MEDICAL COLLEGE OF WISCONSIN
CANCER CENTER

CLINICAL STUDY PROTOCOL

A Pilot Study to Investigate the Hypomethylating Properties of Freeze-dried Black Raspberries (BRB) in Patients with Myelodysplastic Syndrome or Myelodysplastic Syndrome/Myeloproliferative Neoplasm (MDS/MPN)

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BRaspberry

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PROTOCOL SUMMARY

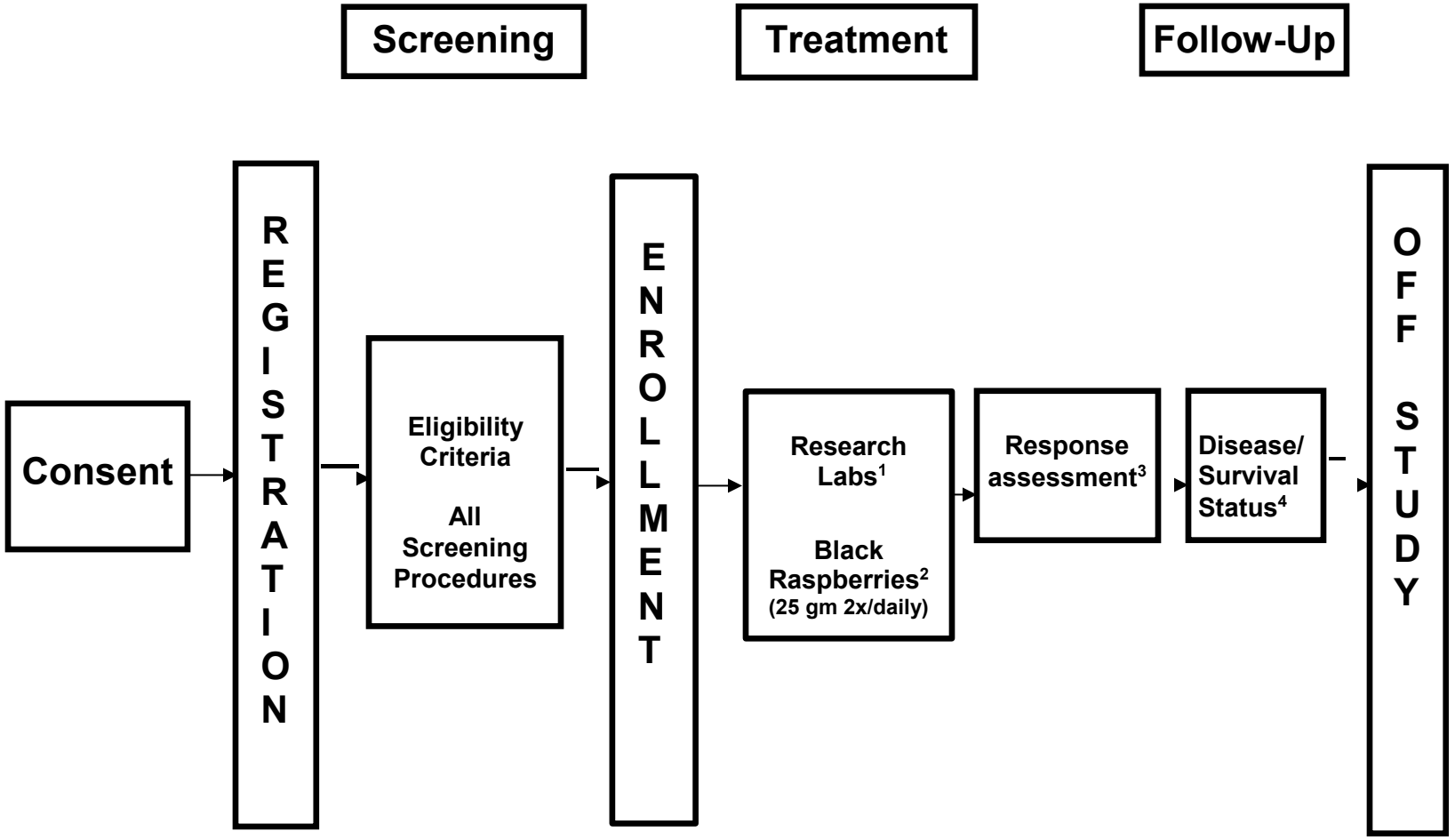
Title	A Pilot Study to Investigate the Hypomethylating Properties of Freeze-dried Black Raspberries (BRB) in Patients with Myelodysplastic Syndrome or Myelodysplastic Syndrome/ Myeloproliferative Neoplasm (MDS/MPN)
Oncore Identifier	IIT-ATALLAHBLACKRASPBERRY
Principal Investigator/ Sponsor-Investigator	Ehab Atallah, MD
Study Site	Froedtert & the Medical College of Wisconsin
Clinical Trial Phase	II
Study Disease	Myelodysplastic Syndrome or Myelodysplastic Syndrome /Myeloproliferative Neoplasm (MDS/MPN)
Eligibility Criteria	**Inclusion Criteria** Patients must have a confirmed diagnosis of myelodysplastic syndrome or myelodysplastic syndrome /myeloproliferative neoplasm (MDS/MPN) proven by bone marrow biopsy/aspirate. Patients with cytopenias (blood cell counts lower than the institutional lower limit of normal within the eight weeks prior to the study) who are receiving or received: • red blood cell transfusions • observation • platelet transfusions • erythropoietin • granulocyte colony-stimulating factors • granulocyte-macrophage colony-stimulating factors • hydrea Age ≥18 years. Predicted life expectancy of at least 12 weeks. Patients should be expected to stay on the same therapy for the period of the study. Patients who do not have an indication for and/or are believed to be unable to tolerate a hypomethylating agent are eligible for the trial. Reproductive requirements: • Female patients must meet one of the following: - Postmenopausal for at least one year before the screening visit, or

	- Surgically sterile, or - If they are of childbearing potential, agree to practice two effective methods of contraception from the time of signing of the informed consent form through 30 days after the last dose of study drug, AND - Must also adhere to the guidelines of any treatment-specific pregnancy prevention program, if applicable, or - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable contraception methods.) • Male patients, even if surgically sterilized (i.e., status postvasectomy), must agree to one of the following: - Practice effective barrier contraception during the entire study treatment period and through 30 days after the last study drug dose, OR - Must also adhere to the guidelines of any treatment-specific pregnancy prevention program, if applicable, OR - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception.) Ability to understand a written informed consent document, and the willingness to sign it. **Exclusion Criteria** A potential subject who meets any of the following exclusion criteria is ineligible to participate in the study. Previously received hypomethylating agents. Allergy to black raspberries. Inability to swallow oral medication. Inability or unwillingness to comply with the BRB administration requirements. Uncontrolled intercurrent illness, including, but not limited to symptomatic congestive heart failure, or psychiatric illness/social situations, that, in the treating investigator's discretion, would limit compliance with study requirements. Concurrent active malignancy is not an exclusion criterion.

	Active infection not well controlled by antibacterial or antiviral therapy. Pregnant or lactating women. Participation in clinical trials with other investigational agents not included in this trial, within 14 days of the start of this trial and throughout the duration of this trial. Patients with allergies to other members of the Rosacea family including but not limited to: apple, almond, apricot, cloudberry, blackberry, etc.
Study Rationale	Hypomethylating agents (HMAs), such as azacitidine and decitabine, are FDA-approved therapies for MDS patients. Approximately 50% of patients respond to HMAs. In addition, HMAs have improved survival and quality of life of patients with MDS when compared with other therapies. Preclinical research shows black raspberries (BRBs) have hypomethylating effects in the colon, blood, spleen and bone marrow of mice treated with BRBs. The aim of this study is to evaluate the hypomethylating properties of BRBs in patients with MDS or MDS/MPN for three cycles (one cycle = 28 days) of BRB supplementation.
Primary Objectives	To evaluate the potential hypomethylating effects of freeze-dried black raspberries (BRBs) in the peripheral blood of patients with myelodysplastic syndrome or myelodysplastic syndrome /myeloproliferative neoplasm (MDS/MPN) after three cycles of BRB administration.
Secondary Objectives	1. To evaluate the toxicity of BRBs in patients with MDS or MDS/MPN. 2. To evaluate the hematological response according to modified IWG criteria (Appendix 2) in patients with MDS or MDS/MPN regardless of the initial blood count.
Study Design	This is a phase II single-group pilot study. This trial is designed to evaluate efficacy and methylation. This study's overarching aim is to evaluate the systemic effects of BRBs in patients with MDS or MDS/MPN.
Study Agent/ Intervention Description	This protocol dictates the use of freeze-dried black raspberry powder. Twenty-five grams of freeze-dried BRBs will be mixed with approximately 8 ounces of water; subjects will consume 25 grams orally two times a day.
Number of Patients	The study team will enroll 18 patients with MDS or MDS/MPN.
Subject Participation Duration	After three and six cycles of treatment, if patients are benefiting from the regimen, they continue treatment for a total maximum of 12 cycles.

