**IGF-MTX Conjugate in the Treatment of Myelodysplastic Syndrome**

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**AML-03: Pilot Study of IGF-Methotrexate Conjugate in the Treatment of Myelodysplastic Syndrome, CMML and Oligoblastic AML.**

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# Synopsis

**Primary Objective:**
The primary objective of this study is to determine the safety and tolerability of utilizing the insulin-like growth factor-1-methotrexate conjugate, 765IGF-MTX for the treatment of advanced, previously treated myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML) and oligoblastic acute myelogenous leukemia (oligoblastic AML or O-AML), including determining the maximum tolerated dose (MTD).

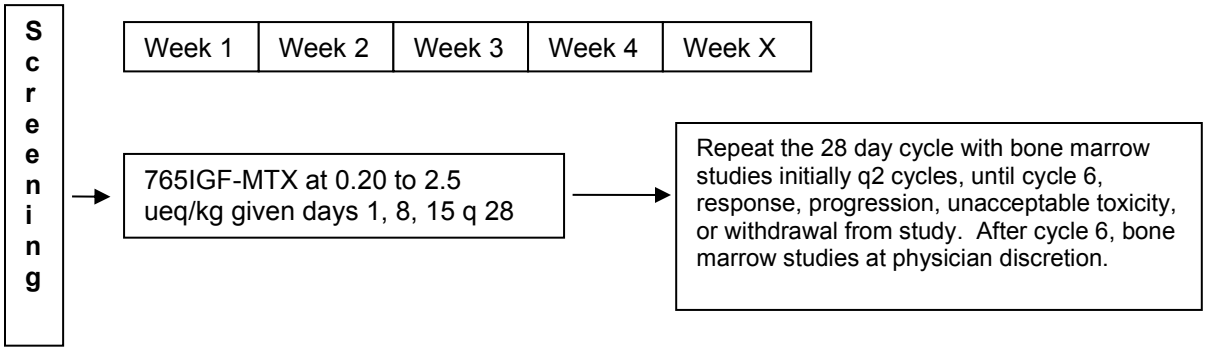
**Secondary Objective:**
The secondary objective of this study is to determine the clinical benefit of 765IGF-MTX as measured by partial and complete remission rates, relapse-free survival, and overall survival in patients with advanced, previously treated MDS, CMML, or O-AML.

**Patient Population:**
- Diagnosis of MDS, CMML or O-AML that is refractory to or intolerant of standard therapy and is no longer likely to respond to such therapy
- Patient must have recovered from the acute toxic effects (≤ grade 1 CTCAE v.4.0) of previous anti-cancer treatment prior to study enrollment
- Age 18 years or older
- ECOG performance status 0, 1, or 2 (appendix III)

**Study Design:**
This pilot study will evaluate use of IGF-Methotrexate conjugate (765IGF-MTX) in patients with advanced, previously treated MDS, CMML and O-AML. 765IGF-MTX at a dose of 0.20 to 2.5 µequivalents per kg is administered as an IV infusion over 1.5 hours on days 1, 8 and 15 of a 28 day cycle. Treatment continues until disease progression, as assessed after 2 cycles, unacceptable toxicity, or patient refusal. Assessment of response will be confirmed by bone marrow studies performed at the end of cycles 2, 4, and 6 (each +/- 3 days).

Once a final maximum tolerated dose level (MTD) is determined, the pharmacokinetics (PK) of 765IGF-MTX will be assessed on days 1 and 2 and days 15 and 16 of cycle 1 at the MTD in three patients.

# Study Schema




Pharmacodynamic samples on day 1 of cycle 1, days 1 and 15 of cycle 2, and day 15 of cycles 4 and 6 before 765IGF-MTX administration.
At the MTD: Pharmacokinetic samples starting on day 1 (including time points on day 2), and day 15 (including time points on day 16) of cycle 1

**Dose Finding Component:** 1 cycle = 28 days

| | Cycle 1 | | | | Cycle 2 | | | | Cycle 3 and beyond[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| day | 1 | 8 | 15 | | 1 | 8 | 15 | | 1 | 8 | 15 | |
| 765IGF-MTX* | X | X | X | | X | X | X | | X | X[1] | X[1] | |
| Clinic visit | X | X | X | | X | X | X | | X | X[1] | X[1] | |

* at assigned dose level (see table below)
[1]Day 8 and day 15 visits at physician's discretion cycle 3 and beyond
[2]Treatment continues until disease progression, unacceptable toxicity, patient refusal or other reason as found in section 7.11

**Phase I Dose Levels**

| Dose Level | 765IGF-MTX Dose | Number of Patients* |
|---|---|---|
| 1 | 0.20 µequivalents per kg | 1-9 |
| 2 | 0.40 µequivalents per kg | 1-9 |
| 3 | 0.80 µequivalents per kg | 1-9 |
| 4 | 1.6 µequivalents per kg | 1-9 |
| 5 | 2.5 µequivalents per kg | 1-9 |

*dose escalation cohorts between 1-9 patients; total of 9 patients in MTD cohort. At the discretion of the principal investigator, intra-patient dose escalation between cycles is also allowed but only after 2 cycles at the assigned dose level and confirmation of safety in at least 3 patients treated at the higher dose level.

# Abbreviations

| Abbreviation | Definition |
|---|---|
| AE | adverse event |
| ALT | alanine aminotransferase |
| AML | acute myelogenous leukemia |
| ANC | absolute neutrophil count |
| AST | aspartate aminotransferase |
| BM | bone marrow |
| CIR | Cumulative Incidence of Relapse rate |
| cm | centimeter |
| CR | complete remission |
| CRi | Compete remission with incomplete hematologic recovery (same as "marrow CR") |
| CMML | Chronic myelomonocytic leukemia |
| CTCAE | (NCI) Common Terminology Criteria for Adverse Events |
| CTEP | Cancer Therapy Evaluation Program |
| dL | deciliter |
| DFS | time to relapse |
| DLT | dose-limiting toxicity |
| ECOG | Eastern Cooperative Oncology Group |
| EGFR | epidermal growth factor receptor |
| EFS | Event-Free Response |
| FDA | Food and Drug Administration |
| h | hour |
| HL | Hodgkin Lymphoma |
| IC50 | 50% Inhibitory Concentration, the concentration needed to inhibit cell growth by 50% |
| IGF-1 | insulin-like growth factor-1 |
| IGF-1R | insulin-like growth factor-1 receptor |
| IHC | immunohistochemistry |
| IND | Investigational New Drug |
| IRB | institutional review board |
| kg | kilogram |
| lb | pound |
| mg | milligram |
| min | minute |
| mL | milliliter |
| $mm^3$ | cubic millimeters |
| MDS | myelodysplastic syndrome |
| MTD | maximum tolerated dose |
| MTX | methotrexate |
| NCI | National Cancer Institute |
| O-AML | Oligoblastic acute myelogenous leukemia |
| ORR | Overall response rate (= CR rate + CRi rate + PR rate) |
| OS | Overall Survival rate |
| PK | Pharmacokinetics studies |
| PD | Pharmacodynamics studies |
| PFS | Progression free survival (Time to disease progression or death from MDS, O-AML, or CMML) |

| | |
|---|---|
| PO | per os (by mouth) |
| PR | Partial Remission |
| RFS | Relapse-Free Survival |
| SAE | serious adverse event |
| US | United States |
| VEGF | vascular endothelial growth factor |
| wt | weight |

# 1. Introduction

## 1.1. Introduction

### 1.1.1. The IGF-1R Pathway

The insulin-like growth factor type 1 receptor (IGF-1R) is a transmembrane heterotetramer tyrosine kinase whose intracellular kinase domain is 84% homologous to that of the insulin receptor (IR).[1] These receptors activate several of the same signaling intermediates. IGF-1R is widely expressed in human tissues and is involved in numerous cellular processes. Binding of its ligands, insulin-like growth factor-1 (IGF-1) and IGF-2 induces biological actions after conformational changes of the receptor, followed by activation of a cascade of intrinsic tyrosine kinase activity. Activated IGF-1R induces recruitment of adaptor proteins belonging to the insulin receptor substrate family (IRS 1/2) or SHC.

Stimulation of two downstream signaling pathways mediate the tumorigenic effects of the IGF cascade in the majority of cancers:

1) the ras -raf-mitogen-activated protein kinase (MAPK)/extra-cellular related kinases (Erk-1/2) pathway, associated with differentiation, growth and proliferation, and

2) the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway, associated with inhibition of apoptosis.[2]

In addition, IGF-1R activation stimulates glucose transport and utilization, amino acid transport and protein synthesis, RNA and DNA synthesis. Angiogenesis regulation is also mediated downstream from the IGF cascade, in concert with vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR).[2,3]

IGF-1, the main circulating IGF-1R ligand, is primarily produced by the liver. Serum IGF binding proteins (IGFBP1-6) regulate IGF 1/2 bioavailability. IGF 1/2 are also produced in other organs where autocrine or paracrine mechanisms of actions are important, and some tumors produce IGF-1/2 in an autocrine or paracrine manner.[4]

### 1.1.2. IGF-1R is overexpressed in Cancer

IGF-1R is overexpressed in many types of cancer, including breast, prostate, lung, colorectal, and hematologic malignancies[5-20]. IGF-1R expression appears to be a critical determinant of resistance to apoptosis, and its down-regulation leads to massive apoptosis.[21,22] Its role in preventing apoptosis may underlie the observation that high/normal serum levels of IGF-1 correlate with an increased risk for developing various cancers, including cancers of the breast[8,9,13,16,20], prostate[8,15], and colon[11,14,18]. Finally IGF-1R activation promotes invasion and metastasis. In vitro and in vivo IGF-1R inhibition leads to anti-proliferative effects and often appears synergistic with chemotherapy.[23,24] Lastly, IGF binding protein-3 (IGF-BP3) a circulating soluble protein that binds IGF-1 and prevents its binding to the IGF-1R, may have a role as tumor suppressor. TGF-β3-induced inhibition of growth of a human breast cancer cell line was mediated by IGF-BP3, and IGF-BP3 has been identified as a target gene regulated by p53.[25]

Consistent with its overexpression in tumor cells, evidence also shows that IGF-1R is important to cancer cell physiology. Multiple myeloma cell lines overexpressing IR and IGF-1R are more responsive to growth stimulation with insulin and IGF-1, as compared to pre-B cell lines[49]. Strong evidence also links IGF-1R overexpression in breast tumors

with radiation resistance and tumor recurrence[50]. Overexpression of IGF-1R has also been shown to inhibit differentiation of rhabdomyosarcoma cells in tissue culture, whereas antisense RNA against IGF-1R promoted differentiation[51]. In melanoma cell lines, there is evidence that IGF-1R expression is critical to sustaining cell growth and resisting apoptosis[52]. IGF-1R expression has also been linked to chemotherapy resistance: cancer cells expressing IGF-1R and activated by IGF-1 have shown enhanced survival against etoposide[53].

#### 1.1.2.1. IGF-1R is overexpressed in MDS and Acute Myelogenous Leukemia

IGF-1R has been shown to be significantly overexpressed in malignant bone marrow nucleated cells in patients with either acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS), as compared to healthy controls.[26] IGF-1R overexpression was greater in AML than MDS.[26] A separate study showed, by immunohistochemical staining that MDS clonal cells markedly overexpressed IGF-1R relative to normal nucleated bone marrow cells in the same individuals (78% vs. 14%, $p < .0001$).[27] Importantly IGF-1R was overexpressed in 25 of 26 MDS patients, and, in the 26th patient, both MDS and normal cells expressed high levels of IGF-1R.[27] Another report indicated that MDS cells could be sorted by flow cytometry based on IGF-1R receptor expression.[28] The expression of IGF-IR in CD34+ cells of 55 MDS patients was significantly higher than that of cells from normal controls (54.0 vs. 4.5%).[28] In the healthy controls, of the 4.5% of cells that were IGF-1R positive, 70% expressed the erythroid lineage marker CD235a, indicating that IGF-1R is mostly expressed during erythropoiesis in healthy bone marrow.

Literature indicates AML cells have been shown to be sensitive to the anti-metabolite methotrexate *in vitro*. The IC50 for methotrexate was 10 nM for the AML cell line HL-60,[29] suggesting that AML and MDS should be responsive to IGF-MTX clinically.

The sponsor, IGF Oncology, has also shown in its laboratories that IGF-MTX inhibits division of 3 of 3 AML cell lines tested: HL-60, HL-60/S4, and Kasumi-1. All three had IC50s of 458-668 nEq/L. IGF-MTX was also synergistic with Azacytidine. In the presence of a concentration of Azacytidine about 1/3 of its IC50, the IC50 of IGF-MTX decreased from 668 to 398 nEq/L in Kasumi-1 cell line and decreased from 466 to 409 nEq/L in HL-60. These results provide further reason to believe IGF-MTX may be effective clinically against the myeloid malignancies MDS, O-AML, and CMML.

