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STUDY INFORMATION

- **Title of Project:**
  CINJ 021905: Salsalate + Venetoclax/Decitabine for Patients with Acute Myelogenous Leukemia or Advanced Myelodysplasia/Myeloproliferative Disease
- [redacted] **r Name**
- [redacted] **Div. & Dept.**
  Division of Hematologic Malignancies, Cancer Institute of New Jersey
- [redacted]
- **Protocol Version Date:**
  V 12.29.2020, Amendment 1

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1.0 Research Design

1.1 Purpose/Specific Aims

To determine if the novel combination of salicylic-salicylic acid (Salsalate), venetoclax and decitabine (or alternate hypomethylating agent [HMA], 5-azacytidine] should be developed as a therapy for patients with advanced myeloid malignancies [acute myelogenous leukemia (AML); high risk myelodysplasia (MDS); or high risk chronic myeloproliferative disease] who are not candidates for standard induction chemotherapy with anthracycline + cytarabine. This is a phase 2 study to determine if the novel combination therapy is tolerated well and if biological endpoints are met. There is no control group.

A. Objectives

Primary Objective: Determine the tolerability of the addition of standard dose Salsalate to the standard treatment combination of venetoclax + HMA (decitabine or 5-azacytidine).

Secondary Objective: Determine the remission rate and, when feasible, perform exploratory studies of: (i) patterns of mutation clearance; and (ii) distribution of cells with low and high reactive oxygen species (ROS) content at various points during therapy.

B. Hypotheses / Research Question(s)

Salicylate has been studied at [redacted] as an adjunct to standard AML induction chemotherapy. In those studies, salicylate, administered as choline magnesium trisalicylate was shown to inhibit the expression of the transcription factor nuclear factor kappa-B (NF-kB) in the nucleus of AML cells. The salicylate was also shown to modulate the expression of NF-kB modulated genes, including XIAP, an inhibitor of apoptosis. (1,2). Salicylates are also known to inhibit mitochondrial oxidative phosphorylation, an effect that is anticipated to enhance the anti-leukemic effect of venetoclax (3,5,6,7,9,10). There were no adverse effects associated with choline magnesium trisalicylate in our studies when administered with standard induction chemotherapy (1,2). Other forms of non-acetylated salicylates have also been administered to patients with myeloid malignancies (8). The safety of salicylates in combination with the induction of enhanced expression of anti-apoptotic genes and the uncoupling of oxidative phosphorylation make them an excellent candidate for study in combination with venetoclax + decitabine or 5-azacytidine, a newly approved therapy for patients with AML who are not candidates for standard induction chemotherapy. Our primary hypothesis is that the combination of a salicylate + venetoclax + decitabine (or in selected patients previously treated with decitabine, 5-azacytidine) will be well tolerated and, if so, should be studied further as therapy for advanced myeloid malignancies. We propose to use salicylic-salicylic acid (Salsalate) in this study because our previously performed study used a salicylate, choline magnesium trisalicylate, which is no longer commercially available. Salsalate (salicylsalicylic acid) is composed of two molecules of salicylic acid joined by an ester link. The ester bond of salsalate occurs at the carboxyl group of one salicylic acid molecule and the hydroxyl group of another. Salsalate is non-acetylated so has no effect on platelet function 8). It is used in the treatment of osteoarthritis, rheumatoid arthritis, and other

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inflammatory conditions. It has fewer gastrointestinal effects than aspirin and is often used as an anti-pyretic for patients receiving chemotherapy.

Salicylate inhibits expression of some nuclear factor kappa-B regulated genes, like XIAP (2). Salicylates also inhibit oxidative phosphorylation (6,7,9). Our secondary hypothesis is that a salicylate in combination with venetoclax + HMA will result in enhanced cytotoxicity to a subset of malignant myeloid cells with low ROS. In patients with AML, this population is reported to be enriched in leukemic stem cells and the prime target of venetoclax (3,10,12). In exploratory studies we will correspond the survival of these cells with disease response and also patterns of myeloid mutation clearance.

1.2 Research Significance

The average age at AML diagnosis is 66 years old. Patients over age 60 with newly diagnosed AML have <20% 3 year survival. Standard cytotoxic induction therapies used in younger patients are often not beneficial when administered to older patients because of intrinsic differences in AML biology in older patients and poor tolerance (based upon age-related co-morbidities). Therefore, patients over age 60 are often treated with lower intensity therapies such as hypomethylating agents like 5-azacytidine or decitabine. These agents yield AML response rates of 20-40%, require 3-5 months to reach best response, and are not curative (11).