Duration of Follow up	Follow-up is required 30 days (+4 days) from treatment discontinuation.
Estimated Time to Complete Enrollment:	The study will reach accrual approximately 24 months from when the first patient is dosed.
Safety Assessments	Patients will be seen every cycle by the study staff for compliance and toxicity evaluation. No toxicity is expected from consumption of freeze-dried BRBs powder at a level of 50 grams per day. However, side effects are always a possibility. The NCI Toxicity Criteria version 4.0 will be employed in this study to record any adverse events.
Efficacy Assessments	Peripheral blood samples will be collected day 1 of cycles 1–4, 7, 10, and end of treatment for evaluation of methylation status of mononuclear cells. Assays will include plasma uptake of berry components, measurement of berry anthocyanins in urine, isolation of peripheral blood monocytes and analysis of DNA methylation.
Unique Aspects of this Study	This study will test the novel suppressive role of black raspberries.

SCHEMA



1. Refer to appendix 4.
2. BRBs will be dispensed every cycle for a maximum of 12 cycles.
3. If the patient is tolerating BRBs well and is benefiting from therapy after cycles 3 and 6, then, the patient will continue treatment for a total maximum of 12 cycles.
4. A follow-up visit is required 30 days (+4 days) from treatment discontinuation.

STUDY CALENDAR

The below study chart is described in detail in Sections 5 and 6.

Study Calendar

Period/Procedure	Screening	Treatment					End of Treatment
Study Day/Visit Day Cycle=28 days	**-28 to 0 Days[1]**	**C1D1**	**C2 & C3, D1 (+/- 4 days)**	**C4 & C7, D1 (+/- 4 days)**	**C10D1 (+/- 4 days)**	**C5-C6 & C8-C9 & C11-C12, D1 (+/- 4 days)[10]**	**Follow-up[2]**
Informed consent	X						
Demographics	X						
Inclusion/exclusion criteria	X						
Medical history/height	X						
Medication history[4]	X						
Drug Administration							
Black raspberries 25 gm (BID)[3]		X	X	X	X	X	
Clinical procedures							
Concomitant medications	X	X	X	X	X	X	X
Diet assessment[14]		X	X	X	X	X	
ECOG Performance Status[16]	X			X			X
Weight		X		X			X
AE assessment[11]		X	X	X	X	X	X
Physical exam	X	X					X
Laboratory procedures							
Complete blood count (CBC) with differential and platelet count	X	X	X	X	X	X	X
Pregnancy test (serum or urine)[5]	X						
Serum chemistry panel[6]	X			X			X
Research serum for anthocyanin level[7, 15]		X					
Research blood methylation samples[8, 15]		X	X	X	X		X
Research urine samples[9, 15]		X					
Disease Response Assessment[12]				X			X
Transfusion history[13]		X	X	X	X	X	X

[1] Screening procedures should occur within 28 days prior to Day 1. BRB treatment should start within 14 days of enrollment.

[2] A follow-up visit is required 30 days (+/- 4 days) from treatment discontinuation.

[3] BRBs will be dispensed every cycle for a maximum of 12 cycles. BRB doses should be separated by at least six hours and the morning dose should be held when the methylation research samples are collected. Refer to appendix 5 for the drug diary.

[4] Capture medications taken within 30 days of day 1 of BRB treatment.

[5] For women of childbearing potential. This is defined as any woman whether or not they have undergone tubal ligation, that meets the following: has not undergone a hysterectomy or bilateral oophorectomy or has not been naturally postmenopausal for at least one year.

[6] Including albumin, total protein, total bilirubin, ALT, AST, alkaline phosphatase, bicarbonate, sodium, potassium, chloride, creatinine, BUN, and glucose.

[7] Blood will be collected within three hours prior to BRB administration and at two hours (+/- 30 minutes) after first dose of BRBs.

[8] Study blood methylation samples will be collected within three hours prior to BRB administration.

[9] Urine will be collected within three hours prior to BRB administration and at three hours (+/- 30 minutes) after first dose of BRBs.

[10] If the patient is tolerating BRBs well and is benefiting from therapy, he or she will continue treatment for a total maximum of 12 cycles.

[11] All adverse events will be followed with appropriate medical management from the date the patient takes their first BRB dose until 30 days following the last dose of treatment. AEs are monitored continuously from the first dose of treatment to 30 days' post last dose

[12] Benefiting from therapy (cycle 4 disease assessment) defined as CR, PR, HI, Stable disease with no disease progression (see appendix 2). Benefiting from therapy (cycle 7 disease assessment) defined as CR, PR, or HI (see appendix 2).)

[13] Capture transfusion history from eight weeks prior to day 1 of BRB treatment.

[14] Refer to appendix 6.

[15] Refer to appendix 4.

[16] Refer to appendix 1.

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LIST OF ABBREVIATIONS

ACF	aberrant crypt foci
AE	adverse event
ALP	alkaline phosphatase
AML	acute myelogenous leukemia
AOM	azoxymethane
BELT	bin-based enrichment level threshold
BID	bis in die or twice a day
BRB(s)	black raspberries
BUN	blood urea nitrogen
CAD	collisionally activated dissociation
CBC	complete blood cell (count)
CpG	—C—phosphate—G—
CRC	clinical research coordinator
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management System
CTO	Clinical Trials Office
DMR	differentially methylated region
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid

ESI	Electrospray Ionization
FAB	French-American-British
FCBP	female of childbearing potential
FDA	Food and Drug Administration
FDR	false discovery rate
GCP	Good Clinical Practice
gm	gram
HGB	hemoglobin
HIV	human immunodeficiency virus
HMAs	hypomethylating agents
HSCT	hematopoietic stem cell transplantation
ICH	International Conference on Harmonization
IND	investigational new drug application
IP	investigational product
IPSS	International Prognostic Scoring System
IRB	Institutional Review Board
IWG	International Working Group
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
IV	intravenous
LDH	lactate dehydrogenase
MBDCap	Methyl-Binding-Domain–Capture
MCW	Medical College of Wisconsin
MCWCC	Medical College of Wisconsin Cancer Center
MDS	myelodysplastic syndrome
MPN	myeloproliferative neoplasm
MS/MS	mass spectrometry

NCI	National Cancer Institute
NMBA	N-nitrosomethylbenzylamine
PBS	Phosphate-buffered saline
PCNA	proliferating cell nuclear antigen
PO	per os (by mouth, orally)
QOL	quality of life
RBC	red blood cell (count)
SAE	serious adverse event
SIM	selective ion monitoring
TBRC	Scientific Review Committee Translational biomedical research center
TSS	transcription start site
UPIRSO	unanticipated problems involving risks to subjects or others
UPLC	ultra-performance liquid chromatography
WBC	white blood cell (count)
Wnt	Wingless-type MMTV integration site family member

1 BACKGROUND

Myelodysplastic Syndromes (MDS)

Myelodysplastic syndromes (MDS) are a group of bone marrow disorders characterized by progressive cytopenias and progression to acute myeloid leukemia (AML). (1) Each year, an estimated 10,000 new patients with MDS above the age of 65 are diagnosed. (2) Clinicians use the French-American-British (FAB) and the World Health Organization (WHO) systems, in addition to the International Prognostic Scoring System (IPSS), to classify MDS. (3) The latter is the most commonly used prognostic scoring system, and it includes the percentage of blasts/morphology, number of cytopenias and cytogenetic abnormalities. The IPSS system divides patients into four prognostic groups — low, intermediate-1, intermediate-2 and high-risk disease. The median survival is 4.8, 2.7, 1.1 and 0.5 years for patients >60 years old with low, intermediate-1, intermediate-2 and high-risk disease, respectively. The median age at diagnosis for patients with MDS is 76 years and 86% of patients are above the age of 65. (2) As most patients with MDS are older, therapy with a low toxicity would be desirable for this patient population.

Current therapy for MDS

Hematopoietic stem cell transplantation (HSCT) remains the only cure for MDS. Clinicians also have other therapy options that correspond with disease risk in treating MDS. For example, supportive care, which may consist of transfusions and growth factors therapy, remains the backbone of low-risk MDS treatment. (4) 5q- syndrome patients are treated with antithymocyte globulin therapy and lenalidomide. (5) Azacitidine or decitabine (6,7) are first-line therapies for high-risk disease patients who can tolerate them. Azacitidine, decitabine, and lenalidomide are FDA-approved therapies for MDS treatment. The first two drugs are hypomethylating agents (HMAs). HMAs have yielded a response rate ranging from 30–50% for an average of 12 months in MDS patients.

