COLUMBIA UNIVERSITY MEDICAL CENTER

Herbert Irving Comprehensive Cancer Center Protocol

A phase II study, with a safety lead-in, to evaluate ATX-101, a peptide drug targeting PCNA, in advanced dedifferentiated liposarcoma and leiomyosarcoma

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TITLE: A phase II study, with a safety lead-in, to evaluate ATX-101, a peptide drug targeting PCNA, in advanced dedifferentiated liposarcoma and leiomyosarcoma

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Study Agent:	ATX-101
IND Status:	IND [redacted] Study May Proceed August 18, 2021

Protocol Signature Page

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable federal, state, and local laws, rules, and regulations relating to the conduct of the protocol. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I will promptly submit the protocol to the applicable IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modification made during the course of the study must first be approved by the IRB, prior to implementation except when such modification is made to remove an immediate hazard to the subject. I certify that I, and the study staff, have received the requisite training to conduct this research protocol. I agree to maintain adequate and accurate records in accordance with Columbia University and Herbert Irving Comprehensive Cancer Center policies, Federal, state and local laws and regulations. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Instructions to Principal Investigator: Sign and Date this signature page and print your name. Return the original, completed and signed to the Clinical Protocol & Data Management Office. Retain a copy in the regulatory binder.

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Signature of Principal Investigator Date

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Principal Investigator Name (Print)

_____

Name of Institution

Protocol Synopsis

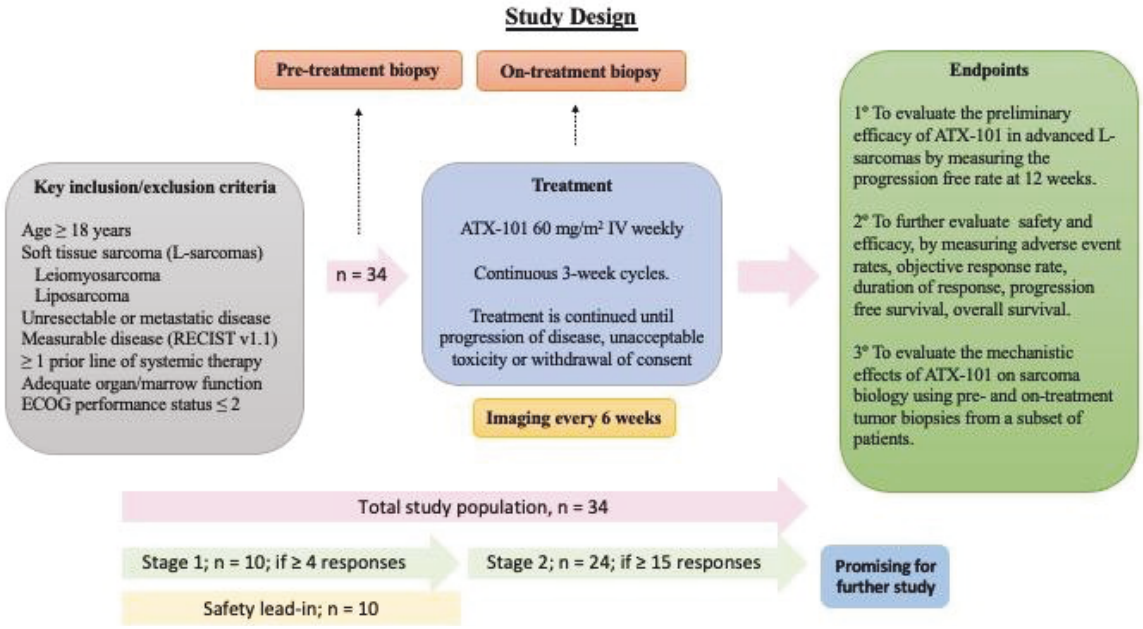
Title	A phase II study, with a safety lead-in, to evaluate ATX-101, a peptide drug targeting PCNA, in advanced dedifferentiated liposarcoma and leiomyosarcoma
Short Title	ATX-101 in advanced sarcoma
Protocol Number	AAAT7079
Phase	Phase II
Methodology	Open-label, single-arm, Simon 2-stage, phase II
Study Duration	18 months
Study Center(s)	Single center