Venetoclax is a selective oral inhibitor of bcl-2 and has been developed as component of induction therapy for elderly patients with AML. Bcl-2 is overexpressed in AML cells and leukemic stem cells (the self-renewing component of the leukemia population). The leukemic stem cells show also a high dependence on oxidative phosphorylation and are often sensitive to bcl-2 inhibition *ex vivo*. Several clinical trials of HMAs (or low dose cytarabine) + venetoclax have been reported and these combinations are now approved by the FDA for AML patients not eligible for intensive induction therapy (3,10,12,21,19).

One recently reported multi-institutional dose escalation study combined a hypomethylating agent (5-azacytidine or decitabine) with venetoclax as therapy for AML patients aged 65 years or older. There were 145 patients treated and median age was 74 years old. Approximately 50% had cytogenetic alterations that are labeled high- risk by a consensus panel (European Leukemia Net). Patients with high-risk cytogenetics generally have low remission rates and durations of response, even with aggressive cytotoxic induction therapy followed by allogeneic hematopoietic stem cell transplant. The CR + CRi (complete remission with incomplete blood count recovery) was 69%. The CR + CRi rate was 73% in the cohort receiving venetoclax 400 mg/d. Patients with high-risk cytogenetics obtained CR + CRi of 60% but duration of response in this group was only 6.7 mos compared to 12.3 mos for patients with intermediate risk cytogenetics. CR + CRi rates of 47% were noted in patients with TP53 mutations. Flt3 mutations did not predict outcome and NPM1 mutations were associated with statistically favorable remission rates and durations of response. Neither age >75 nor secondary AML were adverse prognostic features for obtaining CR + CRi. Overall median duration of response was > 11 months with median OS of 17.5 months. In these studies grade 3 adverse events included fever + neutropenia (43%), thrombocytopenia (24%), bacteremia (8%), and pneumonia (13%)(19,20).

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Another clinical trial of combination decitabine + venetoclax was presented recently at the 2018 American Society of Hematology Meeting (abstract 286 Maiti et al. Interim Analysis of Phase II Study of Venetoclax with 10-Day Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndrome. American Society of Hematology 2018). In that study, a prolonged course of decitabine (10 days/cycle as opposed to the standard 5 days/course) was used in combination with venetoclax. Treated patients included elderly patients with newly diagnosed or relapsed/refractory AML CR + CRi rates for *de novo* AML, secondary AML and relapsed/refractory AML patients were 91%, 72% and 44% respectively. Patients with relapsed/refractory AML had a median duration of response of only ~3 months; patients with *de novo* or secondary AML had median durations of response that exceeded the 12 month period of monitoring.

Both of these studies indicated rapid response rates with best response usually seen by 1-2 cycles of treatment. Both studies also tested for measurable residual disease (MRD). As seen with standard AML therapy, there appeared to be improved response duration in the subset of patients reaching a state of undetectable MRD. In patients treated at the University of Colorado, 22 patients had molecular determination of MRD and 4 had no detectable MRD; none of the 4 with no MRD had relapsed at median f/u > 2 years (5).

These and other studies of older patients with AML indicate that treatment with a HMA in combination with venetoclax can be very effective. Comparison to historic controls treated with standard chemotherapy or single agent hypomethylating agent much improved CR rates and duration of response compared were seen. Even those with traditionally high-risk mutation profiles, secondary AML or age > 75 years old seem to benefit greatly compared to historic controls. However, elderly AML patients with high-risk cytogenetics, relapsed/refractory disease, or prior HMA exposure will have lower CR + CRi rates and durations of response. Even those with newly diagnosed disease who respond are likely to relapse within 2 years. Therefore, modifications of this regimen need to be tested.

Mechanistic studies indicate that leukemic stem cells (the self-renewing population of AML cells) are very dependent on oxidative phosphorylation. It has also been shown that the beneficial effect of venetoclax may in part be based upon this dependence. Well-tolerated drugs known to impact oxidative phosphorylation and/or apoptosis are logical choices for addition to HMAs + venetoclax (3,5,10).

Salsalate is our choice to test in combination with a hypomethylating agent + venetoclax. Salicylates are known to inhibit oxidative phosphorylation and previous clinical trials at Rutgers-CINJ have utilized a salicylate as part of anti-leukemic therapy (1,2,6,7). Choline magnesium trisalicylate used in prior studies is no longer available commercially. In this study we will utilize saliclicsalicylic acid (Salsalate) at a standard dose. The dose has been shown to result in therapeutic salicylate levels and like the choline magnesium trisalicylate is not acetylated and will not result in platelet dysfunction. This medication is often used as part of supportive care (anti-pyretic) during AML induction chemotherapy. We have shown previously that salicylate modulates the expression of many genes in the WNT and NF-kB pathways, including XIAP, an inhibitor of apoptosis (2). It has also been shown by others that salicylates have a direct mitochondrial effect that impedes oxidative phosphorylation (6,7). In this context, we will study Salsalate in combination with decitabine and

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venetoclax as induction therapy for selected elderly patients with advanced myeloid malignancies such as AML, myelodysplasia (MDS) or myeloproliferative diseases with > 10% blast or blast equivalents. Patients who have received decitabine previously will be treated with 5-azacytidine as the HMA. Morphology, standard flow cytometry and next generation sequencing will be used as markers of MRD. When available, single cell DNA sequencing will be used to determine the sensitivity of individual leukemia clones to treatment. Oxidative phosphorylation status of cells will be profiled by flow cytometry to determine if response rates correspond with changes in the profile of oxidative phosphorylation and if Salsalate has independent effects on the profile.

