Masonic Cancer Center
University of Minnesota
Cancer Experimental Therapeutics Initiative (CETI)

Decitabine and Vorinostat with CD3/CD19 Depleted Haploidentical Donor Natural Killer (NK) Cells for the Treatment of High Risk Myelodysplastic Syndromes (MDS)

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REVISION HISTORY

Revision #	Version Date	Revision summary	Consent Revised
	03/31/12	Original to CPRC	n/a
	04/12/12	Original to IRB – In response to CPRC stipulations with additional edits	n/a
1	06/25/12	Revise treatment schedule to accommodate product processing at U of MN for both sites and to avoid weekends; Update statistical section. Original to FDA.	Yes
2	10/03/12	In response to IRB ancillary committee review: Revise donor eligibility to adults only (18 – 75 years of age) In response to study initiation meeting: Synopsis, Sections 7.3 and 10.3: Clarify that the NK cell product will be divided in half prior to IL-2 activation and that the IDS will prepare the IL-2 used in the overnight incubation for all patients (U of MN and Mayo Clinic), for course #2 product MCT will send a post-thaw and post IL-2 cell sample to TTL; Sections 13.2 and 11.3: Revise early study stopping rule to include any non-hematologic, non-infectious grade 4-5 event, regardless of attribution and re-calculate patient number thresholds; Section 4.11 (new) and Checklist: Exclude patient who have received 7+3 or other AML-type induction chemotherapy; Section 11.2: adjust post-IL-2 targeted toxicity assessments to coincide with clinic visits; Other minor clarifications and corrections throughout	Yes
3	01/04/13	Sections 7.3 and 7.4: update lot release information in that the final product must contain at least 20% NK cells (previously at least 30%) as permitted by the FDA and to match the current CMC; additional edits – Section 7.5 re-write IL-2 dose modification guidelines for clarity; Section 8.1.1: clarify follow-up for survival only for the purpose of calendar creation (at Day 90, 180, 270, 360), Section 8.2: delete donor repeat type and screen at day 15; other minor edits	no
4	06/17/13	Section 7.3 and 7.4: change the %NK cells required for lot release to 30% reversing the January 2013 change to 20%; clarify course #2 cell preparation procedures	no
5	5/19/14	Study synopsis, section 4.2 - expand eligibility to include previously treated patients with refractory disease; Section 4.10 – delete prior therapy exclusion criteria – no longer applicable; Synopsis, sections 3, 7 - delete bone marrow biopsy between course 1 and 2 to minimize procedures but add off study marrow if patient discontinues treatment before completing two courses; Section 7.3 – delete the sentence “Final product must contain at least 30% NK cells.” as this is no longer correct based on the current CMC; Section 7.4 – update language to reflect current lot release criteria of > 3-fold NK cell enrichment between apheresis and final product; section 7.10 – redefine the definition of rapidly progressing disease based on peripheral blood since BM examination will not be routinely done between courses 1 and 2; section 10.2.9 – add that Vorinostat may be paid for by research funds; other edits and clarifications – all tracked	yes

Revision #	Version Date	Revision summary	Consent Revised
6	09/05/14	Study synopsis, section 4.1, checklist - expand eligibility to include the following classifications of MDS: • International Prognostic Scoring System- Revised (IPSS-R) Category: High or Very High (Score of 5 or above) • WHO Classification: CMML that is not highly proliferative. WBC < 15k • Therapy related MDS • Severe cytopenias: Severe neutropenia (ANC ≤ 0.8), platelet or PRBC transfusion dependent Update appendices IV (IPSS) to include IPSS-R Section 7.6 and section 8.1 – add a bone marrow biopsy between course 1 and 2 for patients who failed prior treatment (prior to revision #5 all patients had a bm bx mid-treatment, with this revision it is reinstated for a sub-set of patients) other edits and clarifications – all tracked	yes
7	10/28/14	Section 6 – clarify study enrollment/registration is on-study Section 8 – increase the window that pre-treatment evaluations may be done (28 days unless otherwise stated, but 14 days for CBC, plt, CMP, chemistries), previously had been 14 days unless otherwise stated – clarify window is from start of treatment not study enrollment Section 11.4 – update event reporting to IRB to new requirements (UMN only)	no

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Study Synopsis

Study Design:
This is a Phase II therapeutic trial combining Decitabine days 1-5 with oral Vorinostat twice daily days 6-15 followed by a single infusion of CD3-/CD19- enriched donor NK cells on day 17. Subcutaneous Interleukin-2 (IL-2) will be given 2 hours after the NK cell infusion with a 2nd and 3rd dose given on day 19 and day 22. Two courses of treatment will be given separated by 6 - 8 weeks. The intent is to administer all treatment in the outpatient setting.

A single donor apheresis will be collected on day 15 of course 1. Centralized product manufacturing will be done at the U of MN Molecular and Cellular Therapeutics (MCT) laboratory (utilizing overnight courier for Mayo Clinic cells). At MCT the cells will be enriched for NK cells with the large scale CliniMacs device (Miltenyi) then the NK cell product will be divided in two with half stored frozen until course #2. The other half will be activated by incubation with IL-2. On the morning of day 17 (upon arrival back at Mayo, if applicable), the cells will be washed and final lot release done. For course #2, the stored cells will be thawed, IL-2 activated, and infused in a manner identical to course #1.

Clinical response will be formally assessed 4 - 6 weeks after the start of 2nd course based on International Working Group (IWG) criteria.

Primary Objective:
- Evaluate the objective response rate (CR + PR+ Hematologic Improvement (HI)) after 2 courses of Decitabine and Vorinostat with haploidentical NK cells in patients with high risk MDS

Secondary Objectives:
- Assess the safety and tolerability of Decitabine and Vorinostat followed by an infusion of CD3-/CD19- donor NK cells and IL-2 when given in the outpatient setting in patients with high risk MDS
- Assess the proportion of patients who become transfusion independent at 4-6 months post
- Estimate the association of in vivo expansion with clinical response
- Estimate 1 year Overall Survival

Patient Population:
Diagnosis of high risk MDS **requiring treatment** that meets at least **one** of the following classifications:
- International Prognostic Scoring System (IPSS) Category: INT-2 or High Risk
- International Prognostic Scoring System- Revised (IPSS-R) Category: High or Very High (Score of 5 or above)
- WHO Classification: RAEB-1 or RAEB-2
- WHO Classification: CMML that is not highly proliferative, WBC < 15k
- High risk cytogenetic abnormality as defined by presence of Monosomy 7, complex karytope, or monosomal karyotype
- Therapy related MDS
- Severe cytopenias: Severe neutropenia (ANC $\leq$ 0.8), platelet or PRBC transfusion dependent
- WHO Based Prognostic Scoring System (WPSS): High or Very High Risk

Prior treatment:
- Untreated/Minimally Treated: May be untreated or have had a maximum of 2 previous cycles of hypomethylating agents (azacitidine or Decitabine) **without** evidence of treatment failure
- Treatment Failure: Patients who have not responded to or whose disease has progressed on hypomethylating agents (azacitidine or decitabine), 7+3 type induction chemotherapy, or immunosuppressive therapy such as ATG+CSA

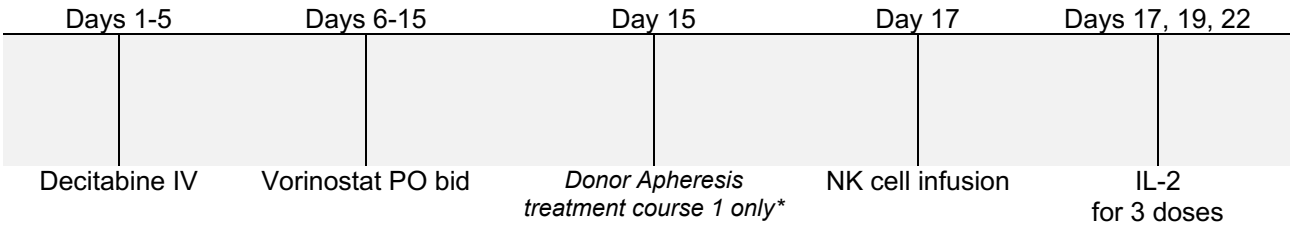
Age ≥ 18 years of age, ECOG 0-2, adequate renal, hepatic and cardiac function as defined in section 4.6
Available related HLA haploidentical NK cell donor age 18-75 years

Accrual Objective:
Stage 1 – 15 evaluable patients; Stage 2 activate if ≥ 6 of stage I patients achieve response. If so, then enroll an additional 31 patients for a total of 46 evaluable patients

Study Schema

Treatment Plan:

All treatment to be administered as an outpatient



Decitabine 10 mg/m^2 IV over 1 hour days 1-5
Vorinostat 200 mg PO twice a day days 6-15
Il-2 activated donor NK cell infusion IV over 15 to 60 minutes day 17
IL-2 6 MU SQ for 3 doses beginning on day 17 (2 hours after NK cell infusion), then day 19 and day 22

- Repeat treatment course 6 to 8 weeks later unless unacceptable toxicity, medically unsafe, or evidence of rapid disease progression as defined in section 7.10
- Assess for disease response 4 to 6 weeks after 2nd treatment course
- Follow for survival and progression to AML for 1 year from enrollment

* A single donor apheresis will be collected on day 15 of course 1. Centralized cell processing will occur at the University of Minnesota utilizing overnight shipping for Mayo products (U of MN products will be held an extra 24 hours to match Mayo's timing). The NK cell product will be divided in two after processing, but before IL-2 activation. Half will be stored frozen until course 2. The remaining cells will be prepared for fresh administration on day 17 of course 1.

1.0 Objectives

1.1 Primary Objective

Evaluate the objective response rate (CR + PR+ Hematologic Improvement (HI)) after 2 courses of Decitabine and Vorinostat with haploidentical NK cells in patients with high risk MDS

1.2 Secondary Objectives

- Assess the safety and tolerability of Decitabine and Vorinostat followed by an infusion of CD3-/CD19- donor NK cells and a short course of IL-2 when given in the outpatient setting in patients with high risk MDS
- Assess the proportion of patients who become transfusion independent (red blood cells and/or platelets)
- Estimate the association of in vivo NK cell expansion with clinical response
- Estimate 1 year Overall Survival (OS)

1.3 Correlative Laboratory Objectives

- Estimate the incidence of successful in vivo expansion of adoptively transferred haploidentical NK cells by NK cell persistence 2 weeks after infusion (Day 28)
- Monitor phenotypic and functional characteristics of natural killer cells and regulatory T cells in vivo

2.0 Background and Scientific Significance

Myelodysplastic syndromes (MDS) are a complex and heterogeneous group of clonal stem cell disorders manifested by diverse clinical and biologic paths with varying needs for transfusions, risk of infection, and risk of progression to acute leukemia. The FDA approval of three new therapeutic agents for MDS over the last few years (azacitidine, lenalidomide, and decitabine) as well as innumerable available clinical trials has changed the treatment paradigm for this spectrum of diseases; however, stem cell transplantation remains the only curative therapy for MDS.