Objectives	Soft tissue sarcoma is a heterogeneous malignancy of mesenchymal origin and more than 70 biologically distinct subtypes exist. Leiomyosarcoma (LMS) and liposarcoma (LPS) (collective referred as L-sarcomas) represent about one-third of all adult soft tissue sarcomas. They are commonly treated with chemotherapy in the first-line setting. Doxorubicin monotherapy, or gemcitabine with docetaxel, provide objective response rates (ORRs) of 15-20%, progression-free survival (PFS) of approximately 5 months and overall survival (OS) of 16 months. ATX-101 is a small molecule peptide comprised of a novel human proliferating cell nuclear antigen (PCNA) interacting motif termed APIM coupled to cellular and nuclear delivery domains. PCNA interacts with many cellular proteins and exerts pleiotropic effects in the cancer cell. Proteins that bind to PCNA via APIM are especially important in the cellular stress and DNA damage responses, as well as intracellular signaling, apoptosis, metabolism and anti-tumor immunity. In preclinical studies, ATX-101 demonstrated single-agent activity and potentiated other cytotoxic and targeted agents across multiple cancer models *in vitro* and *in vivo*, including LMS and LPS. ATX-101 is currently being evaluated in a phase 1 safety and pharmacokinetic study in solid tumors using a 3 + 3 dose escalation design. As of 10/29/2020, ATX-101 has been evaluated across dose levels of 20 mg/m^2 – 60 mg/m^2 IV weekly. Although no MTD was reached, after review of the available safety data, the RP2D was determined to be 60 mg/m2 IV weekly, with no plans to dose escalate further. ATX-101 has been well tolerated, with no grade 3 or higher adverse events (AEs) observed during the phase 1 study. Common AEs include grade 1/2 infusion related reactions (which have been easily managed with supportive care), mild fatigue and diarrhea. In this study, ATX-101 demonstrated encouraging activity as prolonged disease stabilization in patients with progressive, heavily pre-treated malignancies. The current study will evaluate the preliminary efficacy and further establish the safety profile of ATX-101 in advanced LMS and LPS. Because clinical data with ATX-101 is limited, a safety lead-in will be conducted among the first 10 patients enrolled and treated on the study. <u>Primary:</u> To evaluate the preliminary efficacy of ATX-101 in advanced L-sarcomas (LMS, LPS) by measuring the PFR (progression free rate) at 12 weeks (PFR_{12}). <u>Secondary:</u> To perform a safety lead-in among the first 10 patients enrolled and treated on the study to confirm the safety and tolerability

	of the drug in sarcoma patients. To further evaluate the safety and efficacy profile of ATX-101 in LMS and LPS by measuring adverse event rates, objective response rate, duration of response, median progression free survival and median overall survival. Correlative: To evaluate the effects of ATX-101 in sarcoma by evaluating ATX-101's effects on the immune microenvironment, intracellular signaling pathways, and DNA damage response pathways using paired tumor tissue biopsies from a subset of 10 patients and to use the results of these analyses to identify candidate biomarkers for response and resistance to ATX-101 in the sarcoma population.
Number of Subjects	34 patients
Diagnosis and Main Inclusion Criteria	Eligible patients include those 18 years or older with histologically confirmed, unresectable or metastatic LMS or dedifferentiated LPS, progression on or intolerance to at least one prior line of systemic treatment, measurable disease by RECIST version 1.1 criteria, ECOG performance status ≤ 2 and adequate end-organ and bone marrow function.
Study Product, Dose, Route, Regimen	Patients will be treated with ATX-101 60 mg/m^2 IV weekly in continuous 21 day cycles. Patients will receive premedication prior to the ATX-101 infusion to reduce the risk of infusion-related reactions.
Duration of administration	Patients may continue study treatment until evidence of clinical or radiographic disease progression, unacceptable toxicity, withdrawal of consent or study closure.
Reference therapy	Not applicable

Statistical Methodology	The primary endpoint for this phase II study is the progression free rate at 12 weeks (PFR_{12}). This corresponds to the number of patients who are alive and without evidence of disease progression at 12 weeks out of all evaluable patients. Two recent randomized phase 3 studies evaluated novel agents (trabectedin, eribulin) in a population of patients with LPS and LMS. In both studies, the cytotoxic agent dacarbazine was used as the active comparator/control arm. Dacarbazine is a chemotherapy agent often used for later-line treatment of soft tissue sarcoma, including LPS and LMS. In the randomized study of trabectedin versus dacarbazine in patients with LPS and LMS (n=518), median PFS was 4.2 months versus 1.5 months, and the progression-free rate at 3 months (12 weeks) was 56% versus 34%, respectively[1]. In a subset analysis, outcomes were similar in LPS and LMS. Similarly, in the randomized study of eribulin versus dacarbazine (n=452), the median PFS was 2.6 months versus 2.6 months, and the progression-free rate at 3 months (12 weeks) was 33% versus 29%.[2] Based upon the results of these studies, a $PFR_{12} \leq 30\%$ would be considered inactive and unworthy of further study, whereas a $PFR_{12} \geq 55\%$ would suggest clinically meaningful activity, worthy of further evaluation in subsequent studies. PFR_{12} is a commonly used endpoint in phase II sarcoma studies. A Simon optimal 2-stage design is used. The study will enroll 10 patients with LMS and LPS in the first stage. If 4 or more patients meet the PFR_{12} endpoint in the first stage (4/10), the study will proceed to the second stage, enrolling an additional 24 patients for a total sample size of 34 patients. If 15 or more total patients meet the PFR_{12} endpoint in the overall study population (15/34), the agent will be considered promising and worthy of further study. An interim analysis will be performed after the first stage is completed. In the event that the study meets the stage 1 endpoint, but all patients who meet the PFR_{12} endpoint are of the same histology (either LMS or LPS), then the study will proceed to full accrual in that respective histology only. In this event, no further patients will be enrolled from the histology in which no patients met the PFR_{12} endpoint during stage 1. This design provides 85% power and type 1 error (1-sided) of 0.05 to evaluate for an improvement in PFR_{12} from $\leq 30\%$ to $\geq 55\%$.