1.3 Research Design and Methods

A. Research Procedures

In this study we will treat patients with AML, MDS and advanced myeloproliferative disorders with decitabine, venetoclax and salsalte. 5-azacytidine may be substituted for decitabine if the patient has been previously treated with decitabine. Patients will be hospitalized for initiation of therapy (for at least 4 days). Venetoclax dose escalation in cycle 1 will follow the recommended "ramp up" to minimize the risk of tumor lysis. Salsalate will be added on day 5 of decitabine + venetoclax (or 5-azacytidine). There will be only one cycles of treatment with salsalate. The next treatment cycle will be administered under the direction of the treating physician.

Cycle 1:

i. Venetoclax and decitabine (or 5-azacytidine) will initiate concurrently on d1; salsalate will be start on d5.
    1. Decitabine 20 mg/m2/d IV will be administered d1-10. If 5-azacytidine is being used (if the patient has previously been exposed to decitabine) it will be administered at 75 mg/m2/d SC or IV d1-7.
    2. Venetoclax will be dose escalated per package insert - 100 mg d1; 200 mg d2; and 400 mg d3-28.
    3. Salsalate 1500 mg PO bid will start on day 5 of decitabine (or 5-azacytidine) and be administered until and including the day of completion of the decitabine or 5-azacytidine.

Patients with WBC greater than 25,000/uL will be treated with hydroxyurea 2-3g PO bid per treating physician to obtain a WBC<15,000/uL. Once WBC is in target range the protocol treatment can be started. Hydroxyurea may be continued to sustain WBC < 15,000/uL as needed. Standard tumor lysis monitoring and prophylaxis will be used. Allopurinol (or alternate hypouricemic agent) will be started $\geq$24 h prior to starting venetoclax and continued through dose escalation of venetoclax, or longer, as clinically indicated. During dose escalation of venetoclax patients will receive IV fluids, and tumor lysis laboratory studies (basic chemistry profile including calcium, phosphate, uric acid and lactate dehydrogenase) will be obtained every 8 hours.

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Infectious disease prophylaxis may be used at the discretion of the patients treating physician. If anti-fungal prophylaxis is used the dose of venetoclax will be modified as outlined below. Proton pump inhibitors are mandated unless there is a contraindication. Venetoclax dosing will be adjusted if concurrent anti-infectives are CYP3A4 or Pgp inhibitors (see below).

Venetoclax dosing will be modified in the context of concurrent use of CYP3A4 or Pgp inhibitors. Venetoclax will be dosed at 70mg /d (if concurrent posaconazole) or 100 mg/d if there is concurrent use of other strong CYP3A4 inhibitors or 200 mg/d if use of moderate CYP3A4 inhibitors. Venetoclax will be dosed at 200 mg/d in the setting of concurrent use of moderate CYP3A4 inhibitors. Dosage should be modified and timing of dose adjusted if concurrent Pgtp inhibitors are used. All concurrent meds will be reviewed and dosing schedules approved by a research pharmacist.

TABLE 1 Commonly used CYP3A4 inhibitors

Moderate	Strong
Aprepitant	Clarithromycin
Cimetidine	Itraconazole
Cyclosporine	Ketoconazole
Erythromycin	Voriconazole
Fluconazole	Posaconazole
Verapamil	Ritonavir
Isavuconazole	Darunavir
Diltiazem	Atazanavir

If the patient has profound thrombocytopenia (< 10,000/uL at day 21 – 28 in the first cycle) a BM asp and biopsy may be performed at the discretion of the treating physician with the option to hold venetoclax. The start of the second cycle is at the discretion of the treating physician and may include modifications of decitabine/5-azacytidine or venetoclax dosing as per standard recommendations. These additional cycles (starting at cycle 2 are not considered part of this clinical trial).

Any Grade 3 or 4 non-hematologic adverse event thought to be Salsalate-related will require the dose to be held until the event recovers to Grade 2 or less. If the adverse event does not resolve within 1 week, the patient will be removed from the study. For Salsalate these events include renal insufficiency, gastritis, tinnitus, vertigo and unanticipated GI bleeding as common toxicities; the Salsalate-relatedness of other toxicities will be assessed by the research team in real time.

