

**TITLE:** Phase I/II study of IRX5183 in relapsed and refractory acute myeloid leukemia and high risk myelodysplastic syndrome

**Protocol designation:** P\_195183-201-2015

**Principal Investigator:** B Douglas Smith, M.D.  
1650 Orleans Street, Room 246, CRB1  
Baltimore, Maryland 21231  
Telephone 410-614-5068; Fax 410-614-7437  
[bdsmith@jhmi.edu](mailto:bdsmith@jhmi.edu)

**Co-Investigators:** Kelly Norsworthy, M.D.  
1650 Orleans Street, Room 2M48, CRB1  
Baltimore, Maryland 21231  
Telephone 443-694-0509; Fax 410-614-1005  
[knorswol@jhmi.edu](mailto:knorswol@jhmi.edu)

Gabriel Ghiaur, M.D./Ph.D.  
1650 Orleans Street, Room 243, CRB1  
Baltimore, Maryland 21231  
Telephone 410-502-3183; Fax 410-614-7279  
[gghiaur1@jhmi.edu](mailto:gghiaur1@jhmi.edu)

Rick Jones, M.D.  
1650 Orleans Street, Room 244, CRB1  
Baltimore, Maryland 21231  
Telephone 443-287-7104; Fax 410-614-7279  
[rjones@jhmi.edu](mailto:rjones@jhmi.edu)

**Statistician:** Ravi Varadhan, Ph.D., Ph.D.

**Study Sponsors:** Io Therapeutics, Inc.  
1805 East Garry Avenue, Suite 110  
Santa Ana, CA 92705

**Responsible Research Nurse:** Seana Coffin  
Telephone 410-614-2023  
[scoffin1@jhmi.edu](mailto:scoffin1@jhmi.edu)

**Responsible Data Manager:** Colin Huck  
Telephone 410-614-3725  
[chuck1@jhmi.edu](mailto:chuck1@jhmi.edu)

**Regulatory:** Suzanne Bell  
Telephone 919-946-9901  
[sharroll@jhmi.edu](mailto:sharroll@jhmi.edu)

**TABLE OF CONTENTS**

STUDY SCHEMA and SYNOPSIS..... 4

1. OBJECTIVES ..... 7

    1.1. PRIMARY OBJECTIVES: ..... 7

    1.2. SECONDARY OBJECTIVES: ..... 7

2. BACKGROUND ..... 7

    2.1. Acute myeloid leukemia: ..... 7

    2.2. Myelodysplastic syndrome: ..... 9

    2.3. Previous Cell Differentiation Therapies in Myeloid Malignancies: ..... 11

    2.4. Impact of bone marrow microenvironment on sensitivity to retinoids: ..... 12

    2.5. IRX5183: ..... 13

    2.6. Rationale: ..... 15

3. PATIENT SELECTION ..... 16

    3.1. Eligibility criteria: ..... 16

    3.2. Exclusion criteria: ..... 17

    3.3. Pregnancy: ..... 17

    3.4. Inclusion of women and minorities: ..... 18

    3.5. Off-Study Criteria: ..... 18

    3.6. Informed Consent: ..... 18

4. PRETREATMENT PLAN ..... 19

5. TREATMENT PLAN ..... 19

    5.1. IRX5183 Administration: ..... 19

    5.2. Definition of dose-limiting toxicity: ..... 23

    5.3. General concomitant medication and supportive care guidelines: ..... 24

        5.3.1. Differentiation syndrome ..... 24

        5.3.2. Leukocytosis ..... 24

        5.3.3. Hypertriglyceridemia ..... 24

    5.4. Duration of therapy: ..... 25

    5.5. Duration of follow-up: ..... 26

    5.6. Criteria for removal from study: ..... 26

6. DOSING DELAYS/DOSE MODIFICATIONS: ..... 26

    6.1. Dose Modifications for IRX5183: ..... 27

    6.2. Discontinuation of IRX5183 for related adverse events: ..... 29

    6.3. Exceptions to permanent discontinuation: ..... 29

7. TREATMENT EVALUATION AND TOXICITY MONITORING ..... 30

    7.1. Clinical Responses: ..... 30

    7.2. Assessment of Response: ..... 30

    7.3. Measurement of Response / Response Criteria: ..... 31

    7.4. Adverse Events: ..... 31

    7.5. Serious Adverse Event (SAE): ..... 32

    7.6. Toxicity Reporting: ..... 32

    7.7. Toxicity Monitoring: ..... 32

    7.8. Pharmacokinetics: ..... 33

8. CORRELATIVE STUDIES ..... 34

    8.1. Biologic activity: ..... 34

    8.2. Cellular Collection and Processing: ..... 35

9. PHARMACEUTICAL INFORMATION ..... 36

    9.1. IRX5183 drug information: ..... 36

**9.2. Side effects:** ..... 37

    9.2.1. Adverse events in phase I trial in solid tumor patients: ..... 38

    9.2.2. Teratogenic Effects: ..... 38

    9.2.3. Toxicity Profile of Oral Retinoids (Class Effect): ..... 38

    9.2.4. Differentiation syndrome (DS): ..... 39

**10. STUDY CALENDAR** ..... 40

**11. STATISTICAL CONSIDERATIONS** ..... 41

**11.1. Study design & endpoints:** ..... 41

**11.2. Primary endpoints:** ..... 42

**11.3. Secondary endpoints:** ..... 42

**11.4. Sample size considerations:** ..... 42

**11.5. Expected accrual rate:** ..... 43

**11.6. Stratification factors:** ..... 43

**11.7. Analysis of secondary endpoints:** ..... 43

**11.8. Reporting and exclusions:** ..... 44

        11.8.1. Evaluation of toxicity: ..... 44

        11.8.2. Evaluation of response: ..... 44

**12. DATA SAFETY MONITORING PLAN** ..... 45

**12.1. Data Reporting:** ..... 45

**12.2. Data handling and record keeping:** ..... 47

**13. REGULATORY CONSIDERATIONS / ETHICS** ..... 47

**13.1. Institutional review board (IB) / ethics committee (EC) approval:** ..... 47

**13.2. Recruitment:** ..... 46

**13.2. Subject confidentiality:** ..... 48

**13.3. Study records requirements:** ..... 48

**13.4. Evaluation of benefits and risks/discomforts:** ..... 49

**13.5. Risks/benefits analysis:** ..... 49

**13.6. Patient information and consent:** ..... 49

**13.7. Financial disclosure:** ..... 50

**APPENDIX A1. Modified International Working Group (IWG)-2003** ..... 52

**APPENDIX A2. Modified International Working Group (IWG)-2006 response criteria for altering natural history of MDS**...51

**APPENDIX A3. Modified International Working Group (IWG)-2006** ..... 55

**Appendix B:** ..... 56

**A) International Prognostic Scoring System (IPSS) for MDS** ..... 56

**B) Revised International Prognostic Scoring System (IPSS-R) for MDS** ..... 56

**APPENDIX C: Performance Status by ECOG and Karnofsky Grading Criteria** ..... 58

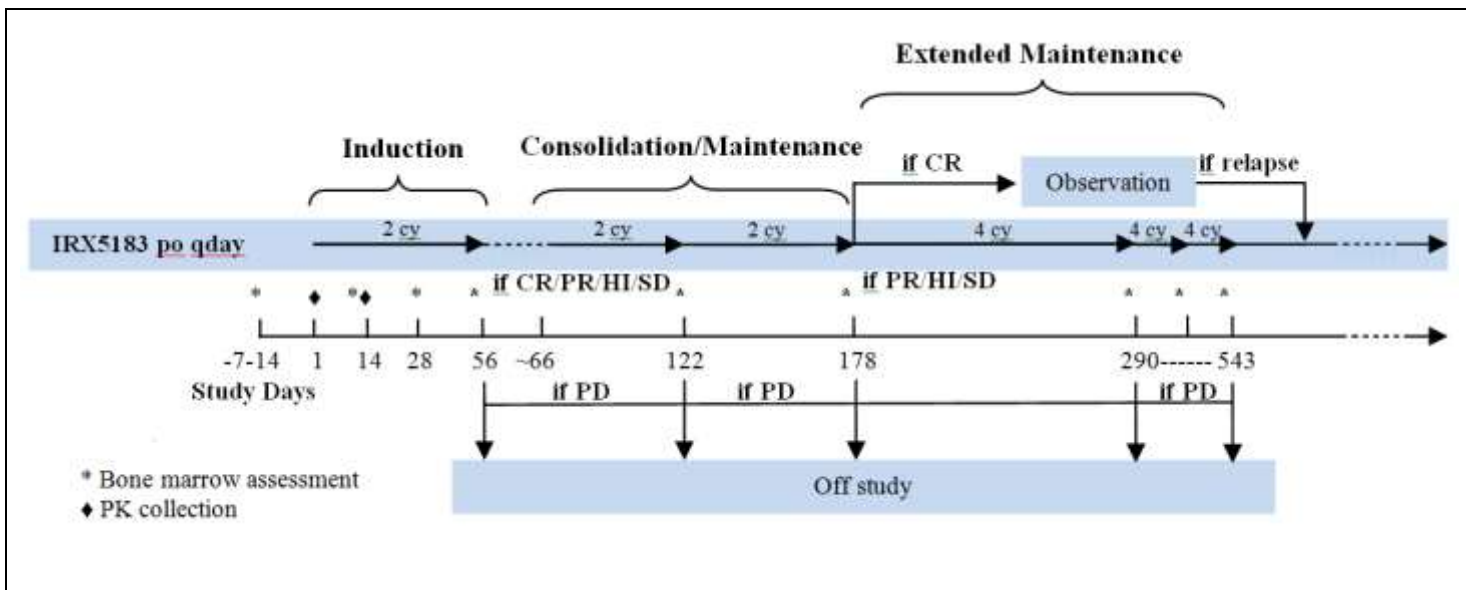
**APPENDIX D: FACT-Leukemia Scoring Guidelines (Version 4)** ..... 59

**REFERENCES** ..... 62

**STUDY SCHEMA and SYNOPSIS**

**Phase I/II study of IRX5183 in relapsed and refractory acute myeloid leukemia and high risk myelodysplastic syndrome**

<b>PROTOCOL NUMBER:</b>	J15219 (IRB00083855)
<b>DATE PROTOCOL FINAL:</b>	07/31/2015
<b>STUDY DRUG:</b>	IRX5183
<b>INDICATION:</b>	Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS)
<p><b>HYPOTHESES:</b></p> <p>The administration of IRX5183 to patients with relapsed/refractory (R/R)-AML and high-risk myelodysplastic syndrome (HR-MDS) will be well tolerated and share a similar toxicity profile of adverse events (AEs) associated with all-trans retinoic acid (ATRA).</p> <p>The net clinical effect of IRX5183 administration in patients with R/R-AML and HR-MDS will be a measurable improvement in hematologic parameters and / or a reduction in the bone marrow blast numbers.</p> <p>We predict that IRX5183 administration will lead to differentiation and apoptosis of leukemia cells in patients with R/R-AML, causing measurable laboratory parameters of differentiation, which will correlate with clinical responses and toxic events.</p>	
<p><b>STUDY OBJECTIVES:</b></p> <p>- <b>Primary objectives:</b></p> <ol style="list-style-type: none"> <li>1) To evaluate the safety and toxicity of IRX5183 in patients with R/R-AML and HR-MDS and determine the recommended phase 2 dose (RP2D).</li> <li>2) To obtain preliminary efficacy data of IRX5183 in patients with R/R-AML and HR-MDS after at least 2 cycles of therapy at the RP2D.</li> </ol> <p>- <b>Secondary objectives:</b></p> <ol style="list-style-type: none"> <li>1) To determine pharmacokinetic (PK) parameters of IRX5183 and assess bioactivity through pharmacodynamics (PD) studies.</li> <li>2) To measure clinical activity of IRX5183 as defined by best International Working Group (IWG) response at any time, time to response, improvement in transfusion requirements, cytopenias, quality of life assessments, event-free survival (EFS), and overall survival (OS) and to determine the toxicity profile of IRX5183 at the optimal dose.</li> </ol>	
<p><b>STUDY DESIGN:</b></p> <p>This is a single-center open-label prospective phase I/II study with an escalation and expansion phase evaluating the use of CYP26 resistant RAR<math>\alpha</math> specific retinoid IRX5183 in 1) patients with relapsed and/or refractory AML and 2) patients with high-risk MDS or CMML.</p> <p>The treatment scheme is summarized below and the dose escalation of IRX5183 is shown in <b>Table 1</b>. IRX5183 will be administered orally daily on days 1-28 of each cycle for 2 cycles of induction. In the phase I part of the study, there will be 3 dose levels (dose level 1 [DL1] with 50 mg, DL2 with 75 mg, and DL3 with 100 mg), with 1 additional dose level to be only used if excessive toxicity noted at the DL1. There will be no intra-patient dose escalation. In the phase II part of the study, we will use the optimal dose identified in phase I and will aim to recruit a total of 27 patients per a Simon's 2 stage design, using a type I error rate of 0.05, power of 0.8, response probability of poor drug 0.05, and response probability of good drug 0.2. The first stage sample size will be 13 and if we do not reach the pre-specified requirement of at least one response (complete remission [CR] or partial response [PR] for AML <i>or</i> CR or PR or hematologic improvement [HI] for MDS), the trial will be aborted. After induction, all patients who do not experience significant toxicity or disease progression will continue on a consolidation/maintenance phase of the study in which 4 additional 28-day cycles of IRX5183 will be administered. This phase will use the same dose used in the induction phase for each individual patient. Patients achieving CR after consolidation/maintenance will enter an observation phase, with a chance to re-enter the study at relapse, and patients with a PR, HI, or stable disease (SD) after consolidation/maintenance will be able to remain on study for extended maintenance for an additional year. Select patients may be eligible to continue beyond this point per the discretion of Io Therapeutics and the principal investigator (PI).</p>	



**Table 1: Dose Escalation of oral IRX5183 administration in phase I part of the study.** \*: DL(-1) will only recruit if the maximally administered dose was reached at DL1. DL: dose level.

Dose level (DL)	Daily dose (mg)
DL(-1)*	25
DL1	50
DL2	75
DL3	100

**STUDY ENDPOINTS**

**- Primary endpoints:**

- 1) The primary endpoint of *phase I* is a description of toxicity of IRX5183 by grading and tabulation using NCI-CTC Version 4.0 criteria, in order to determine DLTs and the RP2D.
- 2) The primary endpoint of *phase II* is best response after at least 2 cycles of therapy per IWG criteria (**Appendix A**).

**- Secondary endpoints:**

- 1) Secondary endpoints for *phase I* are a description of PK parameters of IRX5183 at baseline and day 14, both in the peripheral blood and BM compartments, markers of myeloid differentiation by flow cytometry (e.g. CD15, CD11b), BM blast count, ANC before and after treatment, and changes in detectable cytogenetic abnormalities in the blood and BM compartments at the different dose levels. We will also describe best response per IWG criteria at any time, time to first and best response, and clinical parameters such as transfusion requirements, blood counts, quality of life assessments, and time to either progression or death.
- 2) Secondary endpoints for *phase II* are a description of IWG response and other clinical criteria assessed in the dose escalation phase for the RP2D. We will also measure markers of differentiation assessed in the dose escalation phase for the RP2D, and lastly, the toxicity profile at the RP2D dose.

## 1. OBJECTIVES

### HYPOTHESIS:

We hypothesize that the administration of IRX5183 to patients with relapsed/refractory acute myeloid leukemia (R/R-AML) and high-risk myelodysplastic syndrome (HR-MDS) will be well tolerated and share a similar toxicity profile of adverse events (AEs) to all-trans retinoic acid (ATRA). We hypothesize that the net clinical effect of IRX5183 administration in patients with R/R-AML and HR-MDS will be a measurable improvement in hematologic parameters and/or a reduction in bone marrow (BM) blast numbers. We predict that IRX5183 administration and consequent differentiation and apoptosis of leukemia cells will lead to measurable laboratory parameters of differentiation and these will correlate with clinical responses and toxic events.

### 1.1. PRIMARY OBJECTIVES:

- 1.1.1. To evaluate the safety and toxicity of IRX5183 in patients with R/R-AML and HR-MDS and determine the recommended phase 2 dose (RP2D).
- 1.1.2. To obtain preliminary efficacy data of IRX5183 in patients with R/R-AML and HR-MDS after at least 2 cycles of therapy at the RP2D.

### 1.2. SECONDARY OBJECTIVES:

- 1.2.1. To determine pharmacokinetic (PK) parameters of IRX5183 and assess bioactivity through pharmacodynamics (PD) studies.
- 1.2.2. To measure clinical activity of IRX5183 as defined by best International Working Group (IWG) response at any time, time to response, improvement in transfusion requirements, cytopenias, quality of life assessments, event-free survival (EFS), and overall survival (OS) and to determine the toxicity profile of IRX5183 at the optimal dose.

## 2. BACKGROUND

### 2.1. Acute myeloid leukemia:

Acute myeloid leukemia (AML) describes a group of heterogeneous disorders characterized by malignant transformation of hematopoietic stem cells (HSCs) involving both maturation and apoptotic defects leading to an overabundance of immature myeloid cells and suppression of normal hematopoiesis. Although AML affects people of all ages, the median age at diagnosis is 67 years of age and is most frequently diagnosed (22.9% of new cases) between the ages of 75 and 84. AML is more common in whites (3.4 to 5 per 100,000 females and males, respectively) (Howlader N, 2015).

Biologically, AML is a heterogeneous array of disorders and can arise *de novo* or secondarily as a consequence of exposure to environmental toxins such as benzene, ionizing radiation, or previous chemotherapy (topoisomerase II inhibitors or alkylating agents). Although the complexities of genetic abnormalities resulting in AML are yet to be fully characterized, it is well established that abnormal expression of oncogenes and tumor suppressor control within a pluripotent stem cells lead to abnormal myeloid maturation and resistance to apoptosis (Godwin & Smith, 2003).

Prognosis is dependent on a number of factors including age at diagnosis, specific cytogenetic abnormalities, white blood cell count at time of presentation, previous chemotherapy or exposure to toxins, and previous hematologic problems. Cytogenetic abnormalities such as inversion 16, t(8;21) and t(15;17) are associated with a good prognosis while abnormalities involving loss of 5 or 7, partial deletions of 5 or 7,

and complex cytogenetics are associated with a poor prognosis. Characteristic cytogenetic abnormalities are seen in treatment-related AML with unbalanced translocations or deletions of 5 and 7 seen with alkylating agent exposure and balanced translocations involving the 11q23 MLL gene with topoisomerase II inhibitor exposure. In addition, AML associated with myelodysplasia is associated with a poor prognosis.

#### Conventional Frontline Treatment:

Standard chemotherapy in younger patients with a good performance status involves induction chemotherapy using cytarabine and an anthracycline, either daunorubicin or idarubicin, and can yield a complete remission (CR) rate of 60-70%. Consolidation chemotherapy is then required to remove any microscopic disease (Hiddemann et al., 2005). Despite remission after induction and consolidation chemotherapy, the risk of recurrence is approximately 50-60% (Schumacher, Alvares, Blough, & Mazzella, 2002). Patients who are poorer risk in terms of cytogenetic abnormalities or age may be offered other treatment modalities. For patients who are older or are not able to tolerate intensification of therapy to bone marrow transplant, other drug modalities may be employed including but not limited to more biologically targeted agents such as FLT-3 inhibitors, immunomodulatory therapies, DNA methyltransferase inhibitors, farnesyltransferase inhibitors, etc (Norsworthy, Luznik, & Gojo, 2012). For those patients who are higher risk disease and are younger with a good performance status, proceeding with allogeneic bone marrow transplantation in CR1 or in CR2 is an option.