### 1.1.3. Using the IGF-1R Pathway to Reduce Tumor Growth

IGF binding proteins (IGFBPs) are soluble blood proteins that bind IGF-1. Over 90% of total IGF-1 in circulation is normally bound to IGFBPs, and when bound to the IGFBPs it is not available to bind to the IGF-1R membrane receptor. However, variants of IGF-1 have been identified that have a reduced affinity for IGFBP-1 but still bind strongly to IGF-1R.[30,31] One such variant is long-R3-IGF-1, which has a mutation at position 3 of the IGF-1 sequence (glutamic acid to arginine) and a short amino terminal extension.[30,31] In theory, the long-R3-IGF-1 variant may more efficiently target the IGF-1R receptor *in vivo* because of a lack of interference (binding) by normally reactive IGFBPs. Antibodies against IGF-1R, and tyrosine kinase inhibitors that are relatively specific for IGF-1R have been developed as anticancer agents, and some of these agents have already been tested in clinical trials for solid tumors and shown promising results[32-39]. However, McTavish et al. took a different approach, hypothesizing that one could use the IGF system to deliver an anticancer drug relatively specifically to cancer cells that express

IGF-1R[40]. They developed a novel covalent conjugate that contains the antifolate (and anticancer) drug, methotrexate (MTX), coupled to the long-R3-IGF-1 variant (IGF-MTX conjugate), with the purpose of specifically destroying tumor cells that overexpress the membrane IGF-1 receptor. In *in vivo* studies of mice, they demonstrated that the novel IGF-MTX conjugate was more effective, at significantly lower doses than free MTX, at controlling tumor growth. These data provide proof-of-principle that IGF-chemotherapy conjugates can enhance the therapeutic effects of chemotherapy by specifically increasing anticancer drug localization in tumor cells and tumor-related tissues that overexpress IGF membrane receptors. Accordingly, we propose to examine the therapeutic use of MTX bound to an IGF-1 variant similar in structure to that of the long R3 IGF-1 protein in this pilot clinical trial.

### 1.2. Design of IGF-Methotrexate as a drug conjugate targeting IGF-1R

The conjugate we propose to study is 765IGF-Methotrexate (765IGF-MTX). 765IGF-MTX is a novel anticancer agent containing 765IGF, a variant of human IGF-I similar to long-R3-IGF-1, covalently coupled to the antifolate drug, methotrexate (MTX). 765IGF is 88 amino acids long, and has an 18 amino acid leader sequence that includes 5 lysine residues, followed by the 70 amino acid sequence of human IGF-1, with an arginine residue replacing the native glutamic acid residue at position 21 (position 3 of native IGF-1). The amino acid sequence of 765IGF is as follows:

MVKGKHHHHH HNGKGKSKGP **R**TLCGAELVD ALQFVCGDRG FYFNKPTGYG 50
SSSRRAPQTG IVDECCFRSC DLRRLEMYCA PLKPAKSA 88

The underlined residues 19-88 constitute the complete mature sequence of human IGF-1, (residues 1-70) with a mutant R (arginine) residue in bold, replacing the native glutamic acid residue in that position. Residues 1-18 are nonnative residues and provide a polyhistidine purification tag and five lysine residues for coupling of methotrexate.

Each methotrexate is coupled to 765IGF through one of the two free carboxyl groups of MTX to one of the nine amino groups of 765IGF (8 lysine residues and a free amino terminus). The coupling forms an amide bond between each methotrexate and a lysine side chain or alpha amino group of 765IGF. There may be secondary reactions to other locations on the protein.

The drug has been found to have on average approximately eight methotrexate moieties per 765IGF protein molecule.

As discussed above, a similar conjugate containing long-R3-IGF-1 covalently coupled to MTX was previously made and tested against cancer *in vitro* and *in vivo*.[40] Because IGF-1R and ligand are internalized upon ligand binding, MTX was expected to be internalized by target cells expressing the IGF-1R membrane receptor. The conjugate of MTX with long-R3-IGF-1 was expected to have efficacy at lower doses than a conjugate with wild-type IGF-1, as the long-R3-IGF-1 variant has a reduced binding affinity for circulating IGFBPs, which normally keep the wild-type ligand in circulation and prevent it from binding to the IGF-1R membrane receptor. 765IGF has these same traits of binding to the IGF-1R but poor binding to circulating IGFBPs.

The long-R3-IGF-1-MTX conjugate was significantly more effective than free methotrexate against human prostate tumor xenografts in mice, even at 6.25-fold lower dose.[40] The IGF-

MTX conjugate used in this pilot study is expected to behave similarly due to its high affinity for IGF-1R and low affinity for IGFBPs. [30,31,41]

The 765IGF-MTX dosage used here is based on our completed phase I dose-escalation study using this molecule (765IGF-MTX).

### 1.2.1. **Preclinical Experience with IGF-Methotrexate**

***In Vitro and in Vivo Efficacy Studies***:
IGF-MTX binds specifically to the IGF-1R receptor.
The $K_D$ (dissociation constant) for binding of 765IGF-MTX to the IGF-1R receptor is approximately 20 nM. This is measured by competition binding to MCF7 cells with radiolabeled IGF-1. The $K_D$ of 765IGF, unconjugated to methotrexate, is approximately 5nM. (IGF Oncology's results. See also ref. 40.)

IGF-MTX is cytotoxic against solid tumor cell lines in vitro
765IGF-MTX inhibits proliferation of MCF7 (human breast cancer cell line) with an $IC_{50}$ of about 400-600 nEq/L (nM methotrexate groups) and LNCaP (human prostate cancer cell line) with a similar value. (IGF Oncology's results. See also ref. 40.)

IGF-MTX inhibits tumor growth in vivo at more than 6-fold lower molar dose than free methotrexate.

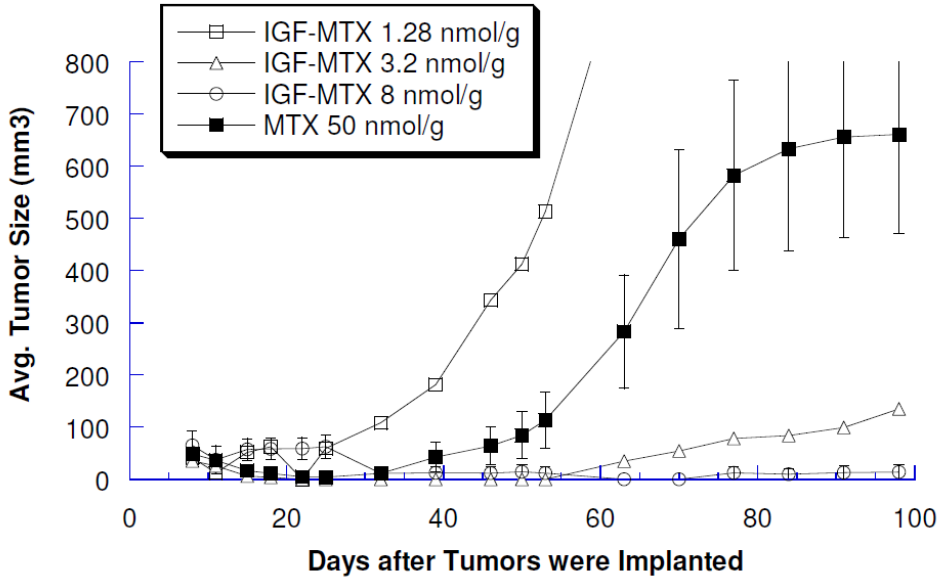



Fig. 1. Inhibition of LNCaP tumor growth in nu/nu mice. Mice were treated once by IV injection with the indicated dosages of free MTX or IGF-MTX conjugate once on day 5. The doses of the IGF-MTX conjugate are expressed in terms of moles of MTX molecules per gram. For clarity, error bars are shown only for the 8 nmol/g IGF-MTX group and the 50 nmol/g MTX group. Data represent means ± SE. The 50 nmol/g MTX group had 8 mice. All other groups had 4 mice per group. The difference between the 8 nmol/g IGF-MTX and 50 nmol/g MTX groups was significant ($P$ = 0.04).[40]

Similarly, IGF-MTX was more effective than free MTX even at 4-fold lower molar dose in treating MCF7 xenografts [40].

IGF-MTX is cytotoxic in vitro against myeloid cancer cell lines and is synergistic with azacytidine

Three AML cell lines were tested in our laboratory for sensitivity to IGF-MTX. The three cell lines were HL-60, HL-60/S4, and Kasumi-1. All three were sensitive to IGF-MTX with IC50s of 458-668 nEq/L (nM of methotrexate groups in the IGF-MTX conjugate). This is about the same as the IC50 against MCF7 breast cancer cell line and LNCaP prostate cancer cell line, both of which are sensitive to IGF-MTX in mouse xenografts.

The effect of IGF-MTX was also additive or synergistic with Azacytidine on at least the Kasumi-1 and HL-60 cell lines (it was not tested the combination on HL-60/S4). In the presence of a concentration of Azacytidine about 1/3 of its IC50, the IC50 of IGF-MTX decreased from 668 to 398 nEq/L in Kasumi-1 cell line and decreased from 466 to 409 nEq/L in HL-60.

***In Vivo Toxicology Studies***

MTD is based on both non-rodent and rodent studies. Formal GLP toxicity *in vivo* studies were completed in rats and Beagle dogs, in which the 765IGF-MTX conjugate was administered intravenously by a single 30 minutes infusion on study Days 1 and 8 as shown in Table 1 for the dogs:

**Table 1.**

| Group / Dose | Target Concentration µeq/ml (µmol MTX groups/mL) | Preparation[1] | | |
|---|---|---|---|---|
| | | Amount of 4 µeq/ml (4 mM MTX groups) 765IGF-MTX Stock Solution (mL) | D5W-Added (mL) | Total Volume (mL) |
| 1. 765IGF-MTX 0.2 µeq/kg (0.2 ul MTX groups /kg) | 0.04 | 1.25 | 123.75 | 125 |
| 2. 765IGF-MTX 2.0 µeqlkg (2.0 µmol MTX groups/kg) | 0.4 | 10 | 90 | 100 |
| 3. 765IGF-MTX 0.5 µeq/kg (0.5 µmol MTX groups/kg) | 0.1 | 3.125 | 121.875 | 125 |
| 4. 765IGF-MTX 6.0 µeq/kg (6.0 µmol MTX groups/kg) | 1.2 | 37.5 | 87.5 | 125 |

Analysis of all generated data, including clinical observations, serial blood glucose determinations, and clinical pathology revealed no drug/treatment-related significant toxicity in dogs treated by intravenous infusion with 765IGF-MTX conjugate in 5% dextrose at 0.2 and 0.5 μeq/kg. In the 0.2 μeq/kg group there was only transient dyspnea and passivity noted, while in the animals treated at 0.5 μeq/kg a single episode of vomiting and diarrhea were noted, and there was also mild reddening and swelling of the skin on the head of the female dog. Treatment of dogs in these two groups was well tolerated, and any reaction to the treatment was transient and resolved by itself.

In animals dosed at 2 μeq/kg the reactions to the treatment included mild to moderate anaphylactoid and hives-type reactions, transient anorexia and weight loss, and hypoglycemia. Reactions to the treatment in the dogs dosed at 6 μeq/kg were similar to the reactions at 2 μmol/kg, but they were more severe and lasted longer. Thus, an MTD of 765IGF-MTX conjugate in Beagle dogs in this study may be considered to be 6 μeq/kg, by a single infusion over 30 minutes.

Recovery from anaphylactoid reactions and hypoglycemia in the female dog dosed at 2 μmol/kg and in both animals dosed at 6 μmol/kg, was assisted by treatments with diphenhydramine and dextrose.

Hypoglycemia in the higher dose groups was an exaggerated pharmacological effect of IGF, which was mitigated by the use of 5% dextrose as a vehicle for delivery of 765IGF-MTX conjugate. The pathogenesis of the anaphylactoid reactions was not clear, but may have been caused by either methotrexate, IGF or the combination thereof. Vomiting, diarrhea, anorexia and weight loss are known reactions to methotrexate.

The highest non-severely toxic dose in beagles was 0.5 μeq/kg. Using the conversion from doses in dogs in uEq/kg to equivalent human dose in μEq/kg, the equivalent human dose to 0.5 μEq/kg in dogs is 0.27 μEq/kg in humans[42,43].

<u>IGF-MTX does not cause significant cytopenia</u>
Cytopenia is a particular concern for MDS, CMML, and O-AML since it is a principal sequela of these diseases. In both rat and dog repeat dose GLP toxicology testing IGF-MTX caused almost no cytopenia, even at the highest doses tested. In both rat and dog toxicology studies IGF-MTX caused a slight dose-dependent reduction in erythrocyte mass, but even at the highest doses tested erythrocyte mass was within normal ranges. In rats but not dogs neutrophils were also slightly decreased by IGF-MTX but remained within normal ranges even at the highest doses. No other hematological parameters were affected by IGF-MTX.

In the completed Phase I dose-escalation study in human solid tumor patients, a dose of 0.8 μEq/kg was found to be tolerated without any serious adverse events. Since MDS patients have greater cytopenia than solid tumor patients, and for safety of MDS patients, we are conducting a new dose escalation in this study beginning at a dose level of 0.2 μEq/kg administered on days 1, 8, and 15. See the schema on page 6 for the dose escalation schema.

