1.5 X ULN after correction of nutritional deficiencies that may contribute to prolonged PT/PTT.

11. As determined by the treating investigator, the patient must have well-controlled blood pressure,

defined as systolic blood pressure <150mmHg and/or diastolic blood pressure <100 mmHg for the majority of measurements.

12. A negative pregnancy test (if female of child bearing potential). For men and women of child-producing potential, willingness to use effective contraceptive methods during the study.

**Exclusion criteria**

1. Pregnant or nursing women. NOTE: Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; or abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

2. Patients who are currently participating in any other clinical trial of an investigational product.

3. Major surgery within 30 days prior to start of treatment

4. Treatment with intravenous antibiotics, antivirals, or antifungals within 14 days prior to start of treatment.

5. Known human immunodeficiency virus infection

6. Active hepatitis B or C infection with abnormal liver functions (i.e., LFTs > 2X upper normal limits)

7. Unstable angina or myocardial infarction within 4 months prior to start of treatment, NYHA Class III or IV heart failure, uncontrolled angina, history of severe coronary artery disease, severe uncontrolled ventricular arrhythmias, sick sinus syndrome, or electrocardiographic evidence of acute ischemia or Grade 3 conduction system abnormalities unless subject has a pacemaker

8. Uncontrolled hypertension or uncontrolled diabetes within 14 days prior to start of treatment

9. Nonhematologic malignancy within the past 3 years with the exception of a) adequately treated basal cell carcinoma, squamous cell skin cancer, or thyroid cancer; b) carcinoma in situ of the cervix or breast; c) prostate cancer of Gleason Grade 6 or less with stable prostate-specific antigen levels; or d) cancer considered cured by surgical resection or unlikely to impact survival during the duration of the study, such as localized transitional cell carcinoma of the bladder

10. Subjects with pleural effusions requiring thoracentesis or ascites requiring paracentesis within 14 days prior to initiation of ABC294640

11. Any other clinically significant medical or psychiatric disease or condition or social situation that, in the Investigator's opinion, may interfere with protocol adherence or a subject's ability to give informed consent

# 4. ENROLLMENT AND TREATMENT

## 4.1 Screening

The screening examination will take place between Day -30 and 0. An informed consent must be signed by the patient before any study-specific screening procedure takes place. Subject data to be collected at the Screening Examination includes:

- Complete medical history

- Concomitant medication assessment
- Baseline Review of Systems and AE documentation
- Physical examination
- Vital signs (temperature, blood pressure, pulse rate, and respiratory rate)
- ECOG Performance Status
- Pregnancy test (for nonsterile women of childbearing potential within 2 days of Treatment Day 1)
- 12-lead electrocardiogram
- Serum chemistry
- Coagulation parameters
- CBC with differential
- Urinalysis
- Bone marrow biopsy and aspirates (should be performed within 2 weeks of treatment day 1)
- SPEP, IFE, UPEP, serum free light chain, LDH, beta-2 microglobulin
- Bone skeletal survey.

Subjects likely to meet eligibility criteria will be offered participation in the study after the investigator verifies that there is a current available slot (phase 1). Subjects will sign informed consent prior to any protocol associated procedure. Screening procedures will 1) ensure that subject meets all the eligibility criteria, 2) obtain disease assessment to allow efficacy measurements, 3) assess baseline toxicity, and 4) provide initial biological samples for pharmacodynamic and correlative studies.

## 4.2 Phase IB: Dose escalation phase

The projected ABC294640 doses for the escalation phase are: 250, 500, and 750 mg BID as determined in the single agent trial for ABC294640. Drug doses are defined in Table 4-1. The dose will be given under fasting conditions or after a light to moderate meal. One patient will be enrolled per week per dose. Subsequent patients on each cohort may not be enrolled less than one week after the previous patient. Patients in each dose cohort must complete Cycle 1 and undergo toxicity evaluation before the next dose level cohort is opened.

**Table 4-1 Dose Escalation Scheme**

| Dose level | ABC294640 Dose (mg AM / PM) | Capsules (AM / PM) |
|---|---|---|
| 0 | 250 / 250 | 1 / 1 |
| 1 | 500 / 500 | 2 / 2 |
| 2 | 750 / 750 | 3 / 3 |

Let the target probability of toxicity be $\phi_T = 0.33$. The following dose-finding algorithm will be employed.

- Treat the first cohort of patients at the 250 mg BID dose level.

- Suppose that the current dose level is $j^0$. Denote the estimated probabilities of toxicity based on the accumulated data to be $\overline{\pi}_1, \overline{\pi}_2, \overline{\pi}_3$ for the 3 dose levels $d_1, d_2, d_3$, respectively. Find the dose level $j^*$ such that

$j^* = \text{argmin} \mid \overline{\pi}_j - \phi_T \mid$.

Then

- if $j^0 > j^*$, de-escalate to dose level $j^0 - 1$;
- if $j^0 < j^*$, escalate to dose level $j^0 + 1$;
- otherwise, stay at dose level $j^0$ for the next cohort of patients.

- Once the maximum sample size is reached, the dose with the toxicity probability closest to $\phi_T$ is declared as the MTD.

If the lowest dose is still too toxic, as indicated by the fact that the posterior probability that the toxicity rate for the lowest dose is greater than 0.33 is >0.9, the trial will be terminated early for safety.

Individual patients may continue to receive therapy at the same dose of ABC294640 provided they do not experience unacceptable toxicity or disease progression. No intrapatient dose escalation is allowed.

Patients experiencing Grade 3 or 4 hematologic toxicity will have the study drug withheld according to the Dose Modification Plan outlined in Table 4-2. If Grade 3 or 4 toxicity continues after 4 weeks, the patient will be removed from the study. The patient will have an End of Treatment visit 4-6 weeks after last dose of study drug.

**Table 4-2 Dose Modification Plan**

| **NCI CTC 4.0.3 Criteria** | **ABC294640 Dose Modification Instructions** | **Need for discontinuation from study** |
|---|---|---|
| Hematologic toxicity (ANC and/or platelet) ≥Grade 3 | Hold study drug. Assess patient weekly. If the toxicity resolves to ≤ baseline by the beginning of subsequent week, restart study drug and reduce dose by 1 dose level (Table 4-1). Omitted doses are not made up. | If the toxicity does not resolve to ≤ baseline after 4 weeks, the patient will be removed from the study. |
| Other non- hematologic toxicity ≥3 | Hold study drug. Assess patient weekly. If the toxicity resolves to ≤ baseline by the beginning of subsequent week, restart study drug and reduce dose by 1 dose level (Table 4-1). Omitted doses are not made up. | Subsequent cycle can be delayed for up to 2 weeks until toxicity is resolved to ≤ baseline. If toxicity does not resolve to ≤ baseline after the extended 2 weeks, discontinue patient from study. |

## 4.3 Phase II

Once the MTD for ABC294640 is found, there will be expansion of the MTD cohort so that a total of 59 individuals will be treated at the MTD of ABC294640. The 3 phase Ib subjects taking the MTD will continue into Phase II portion of the study and will be included in the Phase II analysis. Thus, 56 additional patients will be enrolled in the Phase II portion of the study.

For the phase II portion of the study, we have incorporated safety stopping rules into our design to ensure patient safety (Table below). The first column of the table gives the number of patients treated in each stage. The second column gives the minimum number of patients with unacceptable serious adverse event (SAE) required to stop the trial early for safety. The third column lists the proportion of subjects with related SAEs. Specifically, we will stop the trial early if there is significant toxicity defined as an unacceptable serious adverse event rate of grade 3 or 4 toxicities deemed possibly, probably or definitely related to study drug. We consider an acceptable level to be 15% (null hypothesis) and an unacceptable

rate to be 30% (alternative hypothesis). We will craft a stopping rule for early stopping for strong evidence that the SAE rate is 30% vs. 15%. Given our sample size of 59 and based on 1000 simulations, this stopping rule yields a 83% chance of early termination if the true SAE rate is 0.30 and only a 4% chance of early stopping if the true SAE rate is 0.15.

| Total number of patients treated | Number of patients with grade 3 or 4 related SAEs | Point estimate of SAE rate |
|---|---|---|
| 12 | 8 | 0.67 |
| 24 | 10 | 0.42 |
| 36 | 11 | 0.31 |
| 48 | 12 | 0.25 |
| 59 | 13 | 0.22 |

### 4.4 Safety confirmation and follow-up

Individual patients may continue to receive therapy at the same dose provided they do not experience unacceptable toxicity or disease progression. No intrapatient dose escalation is allowed.

Treatment will continue until documentation of clinically progressive disease, unacceptable side effects, or patient withdraws consent. The patient will have an End of Treatment visit 4-6 weeks after last dose of study drug. Patients will then be followed for survival every 3 months for 2 years following treatment discontinuation and then follow-up per standard practice.

### 4.5 Disease assessment

Disease assessment will occur at the beginning of each cycle. Full myeloma restaging and treatment response assessment will be performed at the end of Cycles 3 and 6 (+/- 7 days) and will consist 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 will be categorized according to appendix E.

## 5. PK and PD Studies

### 5.1 Special Instructions related to administration of ABC 294640 on days of PK Sampling.

PK sampling will occur during Cycle 1 on Day 1. During these study time-points, administration of ABC294640 should only occur at the investigative site.

Blood samples for PK will be drawn on Day 1 of Cycle 1 immediately before dosing and at 1, 2, 4, and 8 hours after dosing.

On Day-1 of Cycle-1, a blood sample will be drawn from each subject for PD and PK analysis prior to drug administration. Then, all subjects will receive their morning dose. PK sampling will continue over an 8 hour period. Patients should take the afternoon dose approximately 12 hours after the morning dose.