Treatment of MDS or MDS/MPN with Hypomethylating Agents

Genetic and epigenetic alterations are the molecular hallmarks of cancer. Genetic alterations include mutations or deletions that alter the primary sequence of DNA. In contrast, epigenetic alterations result from biochemical modification of the composition of chromatin. DNA methylation and alteration of the histone code are two epigenetic changes that can result in transcriptional deregulation and gene silencing. Reversal of this process with hypomethylating agents results not only in gene expression reactivation but in killing of leukemic cells, a phenomenon that has been widely exploited in human clinical trials. (8–10) Hypomethylating agents 5-azacytidine (azacitidine) and 5-aza-2'-deoxycytidine (decitabine) are approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndromes (MDS). Both agents were initially developed as cytarabine derivatives with initially disappointing results, especially at higher concentrations. However, in follow-up studies and with lower-dose schedules, both drugs have shown efficacy in MDS.

In a randomized Phase III trial (AZA-001) conducted by Fenaux et al (6) in patients with high-risk MDS, patients were randomized to receive azacitidine (75 mg/m^2 per day for seven days every 28 days) or conventional care (best supportive care, low-dose cytarabine, or intensive chemotherapy, as selected by investigators before randomization). At a median follow-up of 21.1 months, the median overall survival was 24.5 months (9.9 – not reached) for the azacitidine

group versus 15.0 months (5.6 – 24.1 months) for the conventional care group (p=0.0001). Azacitidine was given for a median of nine cycles (4–15), and 86% of the patients who received azacitidine remained on 75 mg/m^2 per day throughout the study with no dose adjustments. In patients with −7/del(7q), the median overall survival was 13.1 months (3.9 – 24.5 months) in the azacitidine group (n=30) compared with 4.6 months in the conventional care group. The median time to AML transformation was 17.8 months in the azacitidine group compared with 11.5 months in the conventional care group (CCR) (p < 0.0001). The duration of hematological response (CR, PR and any hematological improvement) was also significantly longer in the azacitidine group (median 13.6 months) than in the CCR group (5.2 months; p=0.0002). Median duration of CR plus PR in the azacitidine group was 3.2 months versus 3.0 months (p=0.48) in the CCR group. Factors affecting survival in that study were PS, presence of circulating blasts, RBC TD ≥ 4 units in eight weeks and cytogenetics. Based on these four factors, patients were subdivided into low-intermediate- and high-risk groups with median survival of not reached, 15 months and 6.1 months, respectively. (11) This confirmed that treatment with azacitidine prolongs overall survival and lowers the risk of progression to AML in patients with higher-risk MDS compared with treatment with CCR.

Decitabine was evaluated in two large phase III trials. (12,13) In the first study performed in the US, 170 patients were randomized to receive either BSC or decitabine 15 mg/m^2 three times daily x 3 days repeated every six weeks. The ORR for patients receiving decitabine was 30% (9%CR, 8% PR and13% HI). (12) Using a similar decitabine schedule, Lubbert et al. reported an ORR of 34% (CR13%, PR 6%, and HI 15%). (13) However, in both studies there was no difference in overall survival in patients receiving decitabine in this schedule when compared to BSC. In order to better define the best dose schedule for decitabine, Kantarjian et al. randomized patients with MDS to one of three decitabine schedules: 20 mg/m^2 intravenously daily for five days, 20mg/m^2 subcutaneously daily for five days and 10 mg/m^2 intravenously daily for 10 days. (14) In that study, patients randomized to the 20 mg/m^2 intravenously daily for five days had the highest CR rate (39%) and this was chosen as the basis for a multicenter phase II study, the ADOPT trial. (7) In the ADOPT trial, decitabine 20 mg/m^2 was administered to 99 patients with MDS. As previously mentioned, the ORR was 51% (17% CR, 15 mCR and 18 HI). Most responses were seen after two cycles (82%), and the median duration of response was 10 months. The -one-year survival was 66% and the median overall survival was 19.4 months.

The myelodysplastic syndrome/myeloproliferative neoplasm category was introduced in the thirdrevision on the WHO classification. This group includes chronic myelomonocytic leukemia (CMML), atypical CML, MDS/MPN unclassified and juvenile myelomonocytic leukemia (JMML). Hypomethylating agents have shown in the therapy of those diseases (34-35).

Azacitidine and decitabine are associated with significant toxicities

Although both azacitidine and decitabine are effective therapies for patients with MDS, they are associated with significant toxicities. In the AZA-001 trial (6) previously mentioned, 91%, 85% and 57% of patients developed grade 3 or 4 neutropenia, anemia or thrombocytopenia, respectively. In the ADOPT trial, (7) 31%, 18% and 12% of patients receiving decitabine developed grade 3 or 4 neutropenia, thrombocytopenia and anemia, respectively. In addition, other nonhematologic toxicity occurring in > 10% of patients included fatigue, nausea, fever, diarrhea, constipation, pneumonia, vomiting and chills.

Even though the quality of life of patients receiving azacitidine or decitabine is better when compared with patients receiving BSC only, (15) it is still significantly affected by frequent hospital visits. Azacitidine is administered IV or SQ for five or seven consecutive days each

month as long as the patient is responding and tolerating the therapy. Similarly, decitabine is administered for five consecutive days IV every month as long as the patient is responding and tolerating the therapy. Both those schedules place a large burden on patients and affect their quality of life.

Black raspberries as hypomethylating agents

Black raspberries have been shown to have hypomethylating effects in colon cancer patients. (16) In a study, 20 patients with colorectal cancer were treated with BRBs before surgical resection of the tumor. BRBs exerted hypomethylating effects in the adjacent normal mucosa in all patients and in carcinomas only in patients with > 4 weeks of therapy. (17) These observations on the hypomethylating effects of BRBs with prolonged therapy are consistent with what is seen in patients with MDS treated with hypomethylating agents. In almost all patients, two to three cycles are needed (up to six cycles) before a response is seen in patients receiving either azacitidine or decitabine. This hypomethylating effect in colon cancer is most likely due to local effect of the BRBs on colon cells. However, BRBs are systemically absorbed and have been shown to have a systemic hypomethylating effect. In the colon cancer study previously mentioned, none of the patients in that study had detectable urinary levels of BRB-derived anthocyanins before therapy, while all had detectable levels in urine after administration of BRBs, confirming the systemic absorption of BRBs. In addition, the systemic hypomethylating effects of BRBs have been demonstrated in IL-10 knockout mice. In that study, BRBs reversed aberrant promoter methylation of genes in the Wnt pathway in the colon, bone marrow and spleen of mice. (16) This confirmed the absorption and hypomethylation effect of BRBs in the colon, spleen and bone marrow of mice fed a 5% BRB powder diet for four weeks. (18)

In a study by Silverman et al., the median time to any response was two cycles (range 1–16). Overall, 91% of patients achieved best response by six cycles. Based on that, we will discontinue therapy for patients who do not achieve a response after six cycles. Response defined as CR, PR or HI (see appendix 2). (364) Our aim in this study is to evaluate the systemic effects of BRBs in patients with MDS or MDS/MPN.

Black raspberry dose

One of the most desirable features of a chemopreventive agent is little or no toxicity at concentrations producing chemopreventive efficacy. The toxicity of BRBs has been evaluated in rats fed a synthetic diet (AIN-76A diet) plus either 5% or 10% BRB powder by weight (w/w) for up to nine months. (19) These percentages of BRB powder in a rat diet would be equivalent to approximately 0.9 to 1.8 ounces (25.5 to 51 grams) of BRB powder in the daily human diet, as calculated on a body surface area basis. Histopathologic studies indicated that these BRB diets did not produce toxic effects in any major organs of the animals, and there were no significant differences in either body weight or food consumption between rats on either of the BRB-supplemented diets versus control rats on the AIN-76A-alone diet during the nine-month treatment. (19)

In humans, a phase I trial evaluated the safety and tolerability of BRB powder (45 g as a slurry in water daily for seven days) in healthy participants and results suggested that berries were well tolerated. In a subsequent pilot study of oral BRB powder (32 or 45g/day for six months) in Barrett's esophagus patients, BRBs were tolerated as well. (20) In that study, three of the 20 patients enrolled reported side effects that were likely related to BRBs. The reported side effects were epigastric pain, diarrhea and constipation; all were grade I.