#### Allogeneic Bone Marrow Transplant:

Allogeneic bone marrow transplantation is a treatment modality considered to be a possible curative option for patients with AML. It is typically reserved for patients with intermediate to high risk AML and those whose age and performance status permit the increased intensity of the treatment. According to the Center for International Blood & Marrow Transplant Research (CIBMTR) data, outcomes for an HLA-matched sibling transplant for adult patients 20 years or older in CR1 showed 58% survival at 3 years and in CR2 a 50% survival at 3 years. For a matched-unrelated donor transplant survival outcomes were slightly lower at 49% in CR1 and 47% in CR2 at 3 years (Pasquini MC, 2014). In recent years, the development of approaches to limit graft-versus host disease in half-matched donors have allowed for transplantation of HLA haplo-identical bone marrow with similar outcomes (McCurdy et al., 2015). Limitations of allogeneic bone marrow transplantation include co-morbidities, performance status, treatment-related complications including graft versus host disease and infectious complications. Because of these limitations, transplantation is not an option for everyone and alternative treatment strategies need to be investigated.

#### Conventional salvage/relapse therapy:

Relapse is unfortunately a far too common occurrence for AML patients and there is no one standard of care. Multiple regimens have been studied, but popular ones include MEC (mitoxantrone, etoposide, and cytarabine), HAM (high-dose cytarabine and mitoxantrone), CLAG-M (cladribine, cytarabine, G-CSF, and mitoxantrone), and FLAG-ida (fludarabine, cytarabine, G-CSF, and idarubicin). Response rates can be as high as 40-60% in patients with prolonged first remissions beyond 1 year. However, in patients with shorter CR durations, response rates are dismal at approximately 10-15%, with the exception of core-binding factor AML or CEBPA double mutants (Thol, Schlenk, Heuser, & Ganser, 2015). In addition, many patients in the relapsed and refractory setting are not fit for administration of these chemotherapeutic regimens. Studies looking at hypomethylating agents in the relapsed and refractory setting have shown overall response rates of 15-20% (Itzykson et al., 2015; Ritchie et al., 2013) with overall survival of about



6-8 months; poor risk cytogenetics and high peripheral blast count predicted for poor response. Given these unfavorable response rates, the National Comprehensive Cancer Network (NCCN) recommendations are for a clinical trial in this setting.

## **2.2. Myelodysplastic syndrome:**

Myelodysplastic syndromes (MDS) are a complex and heterogeneous group of disorders characterized by ineffective hematopoiesis manifest by cytopenias, dysplasia, and increased risk of infection. In the general population the incidence is approximately 5 cases per 100,000 people; however, as age increases the incidence rises to approximately 50 cases per 100,000 people age over 70 (Howlader N, 2015). Complete understanding of the pathophysiology of MDS is yet to be elucidated. However, it is generally accepted that there is an initial genetic event within a pluripotent progenitor cell followed by a sequence of inflammation with increased release of cytokines such as TNF-alpha and Interferon-gamma. This all occurs in the setting of an initial pro-apoptotic and proliferative state lending some explanation to the typically seen hypercellular marrow with peripheral cytopenias. Prognosis is predicted by cytogenetics, number of cytopenias, and blast percentage and is calculated by the International Prognostic Scoring System (IPSS) and revised IPSS (IPSS-R) (Appendix B).

### Conventional Treatment of MDS:

There are four major generalizable goals of medical treatment: 1) Control the symptoms caused by cytopenias; 2) Decrease progression to AML; 3) Improve overall survival; 4) Improve overall quality of life (Cheson et al., 2006). For those individuals of advanced age or poor performance status options are limited to supportive care in the form of transfusions, growth factor support with erythropoietin or G-CSF, and prophylactic antibiotics (Blinder & Roboz, 2003). For those individuals of younger age and good performance status treatment is more aggressive and focused on cure with hematopoietic stem cell transplant the only current option (Benesch & Deeg, 2003). Patients who are eligible for curative allogeneic transplant will not be candidates for this trial.

### Supportive Care:

Due to the usual advanced age of patients at presentation, poor performance status, or low risk disease, supportive care in the form of transfusions, prophylactic antibiotics, and growth factor support are sometimes the only option for treatment. Erythropoietin has been used in MDS patients with significant anemia; however, the responders are typically those patients who are already transfusion independent and only yield a hematologic response of approximately 16%. In combination with G-CSF, the response rate can be increased to approximately 36-40% (Stein, 2003). G-CSF is only recommended in situations of repeated neutropenic fevers or infections or in combination with Erythropoietin support. Due to significant costs and low response rate in transfusion-dependent patients, long-term erythropoietin is not of overall long-term benefit.

### Differentiation/Epigenetic Modification Agents:

DNA methylation and histone acetylation are two areas of intense investigation in epigenetic modulation for MDS treatment. DNA methyltransferase inhibitors, such as 5- azacytidine, are now approved for use in high risk MDS patients and yield an overall response rate of approximately 40-63% (Silverman, 2004). These drugs lead to demethylation by incorporating into DNA as nucleoside analogues and subsequently

inhibiting the methyltransferase activity, a process which appears to be both dose and time-dependent (Kizaki & Koeffler, 1992). Histone acetylation and deacetylation also play a significant role in expression of DNA and process is being targeted in investigational MDS therapy. The intricacies of histone deacetylase inhibition are beyond the scope of this background introduction but in brief involve multiple genes affecting differentiation (p21), cell cycle (p21/p27/ upregulation of Cyclin A, and D/CDK4 downregulation), and apoptosis alterations (Bhalla, 2005).

#### Immune Therapy:

Many studies have substantiated the role of the T cell activity in MDS pathogenesis. Anti T-cell therapies have been evaluated by multiple investigators and include such agents as ATG and cyclosporine serving as immunosuppression in patients with MDS (Jonasova et al., 1998; Molldrem et al., 1997). Additionally, the basis for the graft versus leukemia/MDS effect in allogeneic bone marrow transplant lends weight to the argument for immune system involvement in the pathogenesis of MDS as allogeneic bone marrow transplant is the only current curative regimen for MDS.

The role of other methods of immune modulation has been illustrated with trials involving thalidomide and lenalidomide. Thalidomide has been shown to cause a shift of T cell response from a Th1 to a Th2 response and inhibit production and secretion of TNF alpha (Candoni et al., 2004). A 2001 study showed that thalidomide was effective at improving cytopenias in certain MDS patients (Raza et al., 2001). Lenalidomide, a thalidomide analogue with fewer side effects, was also shown to have a hematologic response (Catenacci & Schiller, 2005). These studies, and multiple others, have been done and substantiate the immune modulatory effects of these agents on patients with MDS.

#### Allogeneic Bone Marrow Transplantation:

Allogeneic bone marrow transplant is the only curative treatment option for patients with MDS. The European Society for Blood and Marrow Transplantation (EBMT) reported a 36% disease free survival and a 37% non-relapse mortality at 3 years in a group of 885 patients receiving allogeneic sibling transplants for MDS (de Witte et al., 2000). The same group later reported similar data for unrelated peripheral blood and cord blood transplants (Robin et al., 2015). The CIBMTR reported a 3-year probability of survival of 53% and 49% for recipients of sibling and unrelated donor transplants for early MDS, respectively, and among patients with advanced MDS, probabilities were 45% and 39% (Pasquini MC, 2014). Overall, transplant data shows that patients with low level of disease have a better outcome with transplant (Sierra et al., 2002). In recent years, with the development of post-transplant cyclophosphamide, haplo-identical transplants have been an excellent option for patients with MDS without a matched sibling donor, with one series showing PFS of 41% for patients with MDS and AML (Di Stasi et al., 2014).

Many factors limit the use of transplant. The majority of patients with MDS are older than 60 and often have a poor performance status. Furthermore, there is significant potential for treatment related morbidity and mortality. Because MDS is a heterogeneous population of disease with a wide spectrum of clinical severity where the sole current curative measure of transplantation carries with it significant risk, other options for treatment are in high demand.

#### Treatment of refractory disease:

Patients with MDS unfortunately have dismal outcomes after failure of demethylating agent therapy. One series of 435 patients with high-risk MDS and former refractory anemia with excess blasts in transformation (RAEB-T) after azacitidine treatment failure had an overall survival of only 5.6 months and 2-year survival of 15%. In this study, outcomes were improved when patients received allogeneic stem cell transplant or an investigational therapy (Prebet et al., 2011). Furthermore, a small retrospective analysis showed that treatment with decitabine post-azacitidine failure in patients with MDS or AML resulted in stable disease (SD) in only 20% of patients, with no CRs or PRs, and OS was 5.9 months (Duong et al., 2015). Per these observations and lack of other approved therapies, NCCN guidelines dictate that clinical trials or best supportive care are the best and only options for these patients.

### **2.3. Previous Cell Differentiation Therapies in Myeloid Malignancies:**

Acute promyelocytic leukemia (APL) is a prime example of success with the differentiation therapy all-trans retinoic acid (ATRA). A characteristic genetic finding in APL, the PML/RARA fusion protein, likely mediates the ability of ATRA to induce differentiation of leukemic blasts. However, it is unlikely that APL is unique among neoplastic diseases in its responsiveness to differentiation therapy. With standard induction chemotherapy and observation in patients with APL, the disease free survival at 5 years was less than 20% compared to greater than 70% DFS with ATRA induction and maintenance (Tallman et al., 2002). Thus, ATRA has become the standard of care for treatment of APL. In recent years, we have been able to completely eliminate conventional chemotherapy from the treatment of low-risk APL with the combination of ATRA and arsenic. Lo-Coco et al showed that this therapy was non-inferior to ATRA-chemotherapy, and perhaps even superior with EFS of 97% at 2 years with ATRA-arsenic versus 86% with ATRA-chemotherapy (Lo-Coco et al., 2013). Unfortunately, ATRA has had limited success in non-APL AML (Burnett et al., 2010; Burnett et al., 2007; Estey et al., 1999; Milligan et al., 2006; Schlenk et al., 2004); similarly, other differentiating agents have failed to make a major clinical impact for unclear reasons.

A second example of effective differentiation therapy is the use of myeloid growth factors, namely GM-CSF, in the differentiation of the earliest progenitors of CML. During normal hematopoiesis, homeostasis is maintained by the balance between self-renewal and differentiation; however, specific genetic changes within tumor cells may lead to the preferential enhancement of proliferation or survival by growth factors and result in clonal expansion. Previous laboratory studies have demonstrated that p210 expression in CML increases myeloid cell survival by inhibition of apoptosis (Bedi et al., 1993). Previous clinical studies have explored the role of myeloid growth factors in eradication of CML via differentiation. One phase II trial performed prior to the approval of imatinib showed that interferon in combination with GM-CSF resulted in cure in a small proportion of patients with CML (Zeidner et al., 2014). Another study utilized GM-CSF in the setting of autologous BMT in patients with CML (Gladstone et al., 1999). Autologous bone marrow grafts were treated *in vitro* with GM-CSF and growth factor was administered to patients following grafting. All patients engrafted Ph- indicating that *in vitro* graft treatment preferentially expanded normal hematopoietic progenitors.

Extensive *in vitro* and *in vivo* investigations have shown that combining growth factors with differentiating agents can enhance the anti-leukemic activity. GM-CSF enhances the differentiation activity of various agents such as interferon-alpha, ATRA, arsenic trioxide, and bryostatins (Angstreich et al., 2005; W. Matsui et al., 2005; W. H. Matsui et al., 2002; Smith et al., 2011; Zeidner et al., 2014). For example, bryostatins-1 acts as a partial agonist of protein kinase C (PKC), and previous studies found that it inhibited the clonogenic growth of AML cell lines by inducing cell cycle arrest and differentiation (Berkow & Kraft,

1985; Grant, Pettit, Howe, & McCrady, 1991; Vrana, Saunders, Chellappan, & Grant, 1998). Our group found that the addition of myeloid growth factors markedly enhanced leukemic differentiation, and neutralizing antibodies directed against myeloid growth factors blocked the differentiating potential of bryostatin-1, suggesting that growth factors were necessary for its full anti-leukemic activity (W. Matsui et al., 2005; W. H. Matsui et al., 2002). We studied the combination of bryostatin and GM-CSF in a phase I trial in poor-risk myeloid malignancies (Smith et al., 2011). The combination improved neutrophil counts and led to rare clinical responses, but severe myalgias and arthralgias limited dose escalation. Our group also showed that GM-CSF substantially increases the differentiation potential of the synthetic retinoid bexarotene and the HDAC inhibitor entinostat (formerly MS-275), in leukemia cell lines, as measured by increased expression of differentiation markers by flow cytometry and decreased clonogenic growth (Eunpi et al., 2010).

Our group previously conducted two companion phase II trials investigating dual differentiation and growth factor therapy in patients with high-risk MDS or relapsed/refractory AML by combining GM-CSF with agents known to differentiate AML *in vitro* but with different mechanisms of action: bexarotene (BEX), a retinoid, and entinostat (ENT), a histone deacetylase (HDAC) inhibitor (Eunpi et al., 2010; Norsworthy KJ, 2015). 26 patients received BEX + GM-CSF and 24 patients received ENT + GM-CSF. Of the 13 patients treated with BEX who completed 2 or more cycles, 5 patients had hematologic improvement (HI) for an overall response rate (ORR) of 38%, and 3 had SD. Of the 10 patients treated with ENT who completed 2 or more cycles, 1 patient had a partial response (PR) and 5 had HI for an ORR of 60% and 1 had SD. Both combinations showed a trend in improved neutrophil count with near doubling of mean ANC for BEX and a near four-fold expansion in the ENT study without increase in peripheral blast counts in either group. The drugs were well tolerated with similar toxicity profiles. Neither agent resulted in CRs in the poor-risk cohorts studied, but many had HI or stabilization of disease, supporting the impact of the combination strategy on differentiation.

#### **2.4. Impact of bone marrow microenvironment on sensitivity to retinoids:**

APL and most non-APL AMLs undergo terminal differentiation and elimination by ATRA *in vitro*. However, as mentioned above, ATRA has not proven effective in non-APL AML in randomized clinical trials to date (Burnett et al., 2010; Burnett et al., 2007; Estey et al., 1999; Milligan et al., 2006). Retinoic acid (RA) plays a significant role in the differentiation of hematopoietic stem cells (HSCs). Recent data from our group show that the cytochrome P450 enzyme CYP26 expressed in bone marrow (BM) stromal cells inactivates RA, thereby limiting differentiation of HSCs (Ghiaur et al., 2013). We have also shown that several APL and non-APL AML cell lines are sensitive to RA-induced differentiation and elimination *in vitro*, but this was prevented in the presence of BM stroma; addition of the CYP26 inhibitor R115866 reversed stroma-induced resistance to RA (Su et al., 2015). Use of R115866 in a clinical trial is not possible due to lack of availability.

A second strategy is to use a retinoid resistant to metabolism by CYP26. IRX5183 and AM80 are synthetic retinoids resistant to CYP26 metabolism, 10 times more potent than ATRA as *in vitro* inducers of differentiation in APL cells, and have been studied clinically to treat APL and other malignancies (Osanaï & Petkovich, 2005; Sanford D, 2014). IRX5183 is more selective for retinoic acid receptor alpha than AM80, potentially leading to a milder side effect profile. Preliminary clinical experience with IRX5183 in a phase I/II study in solid tumor patients showed the drug was well tolerated without any serious adverse events; it has also been used in a few patients with newly diagnosed APL (company data). Data from our laboratory show that both AM80 and IRX5183 are resistant to stroma-mediated inactivation, similar to the

combination of ATRA and R115866.

## 2.5. **IRX5183:**

IRX5183 is a synthetic retinoid produced by Io Therapeutics, Inc. that specifically targets retinoic acid receptor alpha (RAR $\alpha$ ). Understanding of the function of the retinoid family of receptors is crucial for comprehension of the function of IRX5183 in treatment of AML. Although an extensive review is beyond the scope of this protocol background, the general principles in retinoid acid receptors are discussed below. In general, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are ligand-dependent transcription factors that are a part of the steroid/thyroid family. RARs play an important role in the differentiation and growth regulation of many adult tissues, especially hematopoiesis, with 6 different domains involved in constitutional transcript activation, DNA binding, ligand binding, dimerization, and ligand-induced transcriptional activation (Morosetti et al., 1996). Metabolites of Vitamin A bind to the RARs but do not bind to the RXRs. The RARs and RXRs have integrated function and alter gene transcription based on their dimerization either as homodimers or heterodimers (Morosetti et al., 1996). An example of the interaction between the RARs and RXRs and treatment with retinoids that bind the RARs is seen with all-trans retinoic acid (ATRA) for acute promyelocytic leukemia. The overwhelming success of ATRA inducing terminal differentiation of blasts in APL is an example of use of agents that affect the RARs and RXRs that is now the standard of care for treatment of APL.

ATRA is known to target RAR $\alpha$ ,  $\beta$ , and  $\gamma$  at pharmacologic concentrations, but only RAR $\alpha$  and  $\gamma$  are expressed in immature and maturing hematopoietic cells. Preclinical studies by Chee et al suggested that ATRA enhances hematopoietic stem cell self-renewal through RAR $\gamma$ , while promoting differentiation of committed myeloid progenitors through RAR $\alpha$  activation (Chee, Hendy, Purton, & McArthur, 2013). They found that IRX5183 decreased clonogenicity and increased mature myeloid markers by flow in AML1-ETO-expressing murine BM progenitors *in vitro*, whereas ATRA had the opposite effect (potentiation of clonogenicity and immature myeloid phenotype by flow), suggesting the efficacy of a RAR $\alpha$  specific agent in non-APL AML. In addition to being a pan-RAR agonist, ATRA is known to induce CYP26 expression, which likely impacts its bioactivity (Ozpolat, Mehta, Tari, & Lopez-Berestein, 2002). In contrast, metabolism studies in liver microsomes from multiple species, including human, indicate that IRX5183 is not subject to oxidative metabolism (Investigator's Brochure, 2008).

Unlike other retinoids with pan RAR activity, IRX5183 did not induce dermal irritation in a mouse model. However, it does retain the hypertriglyceridemic effect of other retinoids. A receptor-specific retinoid such as IRX5183 may induce fewer adverse effects when compared to a receptor-non-specific retinoid such as ATRA, while retaining the modulation activity of the cell cycle. The clinical use of ATRA has been complicated by the development of ATRA resistance due to pharmacokinetic reasons. Chronic oral ATRA therapy in APL patients results in progressively lower blood levels of ATRA presumably because of autoinduction of metabolism (Adamson, 1996). In contrast, structural features incorporated into the IRX5183 molecule have been designed to make the compound resistant to this degradative catabolism. Full pharmacokinetic evaluation in rats demonstrates that, for the most part, maximal concentrations (C<sub>max</sub>) and area-under-the-curve (AUC) values are not changed between days 8 and 30 at any of the doses tested. Similar evaluations in dogs show that IRX5183 C<sub>max</sub> and AUC values are unchanged between days 7 and 28. This pharmacokinetic profile observed in rats and dogs appears to be retained in humans, where C<sub>max</sub> and AUC values on day 14 were increased relative to day 0 after the first dose (Investigator's Brochure, 2008). Therefore, persistently high blood drug levels should be obtained upon chronic oral dosing with IRX5183.

IRX5183 was studied in an animal model of t(8;21) AML1-ETO, where fetal liver cells were transfected and transduced with AML1-ETO9a plasmids and transplanted into irradiated Ptpcr mice (Chee et al., 2013). The mice developed leukemia by 5 weeks post-transplant. IRX5183 or DMSO was given orally at 45 mg/kg daily six days per week for four weeks. The IRX5183 treated mice had significantly lower total white blood cell counts, peripheral blood blasts, and green fluorescent protein positive cells two weeks after completion of treatment. In addition, they had significantly more maturing intermediate myeloid cells and neutrophils. There was no effect on survival of the mice, but treatment ceased after only four weeks of therapy; after drug withdrawal, the blast cell population expanded and ultimately led to death from leukemia.