MDS pathophysiology is complex, diverse, and still not completely understood. Structural alterations in DNA, as evident by the cytogenetic abnormalities seen in a majority of patients, play a role in the pathogenesis of disease due to loss or alteration of genetic material involved in proliferation, differentiation, or apoptosis. Epigenetic changes in the form of modifications to the transcriptional capacity of the cell via processes such as DNA methylation or histone acetylation can also alter gene expression impacting disease biology. [1] As such, DNA hypermethylation of key cellular machinery involved in cell cycle regulation,

apoptosis, and tumor suppressor control is well documented in the pathogenesis of myelodysplastic syndromes as well as other cancers. [1-6]

In addition to the known epigenetic alterations, the data also support evidence of a range of immune dysregulation in MDS subtypes. It is clear from extensive studies with immunosupressive therapies (ATG+ CSA, etc) that there are a subset of MDS patients (younger age, HLA-DR15, possible hypocellular marrows, with low risk IPSS MDS, and shorter duration of disease) where autoimmunity is a primary pathophysiologic defect. [7-11] In these settings, immunosupressive therapy is likely the most appropriate treatment strategy. Additional work investigating NK cell and T regulatory cell function and signatures in MDS suggest a divergent pattern for more advanced states of MDS. The currently available data regarding NK cell dysfunction in MDS, though yet to be fully characterized, appears to be multifactorial due to presence of clonal cytogenetic abnormalities, [12]decreased activating receptors in peripheral blood NK cells (NKp30, NKp46, NKG2D) with NKG2D the primary effector versus decreased expression of bone marrow NK cell activating receptor DNAM-1 contributing to the NK cell dysfunction, [12-14] and a correlation between NK cell dysfunction and increased blasts and more advanced WHO MDS categorization[13]. The influence of T regulatory cells (Tregs), defined as CD4+, $CD25^{high}$, Foxp3+, on immune surveillance and development of autoimmunity or suppression of host immune response has also been widely researched in cancer and more recently in MDS. The overarching theme of T regulatory cell characteristics in MDS describe increased Treg frequency with disease progression and subsequent decrease with response to therapy. Functionally, the Tregs retained their function in late stage MDS, theoretically consistent with their impact on suppressing immune attack on the underlying disease, but in early stage MDS were less efficient at suppression of polyclonal T cell proliferative responses again theoretically consistent with the higher degree of autoimmunity at that disease state. [15-18] Combining these data support the potential role of Tregs in leukemic progression in INT-2/High risk MDS and a more autoimmune milieu in the low/INT-1 patients.

These data highlight the role the immune system may play in the diverse spectrum of MDS by allowing either the development of or promoting the progression of disease. Immune dysregulation is unlikely to account for the entire pathogenesis of these diseases; however, identifying immune abberencies in Treg and NK cells in MDS and developing therapies to abrogate these responses and combining immune focused therapies into standard regimens is a rational next step.

2.1 Conventional Therapy for High Risk MDS

2.1.1 Hypomethylating Agents: Azacitidine and Decitabine

2.1.1.1 Azacitidine as a Single Agent for Myelodysplastic Syndromes

Azacitidine (5-azacytidine; Vidaza; Celgene Corporation, Summit, NJ, United States) was the first drug approved for the treatment of myelodysplastic syndromes by the Food and Drug Administration (FDA) in 2002. Azacitidine is an analog of cytidine, a pyrimidine nucleoside that is a component of human RNA. Although azacitidine falls into the category of hypomethylating agents, its mechanism of action is likely multifactorial.

Based on work by Silverman and colleagues and the outcomes of CALGB 8421, 8921, 9221, and AZA-001, using a standard dose of 75 mg/m2 Days 1-7, azacitidine became a first line therapeutic option for higher risk MDS (INT-2/High by IPSS) requiring therapy. Initial study results revealed an average overall response rate (complete remission + partial remission + hematologic improvements) of approximately 40% but more importantly demonstrated delayed time to leukemia progression, improved quality of life, and improved overall survival compared to conventional care. [19-22] While these study revealed important strides in caring for high risk MDS patients, the delayed time to transformation to AML from 11 months to approximately 18 months, and the 5+ month benefit on survival with azacitidine therapy is far from perfect.

2.1.1.2 Decitabine as Single Agent in MDS

Related to azacitidine, Decitabine (5-aza-2'-deoxycytidine) which is a cytosine analog that eventually incorporates into DNA through a series of metabolic steps differing from azacitidine has been noted to lead to hypomethylation at low doses and direct cytotoxicity at higher doses. [23] Initial studies using a dosing schedule of 15 mg/m2 IV over three hours every 8 hours for a total of 3 days repeated every 6 weeks demonstrated overall response rates of 17% [24]while subsequent studies using more outpatient-friendly regimens (20mg/m2 Day 1-5 SQ, 20mg/m2 Day 1-5 IV, and 10mg/m2 IV Day 1-10) produced higher CR and overall response rates. The dosing schedule of 20mg/m2 intravenously Day 1-5 yielded the highest CR rates (39%). [25] Given these good responses, the above dosing schedule is the one most frequently used in clinical practice.

Independent meta analysis of both azacitidine and decitabine with respect to overall survival and time to AML/death has interestingly shown the advantage in favor of azacitidine [OS: azacitidine (HR 0.56,

95% CI 0.44-0.73) versus Decitabine (HR 0.88, 95% CI, 0.66-1.17)]; time to AML/death: azacitidine (HR of 0.54,95% CI 0.42-0.71) vs. Decitabine (HR 0.85, 95% CI, 0.66-1.07). To identify the reason for this discrepancy, characterization of the study groups within the azacitidine and decitabine trials revealed a similar proportion of IPSS risk groups/FAB groups and median ages; however, the duration of Decitabine therapy was slightly shorter with a median of 3-4 cycles versus 9 with azacitidine. [22] The differing treatment exposure may explain the differing impact on overall survival benefit; however, only randomized studies comparing azacitidine and Decitabine will answer the question of equivalence or superiority. Both agents produce desirable clinical responses and are reasonable therapeutic tools, but given the documented benefits of improved OS and delayed time to AML in the current literature, azacitidine has a mild advantage. However, the higher rates of CR seen in the newer dosing schedule of Decitabine make this an attractive agent when the goal of therapy is disease response in preparation for next steps, such as transplant, and not necessarily as the definitive therapy.

2.1.2 Histone Deacetylase Inhibitor (HDACi) use in MDS

Epigenetic modulation through histone acetylation is another area of intense interest in cancer therapy, and specifically in the myeloid malignancies. Numerous HDAC inhibitors (ex. MS-275, sodium phenylbutyrate, Valproic acid, and Vorinostat, MGCD0103) have been studied extensively in AML and MDS with proposed effects on proliferation, differentiation, and apoptosis. [26-32] Response rates with single agent use of HDAC inhibitors has generally been low at 10-20%. [32] Dose limiting toxicities have been relatively common regardless of the HDAC inhibitor used and typically involve fatigue, nausea, vomiting, and diarrhea. Interestingly, while increases in histone acetylation have been documented in the majority of early phase studies, the clinical responses have not always correlated directly, indicating the possibly of additional mechanisms of action. Because of the relatively low single agent response rates as well as biologic rationale for combination with other drugs, these agents have been subtantially investigated in the combination setting with DNA methyltransferase inhibitors in the AML/MDS and is reviewed extensively in recent publications. [32, 33]

2.1.3 Vorinostat Use in MDS

Vorinostat has been shown to be a popular HDAC inhibitor used in studies with MDS and AML. In early, Phase I testing of single agent Vorinostat (SAHA) in MDS/AML patients evaluating the MTD and safety profile (200mg BID versus 250mg TID - dose divided for improved histone acetylation) yielded an overall response rate appoximating 17% across doses. [26] Subsequent studies combining Vorinostat at various dosing regiments with either azacitidine or Decitabine reported a wide range of

responses from 16-86%, as reviewed in a recent publication by Quintas-Cardama et al. [33] The variable response highlights the uncertainty of best dosing combination and the need for continued work utilizing this promising platform.

2.1.4 Vorinostat Use as an Immunosuppressant

Vorinostat has also recently been cited as a possible immune modulating drug as evidence by elegant mouse work published by Reddy at al. In their mouse models of acute graft versus host disease of allogeneic stem cell transplnation, they showed that administration of Vorinostat soon after infusion of allogeneic stem cells, diminished inflammatory cytokines and subsequent development of acute graft versus host disease, while not impairing T cell responses to host antigens after transplant. [34]

2.2 History of Adoptive NK Cell Therapy for Refractory/Relapsed AML at the U of MN

Using the platform of lymphodepletion with cyclophosphamide, fludarabine with subsequent infusion of CD3 depleted haploidentical natural killer cells and subsequent high dose IL-2 administration, we have treated total of 32 high risk refractory AML patients with the exact same therapy and NK cell enriched products at the University of Minnesota. A total of 10 of these patients achieved a complete remission. Of the ten patients achieving a remission three went on to get a subsequent allogeneic transplant and all had 2-year disease free survival. [35]Three patients died of toxicity. Four patients who were ineligible to go on to allogeneic transplant ultimately relapsed four to eleven months after achieving remission under this protocol. Morbidity associated with this regimen included a very prolonged hospital stay, on average of 4-6 weeks, as well as high fevers, capillary leak with pulmonary edema and peripheral edema, renal toxicity, etc with the high doses of IL-2 used. However, important to note that prolonged hospital admissions with potential higher toxicity is not uncommon for AML patients undergoing salvage chemotherapy. Although the outcomes were relatively favorable for patients with refractory leukemia, the transient nature of response in the absence of definitive therapy such as stem cell transplant and the associated high morbidity indicates that this adoptive cell therapy in its current form was not curative, at least in AML, and would likely be too high risk for an older cohort of MDS patients.

In Vivo Expansion Correlates With Increasing The Intensity Of The Preparative Regimen

Data from prior studies described in the section above suggest that those patients who achieved a remission with were those who expanded NK cells in vivo. In the subsequent trial, MT2003-23, total body irradiation (TBI, 400 cGy) was added to cyclophosphamide and fludarabine (Cy/Flu) and we compared the rates of in vivo NK cell expansion (defined as 100 donor derived NK cells/μL blood) and leukemia clearance (defined as less

1% blasts in the bone marrow). Using this approach (n=13), successful in vivo expansion of adult donor NK cells was detected in 75% of patients with a mean of 606±184 circulating donor derived NK cells/μL blood 14 days after the adult NK cell infusion compared to a rate of only 10% in patients receiving Cy/Flu alone under the predecessor of this protocol, MT2004-25 (Figure 1). In vivo expansion correlated with leukemia clearance.

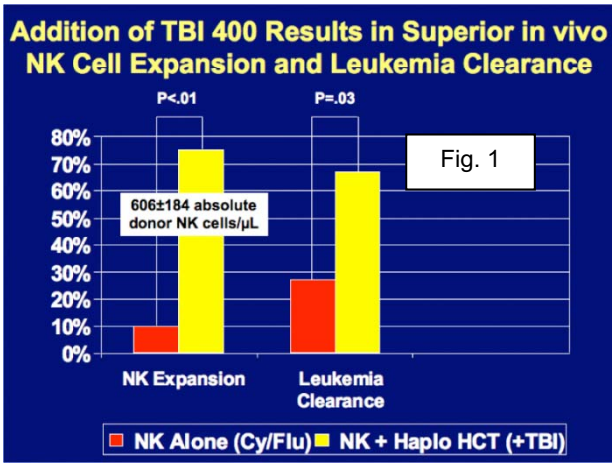


Fig. 1

2.3 Rationale for Proposed Study

Based on the high single agent efficacy of Decitabine as a standard initial MDS therapy, the numerous studies suggesting a synergistic anti-tumor effect in MDS with HDAC inhibitor Vorinostat, and the potential immunosuppressive impact of Vorinostat, we propose the following therapeutic platform. Sequential Decitabine and Vorinostat will provide the basis for both a disease specific therapeutic and immunosuppressive conditioning regimen to allow for transient host acceptance of donor NK cells for added anti-tumor effect. Dosing of sequential Decitabine (10 mg/m^2 Days 1-5) and Vorinostat therapy (200 mg twice daily Days 6-15) is based on Phase I studies of the combination detailing the best delivery and tolerance of the combination with the fewest treatment delays [36] and early phase studies identify responses in MDS and AML patients. [37,38] After haploidentical NK infusion, subsequent lower dose IL-2 will be given to promote NK cell expansion and predicted anti-tumor effect. The less intense conditioning regimen and IL-2 cytokine therapy is predicted to be more tolerable in an older population of patients and feasible in the outpatient setting. However, given the diminished intensity of the regimen, and the need for subsequent cycles of hypomethylating agent therapy to observe consistent clinical responses in the general practice setting, the study will consist of 2 consecutive courses (cycles) of therapy.