### 1.2.2. **Clinical Experience with IGF-Methotrexate**

*Phase I Study of IGF-Methotrexate Conjugate in the Treatment of Advanced Tumors Expressing IGF-1R*
The primary objective of this study was to determine the maximum tolerated dose (MTD) of 765IGF-MTX by evaluation of toxicity during treatment of advanced, previously treated malignancies that express IGF-1R. One inclusion criterion was that a subject's tumor (tissue, bone marrow, or blood) must express IGF-1R, defined as ≥10% of tumor cells expressing IGF-1R by immunohistochemistry (IHC).

Nineteen subjects were enrolled in this dose escalation study. 765IGF-MTX was administered as an IV infusion over 1 hour on days 1, 8 and 15 of a 28 day cycle. Treatment continued until disease progression, unacceptable toxicity, or patient refusal. Assessment of response was confirmed with imaging studies performed at the end of cycle 2 +/- 7 days, and every 2 cycles thereafter. The table below shows, for each dose level tested, the subjects that were tested, the type of malignancies they had, number of cycles they completed, whether they experienced a DLT, and, if applicable, their reason for study discontinuation. One patient with Hodgkin Lymphoma at dose level 3 was treated with 22 doses with apparently stable disease by CT scan, but treatment was discontinued when a lymph node biopsy showed no evidence of cancer.

| Dose Level | Dose (µEq/kg) | Subject # | Malignancy | DLT (Y/N) | # doses | Reason for discontinuation |
|---|---|---|---|---|---|---|
| 1 | 0.05 | 1 | Colon Ca | N | 6 | Progression |
| 2 | 0.10 | 2 | Met adamantinoma | N | 6 | Progression |
| 3 | 0.20 | 3 | Colorectal, met | N | 6 | Progression |
| 3 | 0.20 | 4 | Endometrial Ca | Y | 1 | not evaluable |
| 3 | 0.20 | 5 | Endometrial Ca | N | 18 | stable disease |
| 3 | 0.20 | 6 | Pancreatic Ca | N | 2 | not evaluable |
| 3 | 0.20 | 7 | Thymic Ca | N | 6 | Progression |
| 3 | 0.20 | 8 | Hodgkin Lymphoma | Y | 6 | Progression |
| 3 | 0.20 | 9 | Hodgkin Lymphoma | N | 22* | stable disease |
| 4 | 0.40 | 10 | Colon Ca | N | 5 | Progression |
| 4 | 0.40 | 11 | Thymoma | N | 6 | Progression |
| 4 | 0.40 | 12 | Colon Ca | N | 9 | stable disease |
| 5 | 0.80 | 13 | mCRC | N | 3 | not evaluable |
| 5 | 0.80 | 14 | Pancreatic Ca | N | 6 | Progression |
| 5 | 0.80 | 15 | Colon Ca | N | 3 | not evaluable |
| 5 | 0.80 | 16 | Endometrial Ca | N | 4 | not evaluable |
| 5 | 0.80 | 17 | Colon Ca | N | 6 | Progression |
| 5 | 0.80 | 18 | Breast Ca | N | 6 | Progression |
| 5 | 0.80 | 19 | Basal cell Ca with lung mets. | N | 6 | partial response. |

***** At time of this report is tumor-free by lymph node biopsy.

Adverse Events

Adverse events experienced during the prior solid tumor Phase I on solid tumor patients that were graded as possibly or probably related to the study drug are shown in the Table below.

| **Adverse events possibly related to study drug** | |
|---|---|
| **Event** | **Grade** |
| Hypotension | 3 |
| Fever | 2 |
| Seizure | 2 |
| Sinus tachycardia | 2 |
| Dyspnea | 2 |
| Abdominal pain or cramps | 2 |
| Nausea, Vomiting | 1 |
| Hypoglycemia | 1 |
| Dizziness | 1 |

None of the adverse events were seen in all subjects or all drug administrations in a single subject. The most common adverse events were hypoglycemia, which is an expected consequence of the study drug, chills, and fever. When these occurred, they usually resolved within 2 hours after the end of the infusion. One subject had grade 2 fever beginning with the infusion and lasting overnight. One subject had grade 3 hypotension beginning during the infusion and lasting overnight. Nausea and vomiting were common, but occurred during the infusion, not with a several hour delay as is typical with chemotherapy. In all cases they resolved within 1 hour after the end of the infusion. Abdominal pain or cramping was common but appeared limited to patients with colon carcinoma, so it may have been associated with the study drug binding to and targeting their tumor tissue. The one seizure that was seen occurred during the infusion in a subject during cycle 5, day 1 (the patient's 16th dose of drug) at dose level 0.4 uEq/kg, and resolved in 2 minutes. This patient also experienced the grade 3 hypotension event at the same time and was hospitalized overnight for it.

Other adverse events that have been observed with 765IGF-MTX administration in the present clinical trial have been syncope, tachycardia, fever and chills. These have been observed during the infusion or within 3 hours after infusion.

### 1.3. Study Rationale

Cytotoxic drug conjugates consist of a tumor targeting agent covalently linked to a cytotoxic drug. The conjugated drug is designed to be nontoxic extracellularly, but, after internalization of the conjugate by target cancer cells and subsequent release of the free drug, it can disrupt intracellular enzymes and kill the cancer cell. A recent preclinical study using such an approach produced promising results.[40] The investigators in that study developed a novel covalent conjugate that contains methotrexate (MTX) coupled to the long-R3-IGF-1 variant (IGF-MTX conjugate), with the purpose of specifically targeting and destroying tumor cells that overexpress the membrane IGF-1 receptor. Via *in vivo* studies with mice, they demonstrated that the novel IGF-MTX conjugate was more effective at significantly lower doses than free MTX at controlling tumor growth. These data provide proof-of-principle that IGF-chemotherapy conjugates can enhance the therapeutic effects of chemotherapy by specifically increasing anticancer drug localization in tumor cells and

tumor-related tissues that overexpress IGF-1 membrane receptors. Accordingly, here we propose to examine use of MTX covalently bound to an IGF-1 variant similar in structure to long R3 IGF-1 in this Phase I clinical trial.

Based on the data described in section 1.1.2.1[26-29] we plan to proceed with this pilot study focusing on a select population of patients with specific hematologic malignances, i.e., MDS, CMML, and O-AML, that are refractory to or intolerant of standard treatment. The IGF-MTX drug appears well suited to treat these malignancies for three reasons. (1) The malignant cells of MDS, CMML, and O-AML have been shown to overexpress IGF-1R, the receptor to which IGF-1R binds[26-29]. (2) Our data show malignant myeloid cell lines are sensitive to IGF-MTX. (3) These diseases are characterized by cytopenia, and IGF-MTX appears to cause no cytopenia in humans at the doses that will be used based on experience in the completed solid tumor Phase I clinical study and almost no cytopenia in rats and dogs in toxicology testing, even at the highest doses tested. In rat and dog toxicology studies, IGF-MTX caused only a slight reduction in erythrocyte mass, but even at the highest doses tested erythrocyte mass was within normal ranges. In rats but not dogs neutrophils were also slightly decreased by IGF-MTX but remained within normal ranges even at the highest doses.

Although the primary and secondary goals of this trial are to evaluate the safety and clinical benefit of 765IGF-MTX, this study is also important because other correlative studies will provide critical information. In addition to characterizing pharmacokinetics, pharmacodynamics markers will be collected before and during treatment, including serum IGF-1 and plasma and whole blood IGF-1R levels. Given that 765IGF-MTX cell killing is dependent on ligand-mediated binding and internalization, we plan to assess changes in PD markers during and pre- and post-treatment. Patients' diagnostic tissue (available from the original BM biopsy obtained at time of diagnosis) will be stained to determine if detectable IGF-1R is present on the leukemia or MDS cells and the level of IGF-1R expression. Based on publications that report that all or nearly all MDS and AML patients overexpress IGF-1R on their malignant cells, demonstrated expression of IGF-1R on the patients' diagnostic tissue will not be an inclusion criterion[26-29].

## 2. Objectives

### 2.1. Primary Objective

The primary objective is to determine the safety and tolerability of 765IGF-MTX when used for the treatment of advanced, previously treated MDS, CMML or O-AML, including determining the MTD of IGF-MTX.

### 2.2. Secondary Objectives

2.2.1 Evaluate clinical benefit of 765IGF-MTX by evaluating the overall response rate (ORR) complete and partial remission (CR and PR) rates, complete remission with incomplete hematologic recovery (CRi) rate, relapse-free survival (RFS), cumulative incidence of relapse (CIR), time to relapse, and overall survival (OS) in patients with advanced, previously treated O-AML, CMML or MDS.

### 2.3. Correlative Objectives

2.3.1. Characterize pharmacokinetics (PK) of 765IGF-MTX, 765IGF, methotrexate, and 7-OH methotrexate

2.3.2. Assess potential for QT prolongation

2.3.3. Assess Pharmacodynamic (PD) effects of 765IGF-MTX on soluble IGF-1 and IGF-1R levels

2.3.4. Assess formation of antibodies against 765IGF-MTX

2.3.5. Assess formation of neutralizing antibodies

2.3.6. Assess level of diseased cell IGF-1R expression

## 3. Endpoints

### 3.1. Primary Endpoint

The primary endpoint is safety and tolerability of 765IGF-MTX. This will be assessed by evaluation of adverse effects (AEs) as defined by CTCAE v.4.0.

### 3.2. Secondary Endpoints

3.2.1. Evaluate clinical benefit of 765IGF-MTX by assessment of ORR, RFS, CIR, OS.

3.2.2. Complete remission (CR), Complete remission with incomplete hematologic recovery (CRi) (equivalent to "marrow CR" in Appendix II), PR as defined by the following response criteria for hematologic malignancies (see Appendix I Tables 5 and 6, and Appendix II):

3.2.2.1. Acute leukemia: 2015 SWOG Manual Chapter 11A[44] (Appendix I); European LeukemiaNet criteria[45] and 2003 IWG criteria[46]

3.2.2.2. MDS: 2006 IWG criteria [47] (Appendix II)

### 3.3. Correlative Endpoints

**3.3.1** Pharmacokinetic (PK) parameters as defined by AUC from start of infusion to time of last quantifiable plasma concentration, AUC from start of infusion to infinity, maximum observed plasma concentration, time of maximum plasma concentration, terminal elimination constant of both free MTX and IGF-MTX for 765IGF-MTX, 765IGF, methotrexate, and 7-OH methotrexate.

**3.3.2** Evaluation of potential of 765IGF-MTX for QT prolongation

**3.3.3** Pharmacodynamic parameters (PD) as defined here by serum IGF-1 and plasma and whole blood IGF-1R concentration, and standard of care systemic PD variables (cell count, differential)

**3.3.4** Plasma 765IGF Level and 765IGF-MTX toxicity/response

**3.3.5** Plasma and whole blood IGF-1R level and 765IGF-MTX Toxicity/Response

**3.3.6** Assess formation of antibodies against 765IGF-MTX

**3.3.7** Assess formation of neutralizing antibodies

**3.3.8** IGF-1R expression level in diseased tissue in bone marrow, as measured by IHC, and flow cytometry, and in blood cells as measured by flow cytometry.

## 4. Overall Design and Study Plan

This pilot study will evaluate the safety and clinical benefit of 765IGF-MTX in patients with advanced, previously treated MDS, CMML or O-AML. 765IGF-MTX is administered as an IV infusion over 1.5 hours on days 1, 8 and 15 of a 28 day cycle. Treatment continues until disease progression, unacceptable toxicity, or patient refusal. Assessment of response will be confirmed with bone marrow studies performed at the end of cycle 2, and every 8 weeks +/- 7 days (2 cycles) thereafter up to the end of cycle 6, and at physician's discretion thereafter.

- Dose Finding Component: Up to 5 dose levels will be tested (refer to schema on page 6). The maximum tolerated dose (MTD) will be determined using the modified toxicity probability interval design with dose limiting toxicity (DLT) estimated at 0.33.

  Our modification involves using cohorts of size 1 for the initial doses, which allows rapid escalation through the initial dose levels, and expanding to cohorts of size 3 once any grade 2 or higher toxicity is observed that is considered related to study drug (with the exception of alopecia, nausea, or diarrhea). Additional patient cohorts will not be enrolled until 1 of 1 (with no grade 2 toxicity), 3 of 3, 5 of 6, or 7 of 9 patients at the current dose level complete all planned treatment for cycle 1 (defined as 3 doses of 765IGF-MTX without DLT and are able to start cycle 2 with no more than a 2 week delay. At the discretion of the principal investigator, intra-patient dose escalation between cycles is also allowed but only after 2 cycles at one dose level and confirmation of safety in at least 3 patients treated at the higher dose level.

  If none of the dose levels are acceptable at study completion, an optimal dose level will not be identified and the drug does not warrant further investigation.

- Maximum Tolerated Dose (MTD) Cohort with pharmacokinetics (PKs) and pharmacodynamics (PD): The MTD will be defined as the highest dose associated with DLT in less than 33% of patients treated. Once the MTD is determined, enrollment will continue until 9 patients total are accrued at the MTD. For this group, pharmacokinetics will be performed on at least 3 patients before and for up to 48 hours after drug administration on days 1 and 15 of cycle 1. Pharmacodynamic samples will be assessed on day 1 of cycle 1, and days 1 and 15 of cycle 2 before the infusion of 756IGF-MTX, and one sample will also be drawn within the fourth week of each treatment cycle.

If none of the dose levels are acceptable at study completion, an optimal dose level will not be identified and the drug does not warrant further investigation.