Patients will receive full supportive care therapies per Leukemia Tumor Study Group practices concomitantly during the study. No other chemotherapy, immunotherapy, hormonal cancer therapy, radiation therapy, surgery for cancer, or experimental medications will be permitted while the patients are participating in the treatment component of this study (first 2 cycles).

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CSFs are not permitted as primary prophylactics. If, in the investigator's opinion, it is required in the presence of severe neutropenia (ANC <500/µl for at least 3 days or concurrent neutropenia and infection) then use of CSFs is allowed per treatment plan described above.

Patients that are not hospitalized will be asked to keep a medication diary and instructed to record the number of capsules taken and the time taken. At the end of each cycle of treatment, the actual amount of unused drug will be compared to the amount taken as recorded on the subject's medication diary.

All patients who receive one dose of protocol therapy will be evaluable for assessment of toxicity. Prior to each cycle the treating physician will fully assess the patient's condition with respect to possible treatment related toxicities. All adverse events, whether observed by the physician or reported by the patient, occurring during the active portion of therapy, or up to 30 days after the last dose of treatment will be graded by a numerical score according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0 (http://ctep.cancer.gov/reporting/ctc.html) and recorded in the patient's medical record. For the purposes of reporting laboratory abnormalities, only Grade 3-4 adverse events will be recorded on the adverse event CRF pages. Grade 1-2 laboratory abnormalities will not be recorded on the adverse event CRF pages. Information entered on the adverse event CRF pages will include:

- Specific type and duration of reaction (i.e., start and stop dates, resolution).
- Severity/grade.
- Relationship to study drug (causality, attribution).
- Management of the event, if treated with medication and other actions taken to alleviate the clinical event.
- Whether or not it was considered a SAE.

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

An adverse experience is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to the study therapy, or it has worsened in intensity or frequency following exposure to the study therapy.

All "unexpected" (defined below) and/or "serious" experimental drug related adverse events occurring during the active portion of therapy, or up to 30 days after the last dose of treatment, will be reported to the Office of Human Research Services at (732) 235-7577 or (732) 235-2465. Events will be promptly reported, in writing, to the local IRB in accordance with IRB policy. If a death occurs the IRB will be notified within 24-hours of initial receipt of information. All other SAEs must be reported to the IRB within three to ten days of initial receipt of information. Written follow-up reports are required when additional information is needed to fully characterize the event. Copies of each report sent to the IRB will be kept in the study regulatory file.

In addition to reporting to the local IRB, reporting to external bodies such as industry and/or the FDA may be necessary

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

a) Disease progression/relapse during active treatment,
b) Intercurrent illness that prevents further administration of treatment,
c) Unacceptable adverse event(s).
d) In the event of any drug-related life-threatening toxicity or laboratory abnormality the patient will be withdrawn from further treatment,
e) Patient decides to withdraw from the study,
f) Noncompliance with treatment plan,
g) General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or
h) Protocol violation - any patient found to have entered this study in violation of the protocol might be discontinued from the study at the discretion of the Principal Investigator.
i) The desired level of compliance is $\geq$ 75%. Patients who cannot maintain this level of compliance may be removed from the study.

Evaluations	**Pre-Study (within 2 weeks)**	**Daily during treatment with decitabine or 5-azacytidine**	**Prior to Next Treatment[12]**
Initial History & Physical	X		
Interim History & Physical		X while hospitalized	X
Toxicity Assessment		X	X
Concomitant Medications	X	X	X
ECOG Performance Status	X		X

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CBC, differential, platelets	X[1]	X while hospitalized	X
Serum Chemistries[2]	X	X while hospitalized	X
Tumor lysis monitoring and treatment)		X (q8h during ramp up and q d while hospitalized)	
Liver Enzymes[3]	X	X while hospitalized	X
EKG	X		
Pregnancy Test[4]	X		X
Radiographic Assessments[5]	X		
REDOX Blood Studies[6] (during first week of treatment)	X	X (pre first dose of salsalate and after 4-8 doses salsalate based on blood counts)	
BM asp and biopsy	X		X[11]
Blood flow cytometry/NGS (next generation sequencing)[7]	X	X	
Compliance Assessment			X
Survival[8]			

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1. Within 1 week of enrollment. 2. Includes: Electrolytes, Calcium, BUN, Creatinine, Total Protein, Glucose, Albumin, Total Bilirubin, Alk. Phosphatase, SGOT/SGPT. 3. Includes: Total and Direct. Bilirubin, AST/ALT, Alkaline Phosphatase, Albumin, Total Protein. 4. Women of childbearing potential must have pregnancy test verified by BHCG within 1 week of enrollment. 5. Radiographic assessments will be selected by the attending physician as clinically indicated and in accordance with the criteria for tumor measurement assessments. 6. REDOX analysis (Dr. Herranz lab) will be done on blood samples with CD34 selected blasts at baseline, after 1-5 days of treatment and following administration of of Salsalate with exact timing determined by numbers of leukemic blasts in the blood 7. NGS from BM will be done at baseline and after the second cycle or on any BM with leukemic cells detected as performed for clinical reasons. NGS or Single cell DNA sequencing may also be done by Dr. Khiabanian as feasible from blood based upon disease status and resources. 8. Subjects will be tracked until relapse or change of therapy. 9. Disease status and survival status will be documented every 2 months until disease progression or start of new treatment. 10. End of Treatment = 45 days after the last dose of Salsalate. 11. Bone marrow aspirate can be performed within 14 days of anticipated next treatment cycle.	