In the event a patient vomits within 60 minutes after ingesting the investigational agent on Day 1 of Cycle 1, the PK blood draw schedule will be suspended. The investigational agent will not be administered again. Prior to receiving the investigational agent the following morning, the patient is to be treated with adequate anti-nausea therapy (reference Appendix B for contraindicated medications) prior to study drug administration.

## 5.2 Pharmacodynamic and correlative assays

For our secondary objectives, we will perform pharmacodynamic assays to measure the effects of ABC294640 on S1P in the plasma and in the bone marrow as well as on the c-Myc and Mcl-1 expression on myeloma cells. The key end-points for our pharmacodynamics and mechanistic studies are:

- Plasma PD analysis for sphingosine 1-phosphate (performed using samples collected during the PK sampling and at the beginning of each cycle prior to ABC294640 drug administration). Plasma IL-6 level performed at the beginning of each cycle for the first 6 cycles. Up to 41 different cytokines will be measured using multiplex cytokine assay.

- Sphingolipid concentrations (ceramides, sphingosine and sphingosine 1-phosphate (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). For patients who receive more than 3 cycles of treatment and have a repeated bone marrow biopsy, RNA sequencing and Assay for transposases accessible chromatin sequencing (ATAC seq) will be performed to assess the effects of ABC294640 on global gene and epigenetic expression.

- Expression of sphingolipid signaling genes, SK2 mRNA or activity, c-Myc, Mcl-1 and pS6 (measured in bone marrow CD138+ myeloma cells isolated at the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6) (if sufficient number of bone marrow CD138+ cells can be obtained)

- 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

Pharmacodynamic assays intend to verify if the hypothesized effects of the study drugs are indeed occurring in vivo.

**Table 5-1** - Pharmacodynamic (PD) and correlative assays:

| Assay | Material | Time points | Lab performing the assay |
|---|---|---|---|
| PK study | Peripheral blood plasma | Day 1 of cycle 1 immediately before dosing and at 1, 2, 4 and 8 hours after dosing | Sent-out test (Alliance Pharma) |
| S1P levels | Peripheral blood plasma | In the PK draws and at the beginning of each cycle prior to ABC294640 drug administration (up to cycle#6) | Sent-out test (Alliance Pharma) |

| | | | |
|---|---|---|---|
| IL-6 level | Peripheral blood plasma | At the beginning of each cycle prior to ABC294640 drug administration (up to cycle#6) | Dr. Yubin Kang's lab at Duke |
| Cytokines (up to 41 different cytokines) | Peripheral blood plasma (using existing samples) | In the PK draws (0, 4, 8 hours on cycle 1 day 1 and beginning of each cycle) | Dr. Yubin Kang's Lab and Duke core facility |
| Cell free PINK1/PARk2 expression, Serum bone formation/destruction markers | Peripheral blood plasma | At the beginning of each cycle prior to ABC294640 drug administration (up to cycle#6) | Dr. Yubin Kang's lab at Duke |
| Cell free DNA for next generation sequencing | Peripheral blood plasma | At the beginning of each cycle prior to ABC294640 drug administration (up to cycle#6) | Performed at the discretion of PI and only if the funds are available. |
| 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 | Sent-out test (Medical University of South Carolina) |
| SK mRNA or activity (if sufficient number of bone marrow CD138+ cells can be obtained) | Bone marrow CD138+ cells | At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6 | For mRNA, Dr. Kang's lab. For SK activity, Dr. Charles Smith's lab at Apogee Biotechnology Corp. |
| RNA seq and ATAC seq for determination of the ABC294640 on global gene and epigenetic expression | Bone marrow CD138+ cells | We had one patient in phase I who had received >3 cycles of treatment and had a repeated bone marrow biopsy. RNAseq and ATACseq will be only for this patient | Dr. Yubin Kang's lab and Duke Genomic Core facility |
| Protein level of c-Myc, Mcl-1 and pS6 (Western blot) (if sufficient number of bone marrow CD138+ cells can be obtained) | Bone marrow CD138+ cells | At the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6 | Dr. Yubin Kang's lab at Duke |
| 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 | Dr. Yubin Kang's lab at Duke |
| 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 | Dr. Yubin Kang's lab at Duke |

### 5.3 Time points of sample collection

Please see the Table 5-1 (pharmacodynamics and correlative assays) for the time points at which

samples will be collected. To compare with healthy control plasma samples, we will acquire up to 20 de-identified plasma samples from healthy donors, collected prior to 12/04/2018 under the biorepository protocol Pro00006268, *A Research Specimen Repository and Database to Evaluate Hematologic Malignancies*.

### 5.4 Sample collection and shipment

For peripheral blood samples, at each time point, a total of 8ml of blood will be collected (2x 4ml collected in K3EDTA tubes). Blood samples will be centrifuged as soon as possible at 500 x g for 15 min and the plasma will be transferred to cryovials (in 0.5 ml aliquots) and frozen at -80$^0$ C. These samples will be periodically transferred (shipped on dry ice by FedEx) to Alliance Pharma for analysis. For bone marrow samples, a total of 10 ml of marrow aspirate will be collected in K3 EDTA tube(s). For samples collected at Duke during the daytime, the samples will be transported immediately to Dr. Kang's lab. For samples collected at nighttime, the samples can be stored at 4$^0$C refrigerator and transportation to Dr. Kang's lab the following day. The address for Dr. Kang's laboratory: Room 4040, GSRB1, Duke University, phone: 919 668-4532.

### 5.5 Assays

-- Sphingolipid metabolite measurement (ceramides, sphingosine and sphingosine-1-phosphate):

Bone marrow aspirates will be centrifuged at 500 × g for 15 min and the supernatants will be collected. The samples will then be measured for sphingolipid metabolites related to the ceramide/S1P axis, including ceramides, sphingosine, and S1P. The sphingolipid metabolites will be measured using HPLC-MS/MS as described by Dr. Bielawski et al[101]. The concentration of each sphingolipid metabolite will be normalized against total cellular phosphate and represented as pM/nM of cellular phosphate.

-- SK activity assay

The SK activity in cell lysates prepared as described below, will be measured using HPLC-based SK activity assay as described[61]. In brief, the cell lysates will be incubated with NBD-Sph in the isozyme-selective assay buffers detailed below with 1 mg/ml fatty acid-free bovine serum albumin, 100 μM ATP, and 400 μM $MgCl_2$. The product, i.e., NBD-S1P, is separated from NBD-Sph by HPLC with fluorescence detection. The ratio of NBD-S1P/(NBD-Sph + NBD-S1P) is used as a measure of SK activity. SK-isozyme selective assay buffers each contain 20 mM Tris, pH7.4, 5 mM EDTA, 5 mM EGTA, 3 mM β-mercaptoethanol, 5% glycerol, 1× protease inhibitors (Sigma-Aldrich) and 1× phosphatase inhibitors (Roche Diagnostics, Indianapolis, IN). For the SK1 assay buffer, 0.25% (final) Triton X-100 is added; and for the SK2 buffer, 1 M (final) KCl is added. Assays are run at room temperature, and then a 1.5 volume of methanol is added to terminate the kinase reaction. After 10 min, the samples are centrifuged at 20,000g to pellet the precipitated protein, and the supernatants are analyzed by HPLC. The assay will be performed at Apogee Biotechnology Corporation.

-- Western blot for measurement of c-Myc, Mcl-1 and pS6

Bone marrow CD138$^+$ cells will be isolated from the bone marrow aspirates of these patients using Histopaque-1077 (Sigma) gradient separation followed by human CD138 enrichment kit (EasySep™, StemCell Technologies). The purity of the human CD138$^+$ cells is normally > 95%. The protein expression of c-Myc, Mcl-1 and pS6 will be measured by Western blot as we described[81]. MM cells are harvested, washed with PBS, and re-suspended in lysis buffer containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1% Triton x100, 1% Sodium deoxycholate, and 0.1% SDS. The cells are further lysed by brief sonication. The lysates are centrifuged at high speed for 10min to remove the cell debris. Total protein is quantified using Dc protein estimation kit (Bio Rad) with BSA for standard

curve. Approximately 20µg protein is loaded and run on SDS PAGE. The proteins are transferred onto nitrocellulose membrane. The membrane is blocked with 5% milk in *Tris-Buffered Saline* containing 0.05% of Tween 20 (TBST) and primary antibodies are applied with 1% BSA in TBST for overnight at $4^0$C with gentle rocking. The membrane is then probed with HRP-conjugated secondary antibody and developed using Pierce ECL substrate.

-- Correlative biomarker studies

For the measurement of cell free serum/plasma PINK1/PARK2 (mitophagy markers), cell free DNAs will be isolated from the plasma/serum using cell free DNA isolation columns. PINK1/PARK2 expression will be quantitated by PCR. For the measurement of serum/plasma bone formation/destruction markers, TRAP, Osteocalcin, osterix and others will be measured by ELISA or western blot analysis. For signaling pathway analyses, cell free DNA will be isolated from plasma samples and subject to the next generation sequencing and pathway enrichment analyses. For the measurement of Lyn/Src, osteoclast function and macrophage, CD138+ and CD138- cells will be isolated from the bone marrow aspirate and gene expression will be measured by quantitative RT-PCR. For the measurement of immunologic function and immunologic panel, bone marrow aspirates (CD138+ and CD138- cells) will be cultured with antibodies and cell proliferation will be measured. Additionally, the bone marrow aspirate will be measured for NK cells, NK T cells, T (CD3, CD4, CD8), B cells, MDSCs and co-signaling molecules (PD-1, PD-L1, CTLA-4, TIGIT, TIM3 etc). For simultaneous measurement of up to 41 different cytokines, multiplex cytokine assay will be performed using existing samples at the Duke Immunology core facility. We had one patient in our phase I study who had received >3 cycles of ABC294640 treatment and had a repeated bone marrow biopsy. To determine the effects of ABC294640 treatment on gene and epigenetic expression, we will perform RNA seq and Assay for transposases accessible chromatin with high-throughput sequencing (ATAC seq) using the existing bone marrow samples on this patient.