3.0 Study Design

This is a Phase II therapeutic trial combining Decitabine days 1-5 with oral Vorinostat twice daily days 6-15 followed by a single infusion of CD3-/CD19-enriched donor NK cells on day 17 and a short course of Interleukin-2 (IL-2) to facilitate NK cell survival and expansion. Two courses of treatment will be given separated by 6 - 8 weeks. Clinical response will be formally assessed 4 - 6 weeks after the 2nd course based on International Working Group (IWG) criteria;

however, a bone marrow evaluation will be done if the patient is taken off treatment prior to study completion. **The intent is to administer all treatment in the outpatient setting.**

The trial will use Simon's two-stage design. Fifteen patients will be enrolled in stage 1. If 6 or more of the patients achieve an objective clinical response (CR + PR + hematologic improvement (HI)), stage 2 will be activated and an additional 31 patients enrolled for a total of 46 evaluable patients. Considering a predicted dropout rate of 10% during the two courses of therapy, stage 1 will need a maximum of 17 patients or a maximum of 51 patients if the study proceeds to 2nd stage.

Research and related correlative studies will be done at baseline, between courses 1 and 2, and after completion of course 2 as per section 8.1.2.

4.0 Selection of Patients

Study entry is open to adults regardless of gender or ethnic background. While there will be every effort to seek out and include females and minority patients, the patient population is expected to be no different than that of other studies of high risk myelodysplastic syndromes at the University of Minnesota and the Mayo Clinic.

Inclusion Criteria:

4.1 Diagnosis of high risk MDS that meets one of the following disease classifications and is requiring treatment:

- **4.1.1** International Prognostic Scoring System (IPSS) Category: INT-2 or High Risk (see Appendix IV)
- **4.1.2** International Prognostic Scoring System- Revised (IPSS-R) Category: High or Very High (Score of 5 or above) (Appendix IV)
- **4.1.3** WHO Classification: RAEB-1 or RAEB-2 (Appendix V)
- **4.1.4** WHO Classification: CMML that is not highly proliferative; WBC < 15k
- **4.1.5** High risk cytogenetic abnormality as defined by presence of Monosomy 7, complex karytope, or monosomal karyotype
- **4.1.6** Therapy related MDS
- **4.1.7** Severe cytopenias: Neutropenia (ANC ≤ 0.8), platelet or PRBC transfusion dependent
- **4.1.8** WHO Based Prognostic Scoring System (WPSS): High or Very High Risk (Appendix V)

4.2 Prior Treatment:

- **4.2.1** Untreated/Minimally Treated: maximum of 2 cycles of hypomethylating agents (azacitidine or decitabine) without evidence of treatment failure as defined by progression to more advanced MDS Who classification or AML.

4.2.2 Treatment Failure: Patients who have not responded to or whose disease has progressed on hypomethylating agents (azacitidine or decitabine), 7+3 type induction chemotherapy, or immunosuppressive therapy such as ATG+CSA.

4.2.3 Patients must not have received treatment for their MDS within 4 weeks of beginning this study.

4.3 Age ≥ 18 years of age

4.4 ECOG Performance Status 0-2 (Appendix III)

4.5 Available related HLA-haploidentical NK cell donor by at least Class I serologic typing at the A&B locus

4.6 Have acceptable organ function as defined below within 14 days (28 days for cardiac) of enrollment:

Renal: Creatinine: ≤ 2.0 mg/dL

Hepatic: SGOT/SGPT < 5 x upper limit of institutional normal (ULN)

Pulmonary: oxygen saturation ≥ 90% on room air

Cardiac: LVEF by ECHO or MUGA ≥ 40%, no uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. QTc < 500 msec

4.7 Ability to be off prednisone and other immunosuppressive drugs for at least 3 days prior to the NK cell infusion (excluding pre-meds)

4.8 Women of child bearing potential must agree to use effective methods of contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) from the time of signing the consent form and for 2 months after the last dose of chemotherapy.

4.9 Voluntary written consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn at any time without prejudice to future medical care.

Exclusion Criteria:

4.10 Pregnant or lactating. The agents used in this study are known to be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. Confirmation that the patient is not pregnant must be established by a negative pregnancy test result obtained during screening. Pregnancy testing is not required for surgically sterilized or post-menopausal women.

4.11 New progressive pulmonary infiltrates on screening chest x-ray or chest CT scan that has not been evaluated with bronchoscopy (when feasible)

4.12 Uncontrolled bacterial or viral infections - chronic asymptomatic viral

hepatitis is allowed

4.13 Pleural effusion moderate to large in size that are detectable on chest x-ray

4.14 Known hypersensitivity to one or more of the study agents

4.15 Prior hypomethylating treatment greater than 2 cycles or with documented treatment failure

4.16 Prior use of histone deacetylase inhibitors

4.17 Serious medical or psychiatric illness likely to interfere with participation in this clinical study in the opinion of the enrolling investigator

4.18 Inability to swallow capsules

4.19 Active HIV

4.20 Other active and potentially life threatening malignancy excluding localized basal or squamous cell skin cancer, cervical carcinoma in situ, superficial bladder cancer, localized prostate cancer

5.0 Donor Selection

5.1 Related donor (sibling, parent, offspring, parent or offspring of an HLA identical sibling) 18-75 years of age

5.2 At least 40 kilograms body weight

5.3 In general good health as determined by the evaluating medical provider

5.4 HLA-haploidentical donor/recipient match by at least Class I serologic typing at the A&B locus

5.5 Able and willing to undergo apheresis

5.6 Not pregnant

5.7 Voluntary written consent

6.0 Patient/Donor Enrollment (On Study)

Enrollment onto the study will occur after the patient has signed the patient consent and eligibility is confirmed, but before any treatment has been administered. Any patient signing consent, but not registered in the study, will be recorded as a screen failure in the screening log as detailed in the study's Affiliate Manual.

To be eligible for enrollment to this study, the patient must meet each criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record. The eligibility checklists (patient - Appendix I,

donor – Appendix - II) can be found under attachments within the study in OnCore.

6.1 Registration with Masonic Cancer Center Clinical Trials Office

Upon completion of the screening evaluation, eligibility checklist and obtaining consent, the site study coordinator or designee will enroll the patient and donor into OnCore. The patient will be considered "on study". Complete registration information is found in the study's Affiliate Manual.

OnCore will automatically generate an email alerting key study personnel of the registration.

6.2 Patients Who Do Not Begin Study Treatment

If a patient is registered on study, and is later found not able to begin the preparative regimen (beginning with the first dose of decitabine), for whatever reason, the patient will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and the reason for removal from study will be clearly indicated in OnCore as detailed in the study's Affiliate Manual. The patient will be replaced.

If a patient begins treatment, and then is discontinued for whatever reason, the patient will be replaced as the clinical response (CR + PR + HI) is not evaluable for response. If the patient discontinues therapy due to toxicity, it will count towards the toxicity stopping rules. The patient must be followed for any unresolved toxicity per section 7.10.

7.0 Treatment Plan

Treatment is planned to be given in the outpatient setting and must begin on a Monday to avoid weekends. The administration of the study drugs will follow institutional drug and supportive care guidelines. There will be no dose escalation or de-escalation except for IL-2 where modifications are allowed for unacceptable toxicity.

Study Day	Days of Week	Study Drug	Section of protocol
Days 1-5	Monday-Friday	Decitabine 10 mg/m^2 IV	Section 7.1
Days 6-15	Saturday through the 2nd Monday	Vorinostat 200 mg PO twice a day	Section 7.2
Day 15	Monday	Donor apheresis (course 1 only)	Section 7.3
Day 16	Tuesday	Patient – rest	Section 7.2
Day 17	Wednesday	NK cell infusion	Section 7.4
Days 17, 19, 22	Wednesday, Friday, Monday	IL-2 6 MU SQ beginning 2 hours after the NK cell infusion for 3 doses	Section 7.5

Repeat the treatment course 6 to 8 weeks later unless medically unsafe. Requirements for proceeding to course 2 are defined in Section 7.6.

Clinical response will be formally assessed 4 - 6 weeks after the 2nd course based on International Working Group (IWG) criteria (Appendix VI); however a bone marrow evaluation will be done if the patient is taken off treatment early.

7.1 Decitabine (Days 1-5)

Decitabine 10 mg/m^2 will be administered as a 1 hour intravenous infusion days 1 through 5.

7.2 Vorinostat Administration (Days 6-15)

Vorinostat will be taken at a dose of 200 mg (daily dose of 400 mg) twice daily with food for 10 days. The capsules must be swallowed whole. If a Vorinostat capsule is accidentally opened or crushed, the capsule or the powder must not be touched. Patients will be provided with a daily drug log (appendix VIII) to record their vorinostat dosing as well as any side effects.

Day 16 is a rest day with no treatment.

7.3 Donor Apheresis (Day 15 Course 1 Only) and Cell Product Manufacturing

Peripheral blood cells will be collected by a single apheresis from the haploidentical related donor on day 15 of course 1 only (Monday). Apheresis collections will be performed on the Amicus Apheresis system (Fenwal Inc., Lake Zurich IL, USA) and will use a fixed time of 5 hours with a flow rate of 65 ml/min. The cycle volume of 1400 ml (manufacturer recommendation) will be used. Anticoagulant will consist of either ACD-A or heparin/ACD-A.

Cell product manufacturing will be performed at the University of Minnesota Molecular and Cellular Therapeutics (MCT) laboratory. Mayo Clinic will utilize overnight courier services.[39] The apheresis product and a tube of donor blood (2ml EDTA) will be sent from Mayo Clinic by overnight courier the day of collection (day 15). Refer to the Affiliate Laboratory Manual regarding shipping specifics.

At MCT, the apheresis product will be enriched for NK cells with the large-scale CliniMacs device (Miltenyi) by depletion of CD3+ cells to remove T-lymphocytes and depletion of CD19+ cells to remove B-lymphocytes. The NK cell product will then be divided in two with half stored frozen at MCT until day 17 of course #2.

The remaining NK cell enriched product will be activated by incubation with 1000 U/ml IL-2.

For U of MN patient products, the cells with IL-2 will be processed with lot release testing per the CMC but held for infusion until the morning of day 17.

For Mayo patient products, the cells with IL-2 will be shipped to Mayo's cell product lab on day 16 by overnight courier for arrival the morning of day 17. Partial lot release testing on the pre-IL-2 product including sterility testing (Gram stain, endotoxin), cell viability, flow cytometry analysis will be performed prior to shipping the cells to Mayo. Results will be confirmed with Mayo prior to the NK cell product infusion.

At Mayo, the cells will be washed and final lot release performed.

Samples for TTL in association with original cell production/infusion #1:
A sample of the cell product will be sent from MCT to the Masonic Cancer Center's Translational Therapy Lab (TTL) for cell function and phenotype studies from the following samples:
1) the apheresis unit before processing (100 x 10^6 cells)
2) the post-column pre IL-2 incubation sample (10 x 10^6 cells)
3) the final IL-2 product (10 x 10^6 cells)

Course #2
The frozen NK cells will be thawed by MCT on day 16 and incubated with IL-2. For Mayo patients, the cells with IL-2 will be shipped to Mayo's cell product lab by overnight courier for arrival the morning of day 17. On day 17, the institutional cell product laboratory will wash and release for infusion according to the CMC for infusion on day 17 of the 2nd treatment course.

Samples for TTL in association with infusion #2:
A sample of the cell product will be sent to the Masonic Cancer Center's Translational Therapy Lab (TTL) at the following time points:
1) the post thaw, pre IL-2 incubation sample (10 x 10^6 cells)
2) the final IL-2 product (10 x 10^6 cells)

Refer to the chemistry, manufacturing and control (CMC) section of the Investigational New Drug (IND) application for additional preparation information and lot release criteria.

7.4 Allogeneic $CD3^-$ $CD19^-$ Selected NK Cell Product Infusion (Day 17)

Pre-medication: Patients should be pre-medicated with acetaminophen 650 mg PO and/or diphenhydramine 25 mg PO/IV within 1 hour before and 4 hours after the NK cell infusion. Demerol 25-50 mg IV may be given for chills/rigors during the NK cell infusion. Corticosteroids must not be used.