B. Data Points

Please see above for data points for REDOX analysis, DNA sequencing and disease assessment by bone marrow analysis.

C. Study Duration

Eligible patients will be treated with experimental drug Salsalate + standard venetoclax + decitabine (or 5-azacytidine) for 1 cycles of therapy. Responding patients will then continue on standard of care venetoclax + decitabine (or 5-azacytidine) in the observation phase of the study under supervision of their physician.

D. Endpoints

Primary endpoint: For testing the primary hypothesis, the toxicity of salicylate + venetoclax + decitabine during the first two cycles will be collected as primary endpoints.

Secondary endpoint: We will correspond response rates with the pattern of survival of low oxygen reactive species cells. The administration of drugs will be timed to determine if there is a measurable added effect of salicylate on the oxidative state of cells when used in combination with venetoclax + decitabine. Next generation sequencing will determine also if there is a correspondence of remission with patterns of mutation clearance and/or effects of treatment on the oxidative state of the leukemia cells.

1.4 Preliminary Data

See above (Hypotheses, Research Question). Salicylates have been shown to modulate NF-kB gene expression (studies performed at Rutgers/CINJ) (1,2). Salicylates are known to uncouple oxidative phosphorylation (6,7). In combination these effects are hypothesized to enhance the anti-leukemic effects of combined hypomethylating agent + venetoclax therapy of AML.

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1.5 Sample Size Justification

We propose 20 patients over the course of 3 years to assess toxicity, as outlined above. We anticipate also the use of data to support development of a larger multi-instutional randomized phase 2 trial.

1.6 Study Variables

A. Independent Variables, Interventions, or Predictor Variables

Decitabine (or 5-azacytidine, in patients previously treated with decitabine) in combination with venetoclax is a frequently used AML therapy. This study will introduce salicylic-salicyclic acid (Salsalate) as the experimental agent. Standard doses of decitabine (20 mg/m2/d IV x 10d for the first cycle and 5d for cycle 2) or 5-azacytidine (75 mg/m2/d x 7d SC or IV for 1 cycle). Ramp up dosing of venetoclax and standard dose salsalate will be used.

B. Dependent Variables or Outcome Measures

Remission rate, biochemical response

1.7 Drugs/Devices/Biologics

Product description: Salsalate (salicylic-salicylic acid is commercially available
Preparation (how the dose is to be prepared): The dose will be administered as 3 x 500 mg or 2 x 750 mg bid for a total of 3000 mg/d orally as indicated in the treatment plan.
Storage requirements: The tablets are stored at room temperature.
Route of administration: Oral tablet

1. *Expected toxicities: Constipation, diarrhea, heartburn, tinnitus, nephrotoxicity, liver function test abnormalities, GI intolerance including abdominal pain, nausea & diarrhea, peripheral edema and somnolence.*

   Drug Interactions: no known interactions with hypomethylating agents or venetoclax; Salsalate is metabolized by conjugation to glycine and glucuronide metabolites

A. Drug/Device Accountability and Storage Methods

The investigator is required to maintain adequate records of receipt, dispensing and final disposition of study drug. This responsibility has been delegated to the pharmacy. Include on receipt record (e.g. packing slip) from and to whom study drug was shipped, date, quantity, and batch or lot number. On dispensing record, note quantities and dates study drug was dispensed to and returned by each subject. Empty and partially empty containers of study drug will be disposed of in accordance with institutional policies and procedures.

1.8 Specimen Collection

Exploratory studies include: (i) flow cytometric characterization of malignant cells by reactive oxygen species content using labeling with CELLRox; and (ii) next generation sequencing of malignant cells. The timing of these studies will be determined by the percentage (and total number of malignant cells/uL at the following time points: (i) pretreatment; (ii) after the ramp-up of venetoclax; and (iii) after the initiation of salsalte. These studies are exploratory and logistics regarding cell number and purity are likely to determine the specific timing of studies (based on each individual patient's blood

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counts). The strategy of sequential analysis of the effects of salsalate and chemotherapy on malignant cells before and during induction treatment has been successfully employed in our earlier studies. Dr. Daniel Herranz Benito will supervise the analysis of cells for reactive oxygen species and Dr. Hossein Khiabanian will supervise next generation sequencing. In selected cases with informative bulk next generation sequencing studies, single cell DNA sequencing may be performed. Specimens will be transported by one of investigators to the Biorepository. Specimens will be stored in the aforementioned labs and only accessed by lab staff or investigators. Specimens will not be banked.