-- Healthy control plasma samples. To compare with healthy control plasma samples, we will use the plasma samples collected under the 00006268 biorepository protocol.

# 6. STUDY DRUG: ABC294640

ABC294640 is an orally available inhibitor of the enzyme sphingosine kinase-2.

## 6.1 Formulation

ABC294640 Capsules contain 250 mg of the milled active pharmaceutical ingredient (API) ABC294640 along with the excipients microcrystalline cellulose, USP/NF (Avicel® PH102, FMC) (FMC biopolymer) and colloidal silicon dioxide, NF (Cab-O-Sil® M5P). ABC294640 is a white to off-white powder and for clinical use it is encapsulated in white opaque hard gelatin capsules.

## 6.2 Dosage and administration

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. A dose modification plan is also provided in Section 4.

## 6.3 Storage

Storage conditions: Store at room temperature, 15-30°C (59-86°F) in a secure, locked area, properly labeled and segregated from other materials. This storage area should only be accessible to authorized individuals.

### 6.4 Labeling

The test article will be labeled with the sponsor name and address, description of contents and storage conditions, and will contain the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use."

### 6.5 Test article and accountability

The Investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of the test article including the date, quantity, batch or code number and identification of subjects (patient number and initials) who receive the test article. The Investigator will not supply the test article to any person except those named as sub-investigators and submitted to the local regulatory authority, designated staff and patients in this study. The Investigator will not dispense the test article from any sites other than those submitted to the local regulatory authority. The test article will not be relabeled or reassigned for use by other patients

Drug accountability procedures to be followed by study staff and patients are outlined in Section 7.

Upon completion of the study, unused supplies of the ABC294640 test article will be returned to the sponsor or destroyed as directed.

## 7. METHOD OF DETERMINING TREATMENT COMPLIANCE

Patients will be provided a drug diary at the beginning of each cycle, i.e. when additional drug is dispensed to the patient. Patients will be instructed to complete the drug diary and record their daily administration of ABC294640 during the morning and evening timeframes. Any missed or modified doses should be documented along with the reason for the modification. Patients will be instructed to return their container(s) of ABC294640 at each clinic visit so that the delegated research team can conduct a pill count to assess patient compliance. The research team has the ability to withdraw a patient from the study if that patient demonstrates repeated protocol noncompliance. Noncompliance is defined as intentionally not taking ABC294640 for 1 week in the absence of side effects attributed to ABC294640.

### 7.1 SAFETY PLAN

#### *7.1.1. GENERAL PLAN TO MANAGE SAFETY*

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria and routine monitoring as follows. In addition, an early stopping monitoring plan is outlined in Section 8.6.2.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements (performed by local laboratories). Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation

at each study visit for the duration of their participation in the study. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (within 30–42 days) after last dose of study drug. Patients who have an ongoing treatment-related Grade ≥ 3 or serious adverse event at the time of discontinuation from study treatment will continue to be followed as per Section 7.2.

Women of childbearing potential must have a negative pregnancy test prior to starting therapy and must use adequate contraceptive methods during and for 12 weeks after discontinuing study therapy. If a patient becomes pregnant despite precautions, she should be apprised of the potential risk of fetal morbidity or loss.

### *7.1.2. CONCOMITANT AND EXCLUDED THERAPIES*

All supportive care measures consistent with optimal patient care will be given throughout the study.

Use of anti-neoplastic or anti-tumor agents not part of the study therapy, including chemotherapy, radiation therapy*, immunotherapy, and hormonal anticancer therapy, is not permitted while participating in this study. Use of concurrent investigational agents is not permitted. Use of full dose warfarin is not permitted.
*Localized radiation is allowed.

Patients should receive full supportive care, including anti-emetic drugs, hematopoietic growth factors, transfusions of blood and blood products, fluid and electrolyte replacement, and antibiotics when appropriate. Patients are not permitted to have received cancer chemotherapy, including any other investigational therapy, within two weeks prior to study entry.

Allopurinol (in subjects at risk for TLS due to high tumor burden) is optional and will be prescribed at the Investigator's discretion. These subjects may receive allopurinol 300 mg PO daily. Vitamins and supplements should be recorded on the concomitant medication page. All transfusions and/or blood product related procedures must be recorded on the appropriate form.

Patients cannot receive concomitant therapy with drugs that are sensitive substrates of CYP450 1A2, 3A4, 2C9, 2C19 or 2D6, or strong inhibitors or inducers of all major CYP450 isozymes (including grapefruit juice).

A list of commonly used drugs that are that are sensitive substrates of CYP450 1A2, 3A4, 2C9, 2C19 or 2D6, or strong inhibitors or inducers of all major CYP450 isozymes (with the half-life of each drug identified) is included as Appendix B. Patients who receive any of these medications on a regular basis should be instructed not to take them during their participation in this study. Where possible, alternative medications should be prescribed or recommended. If a patient is receiving a drug that is a sensitive substrate of CYP450 1A2, 3A4, 2C9, 2C19 or 2D6, or strong inhibitor or inducer of a major CYP450 isozyme that cannot be stopped or replaced by another medication for the duration of the clinical study that patient should not be enrolled.

Additionally, due to the protein binding affinity of ABC 294640, patients should not receive concomitant therapy with Coumadin or Coumadin derivatives.

## 7.2 POST-TREATMENT EVALUATIONS

The visit at which a response assessment shows disease progression or the decision was made to discontinue treatment for other reasons, such as toxicity, will be considered the End of Treatment visit.

Patients completing their treatment should return for Post-30 day visit per Study Calendar Section 4.4. Patients who have an ongoing Grade ≥ 3 or serious adverse event that is at least possibly related to treatment will be contacted by the investigator or designee approximately every week until the event is resolved or determined to be irreversible.

Patients will be followed for survival by clinic visit, review of medical record, and/or telephone call every 3 months from treatment discontinuation date, until 2 years post treatment discontinuation.

### 7.3 PATIENT DISCONTINUATION

Patients may discontinue study treatment at any time. Any patient who discontinues treatment will be asked to return to the study center to undergo treatment discontinuation assessments as outlined within Study Calendar Section 4.4 *End of Treatment visit*. The primary reason for discontinuation should be recorded.

Subjects who meet the following criteria should be discontinued from study treatment:

- Grade 4 hypertension or Grade 3 hypertension not controlled with medication
- Nephrotic syndrome
- Grade ≥ 2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
- Any grade arterial thromboembolic event that that cannot be treated with non-coumadin therapy, such as IVC filter, or LMW heparin
- Grade 4 congestive heart failure
- Gastrointestinal perforation
- Tracheoesophageal fistula (any grade) or Grade 4 fistula
- Grade ≥ 3 bowel obstruction that has not fully recovered despite medical or surgical intervention
- Wound dehiscence requiring major medical or surgical intervention
- Documented disease progression per Appendix E
- Clinically significant deterioration of the patient's condition
- Patient noncompliance defined as intentionally not taking ABC294640 for 1 week in the absence of side effects attributed to ABC294640.
- Adverse events requiring treatment discontinuation
- Investigator determination that it is not in the patient's best interest to continue participation
- Pregnancy
- Withdrawal of consent by patient

## 8. STATISTICAL CONSIDERATIONS

### 8.1 PRIMARY OBJECTIVE

**Phase 1b:** 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 ABC294640 in patients with relapsed or refractory multiple myeloma who have previously been treated with a proteasome inhibitor and an immunomodulatory agent.

**Phase 2:** The primary objective of the phase 2 portion of the clinical trial is to evaluate the effects of single agent ABC294640 on treatment response and overall survival (OS) in patients with relapsed or refractory multiple myeloma who have previously been treated with a proteasome inhibitor and an immunomodulatory agent.

## 8.2 PRIMARY ENDPOINTS

### *8.2.1. Maximum tolerated dose*

The primary endpoint of the phase 1b portion of the trial is the maximum tolerated dose (MTD), determined using Bayesian model average continual reassessment method (BMA-CRM) for dose finding.

### *8.2.2. Treatment response*

One of the primary endpoints for the phase 2 portion of the trial is treatment response after Cycle 3 of treatment as defined by the International Myeloma Working Group criteria.

### *8.2.3. Overall survival*

The other primary endpoint for the phase 2 portion of the trial is OS defined as the time interval from trial enrollment to death due to any cause. Survival times will be censored for patients lost to follow-up or still alive at the trial's termination.

## 8.3 SECONDARY ENDPOINTS

### *8.3.1. Safety Endpoints*

Safety assessments will consist of monitoring and reporting 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.

**Dose-limiting toxicity:** For the phase 1b portion of the trial, toxicities will be graded according to the NCI CTCAE version 4.0.3 criteria. DLTs will be defined as any events that are at least (possibly, probably, or definitely) attributable to ABC294640 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

### *8.3.2 PK, PD and correlative endpoints*

- Plasma PD analysis for sphingosine 1-phosphate (performed using the PK samples and at the beginning of each cycle prior to ABC294640 drug administration). Plasma IL-6 performed at the beginning of each cycles for the first 6 cycles. Up to 41 different cytokines will be performed using multiplex cytokine assay.