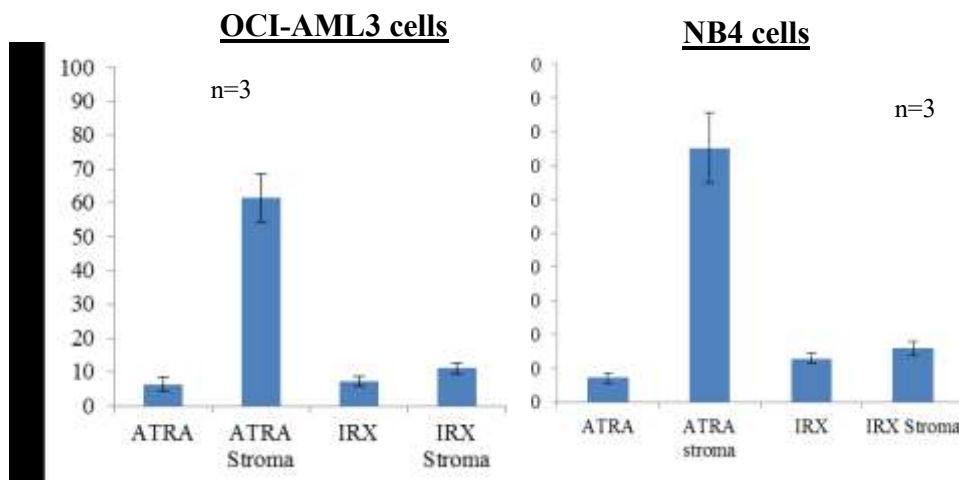
IRX5183 has been used in humans in two separate studies. The first was a single-center, phase I trial evaluating the safety, maximum tolerated dose (MTD), and pharmacokinetics of IRX5183 administered daily for a minimum of 4 weeks, performed in patients with refractory malignancies. The starting dose was 60 mg/m<sup>2</sup>/day as a single daily dose in the morning based on pre-clinical data. A total of 15 patients were accrued, and 12 completed at least one 4-week cycle of therapy. Due to dose-limiting toxicities (DLTs), the dose was reduced to 30 and then 15 mg/m<sup>2</sup>, with 15 mg/m<sup>2</sup> determined to be the MTD. 3 of 4 DLTs were elevated alkaline phosphatase (all in patients with liver metastases and 2 had elevated alkaline phosphatase at baseline). The 4<sup>th</sup> DLT was grade 3 mucosal inflammation. 10 patients came off study because of progressive disease (PD) and no antitumor activity was observed. The drug was absorbed rapidly and reached peak concentrations approximately 1 to 3 hours following dosing. The terminal elimination half-life ranged from 3.3 to 16.1 hours on the first day of dosing. Based on persistently high drug levels, stable anti-proliferative, pro-differentiation and anti-apoptotic effects could be expected with oral IRX5183.

The second trial evaluating IRX5183 in humans was a randomized, open label, single center phase I/II trial studying peripheral blood progenitor cell (PBPC) mobilization in combination with G-CSF, with intra-patient control and an additional control arm of two mobilizations with G-CSF alone, after high dose chemotherapy in patients with multiple myeloma and lymphoma. For the intra-patient control, each patient underwent 2 mobilizations and mobilization variables were compared between each episode. IRX5183 was given at a dose of 60 mg/m<sup>2</sup> daily and G-CSF was given at a dose of 10mcg/kg daily. 8 patients were accrued to the study and patients were administered IRX5183 60 mg/m<sup>2</sup> daily for up to 28 days. No serious adverse events associated with IRX5183 were reported and no DLT was observed. The conclusion from the study was that IRX5183 and G-CSF in combination was unlikely to benefit poor progenitor cell mobilizers, but it may benefit good mobilizers, particularly if pretreated prior to a second mobilization with G-CSF alone.

#### Preclinical studies at Johns Hopkins:

Preclinical studies were conducted in the labs of Gabriel Ghiaur and Richard Jones at Johns Hopkins Oncology Center. Leukemia cell lines NB4 (an APL cell line with t(15;17)), OCI-AML3 (an AML cell line with NPM1 mutation), and Kasumi-1 (AML cell line with t(8;21)) were used in the experiments. Cells were incubated with control media vs. equal concentrations of ATRA or IRX5183 in the presence and absence of BM stroma. Differentiation was assessed via flow analysis for marker CD11b and clonogenic growth was assessed using a colony forming unit assay. ATRA and IRX5183 showed similar increased differentiation and inhibition of clonogenic recovery compared to control in liquid culture, but in the presence of BM stroma, only IRX retained its efficacy (Figure 1, unpublished data). Therefore, IRX5183 is resistant to inactivation by stromal CYP26.

**Figure 1: Stroma blocks ATRA-mediated, but not IRX5183-induced elimination of AML.** Colony forming unit (CFU) experiments with (A) OCI-AML3 and (B) NB4 cells treated with ATRA or IRX5183 at doses of  $10^{-6}$  and  $10^{-7}$ M for the different cell types, respectively, showed a decrease in clonogenic growth compared to control with IRX5183 both off and on stroma.



We additionally incubated NB4 cells with CD437, a RAR $\gamma$  specific agent that is also resistant to metabolism by CYP26, and this resulted in a complete lack of increase in differentiation markers compared to control and no decrease in clonogenic recovery. Therefore, targeting RAR $\gamma$  had no impact on cellular differentiation. qRT-PCR experiments showed that IRX5183 had minimal impact on CYP26 expression in OP-9 stromal cells, unlike ATRA and CD437, suggesting the impact of RAR $\gamma$  in the marrow microenvironment is induction of CYP26, while targeting of RAR $\alpha$  leads to myeloid differentiation. Moreover, IRX5183 is a RAR $\alpha$  specific agent that is able to bypass metabolism by CYP26 in BM stroma. The demonstrated preclinical data in combination with the successful use of retinoids in APL provides the foundation for the proposed trial.

## 2.6. Rationale:

We postulate that, to date, retinoids have not been proven effective in the treatment of non-APL AML due to stromal protection by CYP26. Our lab has published recently that this mechanism of stromal resistance to cancer therapies is not limited to ATRA. There are a multitude of liver enzymes present in the BM stroma, including CYP3A4, which metabolizes BEX and etoposide, among many other agents, and cytidine deaminase, which metabolizes cytarabine (Alonso et al., 2015). BEX + GM-CSF previously showed some differentiating ability in our phase II trial, but may have not been fully effective due to high CYP3A4 expression. IRX5183 is a novel retinoid, which as a single agent bypasses this mechanism of protection afforded leukemia cells in the BM microenvironment. In addition, its RAR $\alpha$  specificity is attractive as RAR $\gamma$  does not appear to have an effect on differentiation and may lead to additional side effects. The preclinical data with IRX5183 inducing differentiation in leukemia cell lines and the prior *in vivo* mouse model give support for proceeding with a trial of single agent IRX5183 in AML and HR-MDS. In addition, the oral bioavailability, and good tolerance of IRX5183 in solid tumor patients previously make it an attractive agent to study. Given that the phase I trial was in solid tumor patients previously, many with underlying liver dysfunction, we would like to perform a phase I/II trial in order to establish the safety and toxicity profile in patients with AML and MDS. Although there is no current data for the use of IRX5183 in myeloid malignancies to describe the baseline response we expect to see

and function as a control to which our results would be compared, there is a plethora of data with other differentiating agents in myeloid malignancies that can serve as expected control response.

### 3. PATIENT SELECTION

#### 3.1. Eligibility criteria:

The eligibility criteria are as follows:

1. Patients must be able to understand and voluntarily sign an informed consent form.
2. Age  $\geq 18$  years at the time of signing the informed consent form.
3. Able to adhere to the study visit schedule and other protocol requirements.
4. Pathologically confirmed disease with A or B as follows:
  - A. AML patients who either have:
    - 1) Relapsed or refractory disease after receiving one or more courses of induction chemotherapy, hypomethylating agent therapy, or bone marrow transplant *or*
    - 2) *de novo* AML but not deemed to be a candidate for conventional therapy based on age, co-morbidities, or patient preference
  - B. MDS, CMML, or MDS/MPN with high risk features as defined below who have relapsed after initial response or are refractory (failure to achieve a CR, PR, or HI) after receiving at least 4 cycles of hypomethylating agents 5-azacitidine or decitabine  $\pm$  other therapies  $\pm$  bone marrow transplant OR with *de novo* MDS but have refused to receive hypomethylating therapy:
    - 1) INT-2 or high IPSS score OR high or very high IPSS-R *or*
    - 2) Secondary MDS (defined as MDS developing in a patient with an antecedent hematologic disorder or any patient with prior chemotherapy or radiation exposure) *or*
    - 3) INT-1 IPSS or intermediate R-IPSS MDS with excess blasts ( $\geq 5\%$  blasts in BM) or transfusion-dependency *or*
    - 4) MDS progressing to oligoblastic AML with 21-30% BM blasts *or*
    - 5) CMML or MDS/MPN with  $\geq 5\%$  marrow blasts, transfusion-dependency, abnormal karyotype, or proliferative features (white blood cell count  $\geq 13,000/\mu\text{L}$ , splenomegaly on physical examination, or extramedullary disease)
5. ECOG performance status of  $\leq 2$  at study entry or Karnofsky  $\geq 60\%$  (**Appendix C**).
6. Laboratory test results within these ranges:
  - ❖ Creatinine level of 3 mg/dL or lower
  - ❖ Total bilirubin  $\leq 3$  mg/dL unless due to Gilbert's syndrome, hemolysis, or ineffective hematopoiesis
  - ❖ AST (SGOT) and ALT (SGPT)  $\leq 3$  x ULN
  - ❖ WBC  $\leq 10,000/\mu\text{L}$
7. Patients must not have received any other treatment for their disease, including hematopoietic growth factors, aside from hydroxyurea for count control, within three weeks of beginning the trial, and should have recovered from all toxicities of prior therapy (to grade 0 or 1).
8. Patients requiring hydroxyurea to bring WBC below  $10,000/\mu\text{L}$  prior to study enrollment will require a 48-hour washout prior to starting the study drug.
9. Women of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to start of IRX5183.
10. Patients must have no clinical evidence of central nervous system (CNS) or pulmonary leukostasis,



disseminated intravascular coagulation, or CNS leukemia.

### 3.2. **Exclusion criteria:**

1. Any serious medical condition or uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, laboratory abnormality, or psychiatric illness/social situations that would limit compliance with study requirements or prevent the subject from signing the informed consent form.
2. Any condition, including the presence of laboratory abnormalities, which places the subject at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study.
3. Use of any other experimental drug or therapy within 21 days of baseline.
4. Known hypersensitivity or history of allergic reactions attributed to compounds of similar chemical or biologic composition to IRX5183.
5. Prior use of other retinoid therapies in the 3 months prior to enrollment in the study.
6. Patients with other active cancers receiving anti-cancer agents, with exceptions being hormonal therapy for breast or prostate cancer and skin cancers treated with local therapies only.
7. Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier (e.g. alopecia, hypothyroid, neuropathy, etc.).
8. Pregnant women are excluded from this study because of potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with IRX5183, breastfeeding should be discontinued if the mother is treated with IRX5183.

### 3.3. **Pregnancy:**

The effects of IRX5183 on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of IRX5183 administration.

Sexually active women of childbearing potential (WOCBP) must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Before enrolling WOCBP in this clinical study, the investigator must review the guideline about study participation for WOCBP, which can be found in the GCP Manual for Investigators. The topics include the following:

- ❖ General Information
- ❖ Informed Consent Form
- ❖ Pregnancy Prevention Information Sheet
- ❖ Drug Interactions with Hormonal Contraceptives
- ❖ Contraceptives in Current Use
- ❖ Guidelines for the Follow-up of a Reported Pregnancy

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion. **All WOCBP MUST have a negative pregnancy test within 72 hours before receiving their first dose of IRX5183.** The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive IRX5183 and must not be enrolled in the study. **In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.**

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (*e.g.*, dose tapering if necessary for subject safety). The investigator must immediately notify Io Therapeutics of this event and record the pregnancy on the Pregnancy Surveillance Form (not an SAE form). Initial information on a pregnancy must be reported immediately to Io Therapeutics, and the outcome information provided once the outcome is known. Completed Pregnancy Surveillance Forms must be forwarded to Io Therapeutics according to SAE reporting procedures. Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (*e.g.*, X-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report and follow-up on information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.

#### 3.4. **Inclusion of women and minorities:**

Both men and women of all races and ethnic groups are eligible for this trial.

#### 3.5. **Off-Study Criteria:**

Patients may be removed from study for any of the following:

- Toxicity that the patient determines to be unacceptable.
- Physician determination that the continued participation in the study is no longer in the best interest of the subject
- 
- Patient elective withdraw of their consent.
- Progression of disease per criteria Appendix A and section 7.3 below.

#### 3.6. **Informed Consent:**

All patients eligible for the study must be evaluated by one of the study investigators. Informed consent must be obtained and the consent form signed. A Johns Hopkins on study form will be completed following fulfillment of the on-study requirements for laboratory work and eligibility criteria.

#### 4. PRETREATMENT PLAN

A complete history and physical examination and list of medications will be documented on each patient within two weeks of starting therapy. A **bone marrow aspirate and biopsy** confirming the suspected diagnosis will be documented within 2 weeks of enrollment.

**Clinical Laboratory Studies** within two weeks of starting therapy will include:

1. Hematologic studies: complete blood count with platelets and differential
2. Renal and electrolyte: chemistry panel (including electrolytes, creatinine, uric acid, albumin, total protein, calcium and phosphate)
3. Hepatic: serum SGOT, SGPT, alkaline phosphatase, total bilirubin
4. Coagulation: fibrinogen, PT/INR, PTT, D-dimer
5. Lipid Panel (fasting): Triglycerides, Cholesterol, LDL, HDL. Retinoids have been shown to increase triglycerides and cholesterol levels; thus, baseline levels will be checked per standard of care.

In addition to baseline laboratory tests and BM aspirate and biopsy, **quality of life assessment** will be performed per the FACTleu questionnaire (available at <http://www.facit.org/FACITOrg/Questionnaires>), administered with the help of the research team.

#### 5. TREATMENT PLAN

##### 5.1. IRX5183 Administration:

IRX5183 is an oral capsule that will be administered once daily. Reported adverse events and potential risks are described in Section 9. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Patients will be instructed to take the daily dose of IRX5183 each morning after food, as close to the same time as possible. IRX5183 will be self-administered at home, except for days 1 and 14 of cycle 1, on which patients will receive their doses in clinic after trough PK levels are drawn. Each patient will keep a pill diary to help with compliance. If a patient forgets to take a dose in the morning and it is before 8:00 p.m., they should be instructed to take their dose. If the subject forgets to take their daily dose and it is after 8:00 p.m., they should be instructed to wait for the next morning to dose. If vomiting occurs after dosing, the subject should not receive another dose, but instead wait until the next morning to dose.

The schedule of IRX5183 administration and the dose escalation schema are shown in figure 2 and table 1, respectively. At any point, patients will be withdrawn from treatment for undue toxicity, disease progression, and/or deterioration of performance status, per guidelines in section 3.5 above. For **induction therapy**, single agent IRX5183 will be administered orally once daily for two 28-day cycles. Bone marrow assessments will be performed after each cycle, but the primary assessment/endpoint is after cycle 2 of induction. IRX5183 may be held for 5-7 days post induction therapy to allow for study drug wash-out if the PI has concerns that the drug is limiting the interpretation of the assessment marrow testing needed to measure response. All study patients who have drug held only for marrow assessment are eligible to resume study drug following the completion of the marrow testing, while awaiting final pathology review and response assessment.

Patients achieving at least SD, i.e. CR, partial remission (PR), hematologic improvement (HI), or SD,

will be eligible to continue the study treatment planned **consolidation/maintenance therapy**, which will consist of up to four continuous 4 week (28 day) cycles using the same dose of IRX5183 used in the induction phase for each individual patient. This will be referred to as **consolidation** therapy if the patient achieves CR after induction, and will be referred to as **maintenance** if the patient achieves a PR, HI, or SD. Consolidation/maintenance therapy may be initiated following the completion of marrow assessment and no longer than 14 days after completion of induction therapy provided there is no evidence of PD. BM testing will be performed after every other cycle in the consolidation/maintenance phase of therapy. IRX5183 may be held for 5-7 days post each cycle of consolidation/maintenance where a BM assessment will be performed if the PI has concerns that the drug is limiting the interpretation of the assessment marrows. The next cycle of therapy may begin anytime the assessment marrows are completed and no longer than 14 days from the completion of the previous cycle provided there is no clinical evidence of PD.

If the patient is in a CR after 4 cycles of consolidation/maintenance, IRX5183 will be withdrawn and the patient will enter **observation** with biweekly counts for the first month, then monthly for at least 6 months, and bimonthly bone marrow assessments for at least 6 months. If their disease relapses, they will be eligible to re-initiate therapy daily in continuous 4 week cycles.

If the patient achieves at least SD, but not a CR, after 4 cycles of consolidation/maintenance, they will continue on **extended maintenance therapy**, receiving continuous 4-week cycles of daily IRX5183 for up to an additional year. Bone marrow assessments will be required at least every 4 months while the patient is on this extended maintenance phase of therapy. Drug may be held 5-7 days post each cycle of extended maintenance where a BM assessment will be performed if the PI has concerns that the drug is limiting the interpretation of the assessment marrows. The next cycle of therapy will be initiated following the completed marrow testing and no longer than 14 days after completion of the prior cycle provided there is no evidence of PD.

If any patient remains with at least SD after the 1 year of extended maintenance therapy, with no undue toxicity and in good condition, they may be eligible for **extended therapy indefinitely**, per discretion of Io Therapeutics and the Principle Investigator. These patients will continue with bone marrow assessments at least every 6 months and blood counts as clinically indicated.

### **Study Phase I:**

In the dose-escalation part of the study, we will abide by a traditional 3+3 design with 3 dose levels (dose level 1 [DL1] with 50 mg IRX5183, DL2 with 75 mg, and DL3 with 100 mg), with 1 additional dose level, DL(-1), to be only used if excessive toxicity is noted at the DL1. There will be no intra-patient dose escalation.

The first patient will enter the trial on DL1. The dose for level 1 will be a flat dose of 50 mg IRX5183 daily per the schedule above. We will enroll 3 patients at a time to determine the toxicity profile of IRX5183. If none of the 3 patients receiving DL1 experiences a DLT, another 3 patients will be treated at the next higher dose level. However, if 1 of the first 3 patients experiences a DLT, 3 more patients will be treated at the same dose level. The dose escalation will continue until at least 2 patients among a cohort of 3 to 6 patients experiences DLTs. If 2 or more patients experience a DLT on DL1, the next patient will be recruited to DL(-1). The RP2D of single agent IRX5183 will generally be the highest dose at which the DLT rate is <33% in at least six patients. If no MTD is determined, the highest dose

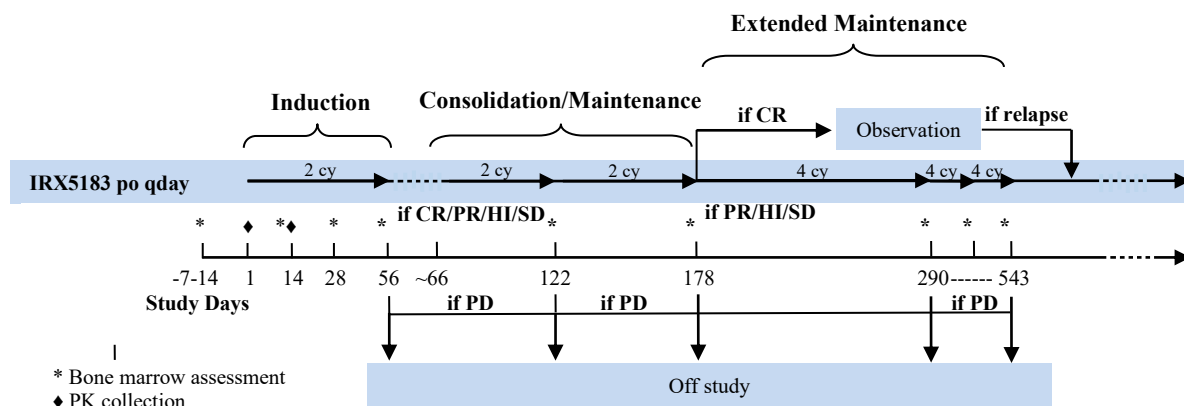
explored will be 100 mg. At the end of phase I, the RP2D will be determined after careful review of all of the clinical data available. The first 3 subjects enrolled to any cohort must have passed the 28 day DLT evaluation point before the 4<sup>th</sup> subject will begin treatment.