1.9 Data Collection

Direct patient data (below) will be collected in the office or hospital by investigators.

Evaluations	Pre-Study (within 2 weeks)	Daily during treatment with decitabine or 5-azacytidine on cycle 1 (as clinically indicated cycle 2)	Prior to Cycle 2
Initial History & Physical	X		
Interim History & Physical		X	X
Toxicity Assessment		X	X

1.10 Timetable/Schedule of Events

Evaluations	Pre-Study (within 2 weeks)	Daily during inpatient treatment with decitabine or 5-azacytidine on cycle 1	Prior to Next Treatment
Initial History & Physical	X		
Interim History & Physical		X	X
Toxicity Assessment		X	X
ECOG Performance Status	X		X
CBC, differential, platelets	X[1]	X	X
Serum Chemistries[2]	X	X	X

[redacted]

[redacted]

Liver Enzymes[3]	X	X	X
EKG	X		
Pregnancy Test[4]	X		X
Radiographic Assessments[5]	X		
REDOX Blood Studies[6]		X (during first week of treatment)	
BM asp and biopsy	X		X
Blood flow cytometry/NGS (next generation sequencing)[7]	X	X	
Compliance Assessment			X
Survival[8]			

1. Within 1 week of enrollment.
2. Includes: Electrolytes, Calcium, BUN, Creatinine, Total Protein, Glucose, Albumin, Total Bilirubin, Alk. Phosphatase, SGOT/SGPT, Uric Acid.
3. Includes: Total and Direct. Bilirubin, AST/ALT, Alkaline Phosphatase, Albumin, Total Protein.
4. Women of childbearing potential must have pregnancy test verified by BHCG within 1 week of enrollment.
5. Radiographic assessments will be selected by the attending physician as clinically indicated and in accordance with the criteria for tumor measurement assessments.
6. REDOX analysis (Dr. Herranz lab) will be done on blood samples with CD34 selected blasts at baseline, after ramp-up of venetoclax and after 1-2 days of SALSALATE as feasible based upon blood counts (leukemia cells in blood) during first cycle
7. NGS from BM will be done at baseline and after the second cycle or on any BM performed for clinical reasons. NGS or Single cell DNA sequencing may also be done by Dr. Khiabanian as feasible from blood based upon disease status and resources.
8. Disease status and survival status will documented every 2 months until disease progression or start of new treatment.

2.0 Project Management

2.1 Research Staff and Qualifications

Study personnel are experts in designing and running clinical trials and do so as part of their positions at Rutgers

2.2 Research Staff Training

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All persons assisting with the research will be informed about the protocol, the procedures, and their duties and functions during research meetings.

2.3 Resources Available

Facilities at CINJ and RWJUH will be used for the purposes of this study.

2.4 Research Sites

Rutgers Cancer Institute of New Jersey and Robert Wood Johnson University Hospital.

3.0 Multi-Center Research

N/A

4.0 Subject Considerations

4.1 Subject Selection and Enrollment Considerations

A. Method to Identify Potential Subjects

Patients will be recruited from the clinical practice of the Hematologic Malignancies Tumor Study Group of Rutgers Cancer Institute of New Jersey.

B. [redacted]

C. Subject Screening

Inclusion Criteria

A patient/subject is eligible for enrollment if all of the following inclusion criteria are met.

Histologically proven AML or advanced myeloid malignancy [myelodysplasia; chronic myelomonocytic leukemia (CMML); or chronic myeloproliferative disease (MPD) each with ≥10% myeloblasts in blood or bone marrow];

For patients with *de novo* AML: must not be a candidate for standard induction therapy based upon age, co-morbidities, patient choice, high risk features known to have poor outcomes with standard induction therapy (ELN high risk disease by cytogenetics, DNA mutation profile or TP53 mutation);

Patients with advanced MDS, secondary AML, relapsed/refractory AML, who have received prior treatment with hypomethylating agents are eligible;

Patients must give informed consent.

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Patients must have an ECOG performance status ≤2 (Appendix A).

Patients must have normal organ function as defined below:

- Total bilirubin < 2 x ULN
- AST(SGOT)/ALT(SGPT) ≤2.5 X institutional ULN
- Creatinine clearance > 30 ml/min

Exclusion Criteria

A patient /subject will not be eligible for this study if any of the following exclusion criteria are met.

WBC uncontrolled (>15,000/uL) despite hydroxyurea x 5 days (see below).
Known CNS AML.