Dose limiting toxicity (DLT) for a patient is defined as one of the following events occurring during cycle 1:

- Grade 4 or greater treatment related hematologic toxicity for > 7 days during the first cycle (28 days) of therapy
- Grade 3 or greater treatment related clinical non-hematological toxicity (excluding ≥ grade 3 nausea, vomiting, or diarrhea without maximal medical intervention and/or prophylaxis) during the first cycle (28 days) of therapy
- Febrile neutropenia during the first cycle (28 days) of therapy

- Platelets less than 10 x $10^9$/L with clinically significant bleeding during the first cycle (28 days) of therapy
  .

Additional patient cohorts will not be enrolled until 1 of 1 (with no grade 2 toxicity), 3 of 3, 5 of 6, or 7 of 9 patients at the current dose level complete all planned treatment for cycle 1 (defined as 3 doses of 765IGF-MTX without DLT and are able to start cycle 2 with no more than a 2 week delay.

The MTD will be defined as a dose level at which fewer than 33% of patients treated experience a DLT.

A minimum of 9 patients are to be treated at the MTD to assure safety and 765IGF-MTX based pharmacokinetics will be performed on at least 3 patients in total.

Pharmacokinetics will be performed before and for up to 24 hours after drug administration on days 1 (for 24 hrs) and 15 (for 24 hrs) of cycle 1. Pharmacodynamic samples will be assessed pre-dosing on day 1 of cycle 1, pre-dosing on days 1 and 15 of cycle 2, and pre-dosing on day 15 of cycles 4 and 6.

## 5. Selection of Patients

Study entry is open to adults ≥ 18 years regardless of gender or ethnic background. While there will be every effort to seek out and include women and minorities, the patient population is expected to be no different than that of other studies performed at the Mayo Clinic.

### 5.1. Inclusion Criteria

5.1.1 Diagnosis of O-AML that is refractory to or intolerant to standard therapy and is no longer likely to respond to such therapy (at least one line of therapy); or Diagnosis of MDS/CMML that is refractory to or intolerant to standard therapy and is no longer likely to respond to such therapy (at least one line of therapy)

5.1.2 Confirmed histologic diagnosis on bone marrow biopsy and aspirate within 28 days of trial entry prior to starting cycle 1.

5.1.3 Platelets > 10 x $10^9$/L

5.1.4 Age ≥ 18 years

5.1.5 ECOG performance status of 0, 1 or 2 (appendix III).

5.1.6 Prior systemic chemotherapy, immunotherapy, or biological therapy, radiation therapy and/or surgery are allowed; prior use of systemic methotrexate > 1 month prior to study entry is allowed. Intrathecal methotrexate is allowed prior to and during treatment per investigator discretion.

Time since prior therapy and the first dose of study drug:

- At least 2 weeks since prior radiation, non cytotoxic small molecule drugs, prior major surgery (defined as a surgery involving a risk to the life of the patient; specifically: an operation upon an organ within the cranium, chest, abdomen, or pelvic cavity), prior systemic FDA-approved therapy

5.1.7 Patient must have recovered from the acute toxic effects (≤ grade 1 CTCAE v.4.0) of previous anti-cancer treatment prior to study enrollment; the only exception is that grade 2 neuropathy is permitted

5.1.8 Adequate organ function within 14 days of study registration defined as:

| System | Laboratory Values |
|---|---|
| **Hematologic** | |
| Platelets | > 10 x $10^9$/L |
| **Hepatic** | |
| Bilirubin total | ≤ 1.5 x ULN |
| Alkaline Phosphatase, AST and ALT | ≤3 X ULN (< 5 x ULN is acceptable if liver has tumor involvement) |
| **Renal** | |
| Serum Creatinine, or<br>Creatinine Clearance, or<br>GFR, or<br>24 hour urine creatinine clearance | ≤ 1.5 x ULN<br>≥ 60 mL/min<br>≥ 60 mL/min<br><br>> 50 mL/min |

5.1.9 Negative serum pregnancy test in females. Male and female patients with reproductive potential must use an approved contraceptive method if appropriate (for example, abstinence, oral contraceptives, implantable hormonal contraceptives, or double barrier methods) during, and for 3 months after the last dose of 765IGF-MTX.

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

*5.2.* **Exclusion Criteria**

5.2.1. ECOG PS >2.
5.2.2. Patients with active extramedullary disease.
5.2.3. Active CNS involvement.
5.2.4. Pleural effusions or ascites.
5.2.5. ≥ Grade 3 peripheral neuropathy within 14 days before enrollment.
5.2.6. Active uncontrolled infection or severe systemic infection (enrollment is possible after control of infection).
5.2.7. Myocardial infarction within ONE months prior to enrollment or has New York Heart Association (NYHA – appendix IV) Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at screening has to be documented by the investigator as not medically relevant.
5.2.8. Pregnant or breastfeeding – methotrexate is Pregnancy Category X - has been reported to cause fetal death and/or congenital abnormalities. Confirmation that the subject is not pregnant must be established by a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.
5.2.9. Uncontrolled diabetes mellitus defined as a Hemoglobin $A_1C$≥ 10% in patients with a prior history of diabetes, prior to study enrollment.

5.2.10. Serious concomitant systemic disorders (e.g., active uncontrolled infection or uncontrolled diabetes) or psychiatric disorders that, in the opinion of the investigator, would compromise the safety of the patient or compromise the patient's ability to complete the study.
5.2.11. Other severe acute or chronic medical or psychiatric conditions, or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study.
5.2.12. Any history of epilepsy or a seizure disorder or any known prior seizures.
5.2.13. Abnormalities on 12-lead electrocardiogram (ECG) considered by the investigator to be clinically significant.
5.2.14. Known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to IGF or methotrexate.

## 6. Registration/Enrollment

Registration/enrollment will occur after the patient has signed the informed consent document and eligibility is confirmed, but before any treatment has been administered. To be eligible for registration/enrollment into this study, the patient must meet each criterion listed on the eligibility checklist and supported by the clinical documentation in the patient's medical record. A copy of the eligibility checklist is maintained by the Clinical Trials Office (CTO) and can be found for each individual subject within the subject shadow chart. Patients must be registered/enrolled on the study within 30 days of signing the informed consent document. If the patient cannot be registered/enrolled within this timeframe, the patient may later be reconsidered for participation as outlined in section 6.3 below.

### 6.1. Registration/Enrollment with the Clinical Trials Office

Upon obtaining informed consent, completion of the screening evaluation, and verifying eligibility, the site study coordinator or designee will enroll the patient into EDC Easy®, the CTO Clinical Trials Management System (CTMS). The eligibility checklist must be completed by the study coordinator and verified/confirmed by the Principal Investigator or her designee (i.e., a sub-Investigator).

### 6.2. Registered/Enrolled Patients Who Do Not Begin Study Treatment

If a patient signs the informed consent document, is subsequently registered/enrolled to the study but is then not able to begin the planned study treatment for any reason, the patient will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and be replaced. Further data will not be collected if the patient has not begun study treatment at the time of removal from trial. The reason for removal from study will be clearly indicated in EDC Easy®.

If a patient begins treatment and is then removed from treatment for any reason, the patient must be followed per section 7.11.

### 6.3. Re-screening Patients

A patient who was once classified as a screening/baseline failure for any reason can later be reconsidered for participation, if deemed clinically beneficial for the patient by the treating physician and Principal Investigator. In such case, the patient must again go through the consent process and must be rescreened. The patient must meet each criterion listed on the eligibility checklist based on the clinical documentation in the patient's medical record. After

the patient has reaffirmed his/her desire to participate, the rescreening process must take place within 14 days of Cycle 1, Day 1 (C1D1) as mandated by the treatment start guidelines. Patients who were previously treated with the study drug but were removed from treatment for any reason cannot be reconsidered for participation at a later date. Patients must be re-registered/re-enrolled on the study within 30 days of re-signing the informed consent document.

## 7. Treatment Plan

### 7.1. Study Drug Administration

After Cycle #1 a new treatment cycle will be initiated when all of the following conditions are met:

- Non-hematologic treatment related toxicities have improved to grade 1 or resolved (CTCAE v.4.0)
- Platelets > 10 x $10^9$/L

Refer to section 7.3 for guidelines for dose modifications based on treatment-related delays and toxicities of the previous cycle.

#### 7.1.1. **765IGF-Methotrexate Administration**

765IGF-MTX will be given at the assigned dose as a 1.5 hour IV infusion in 250 ml of 5% dextrose on days 1, 8, and 15 of a four-week treatment cycle. Every 4 week period will be considered one treatment cycle. Subsequent cycles must meet the criteria found in section 7.3.1 and may begin 1 day earlier to accommodate scheduling issues, or up to 4 weeks later if a patient needs this time to recover to meet criteria for start of a new cycle as specified in section 7.2.1. At the investigator's discretion, the study drug may be administered in 10% dextrose rather than 5% dextrose if it is believed necessary to prevent or control hypoglycemia.

**Pre-medication**

None is required, but can be utilized per clinician discretion. The program of predication below has been used in the prior solid tumor phase I with some of the later enrolled patients to prevent nausea and an allergic reaction. Nausea during infusion was seen in a few patients and a possible allergic reaction to the drug was seen in one patient.

Zofran 8mg PO
Benadryl 50mg PO
Tylenol 650mg PO
Hydrocortisone 100mg IV push
D5 .45 normal saline 100cc/hr to start with premeds

**Monitoring**

Vital signs including blood pressure, pulse, temperature, respirations, and pulse oximetry, and blood glucose will be measured immediately before the infusion and immediately after completion of the infusion in all patients during all cycles. IGF-MTX causes hypoglycemia at higher dose levels, so it is important to monitor blood glucose levels. During the first 28-day cycle, patients will be monitored for blood glucose on infusion days for a total of 3.5 hours. Vital signs listed above will be monitored per the following table:

| Monitoring interval | |
|---|---|
| Immediately before infusion | All vitals |
| To Hour 1.5 (during infusion) | Glucose only, every 15 min |
| Hour 1.5 | All vitals |
| Hours 1.5-3.5 | Glucose only, every 30 min |

The patients will be provided with a home blood glucose monitoring device and instructed to measure and record blood glucose at 7 hours (± 2 hours) and 24 hours (± 6 hours) after the start of the infusion. The same device will be used to monitor blood glucose at the clinic during the 3.5 hour monitoring.

On all subsequent cycles that patients are on treatment (i.e., cycle 2 and beyond), those patients that did not experience a severe adverse event during the infusion and 3.5-hr post-infusion period in cycle 1 will be monitored for a total of 2.5 hours on infusion days (i.e., during the 1.5 hour infusion and one hour after termination of the infusion). Vital signs listed above will be monitored per the following table:

| Monitoring interval | |
|---|---|
| Immediately before infusion | All vitals |
| To Hour 1.5 (during infusion) | Glucose only, every 15 min |
| Hour 1.5 | All vitals |
| Hours 1.5-2.5 | Glucose only, every 30 min |

The patients will be instructed to measure and record blood glucose at 7 hours (± 2 hours) and 24 hours (± 6 hours) after the start of the infusion for each dose.

Patients will be closely monitored for toxicities. Strict I/O will be measured during patient stay both in the ambulatory and inpatient setting (per unit protocol).

**Supportive Measures: See also section 7.7**
Hypoglycemia will be treated with oral glucose or if necessary IV glucose to maintain blood glucose levels above 50 mg/dl. Acetaminophen for fevers, meperidine for chills, anti-emetics for nausea and vomiting, normal saline or furosemide to maintain fluid balance/blood pressure/pulmonary function, electrolyte replacement, albumin to maintain serum albumin at 3 g/dL or greater. Anaphylactoid reactions will be treated with 100 mg methylprednisolone IV, diphenhydramine 25 mg IV, or 0.3 cc epinephrine (1:1000) IV and transfer to an ICU setting for monitoring. Refer to section 7.2.1. regarding management of Allergic Reaction/Hypersensitivity reactions.

**Precautions**
Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Methotrexate has been reported to cause fetal death and/or congenital anomalies. If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and must permanently discontinue study drug. Effective barrier contraception must be practiced during the entire study treatment period and through three months after the last dose of study drug by females

of childbearing potential and male patients (even if surgically sterilized (i.e., status post vasectomy) or the patient must completely abstain from heterosexual intercourse. Refer to section 11.5 for reporting pregnancy in a female patient or the partner of a male patient.

## 7.2. Dose Modifications

### 7.2.1. Start of a New Cycle

Toxicity and adverse will be classified according to NCI's Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40). Assessment of clinical benefit will be performed by investigators at the end of cycle 1 and confirmed with bone marrow studies as per MDS, CMML or O-AML specific response criteria (Appendices I and II) done at the end of cycles #2, 4, and 6 (i.e., at 8, 16, and 24 weeks, each +/- 7 days) and at the discretion of the physician thereafter.