IRX5183 will be initiated at the company’s currently recommended dosage for use in APL patients of 50 mg daily (about 30 mg/m<sup>2</sup>/day). The drug is available in 25 mg capsules, so we will be using a flat dosage. This dose of 50 mg is above the MTD of 15 mg/m<sup>2</sup>/day from the prior phase I trial conducted in solid tumor patients. However, the DLTs seen in this trial were elevations in alkaline phosphatase in patients with known liver metastases. In addition, the phase I/II trial in patients with myeloma and lymphoma used a dose of 60 mg/m<sup>2</sup>, which was well tolerated with no serious adverse events (SAEs). The roughly 2 months of induction therapy followed by consolidation/maintenance described above is modeled after prior studies of ATRA and other synthetic retinoids, in which it can take up to 60 days to see an initial response (Lo-Coco et al., 2013; Sanford D, 2014; Tobita et al., 1997).

**Study Phase II:**

The phase II part of the study will use the optimal dose identified in the phase I part of the study and will aim to recruit a total of 27 patients with AML and HR-MDS at the RP2D (see sample size calculation in section 11). The primary goal of the therapy in this phase of the trial is to determine response after receiving at least 2 cycles of therapy per IWG criteria. Analysis of clinical response will take place after the first 13 patients complete induction therapy, and enrollment will continue to the planned 27 patients if the first stage has at least 1 patient achieve a CR or CRi or PR (AML) or CR or PR or HI (MDS). We will halt enrollment after the first 13 patients complete induction therapy until we have their response data. In addition to IWG response, many secondary endpoints will be assessed as outlined in section 11 below.

**Figure 2: IRX5183 Administration Schema.** Induction phase will consist of 56 days of oral IRX5183 daily. Consolidation/maintenance phase will begin once patient has confirmed SD or better and will continue for up to four 28-day cycles. If patient is in CR after consolidation/maintenance, will hold therapy and monitor, and resume therapy if they relapse. If they have SD or better after consolidation/maintenance, they will continue on maintenance therapy for up to 1 additional year. Select patients may be eligible to continue for longer.



**Table 1: Dose Escalation of oral IRX5183 administration in phase I part of the study.** \*: DL(-1) will only recruit if the maximally

administered dose was reached at DL1. DL: dose level.

<b>Dose level (DL)</b>	<b>Daily dose (mg)</b>
DL(-1)*	25
DL1	50
DL2	75
DL3	100

## 5.2. Definition of dose-limiting toxicity:

For purposes of dose-escalation, dose-limiting toxicity (DLT) will be defined as the occurrence of any of the following during the first 28 days of therapy:

- ❖ Any grade 3 or 4 non-hematologic toxicity with the following exceptions:
  - Transient laboratory abnormalities that can be treated or resolve to grade 2 or less within 1 week from holding study drug.
  - Grade 3-4 non-hematologic toxicities that resolve to  $\leq$  Grade 1 within 1 week of holding study drug
  - Grade 3-4 expected and known drug class-related toxicities associated with differentiation syndrome (see definition below in section 9.2.4) that resolve within 2 weeks of steroid therapy to grade 1 will be excluded as DLT for purposes of dose escalation. This is due to the fact that such events are expected of therapy with retinoids that are effectively causing differentiation. If grade 3 or above differentiation syndrome recurs after this time, it will then be considered a DLT.
  - Asymptomatic Grade 3 hypertriglyceridemia will not be considered a DLT for purposes of dose escalation.
- ❖ Grade 4 hematologic toxicity, including only *treatment-associated* aplasia (to be investigated with a BM test) that persists beyond 4 weeks. Given that severe anemia, neutropenia and thrombocytopenia are features of AML and are commonly encountered in this patient population, they will not be used to define DLT except if associated with prolonged treatment-associated aplasia.
  - If the bone marrow at the end of cycle 1 or 2 of therapy is hypoplastic, as defined by less than 5% cellularity, the patient may have a drug-free window of up to 2 weeks to look for count recovery and treatment will be re-initiated after this time if toxicity returns to  $\leq$  Grade 3

Management and dose modifications associated with the above adverse events are outlined in Section 6.

Toxicities will be assessed during the first cycle of therapy and throughout the trial to make sure there are no late adverse effects. If any chronic toxicity becomes apparent, we will evaluate whether changes in trial design are required.

Dose escalation will proceed within each cohort according to the following scheme.

**Table 2: Dose escalation rules.**

Number of Patients with DLT at a Given Dose Level (DL)	Escalation Decision Rule
0 out of 3 in DL1	Enter 3 patients at the next dose level (DL2). If DL2 the maximally administered dose (MAD), enroll 3 more pts at DL1 and if $< 2$ of 6 have DLT, DL1 will be the RP2D
1 out of 3 in DL1	Enter 3 additional patients at DL1 and if $< 2$ of 6 have a DLT,

	enter 3 patients at the next dose level (DL2)
≥ 2 out of 6 in DL1	Dose escalation will be stopped. DL1 will be declared the MAD, and DL(-1) will be the RP2D if < 2 of 6 had a DLT at that level
0 out of 3 in DL2	Enter 3 patients at the next dose level (DL3). If DL3 the MAD, enroll 3 more pts at DL2 and if < 2 of 6 have DLT, DL2 will be the RP2D
1 out of 3 in DL2	Enter 3 additional patients at DL2 and if < 2 of 6 have a DLT, enter 3 patients at the next dose level (DL3)
≥ 2 out of 6 in DL2	Dose escalation will be stopped. DL2 will be declared the MAD, and DL1 will be the RP2D if < 2 of 6 had a DLT at that level
0 out of 3 in DL3	Enter an additional 3 patients at DL3 and if < 2 of 6 have a DLT, DL3 will be the RP2D
1 out of 3 in DL3	Enter an additional 3 patients at DL3 and if < 2 of 6 have a DLT, DL3 will be the RP2D
≥ 2 out of 6 in DL3	DL3 will be declared the MAD, and DL2 will be the RP2D if < 2 of 6 had a DLT at that level

Note: DL(-1) will be only used if maximally administered dose reached at DL1. If DL(-1) is used and DLT occurred in < 2 of 6 patients, then DL(-1) will be used as the recommended phase 2 dose. If ≥ 2 out of 6 patients in DL(-1) have DLT, then the study will be terminated.

### 5.3. **General concomitant medication and supportive care guidelines:**

The patients will receive appropriate standard of care supportive measures as per the institutional guidelines of the Sidney Kimmel Comprehensive Cancer center, including but not limited to blood and platelet transfusions, antibiotics, anti-emetics, etc. Hematopoietic growth factors will not be used routinely.

#### 5.3.1. **Differentiation syndrome**

Patients with suspected evidence of differentiation syndrome, as defined in section 9.2.4 below will be admitted to the hospital and immediately started on dexamethasone 10 mg IV twice daily and continued until disappearance of signs and symptoms for a minimum of 3 days. In addition, IRX5183 will be held until resolution of symptoms, at least until improvement to grade 2 or below differentiation syndrome, while on concomitant steroids. As above, grade 3-4 signs/symptoms of differentiation syndrome that do not return to grade 1 within 2 weeks of steroid therapy will be considered a DLT.

#### 5.3.2. **Leukocytosis**

Patients who develop leukocytosis on study drug will be administered hydroxyurea at a dose of 500 mg qid for WBC between 20,000 and 50,000/ $\mu$ L and 1 g qid for WBC >50,000/ $\mu$ L. Hydroxyurea may be considered for a WBC between 10,000 and 20,000/ $\mu$ L on a case by case basis, in consultation with the study PI. Hydroxyurea can be discontinued when WBC returns below 10-20,000/ $\mu$ L, per the judgment of the investigator, and must be stopped with WBC <5,000/ $\mu$ L. If a consistent need for hydroxyurea persists beyond induction therapy, the patient will be withdrawn from the protocol.



### 5.3.3. Hypertriglyceridemia

Per the FDA drug label for the similar agent Tretinoin, “up to 60% of patients experienced hypercholesterolemia and/or hypertriglyceridemia, which were reversible upon completion of treatment. The clinical consequences of temporary elevation of triglycerides and cholesterol are unknown, but venous thrombosis and myocardial infarction have been reported in patients who ordinarily are at low risk for such complications.” In attempt to prevent such complications, patients who develop the known drug-class side effect of hypertriglyceridemia grade 3-4 (>500 mg/dL) should be treated with gemfibrozil 600 mg orally twice daily, given 30 minutes before morning and evening meals. IRX5183 should be held in the setting of grade 4 (>1000 mg/dL) hypertriglyceridemia until resolution to grade 3 or less, while on concomitant gemfibrozil. Lovaza (omega-3-acid) 4 grams orally divided qday to bid with meals may be considered on a case-by-case basis in consultation with the PI. Grade 3-4 elevations associated with clinical symptoms, such as pancreatitis, will result in permanent discontinuation of IRX5183.

### 5.4. Duration of therapy:

Treatment is planned for 56 days of induction therapy (as above) and four 28-day cycles of consolidation/maintenance in patients with at least SD after induction, with an opportunity to continue on maintenance if patients remain in at least SD after 4 cycles of consolidation/maintenance (see details in section 5.1 above), or until one of the following criteria applies:

- 1) Disease progression as defined in IWG-2003 (**Appendix A1**) for AML and IWG-2006 for MDS (**Appendix A2**).
- 2) Intercurrent illness that prevents further administration of treatment or inability of the patient to continue treatment for any other reason.
- 3) Unacceptable adverse event(s) requiring temporary or permanent stopping of study drug defined in sections 5.2 and 6.
- 4) Patient decides to withdraw from the study.
- 5) General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- 6) Pregnancy  
All WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.

The investigator must immediately notify Io Therapeutics in the event of a confirmed pregnancy in a patient participating in the study or partner of the patient.

7) Termination of the study by sponsor.

### 5.5. **Duration of follow-up:**

Patients will be followed closely throughout the study period and for 6 months after the last dose of IRX5183 or 6 months after they are removed for the presence of any toxicity (early or late).

During maintenance: Patients will be followed and seen by a member of the study team at least monthly in person, or by phone when study visits are not required.

During the 6 months after the last dose or after removal from study: All patients will be evaluated by the study team at least monthly. Evaluations may be done during routine clinic visits in patients followed longitudinally at JH or via phone for those patients followed outside of JH. The study team will note the follow-up in the medical records and source documents will be sought for any change in condition related to the study or study drug.

Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event OR for at least 6 months after removal from study. The vast majority of patients with relapsed and refractory AML will not survive past 1 year. Therefore, we expect to capture most events of progression, relapse, or death during the maintenance and follow-up phases of the study. For patients who are still alive at end of follow-up period, we will check social security Death Index every 3 months for 2 more years to obtain death dates for OS calculations.

### 5.6. **Criteria for removal from study:**

Patients will be removed from study when any of the criteria listed in Sections 3.5, 5.2, and 5.4 applies. The reason for study removal and the date the patient was removed will be documented in the Case Report Form.

Some patients may have improvement in their baseline PB counts, including a decrease in peripheral blasts following the start of IRX5183 dosing. It is also possible that peripheral counts will worsen or the number of BM blasts will increase with the initiation of therapy. In some cases, patients will not have had sufficient time to develop the required amount of differentiation to impact the counts. It is also possible that increases in blasts or other disease parameters may represent differentiation of leukemia cells in response to IRX5183. In conventional studies, such changes may be worrisome for PD and lead to the premature discontinuation of study drug. Patients with minor blast increases (defined as an increase of  $\leq 50\%$  of baseline bone marrow blast count) or worsening PB counts *prior to* week 8 may continue on study provided that they remain stable clinically. Primary tumor assessments will be made using IWG 2003 and IWG 2006 criteria at the completion of induction therapy (**Appendix A1-A3**).

## 6. **DOSING DELAYS/DOSE MODIFICATIONS:**

Patients will be monitored for toxicity during the induction, maintenance, and follow-up parts of the dose expansion phase in a similar fashion of the dose escalation phase (see calendar in

section 10) with biweekly evaluations during the first cycle of therapy, weekly for the second cycle, and then every other week during the following 4 cycles, and at least monthly after 6 months. Safety data will be captured using CTCAE criteria (version 4). Dose delays and off treatment criteria for IRX5183 associated toxicities are provided below:

Given that severe neutropenia is a common feature of advanced AML and MDS and very mild fluctuations in absolute neutrophil count can lead to significant percent change from baseline, changes in neutrophil count will not be used to hold therapy. If the patient has a neutrophil count at baseline of 1500 or more and the count drops to less than 500 at any point during administration, the nurse will notify the physician who will decide on further action at his or her discretion. Intra-patient dose reductions or escalations are not allowed.

### 6.1. Dose Modifications for IRX5183:

Decisions to hold or discontinue a specific IRX5183 dose will be made on safety criteria. Treatment with IRX5183 will be skipped or discontinued if the subject experiences at least one adverse event, specified below, considered by the investigator to be “possibly,” “probably,” or “definitely” related to IRX5183 treatment.

Any adverse event that will prompt a skipped dose or discontinuation of IRX5183 must be reported. The investigator should contact the study PI to discuss any questions.

The following criteria will be used to determine dose skipping, restarting doses, or discontinuing IRX5183 during the trial:

It is necessary to avoid study drug dosing and initiate appropriate evaluation and/or treatment for the following adverse events:

Any  $\geq$  grade 3 skin related adverse event regardless of causality.

Any  $\geq$  grade 2 non-skin related adverse event, except for easily correctable laboratory abnormalities that do not reflect underlying organ pathology.

Any  $\geq$  grade 3 laboratory abnormality, aside from cytopenias.

Any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued dosing.

It may be necessary to hold study drug to evaluate Grade 1 events that suggest ongoing or incipient differentiation syndrome, including unexplained fever, dyspnea, renal failure, hypotension, and/or unexplained weight gain until diagnosis is determined.

IRX5183 may be restarted if/when the adverse event(s) resolve(s) or returns to baseline or  $\leq$  grade 1 within 2 weeks:

If the adverse event has been determined not to be related to IRX5183: If  $>1$  dose is to be skipped or  $> 1$  week delay is expected due to current events not related to the study, the dosing schedule modifications must be discussed with the principal investigator prior to implementation.

If the adverse event has resolved to  $\leq$  grade 1, IRX5183 dosing may be restarted where the patient left off in their cycle of therapy.

The following criteria will be used to determine dose skipping, restarting doses, or discontinuing IRX5183 during *phase II* of the trial:

**Hepatotoxicity:**

Grade 3-4 hepatotoxicity according to CTCAE is defined as an increase in serum bilirubin of >3 times the upper limit of normal (ULN) or aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (AlkPhos) >5 times the ULN. During the dose expansion phase of the trial, this will be managed with temporary discontinuation and subsequent dose adjustment of IRX5183. As soon as the bilirubin, AST, ALT, and/or AlkPhos returns to Grade 2, IRX5183 will be resumed at less than or equal to 50% of the previous dose (e.g. 25 mg for 50 mg or 75 mg dosing, 50 mg for 100 mg dosing) during the first week. Thereafter, in the absence of worsening previous toxicity, IRX5183 will be resumed at full dosing. In the case of reemergence of hepatotoxicity, the drug will be permanently discontinued. These stipulations are based on prior reports that ATRA-induced hepatotoxicity improves with temporary discontinuation of the drug.

**Other non-hematologic toxicities:**

During phase II of the trial, IRX5183 should be dose reduced by 1 dose level for grade 2 non-hematologic toxicity. If the RP2D is DL(-1), this dose will be 25 mg every other day. For grade 3-4 non-hematologic toxicity, therapy will be discontinued until resolution to grade <2 and then restarted at a 2 level dose reduction. If the RP2D is DL(-1), the patient will go to 25 mg every other day since this is the lowest feasible dose. If symptoms recur to a grade 3-4 the patient will be removed from the trial.

**Myelosuppression:**

If the bone marrow at the end of cycle 1 or 2 of therapy is hypoplastic, as defined by less than 5% cellularity, and the patient has grade 4 hematologic toxicity per CTCAE, the patient may have a drug-free window of up to 2 weeks to look for count recovery. Treatment will be re-initiated after this time. For grade 4 hematologic toxicity that is treatment-related and lasts more than 5 weeks, one DL reduction will be recommended. If the patient is on DL(-1), the dose will go to 25 mg every other day. If myelosuppression lasts beyond 7 weeks or persists for 2 consecutive courses, bone marrow aspirate will be collected and assessed for disease progression. In the case of PD, patients will come off study. If the patient has SD or better, they can go back on therapy on the next lower dose than the previous. If the patient was already taking IRX5183 25 mg every other day, they will stop therapy for up to 2 weeks to allow for count recovery prior to resuming the same dose.

The following criteria will be used to determine dose skipping, restarting doses, or discontinuing IRX5183 during the *all phases* of the trial:

**Differentiation syndrome:**

Grade 3-4 toxicities associated differentiation syndrome (unexplained fever, dyspnea, pleural

and/or pericardial effusion, pulmonary infiltrates, renal failure, hypotension, and unexplained weight gain greater than 5 kg) will be treated with dexamethasone 10 mg IV twice daily as per section 5.3.1 above and IRX5183 will be temporarily discontinued until resolution of symptoms to grade 2 or below, while on concomitant steroid therapy. Signs or symptoms of differentiation syndrome that do not return to grade 1 within 2 weeks of steroid therapy will be considered a DLT and the patient will be removed from the trial. If differentiation syndrome subsequently recurs to 3 or above, the patient will also be removed from the trial.

### **Rash:**

Rashes can be common with retinoid therapies. Therefore, there will be no dose modifications for a grade 1-2 rash. Patients with a grade 1-2 rash should be treated with topical steroids. If the patient develops or progresses to a grade 3 rash, then the patient should be started on oral steroids (specific type and dosing to be determined by the treating physician) and the drug should be held for up to 2 weeks until the rash resolves to  $\leq$  grade 2. If the patient develops a grade 4 rash, they should permanently discontinue IRX5183 and come off study.

### **6.2. Discontinuation of IRX5183 for related adverse events:**

Permanently discontinue IRX5183 for any of the following:

- 1) Persistent adverse reaction(s) thought to be due to IRX5183 therapy, which require holding more than 2 weeks of treatment doses.
- 2) Any grade 3 or 4 event (see exceptions in section 5.2 and 6.1 above).
- 3) Severe or life-threatening adverse reactions, including any of the following:
  - Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations
  - Respiratory failure non-responsive to steroid therapy
    - ❖ Any adverse event, laboratory abnormality or intercurrent illness, which in the judgment of the investigator presents organ specific injury and/or a substantial clinical risk to the patient with continued dosing.
    - ❖ The following neurological adverse event requires permanent discontinuation of IRX5183 and defines unacceptable neurotoxicity:
      - Any motor neurologic toxicity  $\geq$  grade 3 regardless of causality
      - Any  $\geq$  grade 3 treatment related sensory neurologic toxicity

### **6.3. Exceptions to permanent discontinuation:**

- 1) Potentially reversible inflammation ( $<$  grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of myeloid sarcoma or in draining lymph nodes.
- 2) Hospitalization for  $\leq$  grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- 3) Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified based on the potential for continued clinical benefit:
  - ❖ Patients with grade 3-4 differentiation syndrome-related symptoms if symptoms resolve within 2 weeks of high-dose dexamethasone therapy.

- ❖ Grade 2 skin rash treated with topical steroids for less than 4 weeks.

## 7. TREATMENT EVALUATION AND TOXICITY MONITORING

### 7.1. Clinical Responses:

All patients initiated on therapy will be evaluable for toxicity. The primary endpoint of clinical efficacy will be evaluated following at least two cycles of therapy. See Section 7.3 for response criteria.