Further, the daily dose of BRBs at 60 g (20 g x 3) was well tolerated in colon cancer patients

(60g/day for one to nine weeks) and BRBs produced hypomethylating effects in colon cancer patients received average four weeks of BRBs in comparison to those received two weeks of BRBs; suggesting accumulating effects. (17) Also, using a daily dose of 60 g, BRBs were tolerated in patients with familial adenomatous polyposis (FAP) in a nine-month intervention study, and BRBs led to fewer and smaller polyps through hypomethylation. (21) The most recent unpublished data suggest that 50 g BRBs per day (25 g x 2) were tolerated in ulcerative colitis patients for a period of six months. Accordingly, we chose 50 g (25g x 2) daily BRBs to be tested in MDS patients. We anticipated that BRBs will be tolerated and exert hypomethylating effects in MDS patients.

Preclinical Data

As stated above, many berry components have demonstrated chemopreventive activity in animals.

Kresty et al. reported in 2001 (22) that freeze-dried BRBs are a source of multiple nutrient and non-nutritive compounds, including vitamins A, C, E and folic acid; calcium; selenium; β-sitosterol, ellagic acid, ferulic acid, quercetin and certain anthocyanins (see Appendix 3). Many of these agents showed chemopreventive activity, both *in vitro* and in animal models. Their modes of action include inhibition of carcinogen activation and stimulation of carcinogen detoxification. In addition, they reduce the binding of the reactive metabolites of carcinogens to DNA, down regulate genes associated with cancer development, upregulate of genes involved in cell-cycle control and stimulate apoptosis and inhibit angiogenesis (19,23–25).

In addition, ellagitannins in raspberries have been shown to inhibit phorbol ester-induced DNA synthesis and ornithine decarboxylase activity. (26) More specifically, the inhibition of mutagenesis and carcinogenesis by the ellagic acid in ellagitannins involves inhibition of carcinogen activation, stimulation of carcinogen detoxification, interference in the binding of reactive metabolites of carcinogens to DNA and downregulation of certain genes associated with cancer development. (25,27,28)

Black raspberries' chemopreventive effects were clearly demonstrated in a study by Harris et al. The investigators first induced colon tumors in the Fischer 344 rat by intraperitoneal administration of the chemical carcinogen azoxymethane (AOM). BRBs markedly decreased aberrant crypt foci (ACF), the precursor of invasive colon tumors, in the rat. ACF multiplicity decreased 36%, 24% and 21% in the 2.5%, 5% and 10% BRB diet groups, respectively, relative to the AOM-only group. Total tumor multiplicity declined 42%, 45% and 71% in these same groups, and decreases in overall tumor burden (28%, 42% and 75%) were observed (29).

Other studies evidenced that dietary freeze-dried strawberries and BRBs inhibited N-nitrosomethylbenzylamine (NMBA)-induced tumors in the rat esophagus (22,30). Both berry types produced an approximate 40–60% reduction in esophageal tumor multiplicity and were capable of suppressing the development of preneoplastic lesions (hyperplasia and both low- and high-grade dysplasia) into papillomas. The inhibitory effects of the berries were associated with a reduction in the rate of proliferation of preneoplastic cells, as determined by immunohistochemical quantitation of the PCNA labeling index in the esophagi of rats treated with berries plus NMBA versus those treated with NMBA alone. Recently, a laboratory showed that BRBs downregulate COX-2 and iNOS expression and activities in NMBA-treated rat esophagus (31) and BRB extracts have been shown to downregulate the transcription activators, activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB) in cultured mouse epidermal cells. (32) The protective effects of BRBs on esophageal tumorigenesis may be partially due to ellagic acid content, but cannot be attributed to ellagic acid alone. (22)

2 HYPOTHESIS AND OBJECTIVES

2.1 Primary Objectives

To evaluate the potential hypomethylating effects of freeze-dried black raspberries (BRBs) in the peripheral blood of patients with myelodysplastic syndrome or myelodysplastic syndrome/ myeloproliferative neoplasms(MDS/MPN) after three cycles of BRB administration.

2.2 Secondary Objectives

1. To evaluate the toxicity of BRBs in patients with MDS or MDS/MPN.

2. To evaluate the hematological response according to modified IWG criteria (Appendix 2) in patients with MDS or MDS/MPN regardless of the initial blood count.

3. To evaluate the anthocyanin concentrations in peripheral blood of patients prior to and after administration of BRB powder on Cycle 1 Day 1 of treatment.

3 STUDY DESIGN

3.1 General Description

This is a phase II single-group pilot study. This trial is designed to evaluate efficacy and methylation. This study's overarching aim is to evaluate the systemic effects of BRBs in patients with MDS or MDS/MPN. Eighteen patients with MDS or MDS/MPN will be treated with 25 gm (2x/day) of BRB powder taken orally in 8 ounces of water. If the patient is unable to tolerate the taste of the BRB powder mixed in water, he or she may mix it with 8 ounces of milk, ice cream or a smoothie.

Anthocyanins in the blood will be measured within three hours prior to BRB administration, and two hours (+/- 30 minutes) after the first dose. Urine anthocyanins will be measured within three hours prior to BRB administration and three hours (+/-30 minutes) after the first BRB dose. Both of those time points were chosen based on previous studies demonstrating that the maximum concentrations of anthocyanins and ellagic acid in plasma occurred at one to two hours, and maximum quantities in urine appeared from zero to four hours. (20) Methylation status will be measured within three hours prior to BRB administration in cycles 1–4, 7, 10 and at the end of treatment visit.

Patients will receive oral BRBs while receiving their supportive care for a period of at least three cycles. At cycles 4 and 7, day 1, the patient will be assessed for response (Appendix 2) and toxicity. If the patient is tolerating BRBs well and is benefiting from therapy, he or she will continue treatment for a total maximum of 12 cycles. Tolerating well is defined as no grade 3 or 4 toxicity.

*Benefiting from therapy (for cycle 3 disease assessment) defined as CR, PR, HI, stable disease with no disease progression (see Appendix 2).
**Benefiting from therapy (for cycle 6 disease assessment) defined as CR, PR or HI (see Appendix 2).

3.2 Number of Patients

The study team will enroll 18 MDS or MDS/MPN patients.

3.3 Primary Endpoint(s)

To evaluate the potential hypomethylating effects of freeze-dried black raspberries (BRBs) in the peripheral blood of patients with myelodysplastic syndrome or myelodysplastic syndrome/ myeloproliferative neoplasms (MDS/MPN) after three cycles of BRB administration.

3.4 Secondary Endpoint(s)

1. To determine the toxicity of BRB by assessing the incidence of adverse events related to BRB.

2. The patient's best clinical response will be assessed, based on a modified International Working Group (IWG) response criteria (erythroid, platelet, neutrophil) (Appendix 2) in myelodysplastic syndrome or myelodysplastic syndrome/ myeloproliferative neoplasms (MDS/MPN).

3. To evaluate the anthocyanin concentrations in peripheral blood of patients prior to and after administration of BRB powder on Cycle 1 Day 1 of treatment.

3.5 Study Timeline

Patients may continue BRB treatment for a maximum of 12 cycles from the time of study entry.

3.6 Study Completion

The study will reach accrual approximately 24 months from when the first patient is dosed.

4 PATIENT SELECTION

Eligibility Criteria

The written informed consent must be obtained from the patient prior to enrollment and any study-specific procedures. Patients must have baseline evaluations performed prior to enrollment and the first study drug dose. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.1 Inclusion Criteria

A potential subject must meet all the following inclusion criteria to be eligible to participate in the study.

1. Patients must have a confirmed diagnosis of myelodysplastic syndrome or myelodysplastic syndrome/ myeloproliferative neoplasms (MDS/MPN) proven by bone marrow biopsy/aspirate.

2. Patients with cytopenias (blood cell counts lower than the institutional lower limit of normal within the eight weeks prior to the study) who are receiving or received:

   - red blood cell transfusions
   - observation
   - platelet transfusions
   - erythropoietin
   - granulocyte colony-stimulating factors
   - granulocyte-macrophage colony-stimulating factors
   - hydrea

3. Age ≥18 years.
4. Predicted life expectancy of at least 12 weeks.
5. Patients should be expected to stay on the same therapy for the period of the study.
6. Patients who do not have an indication for and/or are believed to be unable to tolerate a hypomethylating agent are eligible for the study.
7. Reproductive requirements:

   Female patients must meet one of the following:
   - Postmenopausal for at least one year before the screening visit, or
   - Surgically sterile, or
   - If they are of childbearing potential, agree to practice two effective methods of contraception from the time of signing of the informed consent form through 30 days after the last dose of study drug, AND
   - Must also adhere to the guidelines of any treatment-specific pregnancy prevention program, if applicable, or
   - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable contraception methods.)

   Male patients, even if surgically sterilized (i.e., status postvasectomy), must agree to one of the following:
   - Practice effective barrier contraception during the entire study treatment period and through 30 days after the last study drug dose, OR
   - Must also adhere to the guidelines of any treatment-specific pregnancy prevention program, if applicable, OR
   - Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception.)