Infusion guidelines: The NK cells will be infused without a filter or pump, slowly by gravity over at least 15 minutes but no longer than 1 hour. The targeted infused cell dose of $CD3^-$ $CD19^-$ selected NK cell product is within the range of 2-3 x 10^7 cells/kg. However, cell infusion will proceed regardless of cell dose if prior defined product collection parameter of > 3-fold NK cell enrichment between the apheresis and final products is met. Flush tubing on completion of the NK cells with normal saline to ensure all of the cells are infused.

Vitals: Obtain vital signs before the NK cell infusion, every 15 minutes during the infusion, and then every 30 minutes x 2, then pre routine. Monitoring time will total at minimum 2 hours after NK cell infusion.

Monitoring: Patients will be monitored for adverse effects of the NK cell infusion such as rash, acute allergic reaction, bronchospasm, respiratory distress, and acute vascular leak syndrome. If life threatening acute reactions occur (defined as CTCAE grade 4 - life-threatening consequences; urgent intervention indicated), the infusion will be stopped. If grade 4 infusion reaction occurs refer to section 8.1.3 regarding testing for HAMA.

Hydration will be administered per institutional guidelines. Any elevation of metabolic monitoring will be treated with more aggressive hydration, while being attentive to fluid overload.

Corticosteroids are to be avoided from the time of NK cell infusion through day 25. If essential, low doses of steroids (e.g. 25 mg hydrocortisone) may be given as clinically indicated during this window.

7.5 IL-2 (begin day 17)

Start subcutaneous IL-2 approximately 2 hours, but no earlier than 2 hours, after the NK cell infusion in the absence of grade 4 infusion related toxicity.

If the patient has experienced grade 4 infusion related toxicity and it resolves to grade 2 or better, the IL-2 may be started up to 48 hours after the NK infusion. If no IL-2 is given, the patient will be replaced for statistical analysis.

IL-2 administration: IL-2 will be given at 6 million units on days 17, 19 and 22. For patients weighing less than 45 kilograms, the IL-2 will be given at a dose of 3 million units/m^2 on days 17, 19 and 22. For the purposes of outpatient scheduling and/or possible delay in treatment start, dosing may be altered to avoid weekends as long as IL-2 is not given on consecutive days and completed within a week.

Pre-medication with acetaminophen 650 mg PO and diphenhydramine 25 mg PO/IV before and 4 hours after each dose of IL-2 is recommended.

Monitoring during IL-2 administration: Patients are monitored for IL-2 related toxicities on a daily basis in clinic until the day after the final IL-2 administration with increased monitoring as clinically required.

IL-2 dose-adjustment for individual patient intolerance: Fevers, rash and flu-like symptoms are expected and should be treated supportively with acetaminophen and diphenhydramine as described above. If a patient cannot tolerate full dose IL-2 due to fevers, rash or constitutional symptoms, a dose decrease to 4 million units is allowed (2 million units/m^2 if < 45 kg). If the patient cannot tolerate the reduced dose, the IL-2 must be permanently discontinued.

IL-2 dosing guidelines for non-hematologic grade 3-4 toxicity (CTCAE v4):
Non-hematologic grade 3 toxicity persisting for > 24 hours: Hold IL-2. If the toxicity resolves to grade 2 or better within 48 hours, the IL-2 can be resumed at a reduced dose (4 million units or 2 million units/m^2 if < 45 kg). If the same toxicity persists, worsens or recurs, the IL-2 must be permanently discontinued. Missed doses are not made up.

Non-hematologic grade 4 toxicity: Permanently discontinue IL-2

7.6 Second Treatment Course

Any patient who failed prior treatment (i.e. met section 4.2.2 of the eligibility criteria at enrollment) will have a bone marrow biopsy and aspirate performed prior to the start of the 2nd treatment course.

A second treatment course will be given 6 to 8 weeks after the start of course 1, identical to the first course substituting the frozen NK cell product for the fresh product, unless medically unsafe.

The following criteria must be met before a new treatment course is started:

- ANC recovery to baseline documented prior to course 1
- Platelet transfusion that can be managed safely in the outpatient setting

- Treatment related non-hematologic toxicity, except fatigue, alopecia, resolved to ≤ grade 2
- Absence of rapid disease progression as defined in Section 7.10

If the above requirements are not met, delay and reassess weekly until recovery occurs. If more than 2 weekly delays occur (>8 weeks from the start of course 1) without hematological recovery and/or non-hematologic treatment related toxicity resolution, the patient will be taken off study. A bone marrow biopsy will be done to assess response to the therapy up to that point or to determine etiology for inadequate recovery of counts.

7.7 Dose Modifications

There are no planned dose modifications or delays except as detailed in section 7.5 in association with the IL-2 administration.
Note: If patients required dose modification of IL-2 during course 1 then the modified dose should be used for course 2.

7.8 Management of Selected Toxicities Associated with NK Cell Infusion

See Appendix VII for expected toxicities of Decitabine, Vorinostat and Interleukin-2.

7.8.1 Acute Allergic Reaction Secondary to the Infusion of Allogeneic NK Cells

Although infusion of donor lymphocytes has not been associated with acute allergic reactions, patients will be closely watched for the occurrence of hypotension, dyspnea and angioedema during and immediately after the infusion.

The NK cell infusion will be stopped if severe acute reactions occurs (defined as CTCAE grade 4 - life-threatening consequences; urgent intervention indicated). A blood sample will be collected for HAMA testing per section 8.1.3.

7.8.2 Vascular Leak Syndrome

Neither administration of allogeneic NK cells nor autologous IL-2 activated NK infusions have been associated with vascular leak syndrome in our previous experience. Nevertheless, patients will be monitored for weight gain (by weights at least 3 times per week) and pulmonary edema during IL-2 administration.

7.8.3 Tumor Lysis Syndrome

Allopurinol will be initiated to according to standard medical practice when the risk of tumor lysis is high.

7.8.4 Prolonged Marrow Suppression

Hematologic toxicity due to allogeneic NK cells may occur later, and therefore hematologic recovery will be assessed beyond the expected chemotherapy-induced nadir.

Pancytopenia will occur with this regimen and should be treated as follows:

Anemia and thrombocytopenia are expected in all patients, and they will receive standard supportive transfusion care according to transfusion committee guidelines or as modified based on clinical parameters. Prolonged anemia or thrombocytopenia are not unexpected and will not count towards toxicity.

Neutropenia is expected in the majority of patients, and they will receive supportive care with prophylactic antibiotics as noted below. G-CSF and granulocytes should be avoided unless clinically indicated in the setting of severe, uncontrolled infection. If the clinical situation warrants growth factor use, then any remaining IL-2 administration will be stopped.

7.9 Supportive Care Guidelines/Concomitant Medication Use

Patients will receive standard supportive care according to institutional guidelines or as modified based on clinical parameters.

Antiemetics will be used according to standard medical practice. Given the modest incidence of nausea, diarrhea, and anorexia with the drug combination, scheduled prophylactic antiemetics will be given during Day 1-15 of therapy.

All patients will receive antibacterial prophylaxis and antifungal therapy while neutropenic (until ANC >1000 x 2 days). Antibacterial options include but are not limited to levofloxacin, azithromycin, Pen V K and antifungal options include fluconazole, voriconazole, posaconazole as indicated. Choice of antibiotics is left to the discretion of the treating physician. Antiviral therapy and trimethoprim/sulfa for PCP prophylaxis are not required unless clinically indicated.

7.10 Duration of Therapy

Patients will receive two courses of study treatment unless one or more of the following occurs:

- Patient withdraws consent or is non-compliant
- Criteria in section 7.6 for a 2nd treatment course are not met
- Medically unsafe, in the opinion of the treating investigator to continue with the treatment plan and based on development of grade 4 non-hematologic toxicities

- Intercurrent illness that prevents further administration of treatment
- Unacceptable toxicity
- Evidence of rapid disease progression defined as ≥ 20% peripheral blood blast percentage suggesting progression to acute leukemia

Any patient who discontinues therapy before the completion of 2 courses should have a bone marrow biopsy and aspirate performed at the time treatment is discontinued. Those who are not evaluable per section 13 will be followed only until the resolution or stabilization of treatment related toxicity. No further follow-up for this study will be required, other than recording the date and cause of death upon knowledge in OnCore.

7.11 Follow-up

All evaluable patients will be followed for response and survival for 1 year from study enrollment unless the patient withdraws consent.

Ongoing therapy with maintenance hypomethylating agents or proceeding to allogeneic stem cell transplantation will be at the discretion of the patient's primary oncologist.

For patients completing 1 year of follow-up no further data collection will be required for this study; however the date and cause of death will be entered in OnCore upon knowledge for IND annual reporting.

An exception to this for all patients is the reporting upon knowledge of any serious, unexpected and at least possibly study treatment related event per sections 11.3 and 11.4.

8.0 Study Calendar

The pre-treatment work-up must occur within 28 days (14 days for CBC, diff, CMP, and chemistries) of treatment start unless otherwise noted. Scheduled evaluations (including research samples) up to day +22 may be performed +/-1 day from the targeted date; evaluations and procedures (including research samples) performed after day +28 may be done +/-3 days of the targeted date. Follow-up for survival may be done +/- 4 weeks of the targeted date. In addition, targeted days may be altered as clinically appropriate.

8.1 Patient

8.1.1 Standard of Care and Investigational Treatment

	Pre-Treatment (within 28 days of treatment start)*	During each treatment course					4- 6 weeks after 2nd treatment course	Follow-up at Day 90, 180, 270, 360
		Day 1[1]	Day 5	Day 15 or 17	During IL-2 administration (Day 17-22)	Day 24, 31, 38, 45		
EVALUATIONS								
Written consent	X							
Medical history	X							
Disease Characteristics	X							
Pathology review of outside dx	X							
Transfusion History	X						X	
Concomitant meds	X	X					X	
Physical exam	X	X	X[6]	X[6]	X[6]	X[6]	X	
Vital signs	X	X		X		X		
Weight	X	X		X	3 x a week during IL-2	X		
Height	X							
ECOG Performance Status	X	X					X	
Survival Status								X[7]
LABORATORY								
CBC w/ diff[2]	X	X	X	X	daily during IL-2	X	X	
Na, K, Cl, CO2, BUN, creatinine, uric acid, phos, LDH, Mg	X	X	X	X		X		
ALT/AST, bili, total protein, albumin, alk phos	X	X		X		X		
Chest X-ray	X							
INR/PTT	X							
Pregnancy test[3]	X							
HLA typing (low resolution)*	X							
Recipient Viral Serology	X							
ECHO/MUGA*	X							
Bone Marrow Bx/Asp[4]	X[4]					X[5]	X[4]	
Investigational TREATMENT								
Decitabine IV		Day 1-5						
Vorinostat PO			Day 6-15					
NK cell infusion (research)				Day 17				
IL-2 tiw for 3 doses (research)					X (start Day 17)			

* CBC, dff, CMP, chemistries within 14 days of study treatment start, HLA typing may be done at any time

1- Course 1 Day 1 evaluations need not be repeated if pre-study evaluations were done within 14 days of 1st treatment
2- CBC with diff weekly throughout all treatment with increased frequency as clinically indicated
3- Women of childbearing potential only; serum or urine
4- Bone marrow aspirate and biopsy with standard flow cytometry, conventional cytogenetics, and MDS/AML FISH panel will be conducted at study initiation and at the completion of the 2nd cycle for standard disease assessment or at time of study withdrawal if early termination.
5- Prior to course 2 ONLY in patients who enrolled in this study as a prior treatment failure per section 4.2.2 or any patient who is discontinuing study treatment after 1 course
6- Clinical assessments will occur at a minimum of weekly up until the NK cell infusion on Day +17 after which clinical assessments will occur daily until the day after the final IL-2 administration.
7- By record review, patient contact, etc.