	For the first stage of enrollment, among the 10 patients enrolled, 5 patients will have LMS, and 5 patients will have LPS. If the study proceeds to the second stage, among the 24 patients subsequently enrolled, 12 patients will have LMS and 12 patients will have LPS.


Study Design
Pre-treatment biopsy
On-treatment biopsy
Key inclusion/exclusion criteria
Age ≥ 18 years
Soft tissue sarcoma (L-sarcomas)
Leiomyosarcoma
Liposarcoma
Unresectable or metastatic disease
Measurable disease (RECIST v1.1)
≥ 1 prior line of systemic therapy
Adequate organ/marrow function
ECOG performance status ≤ 2
n = 34
Treatment
ATX-101 60 mg/m² IV weekly
Continuous 3-week cycles.
Treatment is continued until progression of disease, unacceptable toxicity or withdrawal of consent
Imaging every 6 weeks
Endpoints
1° To evaluate the preliminary efficacy of ATX-101 in advanced L-sarcomas by measuring the progression free rate at 12 weeks.
2° To further evaluate safety and efficacy, by measuring adverse event rates, objective response rate, duration of response, progression free survival, overall survival.
3° To evaluate the mechanistic effects of ATX-101 on sarcoma biology using pre- and on-treatment tumor biopsies from a subset of patients.
Total study population, n = 34
Stage 1; n = 10; if ≥ 4 responses
Stage 2; n = 24; if ≥ 15 responses
Promising for further study
Safety lead-in; n = 10

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1. INTRODUCTION AND RATIONALE

This document is a protocol for a human research study. This study is to be conducted according to US and International standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Columbia University Medical Center institutional research policies and procedures.

1.1 Disease Background

Sarcomas comprise a heterogeneous group of uncommon solid tumors of mesenchymal origin, of which more than 70 subtypes have been defined. In 2020, approximately 13,130 people will be diagnosed with soft tissue sarcoma, and 5,350 will die of this malignancy.[3]

Leiomyosarcoma (LMS) and liposarcoma (LPS), together referred to as "L-sarcomas", comprise about one third of all soft tissue sarcomas. Dedifferentiated LPS represents the most common histological subtype of LPS. The primary management for most localized sarcomas, including LMS and LPS, is surgical resection when feasible. Unfortunately, therapeutic options for patients with metastatic or unresectable disease remain limited. The various subtypes of sarcoma differ greatly in their clinical and molecular characteristics as well as response to traditional cytotoxic agents and radiotherapy. Outcomes are generally poor in the advanced setting, with front-line chemotherapy (doxorubicin or the combination of gemcitabine and docetaxel) eliciting objective responses in about 15-20% of patients, with median overall survival from the diagnosis of metastatic disease limited to 12 to 18 months. [4] The recent approval of trabectedin for advanced liposarcoma and leiomyosarcoma, [1] and pazopanib for advanced non-adipocytic sarcomas, [5] has resulted in modest improvements in progression free survival for these subtypes with no demonstrated benefit in overall survival. Eribulin was shown to modestly improve overall survival as compared placebo for liposarcoma, resulting in FDA approval in liposarcoma.[6] Molecular characterization of certain subtypes of sarcoma has permitted some progress in targeted systemic therapy, most notably with the discovery of activating KIT mutations in gastrointestinal stromal tumors (GIST). However, for the majority of patients with advanced sarcomas, outcomes remain disappointing, and new approaches with targeted agents and immunotherapies are urgently needed.