### 7.2. Assessment of Response:

1. Complete blood counts with WBC differential will be measured twice weekly during the first cycle of treatment, at least weekly throughout the second cycle of therapy, at least every other week during consolidation/maintenance, and at least monthly during extended maintenance, or as often as clinically indicated.
2. Blood chemistries will be measured twice weekly during the first cycle of treatment, at least weekly throughout the second cycle of therapy, at least every other week during consolidation/maintenance, and at least monthly during extended maintenance, or as often as clinically indicated.
3. Fasting lipids with triglyceride evaluation will occur q2 weeks through the first month and then q8 weeks subsequently for assessment of toxicity.
4. Bone marrow aspirate and biopsies for clinical assessment (morphologic analysis, cell count/differential, flow cytometry, FISH/cytogenetics for patients with baseline abnormalities, and molecular studies for patients with baseline abnormalities) and biologic correlates (see section 8 below) will be collected at the following time points:
  - ❖ Day 14 ( $\pm$  3 days) of cycle 1 (not required to stay on study)
  - ❖ At the completion of cycle 1
  - ❖ At the completion of cycle 2
  - ❖ At the end of every two cycles of treatment during consolidation/maintenance
  - ❖ After every 4 cycles of treatment during extended maintenance
  - ❖ Drug levels will be obtained in the peripheral blood (PB) and BM with liquid chromatography mass spectroscopy (LCMS), described in detail in section 7.8.
5. PT/PTT will be followed as needed for those patients who are receiving an anticoagulant during protocol therapy.
6. A track of the total number of transfusions required will be kept.
7. Quality of life assessment per the FACTLeu questionnaire (available at <http://www.facit.org/FACITOrg/Questionnaires>) at the completion of the induction and maintenance phases, with the help of the research team.
8. Response to treatment will be assessed after completion of at least 2 cycles. Patients will be categorized based on standard IWG-2003 response criteria for AML (Appendix A1) and IWG-2006 criteria for MDS (Appendix A2-A3) and patients with at least SD will continue on treatment (see section 5.1).
9. All patients will be evaluated for hematologic improvement (HI) per IWG-2006 MDS criteria (Appendix A2).
10. Those showing CR at the completion of consolidation/maintenance will have treatment ceased to assess duration of response (will check biweekly CBC for the first month, then

monthly for at least 6 months, and bimonthly bone marrow assessments for at least 6 months). Patients will be allowed to resume treatment if they relapse during this observation period.

11. If patients continue with SD, HI, or PR after consolidation/maintenance, they will continue onto extended maintenance for up to an additional year.
12. At the end of extended maintenance, patients may be able to continue on therapy, per the discretion of Io Therapeutics and the PI.
13. Time to first response (CR/CRi/PR for AML, CR/CRi/PR/HI for MDS) and time to best response will be tracked for each patient.
14. Time from treatment initiation to progression (as defined per IWG criteria in Appendix A1-A2) will be tracked for each patient.
15. Time from treatment initiation to death will be tracked for each patient.

### **7.3. Measurement of Response / Response Criteria:**

The primary endpoint of clinical response is defined by the International Working Group recommendations (see Appendix A1-A3) for therapeutic trials in AML and MDS and includes CR, complete remission with incomplete count recovery (CRi, formerly known as morphologic leukemia-free state or marrow CR), partial remission (PR), HI, SD, and PD (Cheson et al., 2003). We will also include an assessment of HI per MDS IWG-2006 recommendations as one of our secondary endpoints in our AML patients (see Appendix A2). In addition to the criteria in Appendix A1, the following will be considered as PD:

- 1) A significant increase in bone marrow blasts beyond 4 weeks of starting the drug:
  - a. if baseline 51-100% blasts – an increase of greater than 50% of baseline
  - b. if baseline 26-50% blasts – an increase up to 2 x the baseline
  - c. if baseline 0-25% blasts – an increase up to 3 x the baseline
- 2) A rising peripheral WBC after adequate dosing with hydroxyurea (see section 5.3 above)

### **7.4. Adverse Events:**

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

AEs are to be coded using an internationally recognized dictionary.

This study will utilize the Common Toxicity Criteria (CTC) version 4.0 for toxicity where applicable and adverse event reporting. In cases where CTC cannot be applied to the toxic event, the investigator will quantify the toxicity based on intensity, as per section 14.1 below.

Patients are to be followed for adverse events for 30 days after the last dose of study drug. Any adverse event occurring in a patient 30 days after stopping the study drug must be reported. The

period after discontinuing study drug may be extended if there is a strong suspicion that the drug has not yet been eliminated or if the nature of the event may suggest long-term effects by the investigational drug, as assessed by the investigator.

#### **7.5. Serious Adverse Event (SAE):**

An SAE is defined in the FDA CFR 312 as any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization, or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon 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.

#### **7.6. Toxicity Reporting:**

Toxicity scores will be assigned using the NCI Common Toxicity Criteria version 4.0 twice weekly by the attending or research nurse through the initial cycle of therapy and weekly thereafter. A copy of the CTC version 4.0 can be downloaded from CTEP online ([http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)). If an unexpected and serious toxicity is seen that results in patients being subjected to unacceptable risk, the trial will be placed on hold while this toxicity is investigated.

#### **7.7. Toxicity Monitoring:**

The Principal Investigator is responsible for the ongoing safety evaluation of the study and shall provide to Io Therapeutics, Inc. all Serious Adverse Events (SAEs) within 24 hours of the Principal Investigator's awareness. All fatal and life-threatening events will be reported immediately by phone. A written report will be submitted within ten working days. The first occurrence of any previously unexpected clinical event, regardless of grade, will be reported by phone within 24 hours. A written report is also required. Unexpected SAEs are defined as those adverse effects not described in the Io therapeutics investigator's brochure. Drug-related SAEs are defined as those adverse events that indicate a possible, probably, or likely relationship between IRX5183 administration and the SAE. In addition to standard reporting to the FDA and clinical trials network, SAEs will be reported to Io Therapeutics, Inc. within 24 hours of the awareness of the SAE. The following guidelines are recommended for phase II studies:

**Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention<sup>1,2</sup>**



<b>FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)</b>		
<p><b>NOTE:</b> Investigators <b>MUST</b> immediately report to the sponsor (NCI) <b>ANY</b> Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)</p> <p>An adverse event is considered serious if it results in <b>ANY</b> of the following outcomes:</p> <p>Death</p> <p>A life-threatening adverse event</p> <p>An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for <math>\geq</math> 24 hours</p> <p>A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions</p> <p>A congenital anomaly/birth defect.</p> <p>Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).</p>		
<p><b>ALL SERIOUS</b> adverse events that meet the above criteria <b>MUST</b> be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.</p>		
<b>Hospitalization</b>	<b>Grade 1 and Grade 2 Timeframes</b>	<b>Grade 3-5 Timeframes</b>
Resulting in Hospitalization $\geq$ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization $\geq$ 24 hrs	Not required	
<p><b>Expedited AE reporting timelines are defined as:</b></p> <ul style="list-style-type: none"> <li>○ “24-Hour; 5 Calendar Days” - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.</li> <li>○ “10 Calendar Days” - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.</li> </ul>		
<p><sup>1</sup>Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:</p> <p><b>Expedited 24-hour notification followed by complete report within 5 calendar days for:</b></p> <ul style="list-style-type: none"> <li>• All Grade 3, 4, and Grade 5 AEs</li> </ul> <p><b>Expedited 10 calendar day reports for:</b></p> <ul style="list-style-type: none"> <li>• Grade 2 AEs resulting in hospitalization or prolongation of hospitalization</li> </ul> <p><sup>2</sup> For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote “1” above applies after this reporting period.</p> <p>Effective Date: May 5, 2011</p>		

## 7.8. Pharmacokinetics:

In phase I, we plan to first determine pharmacokinetic (PK) parameters of IRX5183 on days 1 and 14 ( $\pm$ 3) of cycle 1 in the PB and BM compartments (may be obtained post cycle 1 if day 14 BM specimen not available). In phase II, we will perform confirmatory testing at these time points in a subgroup of patients. Full sampling, processing, storage, and shipment instructions will be provided in the Laboratory manual. Briefly, trough and peak levels of IRX5183 will be determined in the plasma and mononuclear cells (MNC) by obtaining a 4 mL sample of blood (green top tubes-sodium heparin)  $\frac{1}{2}$  hour before the morning dose and then 2 hours ( $\pm$  30 minutes) post drug administration. Phase I PK data showed that  $T_{max}$  generally was in the range of 1-3 hours post dose across individual patients. We will isolate platelet rich plasma (PRP) and

MNC and samples will be frozen in our laboratory, and then shipped and analyzed in a batch on dry ice to the laboratory of Dr. Maureen Kane at the University of Maryland. Drug levels will be determined by liquid chromatography mass spectroscopy (LCMS), per techniques as previously reported (Kane, 2012).

Peak drug levels will also be determined in the BM compartment on day 14 ( $\pm$  3 days) (may be obtained post cycle 1 if day 14 BM specimen not available), approximately 1 to 3 hours after dosing (as close to 2 hours post as possible, but up to 4 hours post drug administration). We will allow for flexibility in timing of the BM due to difficulties with scheduling and wait times, but we will aim for collection as close to that of the PB as possible, for optimal comparison of drug concentrations. We are interested to see whether intracellular levels will differ between LSCs, normal hematopoietic stem cells (HSCs), and bulk AML cells, and how these levels compare to plasma. We will isolate BM plasma and MNC from the BM aspirate and sort for LSCs, normal HSCs, and bulk AML (when possible) and then freeze at -80 degrees Celsius (as per the Lab Manual and section 8.2 below). Depending on the type of AML, we may not be able to isolate CD34<sup>+</sup>CD38<sup>-</sup>ALDH<sup>int</sup> LSCs, in which case we will isolate CD34<sup>+</sup>CD38<sup>-</sup> cells. Samples will be shipped and analyzed in a batch at the end of the study by LCMS in the laboratory of Dr. Kane, as above.

If for any reason during enrollment on the trial, a patient requires a lumbar puncture, we will obtain a specimen for measurement of IRX5183 levels, in order to determine whether the drug penetrates the blood brain barrier. We would obtain 2 mL CSF to be frozen and sent to Dr. Kane on dry ice for analysis by LCMS. We would also collect a 4 mL sample of PB (green top tube-sodium heparin) at the same time point, isolating and freezing PRP and MNC as above. See lab manual for details on processing, storage, and shipment instructions.

## 8. CORRELATIVE STUDIES

### 8.1. Biologic activity:

Assessing the biologic activity of IRX5183 in AML and MDS patients is critical to the interpretation of any clinical activity. Successful induction of terminal differentiation would be expected to be measurable by changes in the malignant cell phenotype as well as possible cytogenetic changes. The following biologic correlatives will be conducted in the laboratories of Drs. Gabriel Ghiur and Richard Jones:

1. ***In vivo* induction of terminal differentiation** - PB and BM markers of myeloid differentiation such as CD15 and CD11b (as directed by disease phenotype) will be measured by flow cytometry prior to the initiation of therapy and repeated on the day 14 ( $\pm$  3) and/or post cycle 1 specimens, with a preference for day 14 ( $\pm$  3), while ongoing differentiation is more likely.
2. **Effect on leukemia stem cells as an indicator of differentiation** – ratio of LSC population to normal HSC population will be determined at baseline, day 14 ( $\pm$  3 days), and later endpoints in select patients. If the patient's leukemia consists of an ALDH intermediate population, we will determine the ratio of CD34<sup>+</sup>CD38<sup>-</sup>ALDH<sup>int</sup> LSCs to

CD34<sup>+</sup>CD38<sup>-</sup>ALDH<sup>high</sup> normal HSCs. We will use techniques as we have previously published (Gerber et al., 2012).

3. ***In vivo* changes in measurable cytogenetic alterations** – baseline clinical fluorescent in situ hybridization (FISH) analysis will be done prior to starting treatment and subsequent analyses will take place with each clinical BM, in order to assess for a cytogenetic response to therapy. When possible, FISH will additionally be used to quantify the cytogenetic changes in PB and marrow myeloid progenitors prior and following therapy on day 14 ( $\pm$  3 days) and/or after cycle 1. The presence of mature neutrophils with the malignant cytogenetic abnormality in the PB will serve as evidence for terminal differentiation of leukemia cells from the therapy.
4. ***In vitro* induction of terminal differentiation** – the susceptibility of malignant progenitors to IRX5183 will be studied *in vitro*. CD34 positive progenitors will be isolated from bone marrow or PB and exposed to IRX5183 in the presence and absence of the patient's own stromal feeder layer. We will look at the impact on differentiation by flow cytometry using various differentiation markers (e.g. CD15, CD11b) and by CFU assay. Techniques will be done as previously reported (Su et al., 2015) and per Laboratory Manual.
5. **Plasma differentiating activity** – the differentiating ability of different dose levels of IRX5183 will be assessed by treating NB4 APL cell lines with patient plasma obtained at day 14 and beyond and then measuring CD11b expression by flow cytometry, compared to control (see Lab Manual).
6. **Cell cycle and apoptosis assays** – cell cycle and apoptosis assays will be performed in CD34<sup>+</sup> cells at baseline and different time points throughout the trial. We will assess viable MNCs with flow cytometry, looking at Annexin-5 and Propidium Iodide (PI) as markers of apoptosis and Ki67/Hoechst to assess cells in G<sub>0</sub>/G<sub>1</sub>/S/G<sub>2</sub>M. In addition, we will assess Bcl-2 expression levels by Western blot. See Lab Manual for full details.

## 8.2. **Cellular Collection and Processing:**

The labs of Drs. Gabriel Ghiaur and Richard Jones will support the planned biologic correlatives through the timely collection and processing of the patient PB and marrow samples. All operating procedures for specimen collection, handling and storage will be standardized. Common standardized operating procedures (SOPs) will be to collect approximately 10 mL PB (1 green top tube-sodium heparin) at the time points of bone marrow assessments (i.e., pre-study, day 14  $\pm$ 3, after cycle 1, cycle 2, cycle 4, cycle 6 (and beyond as per figure 2 in section 5.1 above). Bone marrow aspirate, approximately 10 mL (1 green top tube-sodium heparin), will be collected at the above listed time points, per the protocol in figure 2. For a patient that progresses and is removed from study treatment, additional blood or BM may be obtained at the time of routine collection. Re-adjustment of the direction of bone marrow aspirate needle should take a place after the clinical collection to prevent hemodilution. At the time of collection, tubes must be thoroughly mixed to prevent clotting.

The SKCCC specimen should be delivered to the Ghiaur and Jones laboratory immediately after collection. Specimens should be labeled with the patient's initials, study number, sample collection date and time, and sample source (PB or BM). All data should be kept in the laboratory log.

At each sampling interval, mononuclear cells from PB and marrow will be isolated per procedures in the Laboratory Manual. Samples stored in liquid nitrogen should be clearly marked with the patient's identification number (given at the time of registration), study number, sample collection date, and sample source (PB or BM).

## 9. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 9.2.

### 9.1. IRX5183 drug information:

#### Other Names

NRX195183

AGN195183

CNC195183

VTP195183

#### Pharmacological Classification

Retinoid, antineoplastic agent

#### Position in Class

Receptor-specific agonist (RAR $\alpha$  specific)

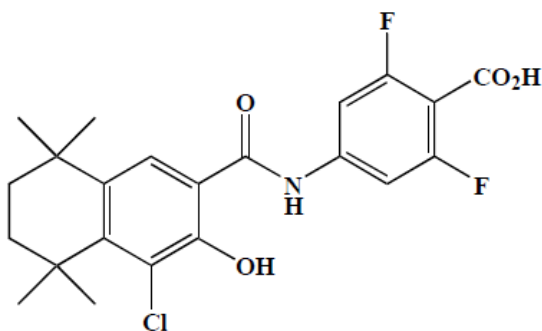
#### Chemical name

4-[[1-(4-Chloro-3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-methanoyl]-amino]-2,6-difluorobenzoic acid

#### Empirical Formula

C<sub>22</sub>H<sub>22</sub>ClF<sub>2</sub>NO<sub>4</sub>

#### Structural Formula



### Dosage Form

The drug is formulated as a soft gelatin capsule containing IRX5183 25 mg per capsule.

### Product Description

White to off-white soft gelatin oblong capsule containing a slightly hazy, yellow solution.

**Table 3: Formulation of NRX 195183 Soft Gelatin Capsules**

Ingredient	% w/w	Amount/Capsule (mg)	Function
<b>Fill Formulation:</b>			
NRX 195183	5	25.0	Active
Propylene Glycol USP	5	25.0	Plasticizer
Polysorbate 80 NF	90	450.0	Solubilizing Vehicle
<b>Gel Mass for Soft Gelatin Capsules</b>	<b>AR*</b>	<b>AR</b>	<b>Capsule Shell</b>

AR=As required per batch record. The following ingredients are used in the Gel Mass: Gelatin NF (150 Bloom Limed Bone, Type B), Glycerin 99.7% UPS, Sorbitol Special, 76%, Purified Water USP, Opatint White G-18000. The following processing aids are used in the capsulation process: Miglyol 812 N (Medium-Chain Triglycerides EP) and Centrocip® 162-US (Lecithin NF).

### Storage Conditions

Store refrigerated (2° - 8°C) in tight containers, protected from light.

### Excretion

Biliary and/or gastrointestinal.

### Elimination Half Life

3.3 to 16.1 hours

### Availability

IRX5183 is not commercially available. Io Therapeutics, Inc. has agreed to provide this agent for this study.

## **9.2. Side effects:**

**9.2.1. Adverse events in phase I trial in solid tumor patients:**

- 1) Dermatologic: Rash (26.7%), mucosal inflammation (6.7%), stomatitis (6.7%)
- 2) Endocrine: Hyperglycemia (26.7%), hypoglycemia (6.7%), hypertriglyceridemia (20%)
- 3) Gastrointestinal: Vomiting (33.3%), nausea (26.7%), dyspepsia (26.7%)
- 4) Hematologic Effects: Anemia (26.7%)
- 5) Hepatic: Elevated alkaline phosphatase (40%)
- 6) Other: Fatigue (40%), anorexia (26.7%)

**9.2.2. Teratogenic Effects:**

There is a high risk that a severely deformed infant will result if IRX5183 is administered during pregnancy.

If, nonetheless, it is determined that IRX 195183 represents the best available treatment for a woman of childbearing potential, it must be assured that the patient has received full information and warnings of the risk to the fetus if she were to be pregnant and of the risk of possible contraception failure and has been instructed in the need to use two reliable forms of contraception simultaneously during therapy and for 1 month following discontinuation of therapy, and has acknowledged her understanding of the need for using dual contraception, unless abstinence is the chosen method.

Within 1 week prior to the institution of IRX5183 therapy, the patient should have blood or urine collected for a serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL. When possible, IRX5183 therapy should be delayed until a negative result from this test is obtained. When a delay is not possible, the patient should be placed on two reliable forms of contraception. Pregnancy testing and contraception counseling should be repeated monthly throughout the period of IRX5183 treatment.

**9.2.3. Toxicity Profile of Oral Retinoids (Class Effect):**

The safety profile of IRX5183 may correspond with the characteristic profile of systemic RAR agonists (ATRA [tretinoin], 13-*cis*-retinoic acid [isotretinoin], acitretin and etretinate) currently in use for acute promyelocytic leukemia (APL), acne, and psoriasis.

The most frequently reported adverse events in this drug class include headache, fever, skin/mucous membrane dryness, bone pain, nausea/vomiting, rash, mucositis, pruritus, increased sweating, visual disturbances, ocular disorders, alopecia, skin changes, changed visual acuity, bone inflammation, and visual field defects.

Headache occurring several hours after drug ingestion is also a common side-effect. Mild analgesics generally suffice for control and the patient usually develops a tolerance to the effect. Bone pain occurs in 10% to 20% of patients and usually remits with continued treatment: occasionally narcotic analgesia may be required to overcome the pain. Skin and mucous membrane effects can be managed with topical lubricants.

Pseudotumor cerebri, a known consequence of Vitamin A toxicity, has been documented in several patients. These persons have required serial lumbar punctures, high dose corticosteroids, and narcotic analgesics.