8.1.2 Patient – Research Related

	Before start of each chemo course	Before NK cell infusion	After each treatment course			
		Day 17	Day 24	Day 31	Day 38	Day 45[3]
Adverse Event Monitoring	Per section 11					
Chimerism – PB (unseparated)*	x		x	x[1]	x[1]	x[1]
60 ml of heparinized blood (green top tubes)*	x	x	x	x	x	x
10 ml of serum (red top tube)* for HAMA[2]		x (post infusion)				
10 ml of serum (red top tube)*	x	x	x	x	x	x
BM 30 ml (heparinized)*	At the time of each bone marrow biopsy for clinical reasons					

1 – continue chimerism monitoring until they disappear
2 –draw sample in the event of a grade 4 infusion related toxicity occurs – refer to section 8.1.3
3 – the day 1 treatment course 2 sample may be eliminated if the patient is ready to start course 2 at day 45
*For the Cancer Center's Translational Therapy Lab (TTL) – refer to section 8.3 for shipping instructions. (U of MN chimerism samples to be sent directly to the Molecular Diagnostics)

As recognized with novel therapies, as in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, up to 3 extra samples for a total of 180 ml of blood may be drawn at additional time points that are not specified above.

8.1.3 Assessment for HAMA in the Event of a Grade 4 Infusion Reaction

If a patient experiences a CTCAE v 4 grade 4 infusion related reaction (life-threatening consequences; urgent intervention indicated) serum will be drawn and be tested for the presence of HAMA. If HAMA is detected, the results will be reported to the FDA in an expedited manner per section 11.4. If not, it will be noted at the time of the IND annual report.

8.2 Donor – Research Related

	Screen	Day 15
Consent	X	
HLA Typing (class 1) SOC - not research	X	
Type and Screen (ABO/RH /Indirect Antiglobulin)	X	X
Donor Infectious Disease Panel	X	X[1]
EBV IgG	X	
BMT/DNA marker	X	
Medical History	X	
Physical Exam	X	X
CBC, diff, platelet	X	X
ALT, creatinine	X	
Pregnancy test, if applicable	X	
60 ml of heparinized blood (green top tubes)*		X
10 ml of serum (red top tube)*		X
Leukapheresis		X

1 - repeat if more than 7 days since baseline testing
*For the Cancer Center's Translational Therapy Lab (TTL) – refer to section 8.3 for shipping instructions.

8.3 Sample Shipping Information

Samples are shipped the day of collection to the Masonic Cancer Center's Translational Therapy Lab (TTL) for overnight delivery in an insulated container with a frozen gel pack Monday-Thursday. Refer to the Affiliate Laboratory Manual for additional details.

Translational Therapy Core Facility
University Of Minnesota
420 Delaware St. SE
Room A410 Mayo Building
Minneapolis, MN 55455
Phone: 612-625-6165
Fax: 612-625-9631

9.0 Study Endpoints

This study is designed as a two stage study of the combination of Decitabine and Vorinostat and haploidentical NK cell infusion. The primary endpoint is clinical response (CR + PR + HI) at the end of the required 2 cycles of therapy. An assessment of response differences between untreated/minimally treated and those with prior treatment failures will be conducted.

The secondary endpoints include evaluation of safety tolerability, the proportion of patients who become transfusion independent, the association of the in vivo NK cell expansion with clinical response, and 1 year Overall Survival.

Clinical responses are defined in Appendix VI.

Determination of toxicities will be important for this new combination. As per NCI CTCAE Version 4.0, the term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either “unrelated or unlikely to be related” to study treatment in the event of an actual relationship developing.

10.0 Drug Formulation And Procurement

10.1 Decitabine

10.1.1 Other Names

Dacogen

10.1.2 Classification

Antineoplastic

10.1.3 Mode of Action

May cause hypomethylation of DNA and cellular differentiation or apoptosis by phosphorylation and direct incorporation into DNA.

10.1.4 Metabolism

Routes of decitabine metabolism and elimination are unknown. Elimination of decitabine is believed to occur through deamination by cytidine deaminase. The effects of renal or hepatic impairment on decitabine pharmacokinetic parameters have not been studied.

10.1.5 Storage and Stability

Store vials at 25°C (77°F); excursions permitted to 15 - 30°C (59 - 86°F).

Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C - 8°C) infusion fluids and stored at 2°C - 8°C (36°F - 46°F) for up to a maximum of 7 hours until administration.

10.1.6 Dose Specifics

For the purposes of this study, decitabine will be given at a dose of 10 mg/m^2 on days 1-5 of each treatment course.

Decitabine for Injection is supplied as a sterile lyophilized white to almost white powder, in a single-dose vial, packaged in cartons of 1 vial. Each vial contains 50 mg of decitabine.

10.1.7 Preparation

Decitabine is a cytotoxic drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing decitabine.

Decitabine should be aseptically reconstituted with 10 mL of Sterile Water for Injection (USP); upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7-7.3. Immediately after reconstitution, the solution should be further diluted with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or Lactated Ringer's Injection to a final drug concentration of 0.1 - 1.0 mg/mL.

Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C - 8°C) infusion fluids and stored at 2°C - 8°C (36°F - 46°F) for up to a maximum of 7 hours until administration.

10.1.8 Administration

Patients may be pre-medicated with standard anti-emetic therapy per institutional guidelines.

10.1.9 Availability

Commercially available

10.1.10 Side Effects

Pregnancy category D
Decitabine may cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant while using Decitabine. Men should be advised not to father a child while receiving treatment with Decitabine, and for 2 months afterwards.

The most commonly occurring adverse reactions include neutropenia, thrombocytopenia, anemia, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea, and hyperglycemia.

10.2 Vorinostat (SAHA)

10.2.1 Other Names

Suberoylanilide hydroxamic acid, zolinza

10.2.2 Classification

Histone deacetylase (HDAC) inhibitor

10.2.3 Mode of Action

Inhibits the enzyme histone deacetylase (HDAC), induces differentiation in some cell types, and has been shown to induce p53-independent apoptosis

10.2.4 Metabolism

The major pathways of Vorinostat metabolism involve glucuronidation and hydrolysis followed by β-oxidation. *In vitro* studies using human liver microsomes indicate negligible biotransformation by cytochromes P450 (CYP).

10.2.5 Elimination

Vorinostat is eliminated predominantly through metabolism with less than 1% of the dose recovered as unchanged drug in urine, indicating that renal excretion does not play a role in the elimination of Vorinostat.

10.2.6 Storage and Stability

Store it at room temperature and away from excess heat and moisture.

10.2.7 Dose Specifics

For the purposes of this study, the Vorinostat dose will be 200 mg twice a day days 6-15 of each treatment course.

10.2.8 Administration

Swallow the capsules whole; do not open, chew, or crush them. Take with food same times each day.

10.2.9 Availability

Commercially available; however study funds may cover the cost of the drug if not paid for by the patient's 3rd party payer.

10.2.10 Drug Interactions

Coumarin-derivative anticoagulants: Prolongation of prothrombin time and International Normalized Ratio have been observed with concomitant use.

10.2.11 Side Effects

Pregnancy category D

Vorinostat can cause fetal harm when administered to a pregnant woman. There are no adequate and well controlled studies of Vorinostat in pregnant women. Results of animal studies indicate that Vorinostat crosses the placenta and is found in fetal plasma at levels up to 50% of maternal concentrations. Doses up to 50 and 100 mg/kg/day were tested in rats and rabbits, respectively (~0.5 times the human exposure based on $AUC_{0\text{-}24\ hours}$). Treatment related developmental defects included decreased mean live fetal weights, incomplete ossification of the skull, thoracic vertebra, sternum, and skeletal variations (cervical ribs, supernumerary ribs, vertebral count and sacral arch variations) in rats at the highest dose of Vorinostat tested. Reductions in mean live fetal weight and an elevated incidence of incomplete ossification of the metacarpals were seen in rabbits dosed at 150 mg/kg/day. The no observed effect levels for these findings were 15 and 50 mg/kg/day (<0.1 times the human exposure based on AUC) in rats and rabbits, respectively.

Common (Occurring in ≥ 20%) : weight decrease, anorexia, diarrhea, nausea, altered taste sensation, fatigue, thrombocytopenia, muscle spasms

Uncommon (in 5-19%): peripheral edema, alopecia, pruritus, dizziness or headache, shivering, raised serum creatinine, cough, upper respiratory infection, anemia

Rare: prolonged QT (3.5 – 6%), DVT/PE

10.3 Interleukin-2 (IL-2)

10.3.1 Other Names

Aldesleukin (modified human recombinant interleukin-2)

10.3.2 Classification

Antineoplastic agent, Immunotherapy, Cytokine

10.3.3 Category

Biologic Response Modifier Agent

10.3.4 Mechanism of Action

- Systemic: Aldesleukin has been shown to possess the biological activity of human native interleukin-2. In vitro studies performed on human cell lines demonstrate the immunoregulatory properties of aldesleukin, including:

  - Enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines;
  - Enhancement of lymphocyte toxicity;
  - Induction of killer cell (lymphokine-activated killer [LAK] cells and natural killer [NK] cells) activity; and
  - Induction of interferon-gamma production.
- The in vivo administration of aldesleukin in select murine tumor models and in the clinic produces multiple immunological effects in a dose-dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines, including tumor necrosis factor, interleukin-1 and gamma interferon. In vivo experiments in murine tumor models have shown inhibition of tumor growth. However, the exact mechanism by which aldesleukin mediates its antitumor activity in animals and humans is unknown.
- Aldesleukin causes a capillary leak syndrome (CLS) as a result of increased capillary permeability, leading to extravasation of plasma proteins and fluid into the extravascular space and contributing to loss of vascular resistance. Interleukin-2 has been reported to reversibly decrease serum cholesterol concentrations. Interleukin-2 has been reported to transiently decrease serum testosterone and dihydroepiandrosterone concentrations and to transiently increase plasma estradiol concentrations. It has also been reported to transiently increase adrenal secretion of ACTH and cortisol.

10.3.5 Metabolism

Systemic: 85 min.

10.3.6 Dose Specifics

For the purposes of this study, subcutaneous IL-2 6 million units per dose will be given on days 17, 19 and 22 of each treatment course. Dose for patients weighing less than 45 kg: 3 million units/m^2 per dose using the same schedule and route

10.3.7 Dose Forms and Strengths

Lyophilized vials containing 22 million units of IL-2

10.3.8 Reconstitution and Syringe Preparation

U of MN patients only (IL-2 for product activation plus 3 injections at 6 million units/dose):

Reconstitute IL-2 vial with 1.2ml sterile water for injection
Add 1.2ml 0.1% albumin in 5% dextrose to IL-2 vial
Yield: 9 million units/ml solution.

Repeat with 2nd vial

Using a 1ml syringe and 18 gauge needle, withdraw 0.56 ml IL-2 from one vial.
Add to an empty, sterile, 10ml vial
Add 3.44ml 0.1% albumin in 5% dextrose.
Yield 5 million units/4ml vial for pheresis product.

Withdraw the remaining contents from the two IL-2 vials into a 10ml syringe, using an 18 gauge needle. Pull the contents from the needle into the syringe.

Attach a syringe tip connector to the 10ml syringe.
Withdraw 0.72 ml dose into a 1ml syringe. Attach luer tip to syringe. Syringe contains a 0.05ml overfill to fill the needle when attached.

Repeat for a total of 3 doses.

Mayo Clinic patients only (IL-2 for pheresis product):
To be prepared by University of Minnesota Medical Center, Fairview IDS

IL-2 will be reconstituted and prepared by Using a 1ml syringe and 18 gauge needle, withdraw 0.56 ml IL-2 from one vial.
Add to an empty, sterile, 10ml vial
Add 3.44ml 0.1% albumin in 5% dextrose.
Yield 5 million units/4ml vial for pheresis product.

Mayo Clinic patients only (IL-2 preparation for patient use):
To be prepared at the Mayo Clinic

Reconstitute IL-2 vial with 1.2ml sterile water for injection
Add 1.2ml 0.1% albumin in 5% dextrose to IL-2 vial
Yield: 9 million units/ml solution

Withdraw the contents from the IL-2 vial into a 10ml syringe, using an 18 gauge needle. Pull the contents from the needle into the syringe.