Serious concomitant systemic disorders (including active infections) that would compromise the safety of the patient or compromise the patient's ability to complete the study, at the discretion of the investigator. Pregnant patients are excluded.

Age <18 years. Because no dosing or adverse event data are currently available on the use of Salsalate in combination with venetoclax and decitabine in patients <18 years of age, children are excluded from this study. Salicylates have also been associated with Reyes syndrome in pediatric patients.

History of allergic reactions attributed to compounds of similar chemical or biologic composition to Salsalate or other agents used in the study. Examples include aspirin.

The effects of venetoclax and decitabine or 5-azacytidine on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and for 24 weeks after. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

Patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy, therefore, known HIV-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with treatment medications or other agents administered during the study.

4.3 Number of Subjects

A. Total Number of Subjects

Twenty at Rutgers CINJ. Total = 20 patients.

C. Feasibility

[redacted]

[redacted]

Based on the number of patients seen at CINJ/RWJUH with AML, MPN, or MDS meeting recruitment goals within the timeframe is expected.

4.4 Consent Procedures

A. Consent Process

Informed consent will be obtained prior to commencing any research procedures. The investigators will seek consent only under circumstances that provide the prospective patient opportunity to consider whether or not to participate and that minimizes the possibility of coercion or undue influence. The information given to the patient, or the representative, shall be in a language understandable to the subject or representative. The informed consent document may not include any exculpatory language through which the subject or representative is made to waive any of the subject's legal rights or releases, or appears to release the investigator, the sponsor or the institution from liability for negligence

C. Documentation of Consent

- **Documenting Consent**
  Individuals will be asked to sign an informed consent document

  Waiver of <u>Documentation</u> Of Consent (i.e., will not obtain subject's signature)
  N/A

4.5 Special Consent/Populations

A. Minors-Subjects Who Are Not Yet Adults

No dosing or adverse event data are currently available on the use of Salsalate in combination with venetoclax and decitabine in patients <18 years of age. Pediatric patients who receive salicylates are also at risk for Reyes syndrome. Therefore, minors will not participate in this study

B. Wards of the State

N/A

C. Non-English-Speaking Subjects

- **Process for Non-English-Speaking Subjects**
  Non English speakers will not be enrolled because this is an investigator-initiated study without funding source.

- **Short Form Consent for Non-English Speakers**
  This will not be used.

D. Adults Unable to Consent / Cognitively Impaired Adults (for interventional studies)

Those unable to consent or cognitively impaired will not be asked to enroll in this trial.

4.6 Economic Burden and/or Compensation for Subjects

[redacted]

[redacted]

The study is an investigator-initiated clinical trial with no financial support. Philantropic funds will be used to pay for study drug (SALSALATE). The additional medications are standard of care and will be financed based upon the patients insurance.

4.7 Risks of Harm/Potential for Benefits to Subjects

A. Description of Risks of Harm to Subjects

MDS, AML and related advanced myeloid disorders are life-threatening diseases. Risks of the disease include bleeding, infection and organ dysfunction. The disease is fatal with standard chemotherapy in > 80% patients eligible for this study over a 2 years. The combination of venetoclax + hypomethylating agent therapy is a breakthrough – greatly improving response rates and, likely, also survival. The potential risks of this study are that there will be toxicity from the study drug related to known SALSALATE toxicities (e.g tinnitus, renal insufficiency) or that it will interfere with the anti-leukemic effect of venetoclax + hypomethylating agent. To protect against or minimize potential risks, we will monitor toxicity real time and have imposed very strict stopping rules.

B. Potential Benefits to Subjects

SALSALATE has been safely administered to patients undergoing intense AML induction therapy. It is also routinely used as a supportive care medication during AML therapy. We believe it has the potential to enhance the anti-leukemic effects of standard venetoclax + hypomethylating agent therapy and improve response rates and duration of remission.

5.0 Special Considerations

N/A

6.0 Data Management Plan

6.1 Data Analysis

1. An unacceptable adverse event is defined as a Grade 3 study drug (SALSALATE) related or possibly related irreversible (i.e. doesn't reverse within one week) event with the exception of:
    a. Grade 3 fever, asthenia or constipation.
    b. Grade 3 nausea, vomiting, diarrhea (not requiring tube feeding, total parenteral nutrition, or prolonged hospitalization;
    c. Grade 3 infection, bleeding or expected complications of cytopenias due to leukemia or decitabine + venetoclax treatment;
    d. Grade 3 or 4 tumor lysis syndrome successfully managed clinically that resolves within 7 days without end organ damage; or
    e. Grade 3 or 4 isolated electrolyte abnormalities that last < 72 hours.

[redacted]

We consider an adverse event rate of 7% or higher to be unacceptably high, so we will stop the trial if, at any point, we are 80% certain that the rate is 7% or higher. In the following table, the shaded cells in the table indicate the region where we are 80% certain that the rate exceeds 7%.