- Sphingolipid concentrations (ceramides, sphingosine and sphingosine 1-phosphate (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)

- Expression of sphingolipid signaling genes, SK2 mRNA or activity, c-Myc, Mcl-1 and pS6

(measured in bone marrow CD138+ myeloma cells isolated at the beginning of treatment, at the last day of cycle #3 and at the last day of cycle #6) (if sufficient number of bone marrow CD138+ cells can be obtained). For patients who receive more than 3 cycles of treatment and have a repeated bone marrow biopsy, RNA sequencing and Assay for transposases accessible chromatin sequencing (ATAC seq) will be performed to assess the effects of ABC294640 on global gene and epigenetic expression.

- 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.

- 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.

## 8.4 DETERMINATION OF SAMPLE SIZE

### *8.4.1. Phase 1b*

The phase 1b trial will explore three dose cohorts of ABC294640. Bayesian model average continual reassessment method (BMA-CRM) will be used for dose finding to determine the MTD. One patient will be enrolled per week per dose. Subsequent patients on each cohort may not be enrolled less than one week after the previous patient. Patients in each dose cohort must complete cycle 1 and undergo toxicity evaluation before the next dose level cohort is opened. Up to 18 patients will be used for phase 1b study. The patients at the MTD will contribute to the total sample size for the phase 2 portion of the trial.

### *8.4.2. Phase 2*

Multiple myeloma patients who relapse or become refractory after receiving previous treatment with a proteasome inhibitor and an immunomodulatory agent usually achieve an response rate of less than 10% and survive less than a year with weekly dexamethasone salvage therapy. Currently several investigational drugs have improved the overall response rate (ORR) to about 20-35% and prolonged the OS to 1.5 years to 2 years. We consider an ORR of 20% and a 6 month improvement in OS for patients treated with single ABC294640 to be sufficiently promising to warrant further drug development.

We therefore design the phase 2 trial to test a null ORR of 10% versus an alternative of 20% and a null median OS of 12 months versus an alternative of 18 months – equivalent to a hazard ratio (HR) of 0.67 – assuming exponential survival, with approximately 36 months total accrual time (including 12 months of accrual for the phase 1b patients treated at the MTD), and 24 months of follow-up following accrual of the final patient.

The exponential survival assumption is strong. Assuming exponential survival, a test of the null hypothesis that median OS = 12 months versus the alternative that median OS = 18 months is equivalent to a test of a null hazard rate of 0.058 versus an alternative hazard rate of 0.039 (HR = 0.67). We will test this hypothesis based on a likelihood ratio test of the exponential hazard rate parameter. Fifty nine subjects yields 80% power to detect a HR of 0.67 based on the stated assumptions and one-sided $\alpha$ = 0.025. Power was estimated using the Southwest Oncology Group's one sample survival power and sample size calculator (http://www.swogstat.org/stat/public/one_survival.htm). This number of patients will yield 73% (or 80%) power to detect an increase of ORR from 10% to 20% (or 21%) with a one-sided chi-square test ($\alpha$ = 0.025).

## 8.5 ANALYSIS PLANS

### *8.5.1. Phase 1b*

The phase 1b trial will explore three dose cohorts of ABC294640 following the Bayesian model averaging continual reassessment design: 250 mg BID (dose 0), 500 mg BID (dose 1), and 750 mg BID (dose 2). The starting dose of 250 mg BID is known to be safely tolerated as a single agent in patients with solid tumors. A dose-limiting toxicity (DLT) is defined per section 2.4. 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. If the lowest dose (250 mg BID) is found to be too toxic, as indicated by the fact that the posterior probability that the toxicity rate for the lowest dose is greater than 0.33 is >0.9, then the trial will close and the drug declared unsafe.

### *8.5.2. Safety analysis*

The primary safety analysis will be conducted using descriptive statistics of the incidence of adverse events (AEs), including DLTs and discontinuations due to AEs. Adverse events will be coded by body system, and summary tables with incidence rates of AEs will be generated. Descriptive statistics of AEs will be reported by dose and for subsets of patients with DLTs, patients who discontinue due to AEs, and patients with related AEs. Severity, duration, investigator attributed relationship to treatment, and outcomes of AEs will be reported. The DLT rate will be estimated with its 95% CI.

### *8.5.3. Phase 2*

Survival times will be displayed graphically using a Kaplan-Meier curve. Median survival will be estimated, and the corresponding 95% CI constructed using Greenwood's variance estimate (Hosmer and Lemeshow, 1999). Assuming exponential survival, a test of the null hypothesis that median OS = 12 months versus the alternative that median OS = 18 months is equivalent to a test of a null hazard rate of 0.058 versus an alternative hazard rate of 0.039 (HR = 0.67). We will test this hypothesis based on a likelihood ratio test of the exponential hazard rate parameter. The overall response rate will tested with a chi-square test, under a null hypothesis that the ORR is 10% and an alternative hypothesis that the ORR is greater than 10%.

### *8.5.4. Analysis of secondary endpoints*

Treatment response will be determined using international multiple myeloma working group response criteria after 3 cycles and 6 cycles of treatment (Appendix E). Frequencies and rates of complete response (CR), partial response (PR), stable disease, and progressive disease will be reported.

## 8.6 INTERIM ANALYSES

### *8.6.1. Efficacy and futility interim analyses*

An interim analysis is planned after the enrollment of 33 efficacy evaluable patients in the Phase II portion of the study. Response data collected after the completion of 3 cycles of treatment will be reviewed. If there are 4 or more responses (PR, VGPR, CR, sCR) to this therapy at this time point, an additional 26 efficacy evaluable patients will be enrolled. Otherwise, the trial is stopped early for futility. If the ORR is 10%, the probability of early stop is 0.58, but if the ORR is 20%, the probability that the trial is stopped early is 0.08.

### *8.6.2 Safety stopping rules*

We have incorporated safety stopping rules into our design to ensure patient safety (Table below) during our phase II portion of the study. The first column of the table gives the number of patients treated in each stage. The second column gives the minimum number of patients with unacceptable serious adverse event (SAE) required to stop the trial early for safety. The third column lists the proportion of subjects with related SAEs. Specifically, we will stop the trial early if there is significant toxicity defined as an unacceptable serious adverse event rate of grade 3 or 4 toxicities deemed possibly, probably or definitely related to study drug. We consider an acceptable level to be 15% (null hypothesis) and an unacceptable rate to be 30% (alternative hypothesis). We will craft a stopping rule for early stopping for strong evidence that the SAE rate is 30% vs. 15%. Given our sample size of 59 and based on 1000 simulations, this stopping rule yields a 83% chance of early termination if the true SAE rate is 0.30 and only a 4% chance of early stopping if the true SAE rate is 0.15.

| Total number of patients treated | Number of patients with grade 3 or 4 related SAEs | Point estimate of SAE rate |
|---|---|---|
| 12 | 8 | 0.67 |
| 24 | 10 | 0.42 |
| 36 | 11 | 0.31 |
| 48 | 12 | 0.25 |
| 59 | 13 | 0.22 |

## 8.7 ANALYSIS POPULATIONS

### *8.7.1 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 has received at least one dose of study medication. For the analysis of tumor response, patients lost to follow-up prior to the 12-week evaluation will be considered failures.

### *8.7.2 Safety population*

The safety population is defined as all patients who receive at least one dose of study drug.

# 9. SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study is collected and reported to the FDA, appropriate IRB(s), DCN, and the sponsor in accordance with CFR 312.32 (IND Safety Reports).

## 9.1 ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of

AE also includes worsening of any pre-existing medical condition, except MM. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of study drug, whether or not related to use of the study drug. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time the subject initiates study treatment through the End of Treatment visit, all AEs must be recorded in the subject medical record and adverse events case report form. AEs will be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

AEs will be assessed according to the CTCAE version 4.0.3 (Appendix A). If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:
- Definite: The AE is clearly related to the study drug
- Probably: The AE is likely related to the study drug
- Possible: The AE may be related to the study drug
- Unrelated: The AE is clearly NOT related to the study drug

## 9.2 SERIOUS ADVERSE EVENTS

An AE is considered "serious" if in the opinion of the investigator it meets one or more of the following criteria:

- Results in death
- Is Life-threatening
- Constitutes a congenital anomaly or birth defect
- Is a medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

## 9.3 ADVERSE EVENT ASSESSMENT

The study period during which all AEs and SAEs must be reported begins after initiation of study treatment and ends 30 days following the last administration of study treatment. After this period, investigators should only report SAEs that are attributed to prior study treatment.

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, severity, suspected relationship to the study, outcome, and actions taken.

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:
"How have you felt since your last clinical visit?"
"Have you had any new or changed health problems since you were last here?"

Pre-existing diseases or conditions will not be considered AEs unless there is an increase in the

frequency or severity, or a change in the quality, of the disease or condition. Progression of cancer also will not be considered an AE.