8. Ability to understand a written informed consent document, and the willingness to sign it.

4.2 Exclusion Criteria

A potential subject who meets any of the following exclusion criteria is ineligible to participate in the study.

1. Previously received hypomethylating agents.
2. Allergy to black raspberries.
3. Inability to swallow oral medication.

4. Inability or unwillingness to comply with the BRB administration requirements.
5. Uncontrolled intercurrent illness, including, but not limited to, symptomatic congestive heart failure, or psychiatric illness/social situations, that, in the treating investigator's discretion, would limit compliance with study requirements. Concurrent active malignancy is not an exclusion criterion.
6. Active infection not well controlled by antibacterial or antiviral therapy.
7. Pregnant or lactating women.
8. Participation in clinical trials with other investigational agents not included in this trial, within 14 days of the start of this trial and throughout the duration of this trial.
9. Patients with allergies to other members of the Rosacea family, including but not limited to apple, almond, apricot, cloudberry, blackberry, etc.

5 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Screening Tests and Procedures

The study-specific assessments are detailed in this section and outlined in the Study Calendar. Screening assessments, unless otherwise specified, must be performed within 28 days prior to the day 1 visit. All on-study visit procedures are allowed a window of ± 4 days unless otherwise noted. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

A written, signed informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated.

All patients who are consented will be registered in OnCore®, the MCW Cancer Center Clinical Trial Management System. The system is password protected and meets HIPAA requirements. Once a patient has been consented, they will be assigned a screening ID by the clinical research coordinator that will be used to identify them throughout the study. The patient identifier will start with BRB and then chronologically numbered there after (BRB-001, BRB-002, BRB-003, etc.).

5.2 Enrollment Process

A subject will be considered enrolled onto the study once they have signed consent, have successfully met all screening criteria, the eligibility criteria has been reviewed and accepted by the PI or a sub-investigator, and entered into OnCore. Contact Dr. Atallah with any eligibility questions at eatallah@mcw.edu.

5.3 Pretreatment Period

The screening procedures and assessments must be completed within 28 days of the Day 1 visit unless otherwise specified in the study parameter schedule.

Refer to the study calendar for specifics:

- Informed consent
- Demographics

- Inclusion/exclusion criteria
- Medical history
- Medication history
- Concomitant medications
- ECOG performance status
- Height
- Physical examination
- Complete blood count (CBC) with differential and platelet count
- Serum or urine pregnancy test (for women of childbearing potential)
- Serum chemistry panel, including:
  Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, blood urea nitrogen (BUN), creatinine, total protein, albumin, potassium, sodium, chloride, bicarbonate, glucose.

5.4 Study Procedures During Treatment

Patients must meet eligibility criteria eligible on Day 1 and be enrolled prior to BRB treatment. BRB treatment should start within 14 days of enrollment.

Study Procedures, Cycle 1 Day 1 (refer to study calendar for specifics)

- Dispense BRB (Appendix 5)
- Concomitant medications
- Diet assessment (Appendix 6)
- Weight
- Adverse event assessment
- Physical exam
- Complete blood count (CBC) with differential and platelet count
- Study serum sample for anthocyanin level
- Study blood methylation samples
- Study urine samples
- Transfusion history

Study Procedures, Cycles 2-3, Day 1 (refer to study calendar for specifics)

These procedures must be completed cycles 2 - 3, day 1 (+/- 4 days).

- Dispense BRB (Appendix 5)
- Concomitant medications
- Diet assessment (Appendix 6)
- Adverse event assessment
- Complete blood count (CBC) with differential and platelet count
- Study blood methylation samples
- Transfusion history

Study Procedures, Cycles 4 & 7, Day 1 (refer to study calendar for specifics)

These procedures must be completed cycles 4 and 7, day 1. (+/- 4 days).

- Dispense BRB (Appendix 5)

- Concomitant medications
- Diet assessment (Appendix 6)
- ECOG performance status
- Weight
- Adverse event assessment
- Complete blood count (CBC) with differential and platelet count
- Serum chemistry panel, including:
  Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, blood urea nitrogen (BUN), creatinine, total protein, albumin, potassium, sodium, chloride, bicarbonate, glucose
- Study blood methylation samples
- Disease response assessment (Appendix 2)
- Transfusion history

Study Procedures, Cycles 5-6 & 8-12, Day 1 (refer to study calendar for specifics)

These procedures must be completed cycles 5–6 and 8–12, day 1 (+/- 4 days).

- Dispense BRB (Appendix 5)
- Concomitant medications
- Diet assessment (Appendix 6)
- Weight
- Adverse event assessment
- Complete blood count (CBC) with differential and platelet count
- Study blood methylation samples (only cycle 10)
- Transfusion history

5.5 Follow-up

Perform follow-up procedures 30 days (+4 days) after study treatment discontinuation.

- Concomitant medications
- ECOG performance status
- Weight
- Adverse event assessment
- Physical exam
- Complete blood count (CBC) with differential and platelet count
- Serum chemistry panel, including:
  Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, blood urea nitrogen (BUN), creatinine, total protein, albumin, potassium, sodium, chloride, bicarbonate, glucose
- Study blood methylation samples
- Disease response assessment
- Transfusion history

5.6 Study Withdrawal Procedures

Patients may continue treatment for one year from the time of study entry.

In the absence of treatment delays due to adverse events, treatment may continue until:

- Disease progression, as defined by the modified IWG criteria (Appendix 2)
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the investigator's judgment.
- Intercurrent illness that prevents further treatment administration
- Patient decides to withdraw from the study
- Significant patient noncompliance with protocol
- Unacceptable adverse event(s) per PI discretion

6 TREATMENT PLAN

6.1 Investigational Agent Administration

Treatment will be administered on an outpatient basis. MCW will obtain black raspberry powder directly from BerriProducts, Inc. in Corvallis, Oregon as study supply. Patients will be treated with 25 gm (2x/day) of BRB powder taken orally in 8 ounces of water. If the patient is tolerating and benefiting from treatment after three and six cycles, then, he or she will continue treatment for a total maximum of 12 cycles.

Study Drug	Dose	Route	Schedule	Cycle Length
Black Raspberries (Freeze-dried powder)	**25 grams**	**Oral**	**Twice a day (each dose separated by at least 6 hours)**	**4 weeks (28 days)**

6.2 Dosing Delays/Dose Modifications

Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

See the table below for dose modification requirements for nonhematological toxicities deemed clinically significant and related to BRB treatment by the treating physician.

Dose Modifications	
Dose Level	**Dose of Study Drug**
0	25 grams twice a day with each dose separated by at least six hours (mixed in 8 ounces of water)[1]
1	≥ grade 1 toxicity related to BRB treatment and unable to tolerate then dose reduce to 25 grams QD
2	≥ grade 2 toxicity or grade 3 allergic reaction related to BRB treatment and/or unable to tolerate, withdraw patient from study
Footnotes[1]	If unable to tolerate the taste, mix BRB with milk, ice cream or smoothie.

Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

6.3 General Concomitant Medication and Supportive Care Guidelines

The treating physician will use standard supportive care to control symptoms and improve quality of life. These may include:

- Transfusions
- Use of growth factors
- Antibiotics

6.4 Dietary Restrictions

There are no dietary restrictions. Patients will take BRB powder orally in 8 ounces of water. If the patient is unable to tolerate the taste of the BRB powder mixed in water, then, he or she may mix it with 8 ounces of milk, ice cream or a smoothie.

6.5 Prohibited Medications

As noted in the eligibility section 4, patients should not be receiving hypomethylating agents. In addition, they should not be receiving other chemotherapy/radiation, or participating in clinical trials with other investigational agents not included in this trial, within 14 days of the start of this trial and throughout the duration of this trial.

6.6 Monitoring Subject Compliance

Black raspberries will be dispensed only to eligible patients under the supervision of the investigator or identified subinvestigator(s). The appropriate study personnel will maintain records of product receipt and dispensing. The patient will complete a drug diary to ensure compliance.

The patient will be seen every cycle by a healthcare professional or study staff for compliance. Patients will also be required to complete a food diary at home while taking BRBs and instructed to return the diary to clinic for assessment every cycle.

6.7 Follow-Up Period

Patients removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event.

7 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

7.1 Defining and reporting an Adverse Event

Definition. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

Prior to the trial. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g. surgery was performed earlier or later than planned).

Reporting source. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

For non-serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Signs or symptoms reported as adverse events will be graded and recorded by the investigator, according to the CTCAE version 4. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

Follow-up of Adverse Events
All adverse events will be followed with appropriate medical management from the first dose of BRBs until 30 days following the last dose of treatment.