A new treatment cycle will only be initiated when all of the following conditions are met:

- non- hematologic treatment related toxicities have improved to ≤ Grade 1 (CTCAE v.4.0) or to the patient's baseline values (except alopecia)
- Platelets per 7.1 (platelets > 10 x $10^9$/L)

If blood counts are below this threshold at the 4 week point, blood work is to be repeated weekly until counts are at an acceptable level. Treatment will be restarted with appropriate dose modifications provided CTCAE hematologic grade ≥4 is not prolonged for more than 6 weeks in the absence of persistent disease. If treatment is unable to restart within 4 weeks of the planned treatment date, the patient will be permanently discontinued from study therapy.

| **CTCAE v.4.0 Adverse Effects Terms & Descriptions (see below for allergic reaction/hypersensitivity)** | **765IGF-MTX Dose Modifications (for treatment-related toxicities deemed clinically significant)** |
|---|---|
| Any persistent (>4 weeks) grade 2 treatment-related non-hematologic toxicity | Drop to next lower dose level or discontinue treatment. |
| Grade 4 or greater treatment-related hematological toxicity for >7 days. | Drop to next lower dose level or discontinue treatment. |
| Grade 3 or greater treatment-related clinical non-hematological toxicity, excluding grade 3 nausea, vomiting, or diarrhea without maximal medical intervention and/or prophylaxis. | Drop to next lower dose level or discontinue treatment. |
| Febrile neutropenia. | Drop to next lower dose level or discontinue treatment. |
| Platelets less than 10 x $10^9$/L | Wait to improve and then drop to next lower dose level or discontinue treatment. |

**CTCAE Grade 1 Allergic Reaction/Hypersensitivity:** (Transient flushing or rash, drug fever < 38°C): Supervise without further treatment for allergic reaction/hypersensitivity.

**CTCAE Grade 2 Allergic Reaction/Hypersensitivity:** (Rash, flushing, dyspnea, urticaria, drug fever greater than or equal to 38°C) and/or asymptomatic bronchospasm: Interrupt the infusion. If symptoms abate, attempt re-infusion at a slower rate. If the symptoms recur,

discontinue infusion and follow for recurrent allergic reaction/hypersensitivity in the next paragraph.

**Recurrent CTCAE Grade 2 or CTCAE Grade 3 or 4 Allergic Reaction/Hypersensitivity:** Stop the infusion. Administer additional doses of H1 and H2 blockers intravenously. Administer IV steroids and consider epinephrine and bronchodilators as clinically indicated.

**CTCAE Grade 3 or 4 Allergic Reaction/Hypersensitivity:** Stop the infusion. Administer additional doses of H1 and H2 blockers intravenously. Administer IV steroids and consider epinephrine and bronchodilators as clinically indicated. Will be permanently discontinued from study treatment.

**Prior to re-challenge of Grade 2 allergic reaction/HSR and with all subsequent cycles**: The following prophylactic premedications are recommended for future infusions: give both an H1 and H2 blocker intravenously plus dexamethasone 20 mg (orally or intravenously) within 30 to 60 minutes before 765IGF-MTX infusion. Dexamethasone could be used at the investigator's discretion in subsequent cycles for recurrent (i.e. occurring despite slowing infusion as discussed above) Grade 2 reactions but would not be mandated.

An appropriate resuscitation plan should be in place and a physician readily available during the period of drug administration. If treatment delay necessitates a period longer than 4 weeks, treatment is stopped and the subject is discontinued from the study.

### 7.3. Permitted Concomitant Medications and Procedures

Myeloid growth factors to treat patients with neutropenia or cytopenia are permitted.

Antiemetic agents may be administered at the discretion of the investigator but are not commonly required as a prophylactic agent. All other manifestations of the patient's malignancy should be treated at the discretion of the investigator.

Medications with potential CNS effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with 765IGF-MTX.

In appropriate settings, such as combinations with agents known to produce frequent thrombocytopenia, restricted uses of anticoagulants should be considered.

All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

### 7.4. The Effects of Other Drugs on Methotrexate

Nonsteroidal anti-inflammatory drugs should not be administered prior to or concomitantly with the high doses of methotrexate, such as used in the treatment of osteosarcoma. Concomitant administration of some NSAIDs with high dose methotrexate therapy has been reported to elevate and prolong serum methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity.

Caution should be used when salicylates are administered concomitantly with lower doses of methotrexate. These drugs have been reported to reduce the tubular secretion of methotrexate in an animal model and may enhance its toxicity.

Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides including BACTRIM. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored.

Methotrexate increases the plasma levels of mercaptopurine. The combination of methotrexate and mercaptopurine may therefore require dose adjustment.

Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria.

Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

The potential for increased hepatotoxicity when methotrexate is administered with other hepatotoxic agents has not been evaluated. However, hepatotoxicity has been reported in such cases. Therefore, patients receiving concomitant therapy with methotrexate and other potential hepatotoxins (e.g., azathioprine, retinoids, sulfasalazine) should be closely monitored for possible increased risk of hepatotoxicity.

Methotrexate may decrease the clearance of theophylline; theophylline levels should be monitored when used concurrently with methotrexate.

Vitamin preparations containing folic acid or its derivatives may decrease responses to systemically administered methotrexate.

Folate deficiency states may increase methotrexate toxicity. Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression in patients receiving methotrexate, probably by decreased tubular secretion and/or an additive antifolate effect.

### 7.5. Concomitant Medications and Non-Drug Therapies

All patients will be asked to provide a complete list of prescription and over-the-counter medications that have been taken within the 4 weeks prior to screening. The investigator must be informed as soon as possible about any new medication(s) taken from the time of screening until the completion of the post-treatment follow-up visit.

### 7.6. Prohibited Concomitant Medications and Concomitant Medications to be used with caution.

The only prohibited concomitant medications are mercaptopurine, and concomitant other anti-cancer therapy (cytotoxic, biologic, or radiation).
Concomitant use of salicylates, sulfonamides, BACTRIM (trimethoprim/sulfomethoxazole) and phenylbutozone is not prohibited, but should be used with caution because of their interactions with high dose methotrexate (doses greater than or equal to 500mg/m2 of methotrexate are considered high), although the methotrexate doses used in this study are not high (the highest dose of 765IGF-MTX used in this study of 2.5 microEq/kg is about equal to 45 mg of methotrexate per meter$^2$).

Patients should not receive other anti-cancer therapy (cytotoxic, biologic, or radiation) while on treatment in this study.

**7.7. Management of Clinical Events and Supportive Care Guidelines**
Optimal patient care is to be provided to all patients.

Patients should receive full supportive care during the study, including transfusion of blood and blood products, treatment with antibiotics, analgesics, or erythropoietin, when appropriate.

Although acetaminophen at doses of ≤2 grams/day is permitted, it should be used with caution in patients with impaired liver function.

Patients with significant third spacing (anasarca, symptomatic pleural effusions, symptomatic ascites) should be monitored closely.

Infusion of IGF-methotrexate for 1.5 hours and follow up observation for an additional 1-2 hours (see section 7.1.1. for further details on duration of follow up observation) will be performed in monitored room with available sealed cart with Diazepam 2 mg vials for immediate intravenous infusion in case of seizures. The following protocol will be in place in case of this drug toxicity:

- Call physician on service.
- Start BLS SPECIFIC CARE:
  - Administer oxygen (high flow if neurological deficits or altered mental status)
  - Place the patient in recovery position, and prevent accidental harm.
  - Anticipate brief combativeness or agitation in post-ictal phase.
- Start ALS SPECIFIC CARE:
  Anticonvulsant Therapy
  - Diazepam (Valium): IV: 2-10 mg, every 5-10 minutes as needed (max of 20 mg), if status epilepticus start phenytoin load 20 mg/kg.

Once the situation is resolved, the duration of further monitoring will be at the discretion of the study physician.

**7.8. Nausea and Vomiting**
Prophylactic antiemetic therapy will not be used in this study unless it becomes clear that 765IGF-MTX causes acute nausea and vomiting. If prophylactic antiemetic therapy is needed, 5-$HT_3$ receptor antagonists (without corticosteroids) should be tried first. Because of the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

Although this study will not initially employ prophylactic antiemetics, there is no prohibition against antiemetic use in the management of a patient who develops nausea or vomiting, or both.

**7.9. Diarrhea**
Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, at the occurrence of the first loose stool and then 2 mg

every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

### 7.10. Duration of Therapy

Patients will receive protocol therapy unless:

- Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
- Patient withdraws consent.
- There is evidence of progressive disease or unacceptable toxicity.
- The treating physician thinks a change of therapy would be in the best interest of the patient.
- More than a 4 week delay between cycles

### 7.11. Follow-up

Administration of study medication will be continued after cycle 2 until disease progression, unacceptable toxicity, or patient withdrawal. A final study visit will occur 30 days (+/-1 week) after the last dose of 765IGF-MTX. This visit will end study participation unless there is ongoing toxicity at least possibly related to study treatment. In this case, the patient will be followed as medically appropriate until resolution or stabilization of the adverse event.

## 8. Study Parameters

### 8.1. Standard of Care Procedures

| | Baseline, within 28 days of enrollment | Each treatment cycle[a] | | | Every 8 weeks (± 1 week) | 30 days (± 1 week) after final dose of 765IGF-MTX[1] |
|---|---|---|---|---|---|---|
| | | Day 1[2] (±1 day) | Day 8 (±1 day) | Day15 (±1 day) | | |
| Signed consent (Registration/ Enrollment must occur within 30 days. | X | | | | | |
| Medical history | X | X | | | | X |
| Review of prior therapy | X | | | | | |
| Bone marrow biopsy and aspirate confirming disease and blast count[3] | X | | | | x[3] | |
| Physical exam | X | X | | | | X |
| Vital signs | X | X | X | X | | X |
| Height, weight | X | X | | | | X |
| Concomitant meds review | X | X | X | X | | X |
| Performance status | X | X | | | | X |
| Symptom and toxicity | X | X | X | X | | x[3] |
| CBC w/ diff | X | X | X | X | | X |
| CMP (with: calcium, glucose, sodium, potassium, CO2, chloride, BUN, creatinine, albumin, ALT, AST, bilirubin, alkaline phosphatase, total protein) | X | X | X | X | | X |
| Uric acid (cycle 1 only) | X | X | | X | | |
| LDH | X | X | | | | |
| HbA1C for diabetic patients | X | | | | | X |
| Serum pregnancy test for females of child-bearing potential | X | | | | | |
| ECG[4] | X | X | | | | X |
| 765IGF-MTX administration | | X | X | X | | |

[a] Subsequent cycles beyond cycle 1 must meet the criteria found in section 7.3 and may begin 1 day earlier or up to 2 days later to accommodate scheduling issues.

[1] For patients who leave treatment with a response, repeat appropriate disease assessment every 6-12 weeks until progression or start of a new treatment.

[2] For cycle 1 only, tests and procedures do not need to be repeated if done within 3 days of day 1.

[3] Bone marrow biopsy should be at end of end of cycle 2, and every 2 cycles thereafter to cycle 6. Patients who achieve response (CR) could have repeat bone marrow biopsies per MD discretion.

[4] ECG performed at screening, at cycle 1 and at the end of study visit 30 days after the final dose.

### 8.2. Research Related Procedures

| | Baseline (within 28 days of Cycle 1, D1 | C1D1 | C1 D15 | C2 D1 | C2 D15 | Every 8 weeks (±1 week) up to week 24 | Cycles 4 and 6, D15 |
|---|---|---|---|---|---|---|---|
| PKs per section 9.1 | | x[1] | x[1] | | | | |
| ECG (QT study) done during PK blood collection per section 9.3. PK patients only.[2] | | x[2] | x[2] | | | | |
| Serum IGF-1 level. All subjects. | | x[3] | | x[3] | x[3] | | |
| Plasma and whole blood IGF-1R level. All subjects. | | x[3] | | x[3] | x[3] | | x[3] |
| Anti-765IGF antibody assay. All subjects. | | x[3] | | x[3] | x[3] | | x[3] |
| Neutralizing antibodies. All subjects. | | x[3] | | x[3] | x[3] | | x[3] |
| IGF-1R expression in bone marrow biopsy and aspirate by IHC and flow cytometry. All subjects. | x[4] | | | | | x[4] | |
| IGF-1R expression in blood cells by flow cytometry. All subjects. | x[5] | | | | | x[5] | |

[1] Done only in the PK patients in the MTD. Pre-765IGF-MTX infusion (at the time of am labs if am labs are within one hour of 765IGF-MTX infusion), 5 min before the infusion ends (+/- 5 minutes), and at the following time points after completion of the infusion: 30 min (+/- 5 minutes), 60 min (+/- 15 minutes), 2 h (+/- 15 minutes), 4 h (+/- 15 minutes), 6 h (+/- 15 minutes), 8 h (+/- 15 minutes and 24 h (+/- 2 hours)). The 8 hour time point will be collected on day 1 but not on day 15.

[2] Done only in PK patients in the MTD. Pre-765IGF-MTX infusion (+/- 5 minutes), and 30 min (+/- 5 minutes) after starting the infusion, at the following time points after completion of the infusion: 60 min (+/- 15 minutes) and 3 hours (+/- 15 minutes). In all patients, ECG is done at baseline, on day 1, cycle 1 (before the infusion), and 30 days (± 1 week) after the final dose (see section 8.1).

[3] Blood draw prior to infusion

[4] Bone marrow biopsy and aspirate will be collected at baseline and every 8 weeks (±1 week) up to week 24, i.e., after cycles 2, 4, and 6, before the first dose of the next cycle. If no bone marrow aspirate viably frozen for flow cytometry is available from a patient in the 28-day baseline period before cycle 1, day 1, the patient may still enroll and no new bone marrow biopsy will be required for a baseline sample.