Attach a syringe tip connector to the 10ml syringe.
Withdraw 0.72 ml dose into a 1ml syringe. Attach luer tip to syringe. Syringe contains a 0.05ml overfill to fill the needle when attached.

Repeat for a total of 3 doses. Label the last filled syringe with the actual dose and as dose #3, in the event this dose is short.

10.3.9 Administration

IL-2 will be administered as an outpatient for three doses no sooner than 2 hours after the NK cell infusion on day 17, and again on days 19 and 22. Pre-medication with acetaminophen 650 mg PO and diphenhydramine 25 mg PO/IV before and 4 hours after each dose of IL-2 is recommended.

10.3.10 Availability

IL-2 will be provided for the purposes of this study.

10.3.11 Warnings and Precautions

- patients with a history of cardiac disease exhibiting a normal thallium stress test
- patients with a history of pulmonary disease exhibiting normal pulmonary function tests
- severe hypotension (due to capillary leak syndrome)
- impaired neutrophil function (reduced chemotaxis)
- increased risk of disseminated infection
- kidney and liver function impairment (avoid concurrent administration with nephrotoxic or hepatotoxic drugs)
- mental status changes
- exacerbation of preexisting autoimmune diseases or initial presentation of autoimmune and inflammatory disorders
- may increase the risk of allograft rejection in transplant patients (due to enhancement of cellular immune function)
- cardiac, pulmonary, renal, hepatic, or central nervous system impairment
- patients with a history of seizures
- Pregnancy Category C

11.0 Adverse Event Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Note: throughout this section the generic term “study drug” refers to the study related treatment (Vorinostat/Decitabine, NK cell infusions and IL-2 injections).

11.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

If either the IND sponsor or the investigator believes the event is life-threatening or serious, the event must be evaluated by the sponsor for expedited reporting (21CRF 312.32(a)).

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, adverse events that occur as part of the disease process (including relapse/progress and death from

such) or underlying medical conditions are considered *unexpected;* however, they will not be reportable per section 11.4.

Unanticipated (unexpected) problems/events as defined by the University Of Minnesota IRB are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 11.4.

11.2 Adverse Event Documentation

Adverse events occurring after the initiation of any study treatment must be documented. Adverse events attributed to a study-related procedure which occur prior to the initiation of study treatment must be documented as well. Refer to appendix VII for a list of expected toxicities.

For the purposes of this study, adverse event documentation requirements will be determined based on grade, expectedness and relationship to study therapy as follows:

<table>
<tr><th></th><th>Grade 1</th><th colspan="2">Grade 2</th><th colspan="2">Grade 3</th><th>Grade 4 and 5</th></tr>
<tr><th></th><th>Expected or Unexpected</th><th>Expected</th><th>Unexpected</th><th>Expected</th><th>Unexpected</th><th>Expected or Unexpected</th></tr>
<tr><td>Unrelated Unlikely</td><td>Not required</td><td>Not required</td><td>Not required</td><td>Not required</td><td>Required</td><td>Required</td></tr>
<tr><td rowspan="2">Possible Probable Definite</td><td rowspan="2">Not required</td><td rowspan="2">Not required</td><td rowspan="2">Required</td><td>Required non-hematological</td><td rowspan="2">Required</td><td rowspan="2">Required</td></tr>
<tr><td>Not required - hematologic events</td></tr>
</table>

All patients will be monitored from the initiation of any study treatment through day 45 after the last dose of study treatment or the start of a new treatment, whichever occurs earlier, as it is expected that most treatment related adverse events will occur during this period. However, the investigator is obligated, upon knowledge of, to report any adverse event considered to be related to study treatment meeting the expedited reporting criteria in section 11.4.

In addition, adverse event collection will include targeted adverse events and unexpected adverse events at specific time points in relation to the NK cell infusion and post NK cell infusion IL-2 injections.

Targeted adverse events (Appendix IX) and unexpected events will be collected at the following time points. Adjust the day to match the time point description. All time points are 24 hours +/- 2 hours.

- Post-Chemo/Pre-NK cell infusion (day 17)
- A single assessment 1-2 hours post NK cell infusion/pre IL-2 injection (day 17)
- pre IL-2 dose 2
- pre IL-2 dose 3
- 48 hours post IL-2 dose 3 (or to coincide with day 24 clinic visit)
- 1 week after last IL-2 dose (or to coincide with day 31 clinic visit)

At each time point, the worst grade of the targeted toxicity since the last assessment or the previous 24 hours (whichever is shorter) will be recorded in addition to any unexpected toxicities felt at least possibly related to the NK cells and/or the IL-2.

In addition, although not always a reportable event, deaths will be recorded in OnCore upon knowledge in the follow-up tab.

11.3 Required Reporting: Mayo Clinic to the Masonic Cancer Center

Serious events: Beginning with the first dose of decitabine and continue through day 45 of the last treatment course, as an affiliate, Mayo will report all events meeting the definition of serious, regardless of attribution or expectedness, within 24 hours of knowledge of the event. After day 45 of the last treatment course, the investigator must report upon knowledge any event that is serious, unexpected and felt to be at least possibly related to study therapy.

Reports are to be submitted to the Study Coordinator at the University of Minnesota Masonic Cancer Center (MCC) using the SAE reporting form found in OnCore. The MCC Study Coordinator will facilitate reporting to the University Of Minnesota IRB and the FDA as required.

Stopping rule events: Any grade 4 or 5 non-hematologic, non-infectious toxicity occurring through day 45 of the last treatment course will count toward the early study stopping rule per section 13 and must be reported to the MCC Study Coordinator using the Early Stopping Rule Report Form found OnCore under the reports tab.

No more than one excess toxicity event will be accumulated per patient within in the same episode of toxicity (i.e. a grade 4 event worsening to a grade 5 is a single event) or when multiple events occur concurrently.

Events that count toward an early stopping rule do not necessarily constitute a serious adverse event requiring expedited reporting and

should be reported as such only if they meet the criteria for expedited reporting as defined in section 11.1.

Lot release issues: Any safety issues associated with the final lot release at the treating institution must be reported immediately to either Dr. Miller or Dr. Warlick at the University of Minnesota (contact information on page 2) with follow-up reporting to MCC Study Coordinator using the Deviation Report found in OnCore.

Affiliate institutions will be responsible for submitting reportable events to their institutional IRB and any other required local regulatory entities.

11.4 Masonic Cancer Center Required Reporting: FDA, IRB, Miltenyi, and MCC's SAE Coordinator

The reporting period for this study is from initiation of any study treatment through day 45 of the last treatment course; however after day 45, the investigator must report upon knowledge any study treatment related event meeting the expedited reporting criteria below.

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy to:
U of MN IRB	Unanticipated death of a locally enrolled subject(s); New or increased risk; Any adverse event that require a change to the protocol or consent form – refer to the IRB website for complete details	5 Business Days	IRB's Report Form	irb@umn.edu With copy to the Mayo Clinic PI or designee	MCC SAE Coordinator MMC 6 mcc-saes@umn.edu
FDA	Unexpected and fatal or life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Day	UMCC SAE form	Submit as an amendment to IND With copy to the Mayo Clinic PI or designee	
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing) 4) positive HAMA in association with a grade 4 infusion reaction	As soon as possible but no later than 15 Calendar-Day			
	Note: Events due to the disease under treatment or an underlying medical condition will not require reporting to the FDA for the purposes of this study				
Miltenyi	Any safety issues associated with cell processing failure or device malfunction	Refer to section 11.5			Not applicable
Masonic Cancer Center SAE Coordinator	Any event that counts toward a study stopping rule (see section 13)	Upon reporting	Study stopping rule form	SAE Coordinator mcc-saes@umn.edu	Not applicable

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports.

The SAE Coordinator will provide the Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

11.5 Reporting of Device/Processing Malfunction

Any clinically significant safety issues associated with cell processing failure or device malfunction regarding the CliniMACS® system must be reported to immediately to either Dr. Miller or Dr. Warlick at the University of Minnesota (contact information on page 2) with follow-up reporting (copy U of MN study coordinator on any correspondence) to:
Miltenyi Biotec, Inc.
Attn: Regulatory Affairs
120 Presidential Parkway
Suite 305
Woburn, MA 01801
There is no specific reporting form, and at a minimum a deviation report must be completed in OnCore.

12.0 Study Data Collection and Monitoring

12.1 Data Management

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database utilizing study specific electronic case report forms. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore forms. Patient demographics, patient specific study treatment calendars, targeted adverse events, reporting of deaths, and other information required for IND annual reporting will be placed in OnCore and other research databases maintained by MCC IT.

12.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRFs) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRFs based on the patient

specific calendar, and updating the patient record until patient death or end of required study participation.

12.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://www.cancer.umn.edu/exfiles/research/dandsmplan.pdf.

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- The PI (Dr. Warlick) will complete and submit a quarterly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable events per the definition of reportable in section 11.4 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND sponsor (Dr. Miller) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

12.4 Monitoring

The PI with the CTO has oversight responsibility for trial monitoring at affiliate sites. Affiliate sites must self-monitor following the University of Minnesota Masonic Cancer Center Data and Safety Monitoring Plan (DSMP - http://www.cancer.umn.edu/exfiles/research/dandsmplan.pdf) and the CTO Affiliate and Satellite Site Monitoring SOPs.

The investigator will permit study-related monitoring, audits, and inspections by the study's Principal Investigator and/or IND sponsor and/or any designees, the local IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related

facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

12.5 Record Retention

The investigator will retain study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient. Please contact the CTO before destroying any study related records.

13.0 Statistical Considerations

13.1 Simon's Two-Stage Design and Sample Size

This is a single clinic Simon's optimum two-stage phase II clinical trial designed to determine the objective response rate (CR+PR+HI) after two courses of Decitabine and Vorinostat with haploidentical NK cell s in patients with high risk MDS. The null hypothesis of the study is $P_{R0} \leq 0.30$ and alternative hypothesis is $P_{R1} \geq 0.50$. Based on this design and the assumption that the majority of patients will complete both cycles of therapy, a maximum of 46 evaluable patients will be used which is sufficient to maintain an overall type I error 5% while providing 80% statistical power.

In the first stage 15 patients will be enrolled, the trial will be terminated if 5 or fewer respond. If 6 patients respond, then the trial goes on to the second stage, and 31 more patients will be studied. If the total number responding is less than or equal to 18, then this approach will be deemed unworthy of further study.

Considering a predicted dropout rate of 10% during the two courses of therapy, in order to have 15 or 46 patients evaluable for the response rate, we will need a maximum of 17 patients (if the study is stopped by 1st stage) or 51 patients (if the study proceeds to 2nd stage).

Only patients completing both course 1 and 2 of planned therapy will be evaluable for response. However, all the patients with complete or partial treatment will be included in the evaluation for toxicities.

13.2 Early Stopping Rule for Toxicities

The complications and adverse events will be closely monitored in this study. The hypothesized rate of having grade 4 or 5 non-hematologic, non-infectious toxicities for the approach is 5%. Enrollment will be stopped and the trial re-evaluated if the grade 4 or 5 toxicity rate shows signs to exceed 15%. This means enrollment will be stopped and the study re-evaluated if there are 3 patients with a non-hematologic, non-infectious grade 4-5 toxic event occurring through day 45 of the last treatment course out of the first 7 patients, 4 out of 18, 5 out of 29, 6 out of 40, and 7 patients at any time. Toxicities such as infections, including but not limited to pneumonia, bacteremia, cellulitis, and neutropenic fever, etc are known results of high risk MDS and will not be included as toxicities attributed to the study therapy. Assessment for toxicities will begin upon initiation of trial therapy and will conclude 2 weeks after last dose of study related treatment. These stopping rules of a statistical sequential probability ratio test designed for no more than 5% the probability of type I error and at least 80% statistical power.