The investigator and his or her team will follow the Medical College of Wisconsin policies related to adverse event reporting.

7.2 Defining and reporting a Serious Adverse Event (SAE)

Serious Adverse Event (SAE) means any untoward medical occurrence that at any dose:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (excluding planned hospitalization).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible
- Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered

serious. For example, a white blood cell count of 1000/mm3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

Reporting SAEs

Serious AEs must be reported from the first dose of BRBs through 30 days after completion of study treatment or until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor-investigator's IRB when required.

AEs which are serious must be reported to the Data Safety Monitoring Committee (DSMC) from the first dose of BRBs through 30 days after completion of treatment on the study.

Reporting to the Data and Safety Monitoring Committee

Regardless of expectedness or causality, all SAEs must also be reported to the DSMC as soon as possible, but no later than five calendar days of the sponsor-investigator's observation or awareness of the event.

Report Method: The investigator will use email to report SAEs to the DSMC. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version 4 as a guideline whenever possible.

The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

7.3 Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

7.4 Procedure for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant, or suspects that she is pregnant, while participating in this study, she must inform the investigator immediately and permanently discontinue the study drug. The sponsor-investigator must notify the DSMC by email. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately notify the DSMC. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

Suggested Pregnancy Reporting Form:

- Pregnancy Report Form (a sample is provided in Appendix 7)

7.5 Common AE List

In a previous pilot study, healthy subjects consumed 45 g of freeze-dried berries for 14 days and the berries were well tolerated.

However, side effects are always a possibility and patients will be asked to report any change in their health status or potential adverse events

Expedited Reporting to the Food and Drug Administration

If the study is being conducted under an IND, the sponsor-investigator is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The sponsor-investigator needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **seven calendar days** after the investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

Suggested Reporting Form:

- US FDA MedWatch 3500A:
  http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

Any other form deemed appropriate by the sponsor-investigator.

8 PHARMACEUTICAL INFORMATION

8.1 Black Raspberries

Product Description

The freeze-dried black raspberry powder is available from BerriProducts, Inc. in 25-gram dosing packets. Each bag contains around 500 freeze-dried black raspberries.

Black raspberries for this powder are grown, harvested mechanically when ripe, washed immediately after harvest and frozen at -20°C.

They are freeze dried under Good Manufacturing Practice (GMP) conditions by Oregon Freeze Dry in Albany, Oregon, the largest U.S. freeze-drying company. After freeze drying, the berries are ground into a powder, and the powder is tested for contamination with fungi, e. coli, salmonella and listeria. In addition, the content of 26 different nutrients are measured in the powder on a routine basis, as described by Stoner. (19)

Classification:

Phytomedicine.

Mechanism of Action:

Their modes of action include inhibition of carcinogen activation and stimulation of carcinogen detoxification. In addition, they reduce the binding of the reactive metabolites of carcinogens to DNA, downregulate genes associated with cancer development, upregulate of genes involved in cell-cycle control and stimulate apoptosis and inhibit angiogenesis.

Metabolism:

Anthocyanins are excreted in intact forms and metabolized into methylated derivatives in human urine. The urinary excretion of anthocyanins reaches maximum concentration during the four- to eight-hour period after black raspberry ingestion.

Contraindications:

Allergic reaction to BRBs

Side Effects:

As noted, side effects are a possibility and potential adverse events (i.e., headache, dizziness, nausea, vomiting, diarrhea, cramping) will be monitored.

Solution Preparation/ Investigational Agent Administration

Twenty-five grams of freeze-dried BRBs will be mixed in 8 ounces of water and consumed twice daily at least six hours a part. If patient is unable to tolerate the taste, he or she may mix the BRB with milk, ice cream or a smoothie.

Storage Requirements

Black raspberries will be stored in Investigational Drug Pharmacy in a freezer at minus 20 degrees Celsius and transported to the clinic in a cooler with an ice pack. Patients need to store BRBs in the freezer at home.

Stability
Freeze-dried products have the same size and shape as the original frozen material and have excellent stability and convenient reconstitution when placed in water.

Route of Administration

Oral administration.

Nursing Implications

Dispensing: Patients will receive oral black raspberries while receiving their supportive care. At office visits, patients will pick up black raspberry doses for the next cycle.

Compliance: Clinical staff will collect phenolic-rich food diaries from patients which reflect the previous cycle.

Handling

No special handling instructions.

Availability

The supplier is BerriProducts, Inc.

Agent Ordering

MCW will obtain black raspberry powder directly from BerriProducts, Inc. in Corvallis, Oregon as study supply.

Contact information: BerriProducts, Inc., 1325 NW Heather Drive, Corvallis, OR 97330. Phone number: 888-761-8407.

Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of black raspberries.

9 REPORTING AND DOCUMENTING RESULTS (MEASUREMENT OF EFFECT)

9.1 Evaluation of Efficacy (or Activity) Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first study drug treatment.

Evaluable for response: Only patients who have undergone at least three cycles of treatment are evaluable for response.

9.2 Methods for Evaluation

All baseline evaluations will be performed as closely as possible to the beginning of treatment. Please refer to the study calendar and Section 3 (study design).

Physical Examination, Laboratory Values, Food Records

After completing a dietary assessment, patients will undergo physical examination and laboratory tests (complete blood count (CBC) with differential, platelet count, and serum chemistry assessment). The treating physician will evaluate the patient every cycle. Peripheral blood samples will be collected cycles 1–4, 7, 10, and EOT for evaluation of methylation status.

9.3 Surrogate Endpoint Biomarkers

Plasma uptake and urine excretion of berry anthocyanins
We will evaluate the uptake of berry compounds into plasma obtained at two hours after the first dose of BRBs. Urine samples will also be collected three hours after the first dose of BRBs. The content of each of the four major anthocyanins in BRBs (i.e., cyanidin-3-rutinoside, cyanidin-3-xylosylrutinoside, cyanidin-3-glucoside and cyanidin-3-sambubioside) will be measured, using HPLC, as described previously. (17) Briefly, separation of plasma and urinary anthocyanins will be conducted on a Symmetry C18 column (4.6 × 75 mm, 3.5 µm; Waters Corp.) using an HPLC system. Cyanidin-3-glucoside will be dissolved in deionized-distilled water containing 5% formic acid. All levels of anthocyanins analyzed in plasma and urine samples will fall within the standard curve range and will be expressed as cyanidin glucoside equivalents in pmol/mL plasma or urine.

DNA methylation analysis by reduced representation bisulfite sequencing (RRBS)
Peripheral blood will be used to isolate CD45+ leukocytes, using CD45 MicroBeads (Miltenyi Biotec). DNA will be extracted from isolated leukocytes (DNA extraction kit, Qiagen). Then, DNA samples will be used for RRBS analysis. In brief, first, genomic DNA is digested using a methylation-insensitive restriction enzyme. The next step is adding an extra adenosine to both the plus and minus strands. End repair and A-Tailing is done within the same reactions, with dCTP, dGTP and dATP deoxyribonucleotides. Then, methylated sequence adapters are ligated to the DNA fragments. The desired size of fragments is then selected to be purified. DNA fragments of 40-220 base pair are representative of the majority of promoter sequences and CpG islands. The DNA fragments are then bisulfite converted, which is a process that deaminates unmethylated cytosine into a uracil. The methylated cytosine remains unchanged, due to the methyl group protecting them from the reaction. The bisulfite converted DNA is then amplified, using PCR with primers that are complementary to the sequence adapters. The fragments are then sequenced. For Illumina sequencing, 36-base single-end sequencing reads are most commonly performed. Alignment to a reference genome allows the programs to identify base pairs within the genome that are methylated. The identified genes will be validated using pyrosequencing.

The research samples will be batched and any leftover blood/urine will be banked for future research. The research samples will be processed and banked at Dr. Li-Shu Wang's lab at the R4655 TBRC, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee WI 53226.

9.4 Response Criteria

The patient's best clinical response will be assessed, based on the modified IWG response criteria (Appendix 2) while receiving BRBs.

9.5 Evaluation of Safety

The study will use the CTCAE v4.0 for reporting of adverse events. Patients will be evaluated every cycle for toxicity.

10 STATISTICAL CONSIDERATIONS

Methods for berry components (ellagic acid and anthocyanins)
A descriptive analysis will be performed for ellagic acid and anthocyanin data. Means, medians and confidence intervals will be calculated for all absolute and relative differences.

Methods for analysis of methylation changes
The primary analysis will consist of six mixed effect models with a random effect for patient, and 4 pairwise comparison of baseline methylation to each treatment times. A Bonferroni correction for 24 total comparisons will be applied to maintain a 5% experimentwise error rate. The primary outcome will be analyzed as the relative methylation rate (difference/baseline). Absolute methylation will also be analyzed, if appropriate for this data. Missing data will be assumed to be missing at random (MAR). Patients with partial data before six6 cycles, e.g., from their visits at cycle 2 or 3, will be included in the analysis.