## 9.4 ADVERSE EVENT CAUSALITY

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

| | |
|---|---|
| **Definitely related** | An adverse event occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary and feasible. |
| **Probably related** | An adverse event with a reasonable time sequence to administration, and which cannot be explained by concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal. Rechallenge information is not required to fulfill this definition. (Note: there are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists, (e.g. bone marrow suppression, fixed-drug eruptions, and tardive dyskinesias) |
| **Possibly related** | An adverse event with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals.<br>Information on drug withdrawal may be lacking or unclear. |
| **Not related** | An adverse event with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying diseases provide plausible explanations. |

## 9.5 ADVERSE REACTION EXPECTEDNESS

**Adverse reactions** are those adverse events considered possibly, probably or definitely related to study medication.

**Expected adverse reaction**s are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

**Unexpected adverse reactions** are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis. )

## 9.6 ADDITIONAL ADVERSE EVENT CONSIDERATIONS

### a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

### *b. Deaths*

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

### *c. Preexisting Medical Conditions*

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

### *d. Hospitalizations for Medical or Surgical Procedures*

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

### *e. Pregnancy*

If a female subject becomes pregnant while receiving investigational therapy or within 12 weeks after the last dose of study drug, a report should be completed and expeditiously submitted to the sponsor, and CATO Pharmacovigilance. Follow-up to obtain the outcome of the pregnancy should also occur. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed should be reported as an SAE.

### *f. Post-Study Adverse Events*

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior investigational agent exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

## 9.7 PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

Investigators must **report all SAEs within 24 hours of first becoming aware** of the event.

The completed SAE report form should be faxed or emailed to the following recipients:

The **Duke Study Team**:

ATTN: Ivy Belskie, RN
*cc: Dr. Yubin Kang*
FAX: (919) 668-1091
Email: Ivy.Belskie@duke.edu; Yubin.Kang@duke.edu

**AND**
All SAEs must be reported promptly to **CATO Pharmacovigilance** within 24 hours of first becoming aware of the event by sending to the pharmacovigilance email box:
RedHill-PhV@cato.com

Terry F Plasse, MD, Medical Director, RedHill Biopharma Ltd. is available for discussion should an investigator or other study staff members require consultation.

Phone: +1-917-913-7315 OR +972-50-588-7376
Email: Terry@Redhillbio.com

At the time of first notification of an SAE, the following information, if available, should be provided by the site to Duke and the pharmacovigilance email:

- Study protocol number
- Site Number
- Subject's study number
- Patient's date of birth
- Patient's gender
- Date of first dose of study treatment
- Date of last dose of test article, if applicable
- Unit dose and schedule of test article
- Adverse event term
- Time and date of occurrence of the event
- A brief description of the event, outcome to date and any actions taken
- The seriousness criteria (on) that were met
- Concomitant medication at onset of the event
- Relevant past history information

- Relevant laboratory test findings
- Investigator's opinion of the relationship to test article (refer to Section 11.6.4.7)
- Investigator's signature

Any missing or additional relevant information concerning the SAE should be provided in a follow-up SAE report.

The Investigator is required to comply with applicable regulations regarding the notification to the IRB. Acknowledgement of IRB review should be forwarded to the Duke study team.

Follow-up Information

After the initial SAE report, the investigator is required to follow and provide further information in regards to the subject's condition. All SAEs should be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. A follow-up SAE report form should be submitted within 24 hours of first awareness.

## 9.8 Quality Control and Quality Assurance

This clinical research study will be monitored internally by the PI and externally by the sponsor or designee. The PI and study staff will continuously monitor and tabulate adverse events at each study visit. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled;
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of AEs and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.

### 9.8.1 Sponsor or designee Oversight

RedHill Biopharma Ltd. (or its designee) will be responsible for activities such as monitoring, Study Trial Master File (TMF) maintenance, data querying and data transfers, pharmacovigilance, statistical analysis and clinical study report writing. The sponsor (or its designee) will also be responsible for FDA Reporting that includes: Expedited Serious Adverse Events Review and Annual IND reports.

## 9.9 Audits

The Duke School of Medicine Office of Audit, Risk and Compliance (OARC) may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the OARC auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. OARC audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator

initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

OARC audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize "best practices" in the research/clinical trials environment.

## 9.10 Data Management and Processing

### *9.10.1 Case Report Forms (CRFs)*

The electronic CRF will be the primary data collection document for the study. The CRFs will be updated in a timely manner following acquisition of new source data. Only approved study staff (i.e. Data managers, Data Coordinators), are permitted to make entries, changes, or corrections in the CRF.

Any paper CRFs created for the study will have errors crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary). The PI or authorized key personnel will maintain a record of the changes and corrections.

In the electronic CRF, an audit trail will be maintained automatically by the electronic CRF management system. Designated personnel will complete user training, as required or appropriate per regulations.

### *9.10.2 Data Management Procedures and Data Verification*

The sponsor or its designee will be responsible for clinical data management.

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

### *9.10.3 Study Closure*

Following completion of the study, the PI will be responsible for ensuring the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

# 10. ADMINISTRATIVE and ETHICAL CONSIDERATIONS

## 10.1 Regulatory and Ethical Compliance

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

## 10.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS Institutional Review Board (IRB) and DCI Cancer Protocol Committee (CPC) for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the CPC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the CPC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

## 10.3 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject or a legally acceptable representative. The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

## 10.4 Study Documentation

Study documentation includes but is not limited to source documents, case report forms (CRFs), monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory

Binder", which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

A case report form (CRF) (including both electronic and paper CRFs) will be the primary data collection document for the study. The CRFs will be updated within two weeks of acquisition of new source data. Only approved study staff (i.e. Data Managers, Data Coordinators), are permitted to make entries, changes, or corrections in the CRF. For paper CRFs, errors will be crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary). The Principal Investigator or authorized key personnel will maintain a record of the changes and corrections. For electronic CRFs, an audit trail will be maintained by the electronic CRF management system.

## 10.5 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using dedicated databases which are located on secure file servers. Access to electronic databases will be limited to Dr. Kang's staff, DCN staff, RedHill staff and designated affiliates.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

## 10.6 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the Duke Quality Assurance Plan.

### 10.7 Protocol Amendments

All protocol amendments must be initiated by the Principal Investigator and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

Though not yet required, the CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, etc.).

### 10.8 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of:

- at least two years after the date on which a New Drug Application is approved by the FDA
- at least two years after formal withdrawal of the IND associated with this protocol
- at least six years after study completion

## 11. SPONSOR AND MULTI-SITE INVESTIGATOR REQUIREMENTS

Dr. Yubin Kang will be responsible for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation, ensuring that the investigation is conducted in accordance with the general investigational plan and protocol, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug.

### 11.1 STUDY INITIATION

Centers participating in the Phase II portion of the study cannot begin enrollment until an initiation letter has been issued from the DCN. Each center is required to participate in an initiation conference call.

Before the start of this study and the shipment of ABC294640 to a participating center, the following documents must be on file at Duke Cancer Center. Participating centers will be responsible for forwarding the initiation documents to DCN.

Initiation documents can be submitted via electronic mail to the regulatory coordinator at Duke. Please ensure that the site and study identifier are clearly identified.

These documents are required to be submitted by each participating center:

1. U.S. Food and Drug Administration (FDA) Form 1572, signed by the Principal Investigator at the participating center.
2. The names of any sub-investigators at the participating center must appear on this form. Investigators must also complete all regulatory documentation as required by local regulations. This includes any required human subjects training required by the site's local IRB.
3. Current curricula vitae and documentation of professional licensure of the Principal Investigator and co-Investigators listed on the 1572.
4. Resumes and human subject protections documentation (e.g. NIH, CITI) for all research personnel

(e.g. study coordinators, data managers and other research personnel).

5. A signed and dated protocol signature page.
6. Written documentation of IRB approval of protocol (identified by title, protocol version and date of approval) for each site.
7. IRB approved study informed consent and HIPAA consent form, if applicable. HIPAA consent language can be included within the study informed consent. Please note that all informed consent forms should be reviewed and approved by the Duke Cancer Network office prior to submission to the site's designated IRB.
8. A signed Confidentiality Agreement.
9. A signed Clinical Trial Agreement for each site.
10. Laboratory certifications (CAP, CLIAs) and laboratory reference value ranges for each laboratory listed on the site's 1572.
11. Any Duke Cancer Network site specific forms as required.

## 11.2 STUDY COMPLETION

The following data and materials are required by DCN before a study can be considered complete or terminated:

1. Copies of protocol amendments and IRB approval/notification, if appropriate.
2. Copies of the IRB final report, documentation of submission to the IRB.
3. A summary of the study prepared by the Principal Investigator (Study report, manuscript and/or abstract).
4. All regulatory documents (e.g., updated curriculum vitae for each Principal Investigator, updated U.S. FDA Form 1572 for each site).

## 11.3 INSTITUTIONAL REVIEW BOARD APPROVAL

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. In addition, any advertising materials, if applicable, must be approved by the IRB. The study will be conducted in accordance with U.S. FDA, applicable national and local health authority, and IRB requirements.

The Principal Investigator at each site is responsible for keeping his/her IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the study drug by the investigator. Some IRBs may have other specific adverse event requirements to which investigators are expected to adhere. If a center's IRB does not require the review of all external adverse events, a written policy or statement outlining the IRB's reporting requirements must be provided to

DCN.

## 11.4 QUALITY ASSURANCE

The PI, sponsor or its designee will be responsible for quality assurance. Steps to assure the accuracy and reliability of data include the review of protocol procedures with the Investigator and associated personnel prior to the study, and periodic monitoring visits by the sponsor and/or its representative, and ongoing data quality reviews of received case report forms. Data will be reviewed for accuracy and completeness and any discrepancies will be resolved with the Investigator or designees as appropriate.

## 11.5 DATA COLLECTION

Data collected will be entered into electronic CRFs managed by the sponsor or designee. The eCRF system is a 21CFR part 11 compliant system. The sponsor and its representatives will be provided access to the data collected, and for monitoring purpose, to source information, in accordance with institutional policy.