The following table summarizes the characteristic toxicities associated with the use of oral retinoids (De Vita Jr VT, 1997; *Physician's Desk Reference*, 2000).

**Table 4: Toxicities reported with the use of oral retinoids**

<b>Cardiovascular effects</b>	Peripheral edema, episodic hypotension, arrhythmias, flushing, edema
<b>Central nervous system effects</b>	Headache, intracranial hypertension (“pseudo tumor cerebri”), fever, fatigue, anxiety, confusion, dizziness, malaise, weakness, insomnia, rigors, paresthesias, depression psychosis
<b>Gastrointestinal effects</b>	Abdominal pain, vomiting, nausea, constipation, diarrhea, nonspecific bowel disorders, weight loss, weight gain, dyspepsia
<b>Gynecologic effects</b>	Increased menstrual bleeding
<b>Hematological effects</b>	Bleeding in other areas
<b>Endocrine effects</b>	Hypertriglyceridemia, hypercholesterolemia, increased LDH, hypercalcemia, hepatic toxicity (raised SGOT, SGPT, bilirubin, alkaline phosphatase), elevated sedimentation rates, elevated CPK, decreased WBC or RBC, and increased WBCs in urine
<b>Musculoskeletal effects</b>	Arthralgias, myalgias, bone pain, skeletal hyperostosis, weakening of the bones or abnormal bony growths
<b>Ophthalmologic effects</b>	decreased night vision, dry eyes, visual field defects, changes in visual acuity, visual disturbances, ocular disorders
<b>Renal effects</b>	Abnormal kidney function, nonspecific findings of the urinary or genital tract
<b>Respiratory effects</b>	Dyspnea, infections, chest discomfort
<b>Skin or mucous membranes</b>	Skin/mucous membrane dryness, skin fragility, rash, pruritus, dry mouth, mucositis, dry lips, and nose, cheilitis, epistaxis, peeling of the palms and soles, sticky skin, increased sweating, sensitivity to the sun, changes in skin pigment, other skin disorders, hair thinning and other hair disorders, nail disorders and genital excoriations
<b>Special senses effects</b>	Ringling in the ears, earaches
<b>Teratogenic effects*</b>	Spontaneous abortion, birth defects

\*See section on teratogenic effects above

#### 9.2.4. Differentiation syndrome (DS):

DS is a common occurrence in patients with APL treated with ATRA. The syndrome has been defined by the presence of the following signs or symptoms: unexplained fever, dyspnea, pleural and/or pericardial effusion, pulmonary infiltrates, renal failure, hypotension, and unexplained weight gain greater than 5 kg, and graded based on the number of these findings (Montesinos et al., 2009). Patients with 4 or more of the aforementioned signs or symptoms are classified as having severe DS, whereas those with 2 or 3 signs or symptoms are classified as having moderate DS. This condition is best treated with high dose dexamethasone at a dose of 10 mg IV twice daily.

Grade 1 DS per CTCAE v4 is defined as “fluid retention; <3 kg of weight gain; intervention with

fluid restriction and/or diuretics indicated.” Grade 2 is defined as “moderate signs or symptoms; steroids indicated.” Grade 3 is defined as “severe symptoms; hospitalization indicated.” Grade 4 is defined as “life-threatening consequences; ventilator support indicated.” The definition per CTCAE is “a disorder characterized by weight gain, dyspnea, pleural and pericardial effusions, leukocytosis and/or renal failure originally described in patients treated with all-trans retinoid acid.”

## 10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy (first dose of IRX5183). In the event that the patient’s condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of therapy. An interim history and physical should be done with each visit. IRX5183 will each be administered on a daily basis with one cycle consisting of 28 days (4 weeks).

Complete blood counts with WBC differential and blood chemistries will be measured twice weekly during the first cycle of treatment, at least weekly throughout the second cycle of therapy, at least every other week during consolidation/maintenance, and at least monthly during extended maintenance, or as often as clinically indicated. Fasting lipids with triglyceride evaluation will occur q2 weeks through the first month and then q8 weeks subsequently, per standard of card assessment on retinoid therapy.

Treatments and Evaluations <sup>▲</sup>	Pre-Study	Cycle 1:				Cycle 2:				Cycles 3-6: <sup>1</sup>				End of Study
		W1 D1&4	W2 D1&4	W3 D1&4	W4 D1&4	W1 D1	W2 D8	W3 D15	W4 D22	W1 D1	W2 D8	W3 D15	W4 D22	
IRX5183 (daily)		X	X	X	X	X	X	X	X	X	X	X	X	
Informed consent	X													
Adverse Events and Toxicity Reporting	X	X X	X X	X X	X X	X	X	X	X	X	X	X	X	X**
Medical history	X	X	X	X	X	X		X		X		X		X##
Physical exam	X	X	X	X	X	X		X		X		X		X##
Height	X													
Transfusion Review	X	X X	X X	X X	X X	X	X	X	X	X		X		
Weight	X	X		X		X		X		X		X		
ECOG Perform Status	X	X	X	X	X	X	X	X	X	X	X	X	X	
FACTleu questionnaire	X								X*				X*	
CBC w/ diff., plts	X	X X	X X	X X	X X	X	X	X	X	X		X		
Comprehensive Panel	X	X X	X X	X X	X X	X	X	X	X	X		X		
Fasting Lipid Panel <sup>2</sup>	X		X		X								X	
Urine Pregnancy	X													
JH Path Review of Diagnosis	X#		X#			X#			X#				X#	X
Peripheral Blood for Research	X#		X#			X#			X#				X#	X
Pharmacokinetics		X♦	X♦			X♦								



Bone Marrow Aspirate Biopsy <sup>3</sup>	X#		X#		X#		X#		X#	X
--	----	--	----	--	----	--	----	--	----	---

<sup>1</sup> If minimal toxicity and at least SD, patients will have the option of continuing on therapy past 6 cycles, for up to an additional year of extended maintenance therapy. At that point, patients will have blood tests at least monthly and will complete a bone marrow biopsy after every 4 cycles of treatment. If the patient doesn't continue on for additional cycles past 6, follow-up in terms of blood counts will be completed per standard guidelines for AML monitoring as clinically indicated.

<sup>2</sup> Evaluations will continue to occur q8 weeks following cycle one.

<sup>3</sup> Patients may have a planned 5-7 day "rest" period between each cycle where patients are scheduled to undergo response assessment by bone marrow sampling (except for after cycle 1). Patients may start their next scheduled cycle of therapy once the marrow testing is completed but no longer than 42 days from the initiation of the previous cycle.

\* To be done at the completion of the 2nd cycle and then at the completion of the 6th cycle.

\*\* Patients will be monitored for toxicity at least 30 days post last dose of study drug.

# To be done at baseline, day 14 ( $\pm$  3 days) of cycle 1, after completion of cycle 1, 2, 4, 6, 10, 14, and 18, and at the end of the study.

## Patients will be monitored at least monthly for 6 months after study completion in person, or by phone if patient followed by a local oncologist.

◆ Draw ½ hour before the morning dose and then 2 hours ( $\pm$ 30 minutes) post drug administration on days 1 and 14 ( $\pm$  3 days). On day 14 ( $\pm$  3 days), bone marrow will be obtained for PK about 2 hours post drug (allowed as soon as 1 hour or as long as 4 hours post). May be obtained post cycle 1, only if day 14 BM specimen not available.

▲ Variations of  $\pm$  3 days of the scheduled visit are permitted. Labs may be drawn more frequently if clinically indicated.

An unscheduled visit can occur at any time during the study. The date for the visit and any data generated must be recorded on the appropriate CRF. Source documents for these unscheduled visits must also be maintained.

Pregnancy tests are only for females of childbearing potential. A female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

## 11. STATISTICAL CONSIDERATIONS

### 11.1. Study design & endpoints:

In the dose-escalation part of the study, there will be 3 dose levels (dose level 1 [DL1] with 50 mg, DL2 with 75 mg, and DL3 with 100 mg), with 1 additional dose level to be only used if excessive toxicity noted at the DL1. There will be no intra-patient dose escalation. Dose-escalation rules are defined through a standard 3+3 algorithm as outlined in Section 5.2.

The phase II expansion part of the study will aim to recruit a total of 27 patients with either R/R-AML or HR-MDS, and there will be an interim analysis after 13 patients for futility through a Simon two-stage minimax design based upon a Type I error rate of 0.05, power of 0.8, response probability of poor drug 0.05, and response probability of good drug of 0.2, based on prior relapsed and refractory AML trials and CTEP data on non-pediatric oncology trials from 2001 to 2012 (Itzykson et al., 2015; Ritchie et al., 2013; Thol et al., 2015; Yoko Korenaga Fukuda, 2014). Stage 1 will enroll 13 patients at the RP2D and will require at least 1 response for

continued enrollment to Stage 2, to reach the total of 27 patients. A total of 4 or more responses out of 27 patients will be considered encouraging evidence for future larger clinical trials of IRX5183.

Response will be defined per IWG criteria as CR, CRi, or PR for AML patients and as CR, CRi, PR, or HI for MDS patients (see definitions in Appendix A) and will be determined with each BM assessment. The composite of these responses across our study population will be referred to as the overall response rate (ORR). Rates of CR, CRi, PR, HI, and the composite endpoint of ORR will be summarized and reported with an exact 95% confidence interval.

### **11.2. Primary endpoints:**

1) Phase I - description of toxicity of IRX5183 by grading and tabulation using NCI-CTC Version 4.0 criteria (section 8). The DLT will be defined as per section 5.2.

2) Phase II – best response after at least 2 cycles of therapy per IWG criteria (**Appendix A**).

### **11.3. Secondary endpoints:**

1) Phase I - Description of PK parameters of IRX5183 at baseline and day 14, both in the peripheral blood and BM compartments, markers of myeloid differentiation by flow cytometry (e.g. CD15, CD11b), BM blast count, ANC before and after treatment, and changes in detectable cytogenetic abnormalities in the blood and BM compartments at the different dose levels. We will also describe best response per IWG criteria at any time, time to first and best response, and clinical parameters such as transfusion requirements, blood counts, quality of life assessments, and time to either progression or death.

2) Phase II – Description of IWG response and other clinical criteria assessed in the dose escalation phase for the RP2D. We will also measure markers of differentiation assessed in the dose escalation phase for the RP2D, and lastly, the toxicity profile at the RP2D dose.

### **11.4. Sample size considerations:**

For the dose-escalation part of the trial, we will enroll up to 18 patients, per standard 3+3 design with 3 DLs (see table 2 in section 5.2). Once we have determined our RP2D, we will proceed to phase II of the trial, where we will recruit an additional 21 R/R-AML or HR-MDS patients at the RP2D, for a total sample size at the RP2D of 27 (inclusive of patients treated at the RP2D in phase I). This sample size was determined using a minimax Simon two-stage design, as described in Section 11.1. Based on prior experience at our institution in this patient population, we will account for a dropout rate of 30%, so we will aim to recruit an additional 8 patients to the dose expansion phase, so that our trial will accrue up to 35 patients to this phase.

In phase II of the trial, the study team will meet regularly to determine whether it is worthwhile to continue enrollment, if an excessive number of patients are unable to complete 2 full cycles of therapy at the RP2D, whether for reasons of DLTs or PD.

**11.5. Expected accrual rate:**

We expect to accrue up to 3 patients with R/R-AML and/or HR-MDS per month once the study is open. Enrollment is expected to be complete within 12-18 months. The duration of therapy and follow-up period is up to 24 months (excluding any patients that may be eligible to continue on therapy after the trial period per discretion of Io Therapeutics and the PI). Therefore, the total expected duration of the study, including recruitment, therapy, and follow-up, is expected to be a maximum of 50 months.

**11.6. Stratification factors:**

There are no planned stratifications for this protocol.

**11.7. Analysis of secondary endpoints:****Pharmacokinetics:**

In the dose escalation phase, we plan to first determine PK parameters of IRX5183 at baseline and day 14 ( $\pm 3$  days) in the PB and BM compartments. In the dose expansion phase, we will perform confirmatory testing in a subgroup of patients at the same time points. PK analyses will be largely descriptive, using Student's t-tests or Wilcoxon rank sum tests comparing drug concentrations at both time points. We will also assess the peak and trough levels in the PB at the above time points.

**Pharmacodynamics:**

Biologic correlates include measurement of markers of myeloid differentiation by flow cytometry (e.g. CD15, CD11b), BM blast count, and ANC at the different dose levels in the dose escalation phase and at the RP2D in the expansion phase at the different time points compared to baseline. In addition, we will look at the ratio of ANC to BM blast count as clinical marker for differentiation at the different time points compared to baseline. Statistics will be descriptive and will consist of paired Student's t-tests or Wilcoxon rank sum tests comparing averages between 2 time points (e.g. after induction therapy compared to baseline).

We will furthermore assess *in vivo* changes in detectable chromosomal abnormalities in patients with baseline cytogenetic alterations that define their malignant clones. This will be measured by fluorescent in situ hybridization (FISH) with cells from the individual cellular compartments quantified as a ratio of malignant to non-malignant cells (i.e., mononuclear cells with the cytogenetic finding to those without). Successful differentiation would be expected to result in a decrease in mononuclear cells harboring the cytogenetic abnormality. These results will be summarized using descriptive statistics and exploratory plots will be used to illustrate results.

**Clinical activity:**

In the dose expansion phase, we will quantify best IWG response at any time, clinical parameters such as weekly transfusion requirements after each cycle of therapy compared to baseline, blood

counts after each cycle compared to baseline, quality of life (QOL) assessment per the FACTleu questionnaire at the completion of the induction and maintenance phases compared to baseline, time from treatment initiation to disease progression, and time to death. We will also assess for best IWG response at any time and HI in our AML patients per the IWG-2006 MDS criteria (Appendix A3). We will look at all clinical parameters both in aggregate and as a subgroup analysis in AML and MDS patients. For response calculations, the report will contain at least a section with all eligible patients. Another section of the report will detail the response rate for evaluable patients only. The response rate analysis based on a subset of patients will explain which patients were excluded and for what reasons. We will provide 95% confidence limits as appropriate. Average weekly transfusion requirements over the duration of a 28-day cycle will be compared to the baseline average weekly transfusion requirement over the 4 to 8 weeks prior to treatment initiation. Event free survival (EFS) and overall survival (OS) will be reported with a 95% confidence interval.

FACTleu version 4 questionnaires will be analyzed per instructions on FACIT.org (Cella et al., 2012). Scoring guidelines are listed in Appendix D. We hypothesize that QOL will improve over the course of the study; however, without a comparison arm, it will be difficult to determine whether this regimen will yield a better or worse QOL outcome than other regimens. Therefore, the questionnaire will serve as more of an exploratory assessment to use when planning further studies. We will look at markers of feasibility, including percentage of completed questions, the time it takes to complete, and how many patients participate in the survey.

### **Toxicity:**

Beyond the dose escalation portion of the study, we will continue to track for adverse events throughout the duration of the trial. We will evaluate the number of patients who experience different toxicities with percentages and descriptive statistics and will explore any potential correlations between IRX5183-induced differentiation, toxicity, and clinical responses (e.g. whether patients who experience toxicities characteristic of DS have a higher chance of clinical response). Therefore, IWG responses and survival endpoints will be qualitatively compared between patients who do and do not have evidence of DS, if we do happen to see this clinical syndrome in our study population.

### **Exploratory assessments:**

We plan to assess whether observed *in vivo* evidence of differentiation per flow cytometry markers, ANC, or BM blast count correlate with ORR, EFS, and/or OS.

## **11.8. Reporting and exclusions:**

### **11.8.1. Evaluation of toxicity:**

All patients will be evaluable for toxicity from the time of their first treatment with IRX5183.

### **11.8.2. Evaluation of response:**

All patients included in the study that complete 2 cycles of therapy will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. The primary endpoint to the dose expansion phase is response per IWG criteria after receiving at least 2 cycles of therapy. Only those patients who have measurable disease present at baseline, have received at least 2 cycles of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated in Appendix A. Patients who exhibit objective disease progression prior to the end of cycle 2 will be considered not evaluable and will be taken off study.

An incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Patients with disease progression, death due to their disease, and death due to toxicity prior to completion of induction therapy will be excluded from the primary analysis. This will prevent dismissal of an active drug based on progression in patients with highly refractory disease who are unable to receive enough of the drug to see a clinical benefit. Patients who are unable to complete induction therapy for any reason will be treated as missing data and additional patients will be recruited in their place to reach our target sample size of 27 for phase II.

## 12. DATA SAFETY MONITORING PLAN

The study principal investigator will be responsible for the conduction of the study, including the monitoring of the study's safety and oversight of the data collection. The PI will follow the Data Safety and Monitoring plan (DSMP) outlined in the Sidney Kimmel Comprehensive Cancer Center's policy.

This is a DSMP Level I study under the SKCCC DSMP (12/6/2012), based on the risk and monitoring needs. The Clinical Research Office will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee. The PI is responsible for internally monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

### 12.1. Data Reporting:

External data monitoring will be performed by the Johns Hopkins Clinical Research Office in accordance with a level I risk/complexity study, as described in the Johns Hopkins Comprehensive Cancer Center (JHCCC) Clinical Trial Monitoring Program guidelines.

#### **Intensity:**

The CTC 4.0 will be used to grade adverse events. If an adverse event cannot be assessed using the CTC, the investigator will classify the intensity of an adverse event according to the following definitions:

**1) Mild:** The subject is aware of signs or symptoms, but they are easily tolerated. Usually does not require additional therapy or discontinuation of study drug.

- 2) Moderate:** The signs and symptoms are sufficient to restrict, but do not prevent usual activity; possibly requires additional therapy but usually does not require discontinuation of study drug.
- 3) Severe:** The subject is unable to perform usual activities and usually requires discontinuation of study drug.

**Drug relationship:**

The investigator is to classify the study product relationship of an adverse event to the investigational product according to the following definitions. Adverse events that are classified as “possible”, “probable”, or “definite” will be considered drug related.

**1) None:** The time course between the administration of study product and the occurrence or worsening of the adverse event rules out a causal relationship and/or another cause is confirmed and no indication of involvement of the study product in the occurrence/worsening of the adverse event exists.

**2) Unlikely:** The time course between administration of the study product and occurrence or worsening of the adverse event makes a causal relationship unlikely; the known effects of the study product or of the substance class provide no indication of involvement in occurrence/worsening of the adverse event and another cause adequately explaining the adverse event is known; regarding the occurrence/worsening of the adverse event, a plausible causal chain may be deduced from the known effects of the study product or the substance class but another cause is much more probable; or another cause is confirmed and involvement of the study product in the occurrence/worsening of the adverse event is unlikely.

**3) Possible:** Regarding the occurrence/worsening of the adverse event, a plausible causal chain may be deduced from the pharmacological properties of the study product or the substance class, but another cause just as likely to be involved is also known; although the pharmacological properties of the study product or the substance class provide no indication of involvement in the occurrence/worsening of the adverse event, no other cause gives adequate explanation.

**4) Probable:** The pharmacological properties of the study product or of the substance class and/or the course of the adverse event suggest involvement of the study product in the occurrence/worsening of the adverse event, although another cause cannot be ruled out.

**5) Definite:** The pharmacological properties of the study product or of the substance class and the course of the adverse event indicate involvement of the study product in the occurrence/worsening of the adverse event and no indication of other causes exists.

**6) Unclassifiable:** [only used for SAE] The available information is not sufficient for causality assessment.

**Outcome:**

The investigator will record the outcome of the AE choosing one of the following categories:  
Recovered/resolved,

Recovering/resolving,  
Not recovered/not resolved,  
Recovered/resolved with residual effects (specify),  
Fatal, or  
Unknown

## 12.2. **Data handling and record keeping:**

### 1) **Case report forms (CRFs) -**

The investigator and study coordinator will document in the patient files (hospital files). Data required according to this protocol are to be recorded on the CRFs developed by the Principal Investigator. Entries on the CRF must be made with a ballpoint pen and must be legible. Any documents related to the study must be archived at the study site or in a central archive. This includes the careful listing of the identities of the patients involved in the study. This list and the signed informed consent statements are key documents in the files to be stored by the investigator.