13.3 Statistical Analysis Methods

Ninety-five percent confidence intervals for the response probability were calculated using methodology reported by Duffy and Santner.[40]
The summary statistics including 95% confidence intervals for toxicity rate and proportion of patients who become transfusion independent will be calculated. The association of in vivo NK cell expansion, as defined as persistence of donor NK cell chimerism at day +8, (yes or no) with clinical response will be estimated by chi-square test. Kaplan- Meier method will be used to estimate 1- year survival from the date of treatment. Given that the majority of patients will go on to receive additional therapy with either ongoing hypomethylating agents versus stem cell transplant, the estimates of 1 year overall survival from the point of study enrollment, will only be partially attributable to the study therapy and thus a minor focus of our statistical analysis.

SAS 9.2 (Cary, NC, USA) will be used for statistical analyses.

14.0 Conduct Of The Study

14.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the

duration of the study and retained according to the appropriate regulations.

14.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

14.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

15.0 References

1. Warlick ED and Smith BD. Myelodysplastic syndromes: review of pathophysiology and current novel treatment approaches. Curr Cancer Drug Targets. 2007;7:541-558.
2. Herman JG, Civin CI, Issa JP, Collector MI, Sharkis SJ, Baylin SB. Distinct patterns of inactivation of p15INK4B and p16INK4A characterize the major types of hematological malignancies. Cancer Res. 1997;57:837-841.
3. Leone G, Teofili L, Voso MT, Lubbert M. DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias. Haematologica. 2002;87:1324-1341.
4. Quesnel B, Guillerm G, Vereecque R, et al. Methylation of the p15(INK4b) gene in myelodysplastic syndromes is frequent and acquired during disease progression. Blood. 1998;91:2985-2990.
5. Aggerholm A, Holm MS, Guldberg P, Olesen LH, Hokland P. Promoter hypermethylation of p15INK4B, HIC1, CDH1, and ER is frequent in myelodysplastic syndrome and predicts poor prognosis in early-stage patients. Eur J Haematol. 2006;76:23-32.
6. Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor prognosis in therapy-related myelodysplasia and acute myeloid leukemia. Leukemia. 2003;17:1813-1819.
7. Molldrem JJ, Caples M, Mavroudis D, Plante M, Young NS, Barrett AJ. Antithymocyte globulin for patients with myelodysplastic syndrome. Br J Haematol. 1997;99:699-705.
8. Jonasova A, Neuwirtova R, Cermak J, et al. Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow. Br J Haematol. 1998;100:304-309.

9. Sloand EM, Wu CO, Greenberg P, Young N, Barrett J. Factors affecting response and survival in patients with myelodysplasia treated with immunosuppressive therapy. J Clin Oncol. 2008;26:2505-2511.
10. Passweg JR, Giagounidis AA, Simcock M, et al. Immunosuppressive therapy for patients with myelodysplastic syndrome: a prospective randomized multicenter phase III trial comparing antithymocyte globulin plus cyclosporine with best supportive care--SAKK 33/99. J Clin Oncol. 2011;29:303-309.
11. Olnes MJ and Sloand EM. Targeting immune dysregulation in myelodysplastic syndromes. JAMA. 2011;305:814-819.
12. Kiladjian JJ, Bourgeois E, Lobe I, et al. Cytolytic function and survival of natural killer cells are severely altered in myelodysplastic syndromes. Leukemia. 2006;20:463-470.
13. Epling-Burnette PK, Bai F, Painter JS, et al. Reduced natural killer (NK) function associated with high-risk myelodysplastic syndrome (MDS) and reduced expression of activating NK receptors. Blood. 2007;109:4816-4824.
14. Carlsten M, Baumann BC, Simonsson M, et al. Reduced DNAM-1 expression on bone marrow NK cells associated with impaired killing of CD34(+) blasts in myelodysplastic syndrome. Leukemia. 2010;24:1607-1616.
15. Kordasti SY, Ingram W, Hayden J, et al. CD4+CD25high Foxp3+ regulatory T cells in myelodysplastic syndrome (MDS). Blood. 2007;110:847-850.
16. Hamdi W, Ogawara H, Handa H, Tsukamoto N, Nojima Y, Murakami H. Clinical significance of regulatory T cells in patients with myelodysplastic syndrome. Eur J Haematol. 2009;82:201-207.
17. Kotsianidis I, Bouchliou I, Nakou E, et al. Kinetics, function and bone marrow trafficking of CD4+CD25+FOXP3+ regulatory T cells in myelodysplastic syndromes (MDS). Leukemia. 2009;23:510-518.
18. Alfinito F, Sica M, Luciano L, et al. Immune dysregulation and dyserythropoiesis in the myelodysplastic syndromes. Br J Haematol. 2010;148:90-98.
19. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002;20:2429-2440.
20. Silverman LR, McKenzie DR, Peterson BL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. J Clin Oncol. 2006;24:3895-3903.
21. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10:223-232.
22. Gurion R, Vidal L, Gafter-Gvili A, et al. 5-Azacitidine Prolongs overall Survival in Patients with Myelodysplastic Syndrome - Systematic Review and Meta-Analysis. Haematologica. 2009.
23. Issa JP and Kantarjian HM. Targeting DNA methylation. Clin Cancer Res. 2009;15:3938-3946.
24. Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006;106:1794-1803.
25. Kantarjian H, Oki Y, Garcia-Manero G, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood. 2007;109:52-57.
26. Garcia-Manero G, Yang H, Bueso-Ramos C, et al. Phase I study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced leukemias and myelodysplastic syndromes. Blood. 2007.

27. Kuendgen A, Schmid M, Schlenk R, et al. The histone deacetylase (HDAC) inhibitor valproic acid as monotherapy or in combination with all-trans retinoic acid in patients with acute myeloid leukemia. Cancer. 2006;106:112-119.
28. Gore SD, Weng LJ, Figg WD, et al. Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. Clin Cancer Res. 2002;8:963-970.
29. Gojo I, Jiemjit A, Trepel JB, et al. Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukemias. Blood. 2007;109:2781-2790.
30. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1:194-202.
31. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Natl Cancer Inst. 2000;92:1210-1216.
32. Prince HM, Bishton MJ, Harrison SJ. Clinical studies of histone deacetylase inhibitors. Clin Cancer Res. 2009;15:3958-3969.
33. Quintas-Cardama A, Santos FP, Garcia-Manero G. Histone deacetylase inhibitors for the treatment of myelodysplastic syndrome and acute myeloid leukemia. Leukemia. 2011;25:226-235.
34. Reddy P, Maeda Y, Hotary K, et al. Histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces acute graft-versus-host disease and preserves graft-versus-leukemia effect. Proc Natl Acad Sci U S A. 2004;101:3921-3926.
35. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105:3051-3057.
36. Stathis A, Hotte SJ, Chen EX, et al. Phase I study of decitabine in combination with vorinostat in patients with advanced solid tumors and non-Hodgkin's lymphomas. Clin Cancer Res. 2011;17:1582-1590.
37. Ravandi F, Faderl S, Thomas D, et al. Phase I Study of Suberoylanilide Hydroxamic Acid (SAHA) and Decitabine in Patients with Relapsed, Refractory or Poor Prognosis Leukemia [abstract]. Blood. 2007.
38. Kirschbaum M, Gojo I, Goldberg S, et al. Phase I Study of Vorinostat in Combination with Decitabine in Patients with Relapsed or Newly Diagnosed Acute Myelogenous Leukemia or Myelodysplastic Syndrome [abstract]. Blood. 2008.
39. Kadidlo D, Bostrom N, Adams S, Fautsch S, McCullough J, Wagner J, Miller J, McKenna D. Validation of Natural Killer Cell Activation During Long-Distance Shipping. Transfusion (2006); 46(9S): 59A-60A.
40. Duffy DE, Santner TJ. Confidence intervals for a binomial para parameterbased on multistage tests. Biometrics 1987; 43: 81–93.
41. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079-2088.
42. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25:3503-3510.

Appendix I – Eligibility Checklist – Patient
Decitabine and Vorinostat with CD3/CD19 Depleted Haploidentical Donor Natural Killer (NK) Cells for the Treatment of High Risk Myelodysplastic Syndromes (MDS) - MT2012-04

Eligibility Checklist – page 1 of 2

Patient initials ☐☐☐☐ (1st 2 initials of first name + 1st 2 initials of last name) Patient ID ☐☐☐-☐☐ (3 letter site code – Seq # (i.e. 01, 02, 03, etc.)

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the patient from study entry.

		Yes	No
1.	Diagnosis of high risk MDS that meets **at least one** of the following disease classifications and is requiring treatment: ☐ International Prognostic Scoring System (IPSS) Category: INT-2 or High Risk (Appendix IV) ☐ International Prognostic Scoring System- Revised (IPSS-R) Category: High or Very High (Score of 5 or above) (Appendix IV) ☐ WHO Classification: RAEB-1 or RAEB-2 ☐ WHO Classification: CMML that is not highly proliferative. WBC < 15K ☐ High risk cytogenetic abnormality as defined by presence of Monosomy 7, complex karytope, or monosomal karyotype ☐ Therapy related MDS ☐ Severe cytopenias: Severe neutropenia (ANC < 0.8), platelet or PRBC transfusion dependent ☐ WHO Based Prognostic Scoring System (WPSS): High or Very High Risk (Appendix V)	☐	☐
2.	☐ Untreated/Minimally Treated: maximum of 2 cycles of hypomethylating agents (azacitidine or decitabine) without evidence of treatment failure as defined by progression to more advanced MDS Who classification or AML. ☐ Treatment Failure: Patients who have not responded to or whose disease has progressed on hypomethylating agents (azacitidine or decitabine), 7+3 type induction chemotherapy, or immunosuppressive therapy such as ATG+CSA. Patients must not have received treatment for their MDS within 4 weeks of beginning the trial.	☐	☐
3.	Age ≥ 18 years of age	☐	☐
4.	ECOG Performance Status 0-2 PS - ☐		
5.	Available related HLA-haploidentical NK cell donor by at least Class I serologic typing at the A&B locus	☐	☐
6.	Acceptable organ function within 14 days of study enrollment defined as: Creatinine ≤ 2.0 mg/dL ☐.☐ ☐☐/☐☐/☐☐ SGOT < 5 x institutional ULN ☐☐☐☐ ☐☐/☐☐/☐☐ SGPT < 5 x institutional ULN ☐☐☐☐ ☐☐/☐☐/☐☐ oxygen saturation ≥ 90% on room air ☐☐☐% ☐☐/☐☐/☐☐ Ejection Fraction ≥ 40% and no uncontrolled angina, ☐☐% ☐☐/☐☐/☐☐ severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. QTc < 500 msec	☐	☐
7.	Able to be off prednisone or other immunosuppressive medications for at least 3 days prior to NK cell (excluding pre-meds)	☐	☐
8.	Women of child bearing potential must agree to use effective methods of contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) from the time of signing the consent form and for 2 months after the last dose of chemotherapy	☐	☐
9.	Voluntary written consent	☐	☐

Decitabine and Vorinostat with CD3/CD19 Depleted Haploidentical Donor Natural Killer (NK) Cells for the Treatment of High Risk Myelodysplastic Syndromes (MDS) - MT2012-04

Eligibility Checklist – page 2 of 2

Patient initials ☐☐☐☐

EXCLUSION CRITERIA

A "YES" response to any of the following disqualifies the patient from study entry.