# 12. GENERAL CONSIDERATIONS

### 12.1 Discontinuation of the Study

The Sponsor reserves the right to discontinue this study for safety or administrative reasons at any time.

### 12.2 Routine Study Termination

The end of this study is defined as the date of the last visit of the last patient (last patient out or last patient last visit) participating in the study. Within 90 days of the end of the clinical study, the sponsor or designee will notify the IRB and regulatory authorities regarding routine termination of the study as required.

### 12.3 Premature Study Termination

The study may be terminated prematurely for any reason and at any time by the sponsor, an IRB/IEC, or a Regulatory Authority. A decision to prematurely terminate the study is binding to all investigators of all study sites. If the study is terminated prematurely, all investigators have to inform their patients and take care of appropriate follow-up and further treatment of the patients to ensure protection of the patients' interests.

### 12.4 Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval of the Sponsor. The protocol amendment must be signed by the Investigator and approved by the IRB or IEC before implementation. Protocol amendments will be filed with the appropriate regulatory agency(s) having jurisdiction over the conduct of the study.

### 12.5 Protocol Deviations for Emergency or Adverse Event

An Investigator shall notify the Principal Investigator and the reviewing IRB of any deviation from the investigational plan to protect the life or physical well-being of a patient in an emergency. Such notice shall be given as soon as possible, but in no event later than five working days after the emergency occurred. Except in such an emergency, prior approval by the Principal Investigator is required for

changes or deviations from a plan, and if these changes or deviations affect the scientific soundness of the plan or the rights, safety, or welfare of patients, notification of the FDA and IRB in accordance with government regulations is also required.

### 12.6 Source Documents

All information recorded in the CRF must be supported by corresponding source documentation. Examples of acceptable source documentation include, but are not limited to, hospital records, clinic and office charts laboratory notes, and recorded data from automated instruments, memoranda, and pharmacy dispensing records. For the proposed study, electronic records will be the primary source documentation, along with written reports for laboratory assays.

During the study, select CRF data may be used as original data collection tools as long as a description of this documentation process is maintained in the Investigator's study files. Before the study starts, a list identifying any data to be recorded directly on the CRFs (i.e., no prior written or electronic record of data) and considered to be source data will be provided.

Clinical laboratory data required by the protocol will be electronically transferred from the central laboratory to the sponsor or its designee. A paper copy of the laboratory results will be provided to the study site and should be retained with each patient's source data.

## 13. APPENDICES

# *APPENDIX A: NATIONAL CANCER INSTITUTE COMMON TOXICITY CRITERIA, VERSION 4.03*

HTTP://WWW.EORTC.BE/SERVICES/DOC/CTC/CTCAE_4.03_2010-06-14_QUICKREFERENCE_5X7.PDF

## *APPENDIX B: LIST OF CYP3A4 INHIBITORS*

From http://www.georgetown.edu/departments/pharmacology/davetab.html

The following are known inhibitors of CYP3A4:

| | |
|---|---|
| Delaviridine | Indinavir |
| Nelfinavir | Ritonavir |
| Saquinavir | Amiodarone |
| Cimetidine | Ciprofloxacin |
| Clarithromycin | Diethyl-dithiocarbamate |
| Diltiazem | Erythromycin |
| Fluconazole | Fluvoxamine |
| Gestodene ++ | Grapefruit juice |
| Itraconazole | Ketoconazole |
| Mifepristone | Nefazodone |
| Norfloxacin | Norfluoxetine |
| Mibefradil | Troleandomycin |
| Atazanavir | Indinavir |
| Telithromycin | Voriconazole |

The following are known inducers of CYP3A4:

| | |
|---|---|
| Rifampicine | Phenytoin |
| Rifabutin | Rifapentine |
| Carbamazepine | Phenobarbital |
| St. John's Wort | |

## *APPENDIX C: ECOG PERFORMANCE STATUS SCALE*

| Grade | Description |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework or office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours |
| 3 | Capable of only limited self-care, confined to a bed or chair >50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

## *APPENDIX D: NEW YORK HEART ASSOCIATION GUIDELINES*

| | |
|---|---|
| **Class I** | People whose physical activity is not limited. Ordinary physical activity does not cause undue fatigue, heart palpitations, trouble breathing, or chest pain. |
| **Class II** | People who have some limitation on physical activity. They are comfortable at rest, but ordinary physical activity causes fatigue, heart palpitations, trouble breathing, or chest pain. |
| **Class III** | People who have a marked limitation on physical activity. They are comfortable at rest, but less-than-ordinary physical activity causes fatigue, heart palpitations, trouble breathing, or chest pain. |
| **Class IV** | People who are unable to carry on any physical activity without discomfort. Symptoms may be present even at rest. If any physical activity is undertaken, discomfort is increased |

## *APPENDIX E: RESPONSE AND RELAPSE CRITERIA FOR MULTIPLE MYELOMA*

| Response category | Criteria |
|---|---|
| Stringent complete response (sCR) | CR as defined below plus<br>• Normal FLC ratio<br>• Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence |
| Complete response (CR) | • Negative immunofixation of the serum and urine and<br>• • ≤ 5% plasma cells in bone marrow and<br>• Disappearance of any soft tissue plasmacytomas. |
| Very good partial response (VGPR) | • Serum and urine M-protein detectable by immunofixation but not on electrophoresis or<br>• 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.<br>• 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<br>• 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%<br>• 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:<br>Increase of ≥25% from baseline in<br>• Serum M-component and/or (the absolute increase must be ≥0.5 g/dl)<br>• Urine M-component and/or (the absolute increase must be ≥200 mg/24hour<br>• 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<br>• Bone marrow plasma cell percentage: the absolute % must be ≥1-%<br>• Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas<br>• 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 |
| **Clinical relapse** | Clinical relapse requires one or more of:<br>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<br>1. Development of new soft tissue plasmacytomas or bone lesions<br>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.<br>3. Hypercalcemia (> 11.5 mg/dl) [2.65 mmol/l]<br>4. Decrease in hemoglobin of ≥ 2g/dl [1.25 mmol/l]<br>5. Rise in serum creatinine by 2 mg/dl or more [177 micro mol/l or more] |

*APPENDIX F: STUDY CALENDAR*

**PHASE Ib AND II PORTION - CLINICAL AND LABORATORY EVALUATIONS**

| 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 | 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 | 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] | | |
| Survival Follow-up | | | | | | | | | | | | | | | | | | | | X |

Footnotes:

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 ABC294640 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 ABC294640 (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 C1D1, these procedures/labs do not need to be repeated. If urine monoclonal protein was performed within 14 days of C1D1, 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 C1D1, 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 C1D1 prior to study drug administration and at approximately 1, 2, 4, and 8, hours after dosing along with PK samples and prior to ABC294640 drug administration on D1 of each subsequent cycle through C6.

## *APPENDIX G: DRUG DIARY*

**ABC294640 Drug Diary (Page 67 of 3)** **Cycle**:_____**Subject ID:_____** *Version 3, 03/22/2017*

Take ____ pills in the morning and ____ pills in the evening approximately 12 hours apart. (Example: 9:00am and 9:00 pm)

Take the pills at least 1 hour before or 2 hours after eating. Bring the pill bottle with any leftover pills (or if empty) and diary with you to *each* clinic visit.

If you have questions, call _____ at the phone number: _____. My doctor's name is: _____.

| Week | Day | Date (DD-MMM-YYYY) | ABC294640 | | | | Comment (include details and reason for missed doses, etc) |
|---|---|---|---|---|---|---|---|
| | | | # of Tablets Taken ___ mg | Exact Time Tablets Taken HH:MM (AM) | # of Tablets Taken ___ mg | Exact Time Tablets Taken HH:MM (PM) | |
| 1 | 1 | | | | | | |
| | 2 | | | | | | |
| | 3 | | | | | | |
| | 4 | | | | | | |
| | 5 | | | | | | |
| | 6 | | | | | | |
| | 7 | | | | | | |
| 2 | 8 | | | | | | |
| | 9 | | | | | | |
| | 10 | | | | | | |
| | 11 | | | | | | |
| | 12 | | | | | | |
| | 13 | | | | | | |
| | 14 | | | | | | |

***To be completed by Research RN:** **wk1** date reviewed: _____ time:_____ Initials:___
**wk**2 date reviewed: _____ time:_____ Initials:_____

**ABC294640 Drug Diary (Page 68 of 3)** **Cycle**:_____**Subject ID:_____** *Version 3, 03/22/2017*

Take ____ pills in the morning and ____ pills in the evening approximately 12 hours apart. (Example: 9:00am and 9:00 pm)

Take the pills at least 1 hour before or 2 hours after eating. Bring the pill bottle with any leftover pills (or if empty) and diary with you to *each* clinic visit.