Patient (hospital) files will be archived according to local regulations. All documents related to the study must be retained until at least 15 years after the end of the study.

### 2) **Laboratory data and reference laboratories -**

For laboratory services provided by the investigator, the investigator must supply the sponsor with a complete list of all normal laboratory values for the laboratory utilized, as well as proof of laboratory certification by the Clinical Laboratory Improvement Amendments (CLIA).

### 3) **Patient registry -**

The investigator should maintain a patient registry of all patients entered into the study in the event a safety issue arises after study completion.

## 13. **REGULATORY CONSIDERATIONS / ETHICS**

### 13.1. **Institutional review board (IB) / ethics committee (EC) approval:**

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB/EC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version date.

Any changes to this protocol after receipt of IRB/EC approval made by the Sponsor or the Investigator must be in the form of a written amendment and the amendment will be appended to

this protocol and submitted to the IRB/EC for approval. Any protocol amendment suggested by the Investigator has to be approved by the Sponsor prior to implementation. Approval of amendments by the IRB is required prior to their implementation unless there are overriding safety reasons. If the change or deviation increases risk to the study population, or adversely affects the validity of the clinical investigation or the subject's rights, full approval must be obtained prior to implementation. For changes that do not involve increased risk or affect the validity of the investigation or the subject's rights, approval may be obtained by expedited review. When appropriate, an amendment may require a change to a written consent form as well. The Investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB/EC prior to use.

### **13.2. Recruitment:**

Potential participants may be identified during chart review in advance of a routine clinic visit or during a routine clinic visit with a provider. Individuals will be approached by the provider or study team to determine willingness to learn more about a study for which they may be eligible. Discussions regarding study participation will take place privately and individuals will be provided with the IRB approved consent form. In addition, potential participants may contact the study team directly. This contact may be in the form of telephone, email, etc. Initial discussions regarding study participation may take place by phone, email, etc., and individuals may be provided with the IRB approved consent form and other IRB reviewed and approved materials (e.g., Patient Handout), as applicable. In all cases, as much time as is needed to consider study participation will be allowed to possible participants; resulting in multiple phone calls, visits, emails, or other communication, as necessary. For individuals who choose to take part, informed consent will happen as per the consent process.

### **13.3. Subject confidentiality:**

The study will conform to the subject's right to protection against invasion of privacy. In compliance with United States federal regulations, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws. Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

### **13.4. Study records requirements:**

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; SAE reports, pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and



at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.]) be retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

PHI will be retained to justify screen failures, and will be stored under the same secure conditions as all study-related data for the length of the trial.

### **13.5. Evaluation of benefits and risks/discomforts:**

**Potential benefits:** Patients will receive evaluation and treatment of their malignancy as a result of participating in this trial. The trial will provide information on how IRX5183 should be administered to patients, but may or may not help a specific patient personally. This treatment may offer temporary control of the disease, but is not expected to be curative by this protocol. Alternative approaches to entering this trial, including supportive care only, will also be discussed before the verbal and written consent is obtained regarding the risks, benefits, and the treatment requirements of this trial.

**Measures for minimizing risk:** Administering IRX5183 to patients may involve risks that are currently unforeseeable. Side effects can be unpredictable in nature and severity, although all care will be taken to minimize them. If patients suffer any physical injury as a result of participating in this study, immediate medical treatment is available at the treatment center. Frequent blood work will be taken to monitor side effects. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. Malignancies with no further standard treatment options generally have a poor prognosis. Therefore, patients may experience significant treatment-related morbidity, and/or complications from progression of their disease.

### **13.6. Risks/benefits analysis:**

Data gathered from both clinical and laboratory evaluations in this trial will be analyzed frequently to ensure safety of patients. Any new or significant finding(s) found during the course of the research will be shared and explained to each participant since that may affect a patient's willingness to participate further. Patient's anonymity will be protected to the maximum extent in all publications and presentations that result from this research.

### **13.7. Patient information and consent:**

The investigator or consent designee will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential benefits and risks of participation to each patient prior to his/her entry into the study (i.e., before examinations and procedures associated with selection for the study are performed). Each patient will have ample opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Following this informative discussion, a patient will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the patient voluntarily agrees to sign the informed consent statement and has done so, may he/she enter the study. The patient will receive a copy of the signed and dated informed consent form.

Those members of the research team (principal investigator, co-investigator, research nurses) who consent patients have been trained in informed consent procedures, are familiar with the protocol, and are listed as a consentor in the application document. Patients are given adequate time and privacy to consider the research study. Before the patient signs the consent, the consentor must be satisfied that the participant understands the information provided, has had an opportunity to discuss the information and ask questions, and is aware that he/she may withdraw from the study at any time. Non-English speaking participants will be consented according to OHRP and JHMIRB policies.

The signed informed consent statement is to remain in the investigator's files. The informed consent form and any other written information provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol which necessitates a change to the content of the written informed consent form. The investigator will inform the patient of changes in a timely manner and will ask the patient to confirm continuation of his/her participation in the study by his/her signature on the revised informed consent form. Any revised written informed consent form must receive the IRB's approval/favorable opinion in advance of use.

### **13.8. Financial disclosure:**

Each investigator (including the principal investigator and any subinvestigators) who is directly involved in the treatment or evaluation of research subjects must disclose certain financial arrangements.

The following arrangements with, and interests of, investigators (including the spouse and dependent children) must be disclosed to the FDA:

- A) Compensation made to the investigator in which the value of compensation could be affected by study outcome (e.g., higher compensation for a favorable outcome than for an unfavorable outcome, or a royalty interest related to product sales);
- B) A proprietary interest by the investigator in the tested product, including, but not limited to, a patent, trademark, copyright or licensing agreement;
- C) Any equity interest in the sponsor of this study, i.e., any ownership interest, stock options, or other financial interest whose value cannot be readily determined through reference to public prices, or any equity interest in a publicly held company that exceeds \$ 50,000 in value held during the time the investigator is carrying out the study and for 1 year following completion of the study;
- D) Significant payments of other sorts, i.e., payments that have a cumulative monetary value of \$25,000 or more, made by the sponsor of a covered study to the investigator or the investigator's institution to support activities of the investigator exclusive of the costs of conducting the clinical study or other clinical studies, (e.g., a grant to fund ongoing research, compensation in the form

of equipment or retainers for ongoing consultation or honoraria) during the time the investigator is carrying out the study and for 1 year following completion of the study.

E) In this context “investigator” is defined as all individuals listed on FDA form 1572 - or for non-IND studies performed outside the U.S. listed in the signature list – directly involved in the treatment or evaluation of research subjects. The term also includes the spouse and each dependent child of the investigator.

A financial disclosure statement must be provided to the sponsor for each investigator (including each subinvestigator in IND studies identified on FDA Form 1572) at a study site before the study can commence. Financial disclosure statements must also be provided at the time the study is closed and at the 1-year anniversary of study closure.

**APPENDIX A1.** Modified International Working Group (IWG)-2003 (A) response criteria, (B) treatment failure, and (C) endpoints in AML.

**A.**

Response Criterion	Time of Assessment	Neutrophils (μL)	Platelets (μL)	Bone Marrow Blasts (%)	Other
Early treatment assessment	7-10 days after therapy	NA	NA	< 5	
Morphologic leukemia-free state	Varies by protocol	NA	NA	< 5	Flow cytometry EMD
Morphologic CR	Varies by protocol	> 1,000	> 100,000	< 5	Transfusion EMD
Cytogenetic CR	Varies by protocol	> 1,000	> 100,000	< 5	Cyogenetics—normal, EMD
Molecular CR	Varies by protocol	> 1,000	> 100,000	< 5	Molecular—negative, EMD
Partial remission	Varies by protocol	> 1,000	> 100,000	> 50 or decrease to 5-25	Blasts < 5% if Auer rod positive

Abbreviations: AML, acute myelogenous leukemia; EMD, extramedullary disease; CR, complete remission.

**B.**

Category	Definition
Resistant disease	Patient survives $\geq$ 7 days post-CT; persistent AML in blood or bone marrow
Aplasia	Patient survives $\geq$ 7 days post-CT; death while cytopenic, with aplastic bone marrow
Indeterminate cause	Patients who die < 7 days posttherapy Patients who die > 7 days posttherapy with no PB blasts, but no bone marrow examination Patients who do not complete the first course of therapy
Morphologic relapse	Reappearance of blasts post-CR in PB or bone marrow
Molecular or cytogenetic relapse	Reappearance of molecular or cytogenetic abnormality

Abbreviations: AML, acute myelogenous leukemia; CT, chemotherapy; PB, peripheral blood; CR, complete remission.

**C.**

Outcome	Response Category	Point of Measurement	Definition
Overall survival	All patients	Entry onto trial	Death from any cause
Relapse-free survival	CR	Leukemia-free state	Disease relapse or patient death from any cause
Event-free survival	All patients*	Entry onto trial	Treatment failure, disease relapse, or patient death from any cause
Remission duration	CR	Date of CR	Disease relapse

NOTE. Complete blood counts should be evaluated at least monthly, or more often if clinically indicated, to establish the durability of responses. Abbreviations: AML, acute myelogenous leukemia; CR, complete remission.

\*Under circumstances where presentation of event-free survival may be appropriate for responders only, this point should be clearly stated.

(Cheson et al., 2003)

**APPENDIX A2. Modified International Working Group (IWG)-2006 response criteria for altering natural history of MDS.**

Category	Response criteria (responses must last at least 4 wk)
Complete remission	<p>Bone marrow: <math>\leq 5\%</math> myeloblasts with normal maturation of all cell lines*</p> <p>Persistent dysplasia will be noted**†</p> <p>Peripheral blood‡</p> <p>Hgb <math>\geq 11</math> g/dL</p> <p>Platelets <math>\geq 100 \times 10^9/L</math></p> <p>Neutrophils <math>\geq 1.0 \times 10^9/L</math>†</p> <p>Blasts 0%</p>
Partial Remission	<p>All CR criteria if abnormal before treatment except:</p> <p>Bone marrow blasts decreased by <math>\geq 50\%</math> over pretreatment but still <math>&gt; 5\%</math></p> <p>Cellularity and morphology not relevant</p>
Marrow CR†	<p>Bone marrow: <math>\leq 5\%</math> myeloblasts and decrease by <math>\geq 50\%</math> over pretreatment†</p> <p>Peripheral blood: if HI responses, they will be noted in addition to marrow CR†</p>
Stable disease	Failure to achieve at least PR, but no evidence of progression for $> 8$ wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	<p>At least 1 of the following:</p> <p>Return to pretreatment bone marrow blast percentage</p> <p>Decrement of <math>\geq 50\%</math> from maximum remission/response levels in <math>\square</math>granulocytes or platelets</p> <p>Reduction in Hgb concentration by <math>\geq 1.5</math> g/dL or transfusion dependence</p>
Cytogenetic response	<p>Complete</p> <p>Disappearance of the chromosomal abnormality without appearance of new ones</p> <p>Partial</p> <p>At least 50% reduction of the chromosomal abnormality</p> <p>For patients with:</p>
Disease progression	<p>Less than 5% blasts: <math>\geq 50\%</math> increase in blasts to <math>&gt; 5\%</math> blasts</p> <p>5%-10% blasts: <math>\geq 50\%</math> increase to <math>&gt; 10\%</math> blasts</p> <p>10%-20% blasts: <math>\geq 50\%</math> increase to <math>&gt; 20\%</math> blasts</p> <p>20%-30% blasts: <math>\geq 50\%</math> increase to <math>&gt; 30\%</math> blasts</p> <p>Any of the following:</p> <p>At least 50% decrement from maximum remission/response in granulocytes or platelets</p> <p>Reduction in Hgb by <math>\geq 2</math> g/dL</p> <p>Transfusion dependency <math>\square</math></p>
Survival	<p>Endpoints:</p> <p>Overall: death from any cause</p>

Event free: failure or death from any cause  
PFS: disease progression or death from MDS  
DFS: time to relapse  
Cause-specific death: death related to MDS

---

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.  
MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival.

\* Dysplastic changes should consider the normal range of dysplastic changes (modification).

† Modification to IWG response criteria.

‡ In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

(Cheson et al., 2006)

### APPENDIX A3. Modified International Working Group (IWG)-2006 response criteria for hematologic improvement

Hematologic improvement*	Response criteria (responses must last at least 8 wk) <sup>†</sup>
Erythroid response (pretreatment, < 11 g/dL)	Hgb increase by $\geq 1.5$ g/dL  Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of $\leq 9.0$ g/dL pretreatment will count in the RBC transfusion response evaluation <sup>†</sup>
Platelet response (pretreatment, < $100 \times 10^9/L$ )	Absolute increase of $\geq 30 \times 10^9/L$ for patients starting with $> 20 \times 10^9/L$ platelets  Increase from $< 20 \times 10^9/L$ to $> 20 \times 10^9/L$ and by at least 100% <sup>†</sup>
Neutrophil response (pretreatment, < $1.0 \times 10^9/L$ )	At least 100% increase and an absolute increase $> 0.5 \times 10^9/L$ <sup>†</sup>
Progression or relapse after HI <sup>‡</sup>	At least 1 of the following:  At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by $\geq 1.5$ g/dL Transfusion dependence

Deletions to the IWG response criteria are not shown.

To convert hemoglobin levels from grams per deciliter to grams per liter, multiply grams per deciliter by 10. Hgb indicates hemoglobin; RBC: red blood cell; HI: hematologic improvement.

\* Pretreatment counts averages of at least 2 measurements (not influenced by transfusions)  $\geq 1$  week apart (modification).

<sup>†</sup> Modification to IWG response criteria.

<sup>‡</sup> In the absence of another explanation, such as acute infection, repeated courses of chemotherapy (modification), gastrointestinal bleeding, hemolysis, and so forth. It is recommended that the 2 kinds of erythroid and platelet responses be reported overall as well as by the individual response pattern.

(Cheson et al., 2006)

**Appendix B:**

## A) International Prognostic Scoring System (IPSS) for MDS

## SCORE VALUES

PROGNOSTIC VARIABLE	0	0.5	1.0	1.5	2.0
Marrow Blasts (%)†	<5	5-10	–	11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenias**	0/1	2/3			

†Patients with 21-30% blasts are considered as MDS or AML (WHO)

\*Cytogenetics: Good = normal, -Y alone, del(5q) alone, del(20q) alone, Poor = complex ( $\geq 3$  abnormalities) or chromosome 7 anomalies; Intermediate = other abnormalities

\*\*Cytopenias: neutrophil count <1800/ $\mu$ l, platelets <100,000, Hb <10 g/dL.

Risk category (% IPSS pop.)	Overall score	Median survival (yr)	25% AML progression (yr)
LOW (33)	0	5.7	9.4
INT-1 (38)	0.5-1.0	3.5	3.3
INT-2 (22)	1.5-2.0	1.1	1.1
HIGH (7)	>2.5	0.4	0.2

## B) Revised International Prognostic Scoring System (IPSS-R) for MDS

PROGNOSTIC VARIABLE	Values/categories	Score
Percentage marrow blasts	$\leq 2$	0
	>2 to <5	1
	5-10	2
	>10	3
Cytogenetics	<i>Very good:</i> -Y, del(11q)	0
	<i>Good:</i> normal, del(5q), del(12p), del(20q)	1
	<i>Intermediate:</i> del(7q), +8, +19, i(17q)	2



	<i>Poor</i> : -7, inv(3)/t(3q)/del(3q), complex: 3 abnormalities	3
	<i>Very poor</i> : complex: > 3 abnormalities	4
Hemoglobin (g/dL)	≥10	0
	8 to < 10	1
	<8	1.5
Platelets (cells/microL)	≥100	0
	50 to 100	0.5
	<50	1
Absolute neutrophil count (cells/microL)	≥0.8	0
	<0.8	0.5

R-IPSS Score	Risk Group	Median Overall Survival (Yrs)
≤1.5	Very low	8.8
>1.5 to 3	Low	5.3
>3 to 4.5	Intermediate	3
>4.5-6	High	1.6
>6	Very high	0.8

**APPENDIX C: Performance Status by ECOG and Karnofsky Grading 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 ( <i>e.g.</i> , light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

**APPENDIX D: FACT-Leukemia Scoring Guidelines (Version 4)**

- Instructions:\*
1. Record answers in "item response" column. If missing, mark with an X
  2. Perform reversals as indicated, and sum individual items to obtain a score.
  3. Multiply the sum of the item scores by the number of items in the subscale, then divide by the number of items answered. This produces the subscale score.
  4. Add subscale scores to derive total scores (TOI, FACT-G & FACT-Leukemia).
  5. **The higher the score, the better the QOL.**

<u>Subscale</u>	<u>Item Code</u>	<u>Reverse item?</u>	<u>Item response</u>	<u>Item Score</u>
<b>PHYSICAL WELL-BEING (PWB)</b>	GP1	4 -	_____	= _____
	GP2	4 -	_____	= _____
	GP3	4 -	_____	= _____
	GP4	4 -	_____	= _____
	GP5	4 -	_____	= _____
	GP6	4 -	_____	= _____
	GP7	4 -	_____	= _____
<i>Score range: 0-28</i>				
<i>Sum individual item scores:</i>				_____
<i>Multiply by 7:</i>				_____
<i>Divide by number of items answered:</i>				_____ = <b><u>PWB subscale</u></b>

**score**

<b>SOCIAL/FAMILY WELL-BEING (SWB)</b>	GS1	0 +	_____	= _____
	GS2	0 +	_____	= _____
	GS3	0 +	_____	= _____
	GS4	0 +	_____	= _____
	GS5	0 +	_____	= _____
	GS6	0 +	_____	= _____
	GS7	0 +	_____	= _____
<i>Score range: 0-28</i>				
<i>Sum individual item scores:</i>				_____
<i>Multiply by 7:</i>				_____
<i>Divide by number of items answered:</i>				_____ = <b><u>SWB subscale</u></b>

**score**

<b>EMOTIONAL WELL-BEING (EWB)</b>	GE1	4 -	_____	= _____
	GE2	0 +	_____	= _____
	GE3	4 -	_____	= _____
	GE4	4 -	_____	= _____
	GE5	4 -	_____	= _____
	GE6	4 -	_____	= _____
<i>Score range: 0-24</i>				
<i>Sum individual item scores:</i>				_____
<i>Multiply by 6:</i>				_____
<i>Divide by number of items answered:</i>				_____ = <b><u>EWB subscale</u></b>

**score**

<b>FUNCTIONAL WELL-BEING (FWB)</b>	GF1	0 +	_____	= _____
	GF2	0 +	_____	= _____
	GF3	0 +	_____	= _____
	GF4	0 +	_____	= _____

Score range: 0-28	GF5	0	+	_____	= _____
	GF6	0	+	_____	= _____
	GF7	0	+	_____	= _____

Sum individual item scores: \_\_\_\_\_

Multiply by 7: \_\_\_\_\_

Divide by number of items answered: \_\_\_\_\_ = **FWB subscale**

**score**

**FACT-Leukemia Scoring Guidelines (Version 4)**

<b><u>Subscale</u></b>	<b><u>Item Code</u></b>	<b><u>Reverse item?</u></b>	<b><u>Item response</u></b>	<b><u>Item Score</u></b>	
<b>LEUKEMIA SUBSCALE (LEUS)</b>	BRM3	4	-	_____	= _____
	P2	4	-	_____	= _____
	BRM2	4	-	_____	= _____
	ES3	4	-	_____	= _____
	LEU1	4	-	_____	= _____
	TH1	4	-	_____	= _____
	TH2	4	-	_____	= _____
	HI12	4	-	_____	= _____
	BMT6	4	-	_____	= _____
	C2	4	-	_____	= _____
	C6	0	+	_____	= _____
	An7	0	+	_____	= _____
	N3	4	-	_____	= _____
	LEU5	4	-	_____	= _____
	LEU6	4	-	_____	= _____
	BRM9	4	-	_____	= _____
	LEU7	4	-	_____	= _____

Score range: 0-68

Sum individual item scores: \_\_\_\_\_

Multiply by 17: \_\_\_\_\_

Divide by number of items answered: \_\_\_\_\_ = **LEU Subscale**

**score**

**To derive a FACT-Leukemia Trial Outcome Index (TOI):**

Score range: 0-124

$$\text{TOI} = \text{_____} + \text{_____} + \text{_____} = \text{_____} = \text{FACT-Leukemia}$$

(PWB score) (FWB score) (LeuS score)

**To Derive a FACT-G total score:**

Score range: 0-108

$$\text{score} = \text{_____} + \text{_____} + \text{_____} + \text{_____} = \text{_____} = \text{FACT-G Total}$$

(PWB score) (SWB score) (EWB score) (FWB score)

**To Derive a FACT-Leukemia total score:**

Score range: 0-176

$$\begin{array}{ccccccccc} \text{_____} & + & \text{_____} & + & \text{_____} & + & \text{_____} & + & \text{_____} & = & \text{_____} & = & \text{FACT-Leukemia} \\ \text{Total} & & & & & & & & & & & & \text{score} \\ \text{(PWB score)} & & \text{(SWB score)} & & \text{(EWB score)} & & \text{(FWB score)} & & \text{(LeuS score)} & & & & \end{array}$$

\*For guidelines on handling missing data and scoring options, please refer to the Administration and Scoring Guidelines in the manual or on-line at [www.facit.org](http://www.facit.org).