According to this table, if there is 1 unacceptable (grade 3) SALSALATE related or possibly related adverse event (defined above) among the first 10 patients, or if there are 2 unacceptable adverse events among the first 20 patients, the trial will be stopped for unacceptable toxicity.

This table was calculated using a Bayesian posterior beta distribution, assuming a uniform prior.

N patients k: number of unacceptable toxicities

	0	1	2	3
3	0.748052	0.973272	0.9987	0.999976
4	0.695688	0.957507	0.99692	0.999887
5	0.64699	0.939179	0.994161	0.999679
6	0.601701	0.918726	0.990312	0.999293
7	0.559582	0.896534	0.985301	0.998664
8	0.520411	0.872948	0.979088	0.997729
9	0.483982	0.84827	0.971658	0.996424
10	0.450104	0.82277	0.963021	0.99469
11	0.418596	0.796683	0.953203	0.992473
12	0.389295	0.770217	0.942247	0.989724
13	0.362044	0.743553	0.930205	0.986401
14	0.336701	0.716847	0.917139	0.982467
15	0.313132	0.690237	0.903119	0.977894
16	0.291213	0.663839	0.888217	0.97266
17	0.270828	0.637756	0.87251	0.966749
18	0.25187	0.612071	0.856078	0.960152
19	0.234239	0.586857	0.838997	0.952867
20	0.217842	0.562173	0.821347	0.944896

[redacted]

6.2 Data Security

Monitoring of this study will occur in accordance with the Cancer Institute of New Jersey's NCI approved Data and Safety Monitoring Plan (DSMP). An "initiation audit" will be conducted at the Cancer Institute of New Jersey in accordance with the DSMP following enrollment of the first two (2) or three (3) patients. Subsequent audits will occur on an annual basis prior to annual IRB continuing review, if the findings from the initiation audit were satisfactory. More frequent audits of patient data and study conduct will occur if necessary. Prior audit findings and/or situations that may arise during the course of the study will determine the need for more frequent auditing. All audit findings will be discussed with the principal investigator and reported to the Cancer Institute of New Jersey's Human Research Oversight Committee and the Rutgers University Institutional Review Board.

6.3 Data and Safety Monitoring

A. Data/Safety Monitoring Plan

A subset of the National Cancer Institute (NCI) CRFs, in electronic format, will be utilized. Completion of the electronic CRFs (eCRFs) will be done in accordance with the instructions in a study specific data capture plan. All eCRFs will be completed by clinical research coordinators of the Office of Human Research Services (OHRS) at the Cancer Institute of New Jersey. The eCRFs will be maintained in a confidential format in a secure database.

Completion of eCRFs will occur in accordance with NCI guidelines. Baseline (pre-study) eCRFs (e.g., enrollment, medical history, concomitant medications, disease assessment, etc.) will be completed no later than 14 days after the start of treatment. Treatment eCRFs (e.g., drug administration, adverse events, chemistries, etc.) will be completed no later than 14 days following each cycle of treatment. Off-treatment information (e.g., follow-up, best response, etc.) will be completed no later than 14 days after the end of protocol treatment.

A research chart (i.e., shadow chart) is maintained at OHRS for each patient enrolled. Copies of significant study source documents will be maintained in the research chart. Examples of source document copies that will be maintained in the research chart include: signed informed consent form, documents that verify eligibility and treatment and documents that verify Grade 3-4 adverse events and response. This information will be updated on a prospective basis and will be confidentially maintained at the Cancer Institute of New Jersey, OHRS.

Data will be analyzed weekly by the PI with review at weekly tumor study group meetings including research nurses assigned to the study.

B. Data/Safety Monitoring Board Details

[redacted]

6.4 Reporting Results

A. Individual Subjects' Results

[redacted]

Results of investigational diagnostic tests, genetic tests, or incidental findings will not be shared with study participants unless that information is the same as would be shared if the patient was not enrolled in this trial.

B. Aggregate Results

Aggregate research results will not be shared with the study participants.

C. Professional Reporting

The policies and procedures of Rutgers University's legal department (see: Investigator's Handbook) will govern publication of the trial. It is expected that the results of this trial will be submitted for publication in a timely manner following the conclusion. The Cancer Institute of New Jersey PI, and all co-authors prior to submission or use, must review any abstract or manuscript.

D. Clinical Trials Registration, Results Reporting and Consent Posting

This research qualifies as a clinical trial

6.5 Secondary Use of the Data

There will be no secondary use of the data.

7.0 Research Repositories – Specimens and/or Data

Specimens will not be stored for use other than outlined in the study.

8.0 Approvals/Authorizations

N/A

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1.1 Appendix A
Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.

[redacted]

2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

[redacted]