[5] Whole blood collection in EDTA tubes.

## 9. Correlative Studies

Information on the collection of blood samples for the correlative studies (collection tubes to be used, volume of blood drawn, collection processing, aliquot procedure and storage, and particular assay to be used) can be found in a laboratory manual to be supplied.

### 9.1. Pharmacokinetics

#### 9.1.1. **Pharmacokinetic Sample Collection**

The pharmacokinetics of 765IGF-MTX will be examined following the doses administered on days 1 and 15 of cycle 1. On these days the 765IGF-Methotrexate will be infused in the morning as described in section 7.1.1. The research team will record the start and stop times of the infusion and volume of the 765IGF-MTX solution infused. Whole blood (6 mL) will be collected from an inserted butterfly needle on the opposite arm or from a peripheral site if the patient has a central venous catheter immediately prior to the 765IGF-MTX infusion, 5 min before the infusion ends, and at the following time points after completion of the infusion: 30 min, 60 min, 2 h, 4 h, 6 h, 8 h, and 24 h, (windows as above [Research-Related Procedures table, footnote 1). The 10 h time point will be collected on day 1 but not day 15. IGF-MTX and MTX unbound to IGF toxicokinetic analysis will be performed. These time points are based on pharmacokinetics in dogs. Please see the laboratory manual for PK sample collection procedures.

#### 9.1.2. **Pharmacokinetic Sample Processing**

Blood samples (6 mL) will be collected in 6-mL EDTA (purple top) tubes. The tube should be gently inverted a few times for complete mixing with the anticoagulant. The exact time of sample collection should be recorded on the tube label and Pharmacokinetics Data Form provided. The tube should be kept on wet ice until centrifugation. Within 120 minutes of blood collection, centrifuge each blood sample at approximately 3,000 X g for 5-10 minutes at 4°C. Aliquots, approximately 0.5 mL each, will be pipetted into 4 separate plastic centrifuge tubes and frozen at -80$^{o}$C until analysis. Please see the laboratory manual for PK sample collection procedures.

765IGF-MTX, 765IGF, methotrexate and 7-OH-methotrexate plasma concentrations will be determined using validated assays under GLP conditions at the Toxicology Research Laboratory, University of Illinois at Chicago.

#### 9.1.3. **Pharmacokinetic Parameter Determination**

The pharmacokinetics of the 765IGF-Methotrexate, 765IGF, methotrexate and 7-OH methotrexate will be analyzed by compartmental and noncompartment approaches. Non-compartmental analysis of the plasma concentration-time data for each of the compounds of interest will be performed using WinNonlin 6.3 (Pharsight, St Louis, MO). Pharmacokinetic parameters to be estimated include: 1). area under the drug plasma concentration-time curve from the start of the infusion to the time of the last quantifiable plasma concentration ($AUC_{0-t}$), 2) AUC from the start of the infusion to infinity ($AUC_{0-\infty}$), 3). maximum observed plasma concentration ($C_{max}$), 4). time of maximum plasma concentration ($T_{max}$) and 5). terminal elimination constant ($\lambda_z$).

Plasma concentration versus time data for the compounds of interest will be fit individually and simultaneously to appropriate models using nonlinear mixed effects modeling as implemented in NONMEM (version7.3). Data will be modeled with individual and population approaches. One, two, and three-compartment models incorporating parent and metabolite disposition will be evaluated. First order conditional estimation,

Monte Carlo expectation maximization and Monte Carlo Bayesian methods will be explored for estimating the maximum likelihood.

If anti-765IGF antibodies are detected in some or all patients, the effect of the presence of these antibodies on the pharmacokinetic parameters will be investigated. This will be done, for instance, by comparing pharmacokinetic parameters in the first dose, when no anti-drug antibodies could be present, with the parameters in later doses after anti-765IGF antibodies have been shown to have developed, and by comparing the parameters in patients who have anti-765IGF antibodies with those that do not.

#### 9.1.4. **Statistical Analysis for Pharmacokinetics**

The parameters for 765IGF-MTX, 765IGF, methotrexate, and 7-OH methotrexate will be expressed by descriptive statistics (geometric mean, median, standard deviation and coefficient of variation). The primary pharmacokinetic parameters investigated for each compound will be $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$ and $\lambda_z$. Descriptive statistics will be calculated for the demographic data. Graphs and correlations will be used to examine the distribution of values and bivariate relationships.

### 9.2. Pharmacodynamic Assessment

Pharmacodynamic samples will be assessed on D1 of cycle 1, D1 and D15 of cycle 2, and D15 of cycles 4 and 6.

Systemic responses are defined as serum concentration of IGF-1 and plasma and whole blood concentrations of IGF-1R, which will be measured for all subjects and used to assess whether 765IGF-MTX has affected the production of these biological markers. Measures of toxicity (e.g., changes WBC counts, differential cell populations, platelets, etc.) are also considered systemic PD variables.

Pharmacodynamic data will be fit to an appropriate model, using maximum likelihood estimation. To determine whether any relationship exists between systemic activity of drug and biomarkers, the individual baseline corrected maximum biomarker concentrations will be plotted against individual 765IGF-MTX pharmacokinetic values, and Pearson's correlation coefficients will be calculated.

### 9.3. Evaluation of potential of 765IGF-MTX for QT prolongation

QT evaluation will be performed in the PK subjects only during PK collection times by ECG immediately prior to the 765IGF-MTX infusion, 30 minutes after starting the infusion, and at the following time points after completion of the infusion: 60 minutes and 3 hours. In all subjects, QT evaluation will be performed by ECG at baseline (within 14 days of enrollment), at cycle 1 day 1 (before the infusion) and at 30 days (±1 week) after the final dose of 765IGF-MTX.

### 9.4. Serum IGF-1 Level and 765IGF-MTX Toxicity/Response

Blood serum samples from before the infusion on D1 of cycle 1, and before the infusion on D1 and D15 of cycle 2 will be collected from each patient to determine if pre- and during treatment serum soluble IGF-1 level is associated with 765IGF-MTX toxicity and/or response. Quantification of IGF-1 in serum will be performed by Q2 Solutions, using Chemiluminescence methodology. A descriptive analysis will be done between clinical response and marker levels. Please see the laboratory manual for biomarker collection procedures.

**9.5. Plasma and whole blood IGF-1R Level and 765IGF-MTX Toxicity/Response**
Plasma and whole blood samples from before the infusion on D1 of cycle 1, and before the infusion on D1 and D15 of cycle 2 and on D15 of cycles 4 and 6 will be collected from each patient to determine if pre- and within-treatment serum and blood IGF-1R level is associated with 765IGF-MTX toxicity and/or response. Quantification of IGF-1R in plasma will be performed by IGF Oncology using western blotting. A descriptive analysis will be done between clinical response and marker levels. Please see the laboratory manual for biomarker collection procedures.

**9.6. Formation of anti-765IG-MTX antibodies.**
Serum samples from before the infusion on D1 of cycle 1, and before the infusion on D1 and D15 of cycle 2 will be collected from each patient and analyzed for anti-drug antibodies by an assay the sponsor has used for detecting anti-765IG-MTX antibodies in preclinical tests in dogs and rats. The assay is a sandwich ELISA involving plating serum in 96-well plates, adding drug to the wells of the plates to bind to any anti-drug antibodies that may be present in the serum, and then detecting bound 765IGF-MTX drug with an anti-IGF-HRP conjugate antibody from R&D Systems, Quantikine human IGF-1 ELISA kit.

That sandwich assay detects antibodies against 765IGF-MTX.

The serum samples will also be analyzed for the presence of neutralizing antibodies. In this assay, serum will be mixed with 765IGF-MTX in an in vitro assay for killing of human MCF7 breast cancer cells to determine whether the addition of patient serum affects the minimum inhibitory concentration of 765IGF-MTX in inhibiting growth of MCF7 cells. This assay will be performed on the same serum samples collected before treatment on Cycle 2, days 1 and 15 and on day 15 of cycles 4 and 6. Please see the laboratory manual for biomarker collection procedures.

***Risk assessment.*** The risk to patients from assaying for anti-765IGF-MTX antibodies is minimal and arises only from an additional blood draw before the infusion on D1 of cycle 1, and D1 and D15 of cycle 2. A small amount of blood (7.5 mL/draw) will be taken, which will have no effects on patient health. The risk to patients from possibly developing anti-765IGF-MTX antibodies is also small and would be lessened by our knowledge as to whether they are developing these antibodies. First, none of the dogs and none of the rats developed anti-drug antibodies in preclinical testing, so it appears unlikely that patients will develop anti-765IGF-MTX antibodies. The risk to patients from developing the antibodies, if it occurs, would be that the antibodies would be expected to possibly reduce the effectiveness of the drug, and would raise a risk of an anaphylactic reaction to administration of the drug. Anaphylactic reactions occur with some biologic medicines, such as Rituximab, and can usually be managed with antihistamines, such as diphenhydramine. Formation of antibodies against 765IGF-MTX may also potentially cause similar side effects of antibodies against IGFR1 receptor, such as the possibility of development of hyperglycemia. Monitoring blood sugar levels will be routinely performed in this study.

**9.7. Neutralizing antibodies and 765IGF-MTX Toxicity/Response**
Serum samples from before the infusion on D1 of cycle 1 and before the infusion on D1 and D15 of cycle 2, and on D15 of cycles 4 and 6 will be analyzed for the presence of neutralizing antibodies. In this assay, serum will be mixed with 765IGF-MTX in an in vitro assay for killing of human MCF7 breast cancer cells to determine whether the addition of patient serum affects the minimum inhibitory concentration of 765IGF-MTX in inhibiting

growth of MCF7 cells. Please see the laboratory manual for biomarker collection procedures.

A descriptive analysis will be done correlating neutralizing antibody presence or levels with clinical response to, and toxicity of, 765IGF-MTX.

### 9.8. IGF-1R expression level in diseased cells of bone marrow aspirate via IHC and flow cytometry

When bone marrow aspirates are collected from patients, a portion will be clotted, fixed, and paraffin embedded; and a second portion will be shipped fresh the same day to Charles River Laboratories (Reno, NV) for flow cytometry assay of IGF-1R and CD34. A pathology report will be prepared for the fixed sample. The fixed samples will be held at Mayo Clinic and shipped together at the end of the study with their pathology reports to Quest Diagnostics for IGF-1R expression level testing by IHC with their test code 19429X.

The fresh second portion of the bone marrow aspirate will be kept at room temperature and shipped on the day of collection in an insulated container at room temperature to Charles River, Inc. (Reno, NV), for testing of IGF-1R and CD34 expression by flow cytometry. This assay will be similar to that of He et al.[28]

### 9.9. IGF-1R expression level in diseased cells of whole blood via flow cytometry

Whole blood will be collected in EDTA tubes (6 ml) and shipped overnight the same day in an insulated container at room temperature to Charles River, Inc., for testing of IGF-1R and CD34 expression by flow cytometry. This assay will be similar to that of He et al.[28] This collection and testing will be at baseline and every 8 weeks after treatment is begun.

## 10. Drug Formulation and Procurement

### 10.1. 765IGF-MTX

#### 10.1.1. Other names

765IGF-methotrexate, IGF-methotrexate, IGF-methotrexate conjugate, IGF-MTX

#### 10.1.2. Classification

Insulin-Like Growth Factor and an enzyme inhibitor of dihydrofolic acid reductase (methotrexate)

#### 10.1.3. How supplied

765IGF-MTX is supplied as a 5 ml sterile solution at 4.0 µeq per ml 765IGF-MTX concentration in aqueous 10 mM HCl in a 10 ml glass vial.

#### 10.1.4. Availability

765IGF-MTX will be provided by IGF Oncology with approval of the FDA for investigational purposes. (IND # 116840).

#### 10.1.5. Description

765IGF-MTX is formulated as a sterile solution at 4.0 µeq/ml (4.0 µmol methotrexate groups per ml) in 10 mM HCl in a 10 ml vial. The appropriate volume of the drug is then added to 250 ml of either 5% of 10% dextrose (per discretion of Principal Investigator) on the day of administration.

10.1.6. **Storage, Handing, and Accountability**

The final product of 765IGF-MTX is stored refrigerated at 4°C. Studies at elevated temperatures suggest the drug is stable for at least 2 years when stored in this manner.

10.1.7. **Administration**

Vials with the dissolved drug are stored refrigerated. Each vial should be mixed with a gentle swirling motion. As with all protein based products, avoid vigorous shaking. The solution must be yellow and translucent. The solution must not be used unless it is translucent and without visible particulate matter.

The aliquot is calculated in µeq/kg dose x patient weight in kg. (All patient doses are calculated in µeq/kg body weight). On the day of administration, the appropriate volume is withdrawn from one or more vials and then diluted into 250 ml of either 5% of 10% dextrose (per discretion of Principal Investigator) for infusion. It is infused over a period of 90 minutes.

10.1.8. **Risks**

The primary side effect predicted in adults receiving 765IGF-MTX is low blood sugar immediately after receiving the drug. This will be treated by intravenous administration of either 5% of 10% dextrose (per discretion of Principal Investigator) or by oral administration of sugar.