**REFERENCES**

- Adamson, P. C. (1996). All-Trans-Retinoic Acid Pharmacology and Its Impact on the Treatment of Acute Promyelocytic Leukemia. *Oncologist*, *1*(5), 305-314.
- Alonso, S., Su, M., Jones, J. W., Ganguly, S., Kane, M. A., Jones, R. J., & Ghiaur, G. (2015). Human bone marrow niche chemoprotection mediated by cytochrome P450 enzymes. *Oncotarget*.
- Angstreich, G. R., Matsui, W., Huff, C. A., Vala, M. S., Barber, J., Hawkins, A. L., . . . Jones, R. J. (2005). Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. *Br J Haematol*, *130*(3), 373-381. doi: 10.1111/j.1365-2141.2005.05606.x
- Bedi, A., Zehnbauer, B. A., Collector, M. I., Barber, J. P., Zicha, M. S., Sharkis, S. J., & Jones, R. J. (1993). BCR-ABL gene rearrangement and expression of primitive hematopoietic progenitors in chronic myeloid leukemia. *Blood*, *81*(11), 2898-2902.
- Benesch, M., & Deeg, H. J. (2003). Hematopoietic cell transplantation for adult patients with myelodysplastic syndromes and myeloproliferative disorders. *Mayo Clin Proc*, *78*(8), 981-990. doi: 10.4065/78.8.981
- Berkow, R. L., & Kraft, A. S. (1985). Bryostatins, a non-phorbol macrocyclic lactone, activates intact human polymorphonuclear leukocytes and binds to the phorbol ester receptor. *Biochem Biophys Res Commun*, *131*(3), 1109-1116.
- Bhalla, K. N. (2005). Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *J Clin Oncol*, *23*(17), 3971-3993. doi: 10.1200/JCO.2005.16.600
- Blinder, V. S., & Roboz, G. J. (2003). Hematopoietic growth factors in myelodysplastic syndromes. *Curr Hematol Rep*, *2*(6), 453-458.
- Burnett, A. K., Hills, R. K., Green, C., Jenkinson, S., Koo, K., Patel, Y., . . . Gale, R. E. (2010). The impact on outcome of the addition of all-trans retinoic acid to intensive chemotherapy in younger patients with nonacute promyelocytic acute myeloid leukemia: overall results and results in genotypic subgroups defined by mutations in NPM1, FLT3, and CEBPA. *Blood*, *115*(5), 948-956. doi: 10.1182/blood-2009-08-236588
- Burnett, A. K., Milligan, D., Prentice, A. G., Goldstone, A. H., McMullin, M. F., Hills, R. K., & Wheatley, K. (2007). A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. *Cancer*, *109*(6), 1114-1124. doi: 10.1002/cncr.22496
- Candoni, A., Silvestri, F., Buonamici, S., Li, D., Reddy, P., Galili, N., . . . Raza, A. (2004). Targeted therapies in myelodysplastic syndromes: ASH 2003 review. *Semin Hematol*, *41*(2 Suppl 4), 13-20.
- Catenacci, D. V., & Schiller, G. J. (2005). Myelodysplastic syndromes: a comprehensive review. *Blood Rev*, *19*(6), 301-319. doi: 10.1016/j.blre.2005.01.004
- Cella, D., Jensen, S. E., Webster, K., Hongyan, D., Lai, J. S., Rosen, S., . . . Yount, S. (2012). Measuring health-related quality of life in leukemia: the Functional Assessment of Cancer Therapy--Leukemia (FACT-Leu) questionnaire. *Value Health*, *15*(8), 1051-1058. doi: 10.1016/j.jval.2012.08.2210
- Chee, L. C., Hendy, J., Purton, L. E., & McArthur, G. A. (2013). ATRA and the specific RARalpha agonist, NRX195183, have opposing effects on the clonogenicity of pre-leukemic murine AML1-ETO bone marrow cells. *Leukemia*, *27*(6), 1369-1380. doi: 10.1038/leu.2012.362

- Cheson, B. D., Bennett, J. M., Kopecky, K. J., Buchner, T., Willman, C. L., Estey, E. H., . . . Reporting Standards for Therapeutic Trials in Acute Myeloid, L. (2003). Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*, 21(24), 4642-4649. doi: 10.1200/JCO.2003.04.036
- Cheson, B. D., Greenberg, P. L., Bennett, J. M., Lowenberg, B., Wijermans, P. W., Nimer, S. D., . . . Kantarjian, H. (2006). Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*, 108(2), 419-425. doi: 10.1182/blood-2005-10-4149
- De Vita Jr VT, H. S., Rosenberg SA, eds. (1997). *Cancer: principles and practice of oncology*. 5th edn. Philadelphia: Lippincott.
- de Witte, T., Hermans, J., Vossen, J., Bacigalupo, A., Meloni, G., Jacobsen, N., . . . Apperley, J. (2000). Haematopoietic stem cell transplantation for patients with myelo-dysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol*, 110(3), 620-630.
- Di Stasi, A., Milton, D. R., Poon, L. M., Hamdi, A., Rondon, G., Chen, J., . . . Ciurea, S. O. (2014). Similar transplantation outcomes for acute myeloid leukemia and myelodysplastic syndrome patients with haploidentical versus 10/10 human leukocyte antigen-matched unrelated and related donors. *Biol Blood Marrow Transplant*, 20(12), 1975-1981. doi: 10.1016/j.bbmt.2014.08.013
- Duong, V. H., Bhatnagar, B., Zandberg, D. P., Vannorsdall, E. J., Tidwell, M. L., Chen, Q., & Baer, M. R. (2015). Lack of objective response of myelodysplastic syndromes and acute myeloid leukemia to decitabine after failure of azacitidine. *Leuk Lymphoma*, 56(6), 1718-1722. doi: 10.3109/10428194.2014.966708
- Estey, E. H., Thall, P. F., Pierce, S., Cortes, J., Beran, M., Kantarjian, H., . . . Freireich, E. (1999). Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin +/- all-trans retinoic acid +/- granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. *Blood*, 93(8), 2478-2484.
- Eunpi, C., Matsui, W., Kowalski, J., Tsai, H.-L., Jones, R. J., & Smith, B. D. (2010). Combination Therapy with Entinostat (MS-275) and GM-CSF to Enhance Differentiation In Myeloid Malignancies. *ASH Annual Meeting Abstracts*, 116(21), 3312-.
- Gerber, J. M., Smith, B. D., Ngwang, B., Zhang, H., Vala, M. S., Morsberger, L., . . . Jones, R. J. (2012). A clinically relevant population of leukemic CD34(+)CD38(-) cells in acute myeloid leukemia. *Blood*, 119(15), 3571-3577. doi: 10.1182/blood-2011-06-364182
- Ghiaur, G., Yegnasubramanian, S., Perkins, B., Gucwa, J. L., Gerber, J. M., & Jones, R. J. (2013). Regulation of human hematopoietic stem cell self-renewal by the microenvironment's control of retinoic acid signaling. *Proc Natl Acad Sci U S A*, 110(40), 16121-16126. doi: 10.1073/pnas.1305937110
- Gladstone, D. E., Bedi, A., Miller, C. B., Noga, S. J., Griffin, C. A., Piantadosi, S., . . . Jones, R. J. (1999). Philadelphia chromosome-negative engraftment after autologous transplantation with granulocyte-macrophage colony-stimulating factor for chronic myeloid leukemia. *Biol Blood Marrow Transplant*, 5(6), 394-399.
- Godwin, J. E., & Smith, S. E. (2003). Acute myeloid leukemia in the older patient. *Crit Rev*

- Oncol Hematol*, 48(Suppl), S17-26.
- Grant, S., Pettit, G. R., Howe, C., & McCrady, C. (1991). Effect of the protein kinase C activating agent bryostatin 1 on the clonogenic response of leukemic blast progenitors to recombinant granulocyte-macrophage colony-stimulating factor. *Leukemia*, 5(5), 392-398.
- Hiddemann, W., Spiekermann, K., Buske, C., Feuring-Buske, M., Braess, J., Haferlach, T., . . . German, A. M. L. C. G. (2005). Towards a pathogenesis-oriented therapy of acute myeloid leukemia. *Crit Rev Oncol Hematol*, 56(2), 235-245. doi: 10.1016/j.critrevonc.2005.07.004
- Howlader N, N. A., Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). (2015). SEER Cancer Statistics Review, 1975-2012, National Cancer Institute.
- Itzykson, R., Thepot, S., Berthon, C., Delaunay, J., Bouscary, D., Cluzeau, T., . . . Gardin, C. (2015). Azacitidine for the treatment of relapsed and refractory AML in older patients. *Leuk Res*, 39(2), 124-130. doi: 10.1016/j.leukres.2014.11.009
- Jonasova, A., Neuwirtova, R., Cermak, J., Vozobulova, V., Mocikova, K., Siskova, M., & Hochova, I. (1998). Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow. *Br J Haematol*, 100(2), 304-309.
- Kane, M. A. (2012). Analysis, occurrence, and function of 9-cis-retinoic acid. *Biochim Biophys Acta*, 1821(1), 10-20. doi: 10.1016/j.bbalip.2011.09.012
- Kizaki, M., & Koeffler, H. P. (1992). Differentiation-inducing agents in the treatment of myelodysplastic syndromes. *Semin Oncol*, 19(1), 95-105.
- Lo-Coco, F., Avvisati, G., Vignetti, M., Thiede, C., Orlando, S. M., Iacobelli, S., . . . Study Alliance, L. (2013). Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med*, 369(2), 111-121. doi: 10.1056/NEJMoa1300874
- Matsui, W., Smith, B. D., Vala, M., Beal, N., Huff, C. A., Diehl, L. F., & Jones, R. J. (2005). Requirement for myeloid growth factors in the differentiation of acute promyelocytic leukaemia. *Br J Haematol*, 128(6), 853-862. doi: 10.1111/j.1365-2141.2005.05395.x
- Matsui, W. H., Gladstone, D. E., Vala, M. S., Barber, J. P., Brodsky, R. A., Smith, B. D., & Jones, R. J. (2002). The role of growth factors in the activity of pharmacological differentiation agents. *Cell Growth Differ*, 13(6), 275-283.
- McCurdy, S. R., Kanakry, J. A., Showel, M. M., Tsai, H. L., Bolanos-Meade, J., Rosner, G. L., . . . Kasamon, Y. L. (2015). Risk-stratified outcomes of nonmyeloablative HLA-haploidentical BMT with high-dose posttransplantation cyclophosphamide. *Blood*, 125(19), 3024-3031. doi: 10.1182/blood-2015-01-623991
- Milligan, D. W., Wheatley, K., Littlewood, T., Craig, J. I., Burnett, A. K., & Group, N. H. O. C. S. (2006). Fludarabine and cytosine are less effective than standard ADE chemotherapy in high-risk acute myeloid leukemia, and addition of G-CSF and ATRA are not beneficial: results of the MRC AML-HR randomized trial. *Blood*, 107(12), 4614-4622. doi: 10.1182/blood-2005-10-4202
- Molldrem, J. J., Caples, M., Mavroudis, D., Plante, M., Young, N. S., & Barrett, A. J. (1997). Antithymocyte globulin for patients with myelodysplastic syndrome. *Br J Haematol*, 99(3), 699-705.
- Montesinos, P., Bergua, J. M., Vellenga, E., Rayon, C., Parody, R., de la Serna, J., . . . Sanz, M. A. (2009). Differentiation syndrome in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline chemotherapy: characteristics,



- outcome, and prognostic factors. *Blood*, 113(4), 775-783. doi: 10.1182/blood-2008-07-168617
- Morosetti, R., Grignani, F., Liberatore, C., Pelicci, P. G., Schiller, G. J., Kizaki, M., . . . Koeffler, H. P. (1996). Infrequent alterations of the RAR alpha gene in acute myelogenous leukemias, retinoic acid-resistant acute promyelocytic leukemias, myelodysplastic syndromes, and cell lines. *Blood*, 87(10), 4399-4403.
- Norsworthy, K., Luznik, L., & Gojo, I. (2012). New treatment approaches in acute myeloid leukemia: review of recent clinical studies. *Rev Recent Clin Trials*, 7(3), 224-237.
- Norsworthy KJ, J. R., Warlick ED, Cho E, Matsui WH, Smith BD. (2015). A phase II study of bexarotene + GM-CSF in refractory AML and high risk MDS. *J Clin Oncol, suppl*, e13542.
- Osanai, M., & Petkovich, M. (2005). Expression of the retinoic acid-metabolizing enzyme CYP26A1 limits programmed cell death. *Mol Pharmacol*, 67(5), 1808-1817. doi: 10.1124/mol.104.005769
- Ozpolat, B., Mehta, K., Tari, A. M., & Lopez-Berestein, G. (2002). all-trans-Retinoic acid-induced expression and regulation of retinoic acid 4-hydroxylase (CYP26) in human promyelocytic leukemia. *Am J Hematol*, 70(1), 39-47. doi: 10.1002/ajh.10099
- Pasquini MC, Z. X. (2014). Current uses and outcomes of hematopoietic stem cell transplantation: 2014 CIBMTR Summary Slides. *Physician's Desk Reference*. (2000). (54 ed.): Medical Economics.
- Prebet, T., Gore, S. D., Esterni, B., Gardin, C., Itzykson, R., Thepot, S., . . . Vey, N. (2011). Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. *J Clin Oncol*, 29(24), 3322-3327. doi: 10.1200/JCO.2011.35.8135
- Raza, A., Meyer, P., Dutt, D., Zorat, F., Lisak, L., Nascimben, F., . . . Zeldis, J. (2001). Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes. *Blood*, 98(4), 958-965.
- Ritchie, E. K., Feldman, E. J., Christos, P. J., Rohan, S. D., Lagassa, C. B., Ippoliti, C., . . . Roboz, G. J. (2013). Decitabine in patients with newly diagnosed and relapsed acute myeloid leukemia. *Leuk Lymphoma*, 54(9), 2003-2007. doi: 10.3109/10428194.2012.762093
- Robin, M., Ruggeri, A., Labopin, M., Niederwieser, D., Tabrizi, R., Sanz, G., . . . Kroger, N. (2015). Comparison of unrelated cord blood and peripheral blood stem cell transplantation in adults with myelodysplastic syndrome after reduced-intensity conditioning regimen: a collaborative study from Eurocord (Cord blood Committee of Cellular Therapy & Immunobiology Working Party of EBMT) and Chronic Malignancies Working Party. *Biol Blood Marrow Transplant*, 21(3), 489-495. doi: 10.1016/j.bbmt.2014.11.675
- Sanford D, L. F., Sanz MA, DiBona E, Coutre S, Altman JK, Wetzler M, Allen SL, Ravandi F, Kantarjian HM, Cortes J. (2014). Single agent tamibarotene has activity in acute promyelocytic leukemia patients previously treated with ATRA and arsenic trioxide, but does not produce durable responses. *Blood*, abstr 3751.
- Schlenk, R. F., Frohling, S., Hartmann, F., Fischer, J. T., Glasmacher, A., del Valle, F., . . . Ulm, A. M. L. S. G. (2004). Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. *Leukemia*, 18(11), 1798-1803. doi: 10.1038/sj.leu.2403528
- Schumacher, H. R., Alvares, C. J., Blough, R. I., & Mazzella, F. (2002). Acute leukemia. *Clin*

- Lab Med*, 22(1), 153-192, vii.
- Sierra, J., Perez, W. S., Rozman, C., Carreras, E., Klein, J. P., Rizzo, J. D., . . . Horowitz, M. M. (2002). Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. *Blood*, 100(6), 1997-2004.
- Silverman, L. R. (2004). DNA methyltransferase inhibitors in myelodysplastic syndrome. *Best Pract Res Clin Haematol*, 17(4), 585-594. doi: 10.1016/j.beha.2004.08.010
- Smith, B. D., Jones, R. J., Cho, E., Kowalski, J., Karp, J. E., Gore, S. D., . . . Matsui, W. H. (2011). Differentiation therapy in poor risk myeloid malignancies: Results of a dose finding study of the combination bryostatins and GM-CSF. *Leuk Res*, 35(1), 87-94. doi: 10.1016/j.leukres.2010.06.001
- Stein, R. S. (2003). The role of erythropoietin in the anemia of myelodysplastic syndrome. *Clin Lymphoma*, 4 Suppl 1, S36-40.
- Su, M., Alonso, S., Jones, J. W., Yu, J., Kane, M. A., Jones, R. J., & Ghiaur, G. (2015). All-Trans Retinoic Acid Activity in Acute Myeloid Leukemia: Role of Cytochrome P450 Enzyme Expression by the Microenvironment. *PLoS One*, 10(6), e0127790. doi: 10.1371/journal.pone.0127790
- Tallman, M. S., Andersen, J. W., Schiffer, C. A., Appelbaum, F. R., Feusner, J. H., Woods, W. G., . . . Wiernik, P. H. (2002). All-trans retinoic acid in acute promyelocytic leukemia: long-term outcome and prognostic factor analysis from the North American Intergroup protocol. *Blood*, 100(13), 4298-4302. doi: 10.1182/blood-2002-02-0632
- Thol, F., Schlenk, R. F., Heuser, M., & Ganser, A. (2015). How I treat refractory and early relapsed acute myeloid leukemia. *Blood*, 126(3), 319-327. doi: 10.1182/blood-2014-10-551911
- Tobita, T., Takeshita, A., Kitamura, K., Ohnishi, K., Yanagi, M., Hiraoka, A., . . . Ohno, R. (1997). Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-trans retinoic acid. *Blood*, 90(3), 967-973.
- Vrana, J. A., Saunders, A. M., Chellappan, S. P., & Grant, S. (1998). Divergent effects of bryostatins and phorbol myristate acetate on cell cycle arrest and maturation in human myelomonocytic leukemia cells (U937). *Differentiation*, 63(1), 33-42. doi: 10.1046/j.1432-0436.1998.6310033.x
- Yoko Korenaga Fukuda, E. H., Shanda Finnigan, S. Percy Ivy, Larry Rubinstein, Naoko Takebe. (2014). Risks and benefits of phase 1 oncology trials, 2001 through 2012. *J Clin Oncol*, 32(5s), abstr 2552.
- Zeidner, J. F., Gladstone, D. E., Zahurak, M., Matsui, W. H., Gocke, C., Jones, R. J., & Smith, B. D. (2014). Granulocyte-macrophage colony stimulating factor (GM-CSF) enhances the clinical responses to interferon-alpha (IFN) in newly diagnosed chronic myeloid leukemia (CML). *Leuk Res*, 38(8), 886-890. doi: 10.1016/j.leukres.2014.05.012