		Yes	No
10.	Pregnant or lactating	☐	☐
11.	New progressive pulmonary infiltrates on screening chest x-ray or chest CT scan that has not been evaluated with bronchoscopy (when feasible)	☐	☐
12.	Uncontrolled bacterial or viral infections - chronic asymptomatic viral hepatitis is allowed	☐	☐
13.	Pleural effusion moderate to large in size that are detectable on chest x-ray	☐	☐
14.	Known hypersensitivity to one or more of the study agents	☐	☐
15.	Prior hypomethylating treatment greater than 2 cycles or with documented treatment failure	☐	☐
16.	Prior use of histone deacetylase inhibitors	☐	☐
17.	Serious medical or psychiatric illness likely to interfere with participation in this clinical study in the opinion of the enrolling investigator	☐	☐
18.	Inability to swallow capsules	☐	☐
19.	Active HIV	☐	☐
20.	Other active and potentially life threatening malignancy excluding localized basal or squamous cell skin cancer, cervical carcinoma in situ, superficial bladder cancer, localized prostate cancer	☐	☐

Date consent form signed: _____

Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this patient is:

☐ Eligible ☐ Ineligible Date registered _____

_____ _____

Signature of person verifying eligibility Date

Appendix II - Eligibility Checklist - Donor

Decitabine and Vorinostat with CD3/CD19 Depleted Haploidentical Donor Natural Killer (NK) Cells for the Treatment of High Risk Myelodysplastic Syndromes (MDS) - MT2012-04

Eligibility Checklist – page 1 of 1

Donor initials ☐☐☐☐ (1st 2 initials of first name + 1st 2 initials of last name)

Donor ID ☐☐☐-☐☐-D (3 letter site code – Pt seq # (i.e. 01, 02, 03, etc.))

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the donor from study entry.

		Yes	No
1.	Related donor (sibling, parent, offspring, parent or offspring of an HLA identical sibling) 18 to 75 years of age	☐	☐
2.	At least 40 kilogram body weight	☐	☐
3.	In general good health as determined by the evaluating medical provider	☐	☐
4.	HLA-haploidentical donor/recipient match by at least Class I serologic typing at the A&B locus	☐	☐
5.	Able and willing to undergo apheresis	☐	☐
6.	Not pregnant	☐	☐
7.	Voluntary written consent	☐	☐

Date consent form signed: _____

_____ _____

Signature of person verifying eligibility Date

Appendix III – ECOG Performance Status Criteria

ECOG Score	Performance Status	Karnofsky Equivalent
0	Asymptomatic	100
1	Symptomatic, fully ambulatory	80-90
2	Symptomatic, in bed < 50% of the day	60-70
3	Symptomatic, in bed > 50% of the day but not bedridden	40-50
4	Bedridden	20-30
5	Dead	

Appendix IV – International Prognostic Scoring System (IPSS) Category and IPSS-R[41]

INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS) FOR MDS

	Survival and AML Evolution Score Value				
Prognostic Variable	0	0.5	1.0	1.5	2.0
Marrow Blasts (%)[1]	< 5	5 –10	—	11–20	21–30
Karyotype[2]	Good	Intermediate	Poor		
Cytopenias[3]	0/1	2/3			

Risk Category	Combined Score
LOW	0
INT-1	0.5–1.0
INT-2	1.5–2.0
HIGH	≥ 2.5

IPSS-R Cytogenetic risk groups*,**

Cytogenetic prognostic subgroups	Cytogenetic abnormalities
Very good	-Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

IPSS-R Prognostic Score Values*

Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good		Good		Intermediate	Poor	Very Poor
BM Blast %	≤2		>2-<5%		5-10%	>10%	
Hemoglobin	≥10		8-<10	<8			
Platelets	≥100	50-<100	<50				
ANC	≥0.8	<0.8					

IPSS-R Prognostic Risk Categories/Scores*

RISK CATEGORY	RISK SCORE
Very Low	≤1.5
Low	>1.5 - 3
Intermediate	>3 - 4.5
High	>4.5 - 6
Very High	>6

*Greenberg, Tuechler, Schanz et al, Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndrome, Blood 120: 2454, 2012.
**Schanz J et al, J Clin Oncology 2012; 30:820

Source: http://ipss-r.com/ (accessed September 5, 2014)

Appendix V – WHO Based Prognostic Scoring System[42]

Table 2. WHO Classification–Based Prognostic Scoring System for MDS

Variable	0	1	2	3
WHO category	RA, RARS, 5q–	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype*	Good	Intermediate	Poor	—
Transfusion requirement†	No	Regular	—	—

NOTE. Risk groups were as follows: very low (score = 0), low (score = 1), intermediate (score = 2), high (score = 3 to 4), and very high (score = 5 to 6).
Abbreviations: MDS, myelodysplastic syndrome; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; 5q–, myelodysplastic syndrome with isolated del(5q) and marrow blasts less than 5%; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts; RAEB-1, refractory anemia with excess of blasts-1; RAEB-2, refractory anemia with excess of blasts-2.
*Karyotype was as follows: good: normal, –Y, del(5q), del(20q); poor: complex (≥ three abnormalities), chromosome 7 anomalies; and intermediate: other abnormalities.
†RBC transfusion dependency was defined as having at least one RBC transfusion every 8 weeks over a period of 4 months.

Appendix VI – MDS Response Criteria

Based on International Working Group 2006 Modified Criteria

- Complete Remission (CR): ≤ 5% myeloblasts with normal maturation of all cell lines
  - Persistent dysplasia will be noted
  - Peripheral Blood:
    - Hgb ≥ 11g/dl
    - Platelets ≥ 100 X 10^9 /L
    - Neutrophils ≥ 1000
    - Blasts = 0%
- Partial Remission (PR): all CR criteria if abnormal before except:
  - BM blasts decreased by ≥ 50% over pre-treatment but still >5%
- Marrow CR: Bone marrow with ≤ 5% myeloblasts and decrease by ≥50% over pretreatment with incomplete peripheral blood count normalization
- Hematologic Improvement (HI):
  - Hgb: Increase by ≥ 1.5g/dl or decreased PRBC transfusions by at least 4/8 week period (only PRBC given for Hgb<9.0g/dl)
  - Platelet Response: Absolute increase of ≥ 30 X 10^9/L for those starting at >20 X 10^9/L For those < 20 X 10^9/L at baseline increase by 100%.
  - Neutrophil Response: at least 100% increase and an absolute increase of >0.5 X 10^9/L
- Stable Disease: Failure to achieve at least a PR but no evidence of disease progression for >8 weeks
- Failure: Death during treatment or disease progression characterized by worsening cytopenias, increase percentage of marrow blasts, or progression to a more advanced MDS FAB subtype
- Cytogenetic Response:
  - Complete: Disappearance of any pre-treatment chromosomal abnormalities without the appearance of new ones
  - Partial: At least 50% reduction of chromosomal abnormality
- Disease Progression: Compared to pre-treatment values
  - Less than 5% blasts: greater than 50% increase to >5% blasts
  - 5-10% blasts: greater than 50% increase to >10% blasts
  - 10-20% blasts: greater than 50% increase to >20% blasts

Appendix VII – Expected Toxicities of Study Treatment

Decitabine		
Common	**Less Common**	**Rare**
• low platelet count with increased risk of bruising/bleeding* • low white blood cell count with increased risk of infection* • low red blood cell count (anemia) with symptoms like tiredness, low energy, or shortness of breath* • nausea • vomiting • diarrhea • constipation • tiredness • fever • pain or swelling in the arms or legs	• blurred vision • pain • high blood sugar* • headache • bruises or bleeding • swollen lymph nodes ("glands") • sores in mouth, on tongue, or on lips* • infection • indigestion or sour stomach • abnormal blood tests which suggest that the drug is affecting the liver	• confusion • trouble sleeping • fluid in the lungs* • severe allergic reaction, with symptoms like flushing, hives, trouble breathing or swallowing, dizziness, swelling of mouth or throat (while drug is being infused) • serious infections • death from infection, bleeding, or other cause

Vorinostat		
Common	**Less Common**	**Rare**
• feeling tired • nausea/vomiting* • diarrhea • loss of appetite • changes in how foods taste • weight loss • muscle aches • low blood platelet count with increased risk of bleeding • high blood sugar levels • abnormal blood or urine tests which suggest that the drug is affecting the kidneys	• muscle spasms • hair loss (including face and body hair) • dry mouth • constipation • fever, chills • feeling dizzy • swelling in the hands, feet, legs, or ankles • headache • itching • low red blood cell count (anemia) • cough • upper respiratory infection	• blood clots in the lungs or legs • changes in electric impulses of the heart, abnormal heart rhythm • slow or fast heart rate • death due to infection or other cause

Interleukin-2 (IL-2)		
Common	**Less Common**	**Rare**
• flu-like syndrome (may include fever, chills, tiredness, headache, muscle and joint pain) • low blood pressure • nausea/vomiting • diarrhea • feeling weak • confusion • shortness of breath • flushing of the face • rash or dry, itchy skin • low urine output • abnormal blood tests which suggest that the drug is affecting the liver or kidneys	• feeling dizzy • feeling drowsy • sores in the mouth or on the lips • loss of appetite • abdominal pain • increased heart rate or change in heart rhythm • swelling in the face, hands, or feet • trouble sleeping • trouble concentrating • runny nose • low platelet count with increased risk of bleeding* • low red blood cell count (anemia) with symptoms like tiredness, weakness, shortness of breath • low white blood cell count with increased risk of infection	• allergic reaction* • cough • confusion • psychosis (loss of touch with reality) • depression • seizures • coma • damage to bowel, ruptured or bleeding bowel • serious infection • kidney failure • heart attack • stroke • breathing problems • death from low blood pressure, capillary leak syndrome, heart problems, or other complications

Refer to section 7.8 for NK cell expected toxicity

Appendix VIII – Vorinostat Drug Log

Patient Number: _____ Course Number: ☐

Record the date and time each dose of vorinostat is taken. If not taken, record dose as 0 for that day and indicate reason not taken.

Contact the study doctor or research nurse with any questions or concerns.

Day #	Date	Dose 1 Time taken	Dose 2 Time taken	**Notes** (side effects, reason not taken, etc)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Return this form with any leftover tablets and drug containers at the time of your next treatment appointment.

Appendix IX – Targeted Toxicity Form NK Cells/IL-2

Patient Initials: _____ **Date of Assessment:** _____ **Assessment Time point:** _____

ADL = activities of daily living

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction	None	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment; prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion);	Life-threatening consequences; urgent intervention indicated (collect sample for HAMA)
Dyspnea	None or no change	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	……	Decreased O_2 saturation with exercise (e.g., pulse oximeter < 88%) intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or PaO_2 ≤ 55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)
Fever	None	38.0 - 39.0 degrees C (100.4 -102.2 F)	> 39.0 - 40.0 degrees C (102.3 - 104.0 degrees F)	> 40.0 degrees C (>104.0 degrees F) for ≤ 24 hrs	> 40.0 degrees C (>104.0 degrees F) for > 24 hrs
Febrile neutropenia		……	……	temperature of >38.3 degrees C (101 degrees F) or a temp of ≥38 degrees C (100.4 degrees F) for > 1hour	Life-threatening consequences; urgent intervention indicated
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	……
Hypertension	None	Pre-hypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg); medical intervention indicated; recurrent or persistent ≥ 24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated Pediatric: recurrent or persistent (≥ 24 hrs) BP >ULN; monotherapy indicated	Stage 2 hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated. Pediatric: Same as adult	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated. Pediatric: Same as adult
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Edema	None	Localized to dependent areas, no disability or functional impairment	Moderate localized edema and intervention indicated; limiting instrumental ADL	Severe localized edema and intervention indicated; limiting self care ADL	……
Pneumonitis	None	Asymptomatic; clinical or diagnostic observations only;	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g. intubation or tracheotomy)
Injection Site Reaction	None	Tenderness with or without associated symptoms	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated
Rash	None	Covering < 10% body surface area (BSA)	Covering 10-30% body surface area (BSA)	>30% body surface area (BSA)	Generalized exfoliative, ulcerative, or bullous dermatitis
Creatinine Increased	None	>1 - 1.5 x baseline; >ULN - 1.5 x ULN	>1.5 - 3.0 x baseline; >1.5 -3.0 x ULN	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN
Weight Gain	None	5 - <10% from baseline	10 - <20% from baseline	≥20% from baseline	……
Other- Specify _____		Mild	Moderate	Severe	Life- threatening

Person Completing Form: _____ PI Signature: _____

All Events are Expected and Attributable Except: _____