If you have questions, call _____ at the phone number: _____. My doctor's name is:

| Week | Day | Date (DD-MMM-YYYY) | ABC294640 | | | | Comment (include details and reason for missed doses, etc) |
|---|---|---|---|---|---|---|---|
| | | | # of Tablets Taken ___ mg | Exact Time Tablets Taken HH:MM (AM) | # of Tablets Taken ____ mg | Exact Time Tablets Taken HH:MM (PM) | |
| 3 | 15 | | | | | | |
| | 16 | | | | | | |
| | 17 | | | | | | |
| | 18 | | | | | | |
| | 19 | | | | | | |
| | 20 | | | | | | |
| | 21 | | | | | | |
| 4 | 22 | | | | | | |
| | 23 | | | | | | |
| | 24 | | | | | | |
| | 25 | | | | | | |
| | 26 | | | | | | |
| | 27 | | | | | | |
| | 28 | | | | | | |

_____.

***To be completed by Research RN:*** **wk3** date reviewed: _____ time:_____ Initials:___
**wk4** date reviewed: _____ time:_____ Initials:_____

If you need additional room to write your information or comments, please use the table below. Write any additional notes regarding your answers above.

| Date | Additional Notes |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |

## REFERENCES

1. Ludwig, H.*, et al.* Survival and years of life lost in different age cohorts of patients with multiple myeloma. *J Clin Oncol* **28**, 1599-1605 (2010).
2. Kyle, R.A. & Rajkumar, S.V. Multiple myeloma. *Blood* **111**, 2962-2972 (2008).
3. Cronin, C.C. & Harris, A.M. Atrial fibrillation and interatrial septal aneurysm in a patient with Marfan's syndrome. *Int J Cardiol* **34**, 115-117 (1992).
4. Palumbo, A.*, et al.* Oral melphalan, prednisone, and thalidomide in elderly patients with multiple myeloma: updated results of a randomized controlled trial. *Blood* **112**, 3107-3114 (2008).
5. Wijermans, P.*, et al.* Phase III Study of the Value of Thalidomide Added to Melphalan Plus Prednisone in Elderly Patients With Newly Diagnosed Multiple Myeloma: The HOVON 49 Study. *J Clin Oncol*.
6. Child, J.A.*, et al.* High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med* **348**, 1875-1883 (2003).
7. Blade, J.*, et al.* High-dose therapy intensification compared with continued standard chemotherapy in multiple myeloma patients responding to the initial chemotherapy: long-term results from a prospective randomized trial from the Spanish cooperative group PETHEMA. *Blood* **106**, 3755-3759 (2005).
8. Attal, M.*, et al.* A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. *N Engl J Med* **335**, 91-97 (1996).
9. Attal, M.*, et al.* Single versus double autologous stem-cell transplantation for multiple myeloma. *N Engl J Med* **349**, 2495-2502 (2003).
10. Richardson, P.G.*, et al.* A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* **348**, 2609-2617 (2003).
11. Richardson, P.G.*, et al.* Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* **352**, 2487-2498 (2005).
12. Kuhn, D.J.*, et al.* Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. *Blood* **110**, 3281-3290 (2007).
13. Barlogie, B.*, et al.* Incorporating bortezomib into upfront treatment for multiple myeloma: early results of total therapy 3. *Br J Haematol* **138**, 176-185 (2007).
14. Reeder, C.B.*, et al.* Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. *Leukemia* **23**, 1337-1341 (2009).
15. Knop, S.*, et al.* Bortezomib, IV cyclophosphamide, and dexamethasone (VelCD) as induction therapy in newly diagnosed multiple myeloma: results of an interim analysis of the German DSMM Xia trial. *J Clin Oncol* **27**, abstr 8516 (2009).
16. Palumbo, A.*, et al.* Bortezomib as induction before autologous transplantation, followed by lenalidomide as consolidation-maintenance in untreated multiple myeloma patients. *J Clin Oncol* **28**, 800-807 (2010).
17. Rajkumar, S.V.*, et al.* Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. *Lancet Oncol* **11**, 29-37 (2010).
18. Kumar, S.K.*, et al.* Bortezomib, dexamethasone, cyclophosphamide and lenalidomide combination for newly diagnosed multiple myeloma: phase 1 results from the multicenter EVOLUTION study. *Leukemia*.
19. Furuya, H., Shimizu, Y. & Kawamori, T. Sphingolipids in cancer. *Cancer Metastasis Rev* **30**, 567-

576 (2011).
20. Santos, C.R. & Schulze, A. Lipid metabolism in cancer. *FEBS J* (2012).
21. Snider, A.J., Alexa Orr Gandy, K. & Obeid, L.M. Sphingosine kinase: Role in regulation of bioactive sphingolipid mediators in inflammation. *Biochimie* (2010).
22. Nixon, G.F. Sphingolipids in inflammation: pathological implications and potential therapeutic targets. *Br J Pharmacol* **158**, 982-993 (2009).
23. Maceyka, M., Milstien, S. & Spiegel, S. Sphingosine-1-phosphate: the Swiss army knife of sphingolipid signaling. *J Lipid Res* **50 Suppl**, S272-276 (2009).
24. Cowart, L.A. Sphingolipids: players in the pathology of metabolic disease. *Trends Endocrinol Metab* **20**, 34-42 (2009).
25. Saddoughi, S.A., Song, P. & Ogretmen, B. Roles of bioactive sphingolipids in cancer biology and therapeutics. *Subcell Biochem* **49**, 413-440 (2008).
26. Billich, A. & Baumruker, T. Sphingolipid metabolizing enzymes as novel therapeutic targets. *Subcell Biochem* **49**, 487-522 (2008).
27. Cuvillier, O. Sphingosine in apoptosis signaling. *Biochim Biophys Acta* **1585**, 153-162 (2002).
28. Cuvillier, O.*, et al.* Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature* **381**, 800-803 (1996).
29. T, P.L. & Wang, H.G. Editorial [hot topic: therapeutic targeting of the sphingolipid "biostat" in hematologic malignancies (guest editors: Thomas p. Loughran and Hong-gang wang)]. *Anticancer Agents Med Chem* **11**, 780-781 (2011).
30. Ponnusamy, S.*, et al.* Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. *Future Oncol* **6**, 1603-1624 (2010).
31. Leung, R.W., Alison, J.A., McKeough, Z.J. & Peters, M.J. A study design to investigate the effect of short-form Sun-style Tai Chi in improving functional exercise capacity, physical performance, balance and health related quality of life in people with Chronic Obstructive Pulmonary Disease (COPD). *Contemp Clin Trials* **32**, 267-272 (2011).
32. Ekiz, H.A. & Baran, Y. Therapeutic applications of bioactive sphingolipids in hematological malignancies. *Int J Cancer* **127**, 1497-1506 (2010).
33. Leong, W.I. & Saba, J.D. S1P metabolism in cancer and other pathological conditions. *Biochimie* **92**, 716-723 (2010).
34. Isakoff, S.J.*, et al.* A phase II trial of the PARP inhibitor veliparib (ABT888) and temozolomide for metastatic breast cancer. *J. Clin. Oncol.* **28(suppl)**, abstr 1019 (2010).
35. Mathias, S., Pena, L.A. & Kolesnick, R.N. Signal transduction of stress via ceramide. *Biochem J* **335 ( Pt 3)**, 465-480 (1998).
36. Perry, D.K. & Hannun, Y.A. The role of ceramide in cell signaling. *Biochim Biophys Acta* **1436**, 233-243 (1998).
37. Perry, D.K. & Kolesnick, R.N. Ceramide and sphingosine 1-phosphate in anti-cancer therapies. *Cancer Treat Res* **115**, 345-354 (2003).
38. Kolesnick, R. & Fuks, Z. Radiation and ceramide-induced apoptosis. *Oncogene* **22**, 5897-5906 (2003).
39. Olivera, A. & Spiegel, S. Sphingosine kinase: a mediator of vital cellular functions. *Prostaglandins* **64**, 123-134 (2001).
40. Spiegel, S. & Milstien, S. Sphingosine 1-phosphate, a key cell signaling molecule. *J Biol Chem* **277**, 25851-25854 (2002).
41. Strub, G.M., Maceyka, M., Hait, N.C., Milstien, S. & Spiegel, S. Extracellular and intracellular actions of sphingosine-1-phosphate. *Adv Exp Med Biol* **688**, 141-155 (2010).
42. Pitman, M.R. & Pitson, S.M. Inhibitors of the sphingosine kinase pathway as potential therapeutics. *Curr Cancer Drug Targets* **10**, 354-367 (2010).
43. Cuvillier, O.*, et al.* Activation of sphingosine kinase-1 in cancer: implications for therapeutic