Power calculation
Strong changes in methylation levels have been observed in pilot data, with mean differences exceeding 20% difference, with standard deviations ranging from six to eight. This study calls for comparing the methylation differences from baseline to each treatment time points, and repeated over six different genes of interest, for a total of 24 comparisons. Based on a two-sided paired *t*-test with 5% type I error rate with Bonferroni adjustment for 24 comparisons, a standard deviation of 8.0 and methylation difference of 12%, a sample size of 16 will be sufficient for 95% power. To date, seven patients were enrolled to the trial and they all finished three cycles of BRB treatment. Based on the current compliance to the treatment protocol, no one dropped and no one was noncompliant. Therefore, we assume that there will be no more than 5% non-compliance to the treatment protocol. An addition of 5% will be recruited, so power can be maintained using nonparametric methods (Wilcoxon Signed-Rank test). Therefore, this brings the final sample required sample size to 18 patients to maintain the power of the study.
Toxicity monitoring plan (stopping rule)
Severe toxicity is not expected with BRB. As a precaution for unexpected high toxicity, the trial enrolment will be pause and the protocol reviewed if a toxicity rate exceeding 15% is observed. The following table gives the stopping rule a Pocock-type boundary for 15% toxicity rate:

# enrolled	3	5	9	12	16	18
Pause if toxicities equal or greater than	3	4	5	6	7	8

Ex: Five toxicities with 10 enrolled would call for a pause for review.

11 DATA AND SAFETY MONITORING PLAN (DSMP)

11.1 Data and Safety Management Overview

The Medical College of Wisconsin (MCW) Data Safety Monitoring Committee (DSMC) and the MCW Institutional Review Board (IRB) will approve protocol-specific DSM plans described below. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with regular safety and progress reports submitted to the DSMC.

The DSMP for this study will involve the following entities:

11.2 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist and the study biostatistician. While patients are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status. This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed. All meetings including attendance are documented.

11.3 Quality Assurance

The MCWCC Clinical Trials Office provides ongoing quality assurance audits
Intermediate risk trials are reviewed every year

- 20% of subject files will be selected randomly for review (a maximum of 10 subjects at each monitoring time point). Consent/eligibility and objective-based data will be reviewed for those files selected.
- One file will be selected randomly for a comprehensive review at each monitoring time point.
- Regulatory documents (IRB submissions, reportable events, etc.) will be reviewed at each monitoring time point.

11.4 Clinical Trials Office

The MCWCC Clinical Trials Office [CTO] provides administrative assistance and support to the DSMC.

11.5 DSMC

The Medical College of Wisconsin Cancer Center places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW includes a plan for safety and data monitoring.

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC). A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.)
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

12 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

12.1 Ethical Standard

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

12.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

12.3 Institutional Review Board

The protocol, the proposed informed consent form and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the MCW Institutional Review Board. Prior to obtaining MCW approval, the protocol must be approved by the Medical College of Wisconsin Cancer Center Scientific Review Committee. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

12.4 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks

are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB-approved and the subject (and Legally Authorized Representative, if necessary) will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

Subject confidentiality is strictly held in trust by the sponsor-investigator, participating investigators, and any staff. This confidentiality includes the clinical information relating to participating subjects, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the principal investigator.

The conditions for maintaining confidentiality of the subjects' records are required for the life of the data. These rules apply equally to any and all MCWCC projects.

12.5 Prestudy Documentation

Prior to implementing this protocol at MCWCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MCW IRB.
The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

12.6 Protection of Human Subjects

Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

Protection of Privacy

As noted, patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document.

12.7 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable

opinion by the IRB and the appropriate regulatory authority(ies).

12.8 Onsite Audits

Auditing is essential to ensure that research conducted at the Medical College of Wisconsin (MCW) Cancer Center is of the highest quality and meets MCW and regulatory agency standards.

Regulatory authorities, the IRB and/or sponsor may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

13 DATA HANDLING AND RECORD KEEPING

13.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

13.2 Case Report Forms

The principal investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study-specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs, in accordance with the study calendar, using single data entry with a secure access account.

All source documentation and data will be available for review/monitoring by the MCWCC DSMC and regulatory agencies.

13.3 Handling and Documentation of Clinical Supplies

The Investigational Pharmacy will maintain complete records showing the receipt, dispensation, return, or other disposition of black raspberries. The date, quantity and batch or code number of the agent, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The principal investigator shall not make the agent available to any individuals other than to qualified study patients. Furthermore, the principal investigator will not allow the agent to be used in any manner other than that specified in this protocol.

13.4 Study Record Retention

The principal investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity and use by subjects, as well as written records of the disposition of the drug when the study ends.

In accordance with FDA regulations, the investigator shall retain records for a period of two years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

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APPENDIX 1. PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Descriptions
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity; minor signs or symptoms of disease
1	Symptoms, but ambulatory Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work)	80	Normal activity with effort; some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed < 50% of the time Ambulatory and capable of all self-care, but unable to carry out any work activities Up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs
		50	Requires considerable assistance and frequent medical care
3	In bed > 50% of the time Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated Death not imminent
4	100% bedridden Completely disabled Cannot carry on any self-care Totally confined to bed or chair	20	Very sick, hospitalization indicated Death not imminent
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

APPENDIX 2. MODIFIED IWG RESPONSE CRITERIA

Hematologic Improvement (HI)	Response Criteria (responses must last at least 8 weeks)
Erythroid response (pretreatment, < 11 g/dL)	- Hgb increase by ≥ 1.5 g/dL. - Relevant reduction of RBC transfusion units by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. - Only RBC transfusions given for an Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation.
Platelet response (pretreatment, < 100 x 109/L)	- Absolute increase of ≥ 30 x 109/L for patients starting with > 20 x 109/L platelet. - Increase from < 20 x 109/L to > 20 x 109/L and by at least 100%.
Neutrophil response (pretreatment, < 1.0 x 109/L)	At least 100% increase and an absolute increase > 0.5 x 109/L.
Progression or relapse after HI	At least one of the following: - At least 50% decrement from maximum response levels in granulocytes or platelets. - Reduction in Hgb by ≥ 1.5 g/dL. - Transfusion dependence in transfusion independent patients.

Category	Response criteria (responses must last at least 4 weeks)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines Persistent dysplasia will be noted Peripheral blood: Hgb ≥ 11 g/dL Platelets ≥ 100 × 10^9/L Neutrophils ≥ 1.0 × 10^9/L Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except: Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5% Cellularity and morphology not relevant
Marrow CR	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment Peripheral blood: if HI responses, they will be noted in addition to marrow CR
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 weeks.
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Cytogenetic response	Complete: Disappearance of the chromosomal abnormality without appearance of new ones Partial: At least 50% reduction of the chromosomal abnormality
Disease Progression	For patients with: • Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts • 5%-10% blasts: ≥ 50% increase to > 10% blasts • 10%-20% blasts: ≥ 50% increase to > 20% blasts • 20%-30% blasts: ≥ 50% increase to > 30% blasts Any of the following: • At least 50% decrement from maximum remission/response in granulocytes or platelets • Reduction in Hgb by ≥ 2 g/dL • Transfusion dependence

World Health Organization (WHO) Classification-Based Prognosis Scoring System (WPSS calculator): http://www.myelodysplastischesyndrome.de/service/wpss_kalkulator/

APPENDIX 3. BLACK RASPBERRIES NUTRIENT LEVELS AND POTENTIAL CHEMOPREVENTIVE COMPONENTS

Dietary Components	Berry Samples Analyzed (mg/100 g)	
	BRB1	BRB2
Minerals		
Calcium	245.0	215
Copper	0.522	0.554
Iron	13.2	10.1
Magnesium	169.0	153.0
Manganese	3.60	4.68
Phosphorus	222.0	170.0
Potassium	1200.0	1300.0
Zinc	2.69	2.17
Selenium	<5.0	<5.0
Folate	0.07	0.06
Vitamins		
Ascorbic Acid	<1.0	4.4
α-carotene	<0.02	<0.02
β-carotene	<0.02	<0.02
Sterols		
β-sitosterol	89.1	80.1
Campesterol	4.3	3.4
Stigmasterol	<3.0	<3.0
Cholesterol	<1.0	<1.0
Phenolics		
Ellagic Acid	185.0	166.3
Ferulic Acid	32.4	17.6
p-Coumaric Acid	7.94	9.23

Abbreviations: BRB, freeze-dried black raspberries. (components reported in mg/100 g sample, except selenium levels reported in mcg/100g)]. BRB1 and BRB2 are two separate lots of freeze-dried BRBs obtained in 1998 and 1999, respectively. Subsequent lots obtained since 1999 have similar quantities of the components listed.