The following toxicities are predicted in adults receiving methotrexate:
Immediate side effects include allergic reactions associated with low blood pressure, shortness of breath, hypotension, , back pain and hives. These will be treated with medications, fluids and oxygen. If symptoms persist, the patient may have to be hospitalized for further treatment.

Other adverse events that have been observed with 765IGF-MTX administration have been nausea, syncope, tachycardia, fever and chills, seizure, and abdominal cramps. These have been observed during the infusion or within 3 hours after infusion.

Delayed toxicities of methotrexate which occur within hours to days include skin rashes, diarrhea, fever, anorexia, nausea, vomiting, mouth sores, fatigue, malaise, myalgias, arthralgias, chills, high blood sugar level. These will be treated with medications as needed. Work up for infections will be conducted as needed.

Possible late toxicities include abnormal liver function tests. These effects occur several days to a week after treatment. See section 10.1.9 Warnings **and Precautions**.

Rare toxic effects of methotrexate include encephalopathy, sensory changes, kidney problems, and lung problems. See section 10.1.9 **Warnings and Precautions**.

10.1.9. **Warnings and Precautions**

Methotrexate has been reported to cause fetal death and/or congenital anomalies. Therefore, it is not recommended for women of childbearing potential unless there is clear medical evidence that the benefits can be expected to outweigh the considered risks. If a woman becomes pregnant or suspects that she is pregnant

while participating in this study, she must inform the investigator immediately and must permanently discontinue study drug.

Methotrexate elimination is reduced in patients with impaired renal function, ascites, or pleural effusions. Such patients require especially careful monitoring for toxicity, and may require dose reduction or, in some cases, discontinuation of methotrexate administration.

Unexpectedly severe (sometimes fatal) bone marrow suppression, aplastic anemia, and gastrointestinal toxicity have been reported with concomitant administration of methotrexate (usually in high dosage) along with some nonsteroidal anti-inflammatory drugs (NSAIDs).

Methotrexate causes hepatotoxicity, fibrosis and cirrhosis, but generally only after prolonged use. Acutely, liver enzyme elevations are frequently seen. These are usually transient and asymptomatic and also do not appear predictive of subsequent hepatic disease. Liver biopsy after sustained use often shows histologic changes, and fibrosis and cirrhosis have been reported.

Methotrexate-induced lung disease, including acute or chronic interstitial pneumonitis, is a potentially dangerous lesion, which may occur acutely at any time during therapy and has been reported at low doses. It is not always fully reversible and fatalities have been reported. Pulmonary symptoms (especially a dry, nonproductive cough) may require interruption of treatment and careful investigation.

Diarrhea and ulcerative stomatitis require interruption of therapy: otherwise, hemorrhagic enteritis and death from intestinal perforation may occur.

Like other cytotoxic drugs, methotrexate may induce "tumor lysis syndrome" in patients with rapidly growing leukemias. Appropriate supportive and pharmacologic measures may prevent or alleviate this complication.

Severe, occasionally fatal, skin reactions have been reported following single or multiple doses of methotrexate. Reactions have occurred within days of oral, intramuscular, intravenous, or intrathecal methotrexate administration. Recovery has been reported with discontinuation of therapy.

Potentially fatal opportunistic infections, especially *Pneumocystis carinii pneumonia*, may occur with methotrexate therapy.

## 11. Adverse Event Documentation and Reporting

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

Note: throughout this section the generic term "drug" refers to 765IGF-MTX.

### 11.1. Definitions

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

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

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

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

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

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

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

**Preplanned hospitalization:** A hospitalization planned before signing the ICF is not considered an SAE, but rather a therapeutic intervention. However, if during the preplanned hospitalization an event occurs, which prolongs the hospitalization or meets any other SAE criteria, the event will be considered an SAE. Surgeries or interventions that were under consideration, but not performed before signing the ICF, will not be considered serious if they are performed after signing the ICF for a condition that has not changed from its baseline level. Elective hospitalizations for social reasons, solely for the treatment of prior conditions or due to long travel distances are also not SAEs.

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

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

or underlying medical conditions are considered *unexpected;* however, they will not be reportable per section 11.3.

**Unanticipated (unexpected) problems/events** defined as those that are *not* already described as potential risks in the consent form, are *not* listed in the Investigator's Brochure, and are *not caused by* an underlying disease.

**Note:** The major discord between the FDA and IRB definitions is whether or not the underlying disease is included when considering expectedness.

**UPIRSO:** Federal regulations [45CFR46.103(b)(5) and 21CFR56.108(b)(1)] require the IRB to ensure that researchers promptly report "any unanticipated problems involving risk to subjects or others" (UPIRSOs). The Mayo Clinic IRB defines a UPIRSO as <u>any problem or event which in the opinion of the local researcher was *unanticipated, reflects new or increased risk to the subjects and at least possibly related* to the research procedures</u>.

In addition, the Mayo Clinic IRB has defined the following problems/events as reportable using the Prompt Report form found on the IRB website:

- Any accidental or unintentional change to the IRB-approved protocol that increases risk or has the potential to recur
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research
- Any breach in confidentiality that may involve risk to the subject or others
- Any complaint of a subject that cannot be resolved by the research staff
- Any other possibly related event which in the opinion of the investigator constitutes an unanticipated risk

**Expedited (Rapid) Reporting:** Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 11.3. For the IRB this is 5 working days. For studies under an IND, it is 7 or 15 calendar days.

### 11.2. Adverse Event Documentation

Adverse events occurring from the first dose of 765IGF-MTX up to and including 30 days after administration of the last dose must be documented. Adverse events attributed to a study-related procedure which occur prior to the initiation of study treatment must be documented as well.

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

| | **Grade 1** | **Grade 2** | | **Grade 3** | | **Grade 4 and 5** |
|---|---|---|---|---|---|---|
| | **Expected or Unexpected** | **Expected** | **Un-expected** | **Expected** | **Un-expected** | **Expected or Unexpected** |
| **Unrelated Unlikely** | Not required | Not required | Not required | Not required | Required | Required |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Possible Probable Definite** | Not required | Required | Required | Required | Required | Required |

All patients will be monitored with appropriate event documentation in EDC Easy® through the final study visit per section 7 as it is expected that most treatment related adverse events will occur during this period.

However, all toxicities (grades 1, 2, 3, 4, 5) must be reported within internal study forms, and include the following descriptors:

- Toxicity
- Grade
- Date
- Expected or Unexpected
- Attribution (unrelated, unlikely, possible, probably, definite)

For grade 2 or higher toxicities, principal investigator and/or treating physician will be notified the same day as study visit.

### 11.3 Required Reporting, IRB, FDA, IGF Oncology, LLC

| Agency | Criteria for reporting | Timeframe | Form to Use | Submission address/ fax numbers | Copy AE to: |
|---|---|---|---|---|---|
| **Study Site IRB** | **UPIRSO**: Events which are unanticipated, involved risk to subjects or others, and was at least possibly related to study procedures/ treatment | 5 Working Days | EDC Easy® (Paper report can be generated from system)/ Mayo Clinic IRB Prompt Report Form | Study Site IRB | Study Site Regulatory Coordinator |
| | **Other Problems or Events** meeting the definition of UPIRSO in section 11.1 | 5-15 Working Days per IRB requirements | Mayo Clinic Rochester IRB/Prompt Report Form | | |
| **FDA** | Unexpected and fatal or life threatening suspected adverse reaction | As soon as possible but no later than 7 Calendar Days | Medwatch/ EDC Easy® (Paper report can be generated | Fax SAE to IND/IDE Regulatory Health Project Manager, Techiya (Thea) Toaff, R.N., B.S.N., Division of Oncology Products 1 at 1-301-796-9845. | |

| | | | | | |
|---|---|---|---|---|---|
| **FDA (continued)** | 1) Serious and unexpected suspected adverse reaction or<br>2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or<br>3) findings from other sources (other studies, animal or in vitro testing) | As soon as possible but no later than 15 Calendar Days | from system) | Email:techiya.toaff@fda.hhs.gov<br><br>Follow-up with written report submitted as an amendment to:<br><br>MedWatch,<br>5600 Fishers Lane,<br>Rockville, MD 20852-9787 | |
| | All other events per CRF 312.33 | At time of IND annual report | Summary format | Submit as part of the IND annual report | |
| **Note: Events due to the disease under treatment or an underlying medical condition will not require expedited reporting to the FDA for the purposes of this study** | | | | | |
| **IGF-Oncology, LLC** | All serious adverse events, regardless of expectedness or relationship with any study drug per section 11.4 | Within 24 hours of knowledge | SAE Initial and Follow-up Report Forms provided to each site<br><br>MedWatch/ EDC Easy® (Paper report can be generated from system) | **IGF Clinical Operations Team** *(SAE and Pregnancy Reporting Contact Information):*<br><br>**Hugh McTavish, Ph.D., IGF Oncology, President, CEO**<br>hmctavish@igfoncology.com<br>Phone: 651-492-0283<br><br>**Arkadiusz Dudek. M.D., IGF Oncology, Chief Medical Officer**<br>adudek021@outlook.com<br>Phone: 612-718-1960<br><br>**William E. Gannon Jr., M.D. Chief Medical Monitor**<br>WGannon@capcitytek.com<br>Phone: 703-447-2615<br><br>**Kathleen Littrell, IGF Oncology, Sr. Project Management**<br>Klittrell@igfoncology.com<br>Phone: 919-606-8581<br><br>**Linda Sneed, IGF Oncology, Sr. Clinical Research Associate**<br>lpsneed@IGFOncology.com<br>Phone: 423-747-8515 | |
| | Pregnancy in a female patient or the partner of a male patient per section 11.5 | | Pregnancy Reporting Form | | |

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

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

### 11.4 Procedures for Reporting SAEs to IGF Oncology, LLC

Adverse events (AEs) may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures must be reported to the IGF Oncology Clinical Operations team. AEs which are serious must be reported to the IGF Oncology Clinical Operations team (see contact information in the chart above) from first dose of 765IGF-MTX up to and including 30 days after administration of the last dose of 765IGF-MTX. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. Any SAE that occurs at any time after completion of 765IGF-MTX treatment or after the designated follow-up period that the investigator and/or sub-investigator considers to be related to any study drug must be reported to the IGF Oncology Clinical Operations team.

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

This is an IGF Oncology sponsored study. The principal investigators conducting the study are Mrinal S. Patnaik, M.B.B.S., at Mayo Clinic and Dr. Yan Ji, at Health Partners Regions Cancer Care Center.

Investigator must report all SAEs, regardless of expectedness or relationship with any study drug, to the IGF Oncology Clinical Operations team (contacts noted in above chart ) as soon as possible, but no later than 5 calendar days of the sponsor-investigator's observation or awareness of the event. In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to the IGF Oncology Clinical Operations team (contacts noted on page 42) and reports will be sent to all sites participating in the study. Subinvestigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the IGF Oncology Clinical Operations team, unless otherwise agreed between the sponsor-investigator and subinvestigator(s). The IGF Oncology Clinical Operations team may request follow-up information to a reported SAE, which the sponsor-investigator will be responsible for providing to the IGF Oncology Clinical Operations team.

The SAE report must include event term(s), serious criteria, and the investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration.

Intensity for each SAE, including any lab abnormality, will be determined by using the NCI CTCAE v.4.0.

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug.

Sponsor-investigator must also provide the IGF Oncology Clinical Operations team with a copy of all communications with applicable regulatory authorities related to the study or study

drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communication.

Data will be entered in the SAE section of EDC Easy®, from which a paper report can be generated at any time. In addition, IGF Oncology will provide the investigators with both an Initial SAE Report Form, (to be completed at the time of the SAE) and an SAE Follow-up Report form (to be completed when all follow-up information has been gathered). The SAE Report forms should be E-mailed to the IGF Oncology Clinical Operations team (contact E-mails are noted on the chart above (page 42).

### 11.5 Procedures for Reporting During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and must permanently discontinue study drug(s). All pregnancies and suspected pregnancies must be reported to the IGF Oncology Clinical Operations team immediately using a Pregnancy Report Form (see Section 11.3 for contact information). The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and the IGF Oncology Operations team will request this information from the investigator.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, this must be reported to the IGF Oncology Clinical Operations team immediately using a Pregnancy Report Form (see Section 11.3 for contact information). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

## 12. Study Data Collection and Monitoring

### 12.1. Data Management

This study will report clinical data using the EDC Easy® Data Management System utilizing study specific case report forms. Key study personnel are trained on the use of case report forms and will comply with protocol specific instructions for data collection.

Patient demographics, patient specific study treatment calendars, adverse events and other information required for IND annual reporting will be maintained with EDC Easy®.

### 12.2. Case Report Forms

Participant data will be collected using protocol specific case report forms (CRFs). The CRFs will be approved by the study's Principal Investigator and the study biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into EDC Easy® at time of study entry, completing CRFs based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

### 12.3. Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with Mayo Clinic Cancer Center's Data & Safety Monitoring Plan (DSMP).

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

- The PI will complete and submit a quarterly Trial Progress Report to the Cancer Center Data and Safety Monitoring Committee (DSMC) with the understanding the Cancer Center Protocol Review Committee (CCPRC) may require more frequent reporting.

- The PI will comply with at least twice yearly monitoring of the project by the Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 11.3 to the Cancer Center's SAE Coordinator, the Mayo Clinic IRB, and the FDA.