targeting. *Curr Mol Pharmacol* **3**, 53-65 (2010).
44. Gangoiti, P.*, et al.* Control of metabolism and signaling of simple bioactive sphingolipids: Implications in disease. *Prog Lipid Res* **49**, 316-334 (2010).
45. Baud, V. & Karin, M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov* **8**, 33-40 (2009).
46. Ader, I., Brizuela, L., Bouquerel, P., Malavaud, B. & Cuvillier, O. Sphingosine kinase 1: a new modulator of hypoxia inducible factor 1alpha during hypoxia in human cancer cells. *Cancer Res* **68**, 8635-8642 (2008).
47. Ahmad, M., Long, J.S., Pyne, N.J. & Pyne, S. The effect of hypoxia on lipid phosphate receptor and sphingosine kinase expression and mitogen-activated protein kinase signaling in human pulmonary smooth muscle cells. *Prostaglandins Other Lipid Mediat* **79**, 278-286 (2006).
48. Anelli, V., Gault, C.R., Cheng, A.B. & Obeid, L.M. Sphingosine kinase 1 is up-regulated during hypoxia in U87MG glioma cells. Role of hypoxia-inducible factors 1 and 2. *J Biol Chem* **283**, 3365-3375 (2008).
49. Schwalm, S.*, et al.* Sphingosine kinase-1 is a hypoxia-regulated gene that stimulates migration of human endothelial cells. *Biochem Biophys Res Commun* **368**, 1020-1025 (2008).
50. Le Scolan, E.*, et al.* Overexpression of sphingosine kinase 1 is an oncogenic event in erythroleukemic progression. *Blood* **106**, 1808-1816 (2005).
51. Xia, P.*, et al.* An oncogenic role of sphingosine kinase. *Curr Biol* **10**, 1527-1530 (2000).
52. Maceyka, M.*, et al.* SphK1 and SphK2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. *J Biol Chem* **280**, 37118-37129 (2005).
53. Okada, T.*, et al.* Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis. *J Biol Chem* **280**, 36318-36325 (2005).
54. Doll, F., Pfeilschifter, J. & Huwiler, A. The epidermal growth factor stimulates sphingosine kinase-1 expression and activity in the human mammary carcinoma cell line MCF7. *Biochim Biophys Acta* **1738**, 72-81 (2005).
55. Hait, N.C.*, et al.* Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. *J Biol Chem* **280**, 29462-29469 (2005).
56. French, K.J.*, et al.* Discovery and evaluation of inhibitors of human sphingosine kinase. *Cancer Res* **63**, 5962-5969 (2003).
57. French, K.J.*, et al.* Antitumor activity of sphingosine kinase inhibitors. *J Pharmacol Exp Ther* **318**, 596-603 (2006).
58. Maines, L.W.*, et al.* Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. *Dig Dis Sci* **53**, 997-1012 (2008).
59. Maines, L.W., French, K.J., Wolpert, E.B., Antonetti, D.A. & Smith, C.D. Pharmacologic manipulation of sphingosine kinase in retinal endothelial cells: implications for angiogenic ocular diseases. *Invest Ophthalmol Vis Sci* **47**, 5022-5031 (2006).
60. Smith, C.D., French, K.J. & Zhuang, Y. Sphingosine Kinase Inhibitors. in *USPTO*, Vol. US 7,338,961 B2 (Apogee Biotechnology Corporation, US 7,338,961, 2008).
61. French, K.J.*, et al.* Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2. *J Pharmacol Exp Ther* **333**, 129-139 (2010).
62. Gao, P., Peterson, Y.K., Smith, R.A. & Smith, C.D. Characterization of isoenzyme-selective inhibitors of human sphingosine kinases. *PLoS One* **7**, e44543 (2012).
63. Antoon, J.W.*, et al.* Pharmacological Inhibition of Sphingosine Kinase Isoforms Alters Estrogen Receptor Signaling in Human Breast Cancer. *J Mol Endocrinol* (2011).
64. Antoon, J.W.*, et al.* Antiestrogenic effects of the novel sphingosine kinase-2 inhibitor ABC294640. *Endocrinology* **151**, 5124-5135 (2010).
65. Antoon, J.W.*, et al.* Targeting NF-kB mediated breast cancer chemoresistance through

selective inhibition of sphingosine kinase-2. *Cancer Biol Ther* **11**(2011).
66. Beljanski, V., Knaak, C. & Smith, C.D. A novel sphingosine kinase inhibitor induces autophagy in tumor cells. *J Pharmacol Exp Ther* **333**, 454-464 (2010).
67. Beljanski, V., Knaak, C., Zhuang, Y. & Smith, C.D. Combined anticancer effects of sphingosine kinase inhibitors and sorafenib. *Invest New Drugs* (2010).
68. Beljanski, V., Lewis, C.S. & Smith, C.D. Antitumor activity of sphingosine kinase 2 inhibitor ABC294640 and sorafenib in hepatocellular carcinoma xenografts. *Cancer Biol Ther* **11**, 524-534 (2011).
69. Chumanevich, A.A.*, et al.* Suppression of colitis-driven colon cancer in mice by a novel small molecule inhibitor of sphingosine kinase. *Carcinogenesis* **31**, 1787-1793 (2010).
70. Fitzpatrick, L.R., Green, C.L., Maines, L.W. & Smith, C.D. Experimental osteoarthritis in rats is attenuated by ABC294640, a selective inhibitor of sphingosine kinase-2. *Pharmacology* **87**, 135-143 (2011).
71. Fitzpatrick, L.R.*, et al.* Attenuation of arthritis in rodents by a novel orally-available inhibitor of sphingosine kinase. *Inflammopharmacology* **19**, 75-87 (2010).
72. Maines, L.W., Fitzpatrick, L.R., Green, C.L., Zhuang, Y. & Smith, C.D. Efficacy of a novel sphingosine kinase inhibitor in experimental Crohn's disease. *Inflammopharmacology* **18**, 73-85 (2010).
73. Smith, C.D., Holschbach, M.A., Olsewicz, J. & Lonstein, J.S. Effects of noradrenergic alpha-2 receptor antagonism or noradrenergic lesions in the ventral bed nucleus of the stria terminalis and medial preoptic area on maternal care in female rats. *Psychopharmacology* **224**, 263-276 (2012).
74. Chauhan, D. & Anderson, K.C. Mechanisms of cell death and survival in multiple myeloma (MM): Therapeutic implications. *Apoptosis* **8**, 337-343 (2003).
75. Oancea, M., Mani, A., Hussein, M.A. & Almasan, A. Apoptosis of multiple myeloma. *Int J Hematol* **80**, 224-231 (2004).
76. Li, Q.F., Wu, C.T., Guo, Q., Wang, H. & Wang, L.S. Sphingosine 1-phosphate induces Mcl-1 upregulation and protects multiple myeloma cells against apoptosis. *Biochem Biophys Res Commun* **371**, 159-162 (2008).
77. Garcia-Bernal, D.*, et al.* Sphingosine-1-phosphate activates chemokine-promoted myeloma cell adhesion and migration involving alpha4beta1 integrin function. *J Pathol* **229**, 36-48 (2013).
78. Yasui, H.*, et al.* FTY720 induces apoptosis in multiple myeloma cells and overcomes drug resistance. *Cancer Res* **65**, 7478-7484 (2005).
79. Yabu, T.*, et al.* Thalidomide-induced antiangiogenic action is mediated by ceramide through depletion of VEGF receptors, and is antagonized by sphingosine-1-phosphate. *Blood* **106**, 125-134 (2005).
80. Li, Q.F.*, et al.* Activation of sphingosine kinase mediates suppressive effect of interleukin-6 on human multiple myeloma cell apoptosis. *Br J Haematol* **138**, 632-639 (2007).
81. Kummetha Venkata, J.*, et al.* Inhibition of sphingosine kinase 2 down-regulates the expression of c-Myc and Mcl-1 and induces apoptosis in multiple myeloma. *Blood* (2014).
82. Chng, W.J.*, et al.* Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. *Cancer Res* **67**, 2982-2989 (2007).
83. Tiedemann, R.E.*, et al.* Kinome-wide RNAi studies in human multiple myeloma identify vulnerable kinase targets, including a lymphoid-restricted kinase, GRK6. *Blood* **115**, 1594-1604 (2010).
84. Derenne, S.*, et al.* Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x(L) is an essential survival protein of human myeloma cells. *Blood* **100**, 194-199 (2002).
85. Zhang, B., Gojo, I. & Fenton, R.G. Myeloid cell factor-1 is a critical survival factor for multiple

myeloma. *Blood* **99**, 1885-1893 (2002).
86. Wuilleme-Toumi, S.*, et al.* Mcl-1 is overexpressed in multiple myeloma and associated with relapse and shorter survival. *Leukemia* **19**, 1248-1252 (2005).
87. Morales, A.A., Gutman, D., Lee, K.P. & Boise, L.H. BH3-only proteins Noxa, Bmf, and Bim are necessary for arsenic trioxide-induced cell death in myeloma. *Blood* **111**, 5152-5162 (2008).
88. Gomez-Bougie, P.*, et al.* Noxa up-regulation and Mcl-1 cleavage are associated to apoptosis induction by bortezomib in multiple myeloma. *Cancer Res* **67**, 5418-5424 (2007).
89. Alves, N.L.*, et al.* The Noxa/Mcl-1 axis regulates susceptibility to apoptosis under glucose limitation in dividing T cells. *Immunity* **24**, 703-716 (2006).
90. Shou, Y.*, et al.* Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. *Proc Natl Acad Sci U S A* **97**, 228-233 (2000).
91. Chng, W.J.*, et al.* Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma. *Leukemia* **25**, 1026-1035 (2011).
92. Zingone, A. & Kuehl, W.M. Pathogenesis of monoclonal gammopathy of undetermined significance and progression to multiple myeloma. *Semin Hematol* **48**, 4-12 (2011).
93. Kuehl, W.M. & Bergsagel, P.L. MYC addiction: a potential therapeutic target in MM. *Blood* **120**, 2351-2352 (2012).
94. Chesi, M.*, et al.* AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. *Cancer Cell* **13**, 167-180 (2008).
95. Rutsch, S.*, et al.* IL-6 and MYC collaborate in plasma cell tumor formation in mice. *Blood* **115**, 1746-1754 (2010).
96. Holien, T., Vatsveen, T.K., Hella, H., Waage, A. & Sundan, A. Addiction to c-MYC in multiple myeloma. *Blood* **120**, 2450-2453 (2012).
97. Pene, F.*, et al.* Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene* **21**, 6587-6597 (2002).
98. Daimon, T., Zohar, S. & O'Quigley, J. Posterior maximization and averaging for Bayesian working model choice in the continual reassessment method. *Stat Med* **30**, 1563-1573 (2011).
99. Yuan, Y. & Yin, G. Robust EM Continual Reassessment Method in Oncology Dose Finding. *Journal of the American Statistical Association* **106**, 818-831 (2011).
100. Yin, G., Yuan Y. Bayesian Model Averaging Continual Reassessment Method in Phase I Clinical Trial. *Journal of the American Statistical Association* **104**, 954-968 (2009).
101. Hammad, S.M.*, et al.* Blood sphingolipidomics in healthy humans: impact of sample collection methodology. *J Lipid Res* **51**, 3074-3087 (2010).