Food Category	Specific Foods with High Phenolic Content
Fruits	Apples, apricots, berries (all), cherries, cranberries, currants, grapes, grapefruit, nectarines, oranges, peaches, peppers (bell and chili), plums, prunes, raisins, tangerines
Vegetables	Cucumbers, tomatoes
Other foods	Almonds, pickles, peanuts, walnuts
Beverages	Cider, coffee, fruit juices, tea, wine
Spices	Chili powder, cloves, paprika
Other	Cider and wine vinegar

APPENDIX 4. SPECIMEN COLLECTION

Specimen Collection & Handling

Anthocyanin collection:
Collect a minimum of 5 ml of whole blood in an EDTA vacutainer (lavender top) tube on cycle 1 day 1 within 3 hours prior to BRB administration and 2 hours (+/- 30 minutes) post BRB dose. No preprocessing is required.

Urine sample collection:
Collect in a urine collection container within three hours prior to BRB administration and at three hours (+/- 30 minutes) post BRB dose on cycle 1 day 1. No preprocessing is required.

Methylation collection:
Collect a minimum of 15 ml of whole blood in an EDTA vacutainer (lavender top) tubes within three hours prior to BRB administration on cycle 1 day 1 and day 1 of cycles 2–4, 7, 10 and at end of treatment. No preprocessing is required.

Specimen Labeling:
All tubes/containers must be labeled with the patient's initials, patient study ID number, patient's date of birth and date/time of specimen collection.

Transporting and Storage Requirements:
For all transportations, a requisition should accompany all samples. A copy of the requisition will be retained at the site. Only transport samples Monday through Friday. Samples should be kept refrigerated until transport. The samples will need to be transported on ice. Samples should be packed and transported according to IATA regulations. Personnel from Dr. Li-Shu Wang's Lab will pick up the study samples only Monday through Friday. Contact 414-955-2579 for sample pickup.

Specimen Requisition Form

A Pilot Study to Investigate the Hypomethylating Properties of Freeze-dried Black Raspberries in Patients with Myelodysplastic Syndrome or Myelodysplastic Syndrome/Myeloproliferative Neoplasm (MDS/MPN)

Patient Information:

Patient initials: _____

Birth Date: _____

Study ID #: _____

Research Sample Information:

Date of Collection:_____Time of Sample Collection: _____

Study time point:_____Physician: _____

BRB administration time: _____

Person Completing Requisition: _____

Phone Number:_____Email: _____

Sample Type:

☐ Anthocyanin

☐ Urine Sample

☐ Methylation

APPENDIX 5. DRUG DIARY

Black Raspberry Drug Diary

Patient #: _____ Cycle #: _____

Directions:

- Mix 25 gm of black raspberry powder in 8 ounces of water, twice a day, at least six hours a part.
- Store black raspberry powder in the freezer.
- Bring this diary to all oncology doctor visits.

Date	Time of AM Dose	Time of PM Dose	Comments

Patient Signature & Date: _____

APPENDIX 6. PHENOLIC-RICH FOOD DIARIES

Patient #: _____

Cycle #: _____

Dates Tracked:

Polyphenol Weekly Tracking

	Amount Consumed (in cups)						
Food Item	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Black chokeberry							
Dark chocolate							
Flaxseed meal							
Black elderberry							
Chestnut							
Lowbush blueberry							
Black currant							
Black olive							
Highbush blueberry							
Hazelnut							
Pecan nut							
Plum							
Green olive							
Sweet cherry							
Globe artichoke heads							
Blackberry							
Strawberry							
Red chicory							
Red raspberry							
Prune							
Black grape							

Patient Signature & Date: _____

APPENDIX 7. PREGNANCY FORM

Pregnancy occurring in participant in a Clinical Trial of Investigational Medicinal Product, while not considered an adverse event or serious adverse event, requires monitoring and follow up.

The investigator must collect pregnancy information for female trial subjects or female partners of male trial subjects. This includes subjects who become pregnant while participating in a clinical trial or during a stage where the fetus could have been exposed to the investigational medicinal product (e.g., if the active substance or one of its metabolites has a long half-life).

Any pregnancy should be reported by the PI to the sponsor using either a study specific or generic pregnancy reporting form (see below).

The pregnancy should be followed up by the investigator until delivery. It may be necessary to monitor the development of the newborn for an appropriate period post-delivery. Any occurrences that result in a Serious Adverse Event should be reported as per the SAE reporting procedure.

The below form will be used.

Study Drug: Study/Protocol N°:	PATIENT ID Patient's Initials 1. 2. fam. If applicable Centre No. Patient No	REPORT TYPE ☐ Initial ☐ Follow-Up

STUDY PREGNANCY FORM **Page 1 of 3**

1. Country:	2. LOCAL CASE ID:

I. MATERNAL INFORMATION

3. DATE OF BIRTH day monthyear	4. AGE yrs./mo.	5. RACE ☐ Caucasian ☐ Oriental ☐ Black ☐ Other	6. HEIGHT cm	7. WEIGHT kg

8. Date of Last Menstrual Period day month year	9. Expected Date of Delivery day month year
10. Method of Contraception	***11. Contraception used as instructed*** ☐ **yes** ☐ **no** ☐ **uncertain**

II. HISTORY

12. PATIENT'S PAST MEDICAL HISTORY (include information on familial disorders, known risk factors or conditions that may affect the outcome of the pregnancy e.g. alcohol, smoking, other substance consumption, hypertension, eclampsia, diabetes including gestational, infections during pregnancy, environmental or occupational exposure that may pose a risk factor).

13. PREVIOUS OBSTETRIC HISTORY – provide details on all previous pregnancies below, including abortion or stillbirth (use page 3 if needed)

	Gestation week	Outcome including any abnormalities
1		
2		
3		
4		
5		

14. DRUG INFORMATION – please list the Novartis drug(s) first and all other therapies taken prior to or during pregnancy

Drug Names	Daily Dose	Route	Treatment Dates		Indication	(specify week of pregnancy)	
			Start	Stop		Start	Stop

2. LOCAL CASE ID:

III. PREGNANCY INFORMATION

18. PRENATAL
Have any specific tests, e.g. amniocentesis, ultrasound, maternal serum AFP, been performed during the pregnancy so far?

☐ **No** ☐ **Yes** ☐ **Not known**

If yes, please specify test date and results:

19. PREGNANCY OUTCOME
Delivery

☐ **Normal** ☐ **Forceps/Ventouse** ☐ **Caesarean section**

Maternal complications or problems related to birth:_____

Abortion

☐ **Therapeutic** ☐ **Planned** ☐ **Spontaneous** **Please, specify reason and any abnormalities (if known)**

_____

☐ **Unspecified**

Date of abortion/delivery day month year
at week _____

20. **MATERNAL PREGNANCY ASSOCIATED EVENTS:**

If the mother experiences a serious adverse drug reaction (ADR) during a pregnancy, please complete a SAE form and submit as requested.

IV. CHILD INFORMATION

21. **Neonate**

☐ **Normal** ☐ **Abnormal** ☐ **Stillbirth** **please specify any abnormalities:** _____

Sex	Height	Weight	Apgar Scores	Head circumference
☐ **Male** ☐ **Female**	**inches**	**pounds**	**1 min.** **5 mins.** **10 mins.**	**inches**

For additional information, please use page 3 (please provide copies of relevant documentation)

V. ASSESSMENT OF PREGNANCY OUTCOME

22. SERIOUSNESS CRITERIA

☐ **Non Serious**

☐ **Mother** died day month year

☐ **Stillbirth** / Neonate died day month year

☐ Involved or prolonged inpatient hospitalization ☐ Life-threatening

☐ Results in persistent or significant disability/incapacity

Other Seriousness Criteria: ☐ Congenital anomaly/birth defect ☐ Other significant medical events

23. ASSESSMENT OF CAUSALITY
Please indicate the relationship between pregnancy outcome and Novartis study drug

☐ Not suspected ☐ Suspected

INFORMATION SOURCE

24. NAME, ADDRESS AND TELEPHONE NUMBER OF INVESTIGATOR	25. REPORTING DATE BY INVESTIGATOR/PERSON REPORTING EVENT
Signature:	daymonthyear

2. LOCAL CASE ID:

FOR ADDITIONAL INFORMATION:

INFORMATION SOURCE	
32. NAME, ADDRESS AND TELEPHONE NUMBER OF INVESTIGATOR Signature:	33. REPORTING DATE BY INVESTIGATOR/PERSON REPORTING daymonthyear

PLEASE SEND FORM TO DSMC