In addition, at the time of the continuing review with the Mayo Clinic IRB, a copy of the report with any attachments will be submitted to the Protocol Review Committee (PRC).

**IND Annual Reports**
In accordance with regulation 21 CFR § 312.33, the sponsor will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect. Included as a part of the annual report for this IND will be initial reporting of all deaths due to disease at any time and all deaths (regardless of cause) occurring more than 2 years after 765IGF-MTX infusion.

### 12.4. Monitoring
The investigator will permit study-related monitoring, audits, and inspections by the study's Investigator and/or any designees, the local IRB, government regulatory bodies, and Mayo Clinic compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

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

In addition, the CTO will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

The CTO must be contacted before destroying any study related records.

## 13. Statistical Considerations

A brief overview of the statistical analysis plan is presented below. Complete details of the planned analysis will be documented in a full Statistical Analysis Plan, which will be finalized before locking the study database.

### 13.1. Sample Size
Given the design and primary objectives of this study, the sample size will be determined by the guidelines governing dose escalation/de-escalation and the identification of DLTs. As a result, the final sample size is not fixed and will not be determined by consideration of inferential analyses conducted via statistical hypothesis testing.

### 13.2. Statistical Methods
Statistical analyses will be performed using SAS software version 9.4 or higher. In general, data summaries will be compiled by 765IGF-MTX dose level and overall and will include the mean,

standard deviation, median, minimum and maximum values for continuous data; the median, 25th and 75th percentiles, minimum and maximum values for time-to-event endpoints; and the number and percentage of patients in each category for categorical data. Pointwise 95% confidence intervals (CI) will also be estimated for the mean (continuous data), median (time-to-event endpoints) or percentage of patients (categorical data).

Baseline value of a characteristic is defined as the last measured value prior to the first dose of 765IGF-MTX.

Descriptive statistics such as Spearman or Pearson correlation coefficients will be estimated with 95% CIs to assess the relationship between anti-765IGF antibody and anti-765IGF-MTX antibody levels with clinical response and toxicity.

#### 13.2.1. **Efficacy Endpoints**

The numbers of overall complete remissions (patients with a best objective response of CR or CRi), and overall remissions (patients with a best objective response of CR, CRi or PR) will be summarized by observed rates and estimated 95% CIs. If there are sufficient numbers of patients achieving remissions for an analysis to be informative, logistic regression will be used to model the probability of achieving a remission as a function of the assigned 765IGF-MTX dose level for Cycle 1. Other factors may be added to the model to investigate their prognostic value in predicting remission.

In general, missing values will not be replaced by imputed values. Patients who, for any reason, do not supply bone marrow for response assessment are counted among those not achieving a response.

Kaplan-Meier time-to-event analyses will be conducted on overall survival and progression-free survival. Mortality (all causes) at certain landmark time points (e.g., 90 and 180 days) will also be estimated together with 95% CIs. Cox proportional hazards models will be fit to these time-to-event endpoints with the assigned 765IGF-MTX dose level for Cycle 1 serving as the independent variable in the model.

#### 13.2.2. **Pharmacokinetic Endpoints**

Dose linearity for the pharmacokinetic parameters AUC and $C_{max}$ will be tested using a standard power model such as, $AUC = \alpha + dose^{\beta}$, which is equivalent to $\log(AUC) = \log(\alpha) + \beta \cdot \log(dose)$. Linearity (also called dose proportionality) will be accepted if $\beta$ is not significantly different from 1 at a nominal 10% level of significance.

#### 13.2.3. **Safety Endpoints**

Incidence rates of treatment-emergent adverse events will be summarized within treatment group at the MedDRA preferred term and primary system organ class levels. Similar summaries will be made for subsets of AEs such as (1) those judged by the Investigator to be related to study treatment, and (2) serious adverse events (SAEs). Other routine safety assessments (e.g., clinical laboratory parameters, ECG parameters, and vital signs) will be summarized by standard deviation, median, minimum and maximum observed values and changes from baseline values. NCI-CTCAE severity grades for laboratory parameters and other endpoints measured on an ordinal categorical scale may be summarized using shift tables (a two-way frequency table pairing baseline value with the most extreme post-baseline result).

Additional exploratory analyses may be performed to assist the sponsor in planning future studies.

**13.3. Analysis plan for primary objective:**

The primary objective is to determine safety and tolerability of 765IGF-MTX by evaluation of toxicity for the treatment of advanced, previously treated hematologic malignancies. Preliminary information will be collected for treatment related toxicity and tolerance. Toxicity will be graded using the NCI's Common Terminology Criteria for Adverse Events version 4 (CTCAE v.4.0). Treatment tolerance will be based on the number of dose-limiting toxicities, treatment delays and dose reductions. Information will be presented in a tabular and descriptive manner.

**13.4 Analysis plan for secondary objectives:**

**13.4.1 Objective Response Rate, Relapse-Free Survival, Overall Survival and Cumulative Incidence of Relapse;** descriptive statistics will be used to describe clinical benefit measured by the above parameters.

**13.4.2 Describe clinical response (CR) as defined by hematologic-specific response criteria:** Disease response will be assessed every 8 weeks +/- 7 days while on study treatment using appropriate hematologic-specific response criteria (Appendices I and II). Due to the small sample sizes and heterogeneous patient population, clinical responses will be reported by dose and disease (MDS, CMML, or O-AML) type.

**13.5 Analysis plan for correlative objectives:**

**13.5.1 Describe changes in pharmacokinetic parameters:** The pharmacokinetic parameters for 765IGF-MTX, 765IGF, methotrexate, and 7-OH methotrexate will be expressed by descriptive statistics (geometric mean, median, standard deviation and coefficient of variation). The primary pharmacokinetic parameters investigated for each compound will be $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$ and $\lambda_z$. Descriptive statistics will be calculated for the demographic data. Graphs and correlations will be used to examine the distribution of values and bivariate relationships.

**13.5.2 Describe changes in pharmacodynamics parameters:** The pharmacodynamics parameters of IGF-1, IGF-1R, formation of antibodies against 765IGF-MTX, and formation of neutralizing antibodies will be expressed by descriptive statistics (geometric mean, median, standard deviation and coefficient of clinical response). Percent change from pre- and post-treatment levels will be correlated with response using summary statistics and the two-sample t-test and nonparametric Wilcoxon rank-sum test if warranted. Graphs and correlations will be used to examine the distribution of values and bivariate relationships.

13.5.3 **Evaluation of 765IGF-MTX for potential of QT prolongation:** Compare baseline ECG profile to post-765IGF-MTX ECG profiles to determine if the drug prolongs QT interval.

13.5.4 **Serum IGF level and 765IGF-MTX Toxicity/Response:** Determine if pre- and within-treatment serum soluble IGF-1 level is associated with 765IGF-MTX toxicity and/or response. A descriptive analysis will be done between clinical response and marker levels.

13.5.5 **Plasma and blood IGF-1R level and 765IGF-MTX Toxicity/Response:** Determine if pre- and within-treatment plasma and whole blood IGF-1R level is associated with 765IGF-MTX toxicity and/or response. A descriptive analysis will be done between clinical response and marker levels.

13.5.6 **Antibody formation against 765IGF-MTX:** Serum samples from before infusion on D1 of cycle 1, and before infusion on D1 and D15 of cycle 2 and day 15 of cycles 4 and 6 will be collected and analyzed for anti-765IGF-MTX antibodies. A descriptive analysis will be done correlating anti-765IGF-MTX antibody presence or levels with clinical response and toxicity.

**13.5.7 Neutralizing antibodies and 765IGF-MTX toxicity/response:** Serum samples from before infusion on D1 of cycle 1 and before infusion on D1 and D15 of cycle 2 and D15 of cycles 4 and 6 will be collected for presence of neutralizing antibodies. A descriptive analysis will be done correlating presence or levels of neutralizing antibodies with clinical response and toxicity.

**13.5.8 IGF-1R expression level in bone marrow biopsies via IHC and flow cytometry and IGF-1R expression level in blood cells by flow cytometry:** IGF-1R expression at baseline will be performed on paraffin-embedded fixed bone marrow aspirates by Quest Diagnostics by IHC and IGF-1R expression at baseline will be performed on fresh viable bone marrow aspirates and fresh whole blood by Charles River, Inc. by flow cytometry. If sample sizes are small and heterogeneous, clinical responses will be reported by dose and disease (MDS, CMML, or O-AML) type

# 14 Conduct of the Study

## 14.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

## 14.2 Ethical Considerations

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

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# Appendix I – Response Criteria for Acute Leukemia

## Acute Myeloid Leukemia[45]

**Table 5. Response criteria in AML**

| Category | Definition |
|---|---|
| Complete remission (CR)* | Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0 × $10^9$/L (1000/μL); platelet count > 100 × $10^9$/L (100 000/μL); independence of red cell transfusions |
| CR with incomplete recovery (CRi)† | All CR criteria except for residual neutropenia (< 1.0 × $10^9$/L [1000/μL]) or thrombocytopenia (< 100 × $10^9$/L [100 000/μL]) |
| Morphologic leukemia-free state‡ | Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required |
| Partial remission (PR) | Relevant in the setting of phase 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50% |
| Cytogenetic CR (CRc)§ | Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow |
| Molecular CR (CRm)‖ | No standard definition; depends on molecular target |
| Treatment failure | |
| Resistant disease (RD) | Failure to achieve CR or CRi (general practice; phase 2/3 trials), or failure to achieve CR, CRi, or PR (phase 1 trials); only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination |
| Death in aplasia | Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia |
| Death from indeterminate cause | Deaths occurring before completion of therapy, or < 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available |
| Relapse¶ | Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease |

**Table 6. Outcome measures in AML**

| Category | Definition |
|---|---|
| Overall survival | Defined for all patients of a trial; measured from the date of entry into a study to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive |
| Relapse-free survival* | Defined only for patients achieving CR or CRi‡; measured from the date of achievement of a remission until the date of relapse or death from any cause; patients not known to have relapsed or died at last follow-up are censored on the date they were last examined |
| Event-free survival | Defined for all patients of a trial; measured from the date of entry into a study to the date of induction treatment failure, or relapse from CR or CRi,‡ or death from any cause; patients not known to have any of these events are censored on the date they were last examined |
| Cumulative incidence of relapse (CIR)† | Defined for all patients achieving CR or CRi‡ measured from the date of achievement of a remission until the date of relapse; patients not known to have relapsed are censored on the date they were last examined; patients who died without relapse are counted as a competing cause of failure |

*Relapse-free and disease-free survival have been used with the same definition

†It is important to provide estimates of cumulative incidence of death (CID) as well, since just considering the results of CIR may be misleading if for instance CIR is lower for one group but CID is actually higher for that same group.

‡In studies where the criterion CRi is used, relapse-free survival should be defined for all patients achieving CR or CRi; for event-free survival, relapse should be considered from CR and CRi.

| | |
|---|---|
| ***FAILURE - aplasia*** | *Aplasia:* Patient survives ≥ 7 days following completion of initial treatment course then dies while cytopenic, with the last post-induction bone marrow aplastic or hypoplastic (i.e. < 20% cellularity) and without leukemia blasts. |
| ***FAILURE - indeterminate*** | *Indeterminate:* (a) Patient survives < 7 days after completion of initial treatment course; **or** (b) patient survives ≥ **7** days following completion of initial treatment course then dies with no persistent leukemia in the peripheral smear but no post-induction bone marrow examination. |
| ***RELAPSE FROM CR*** | *Relapse:* Reappearance of leukemia blasts in the peripheral blood; or > 5% blasts in the bone marrow not attributable to another cause (e.g., recovery of normal cells following chemotherapy-induced aplasia); or appearance or reappearance of extramedullary disease. |

# Appendix II – Response Criteria for MDS[47]

Table 3. Proposed modified International Working Group response criteria for altering natural history of MDS[7]

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

## Appendix III – ECOG Performance Status

| ECOG Score | Performance Status |
|---|---|
| 0 | Asymptomatic |
| 1 | Symptomatic, fully ambulatory |
| 2 | Symptomatic, in bed < 50% of the day |
| 3 | Symptomatic, in bed > 50% of the day but not bedridden |
| 4 | Bedridden |
| 5 | Dead |

## Appendix IV – New York Heart Association Classification

| Class | Functional Capacity | Objective Assessment |
|---|---|---|
| I | Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain. | No objective evidence of cardiovascular disease. |
| II | Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain. | Objective evidence of minimal cardiovascular disease. |
| III | Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain. | Objective evidence of moderately severe cardiovascular disease. |
| IV | Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased. | Objective evidence of severe cardiovascular disease. |

## Appendix V – Prohibited Concomitant Medications

| |
|---|
| Mercaptopurine |
| Concomitant other anti-cancer therapy (cytotoxic, biologic, or radiation) |

## Appendix VI – Medications to use with caution, see Sections 7.4 and 7.6

| |
|---|
| NSAIDs |
| Phenytoin |
| BACTRIM ( trimethoprim/sulfamethoxazole) |
| Sulfonamides |
| Phenylbutazone |
| Salicylates |
| Oral antibiotics |
| Penicillins |
| Azathioprine |
| Retinoids |
| Sulfasalazine |
| other potential hepatotoxins |
| Theophylline |
| Folic acid vitamin supplement |