1.2 ATX-101, a peptide drug targeting PCNA

ATX-101 is a therapeutic peptide that targets proliferating cell nuclear antigen (PCNA) and more specifically, PCNA-related cellular stress responses and related downstream pathways. ATX-101 contains a novel human PCNA interacting motif termed APIM coupled to cellular and nuclear delivery domains.

PCNA is a widely expressed and primarily nuclear 261 amino acid protein which is a cofactor of the deoxyribonucleic acid (DNA) polymerases. PCNA is a member of the conserved sliding clamp family of proteins, and acts as a ring-shaped homotrimer that encircles and freely slides along double stranded DNA. [7, 8] This protein serves as a scaffold or binding platform protein that is important in several nuclear processes. [9] It plays a key role in orchestrating normal DNA replication, but also acts as an important platform for recruiting components of the replication surveillance machineries, the DNA damage response and DNA damage bypass

pathways, which permit replication through problematic or damaged regions. [10] PCNA has recently also been linked to various cytosolic functions such as the regulation of intracellular signaling, apoptosis, metabolism and anti-tumor immunity. [11-14]

The multi-functionality of PCNA is based on its ability to bind many proteins. These interactions are mediated via two known PCNA-interacting sequences that have been shown to be present in numerous proteins: the PCNA-interacting peptide (PIP)-box [15] and the Alk B homolog 2 protein (ABH2) PCNA-interacting motif (APIM). [16] Evidence suggests that proteins that bind to PCNA via a PIP-box are principally those involved in DNA replication, whereas proteins that bind to PCNA via APIM are mainly those that are important in the cellular stress responses. [16-20]

PCNA in normal cells is subject to post-translational modifications (PTM) upon cellular stress, which results in increased affinities for APIM-proteins, and this is part of the normal cellular response to stress. However, DNA damage and stress responses are regulated in highly interconnected networks that control cell fate via multiple levels of feedback and crosstalk, and while these processes are functional in normal cells, defects in these systems are a reason for uncontrolled growth and thus cancer. Therefore, any perturbation of DNA damage and stress responses will be more detrimental for cancer cells than normal cells.

Human ABH2 (hABH2) is a DNA repair protein associated with the replication machinery and plays a role in removing alkylation damage from DNA. [21] Within the 10 N-terminal amino acids of hABH2, a conserved five amino acid sequence that mediates direct interaction with PCNA was identified and termed APIM. [16] Several lines of evidence suggest that the APIM is especially important during the cellular response to DNA damage, suggesting that APIM-mediated PCNA binding of many proteins involved in DNA repair and cell cycle control is important during genotoxic stress. [16-18, 20, 22-25] Upon stress there is a “switch” in the repertoire of PCNA interaction partners from replicative proteins to proteins handling DNA damage and many of the proteins containing APIM are in the latter category. [9, 18] PCNA controls key housekeeping processes by interacting with a set of cellular proteins via the PIP-box. Upon cellular stress such as DNA damage, PCNA is post-translationally modified and interacts with APIM-containing proteins.

1.3 Rationale for the use of ATX-101 in cancer

ATX-101 has been designed to target PCNA and reduce the binding of APIM-containing proteins to PCNA. Blocking PCNA’s interactions through APIM prevents interactions that are vital for the cellular response to stress. This impairs the cell’s ability to survive stress resulting in cell death by apoptosis. [16, 22, 23, 26] Selective blocking of the interaction site for APIM on PCNA by exogenously administered peptides comprising the APIM-sequence is a novel intervention modality, in which cancer cells would be rendered more sensitive to chemotherapeutic agents.

1.4 Rationale for Selection of Subtypes

Clinical trials in sarcoma must balance the biological heterogeneity of over fifty subtypes with the feasibility of conducting clinical trials in rare diseases.

LPS are genomically complex tumors, with few recurrent, targetable molecular alterations. They harbor the highest burden of structural aberrations across all of human cancer with frequent whole genome duplication. LMS is also associated with high levels of replicative stress and harbors characteristic defects in DNA repair pathways. [27] LPS and LMS are frequently studied together in interventional clinical trials, including the recent, large, randomized phase III studies of trabectedin versus dacarbazine, and eribulin versus dacarbazine, both of which were conducted in LPS and LMS.[1, 6] In both of these studies, dacarbazine, a cytotoxic, was used as an active comparator. In addition, preclinical studies described below support the further study of ATX-101 in LPS and LMS.

1.5 Preclinical Experience with ATX-101

Preclinical studies have demonstrated ATX-101's is effective for inhibiting the growth of cancer cells across a broad range of cancer cell lines in vitro, as well as in different in vivo models. The APIM consensus sequence is validated as a functional PCNA-binding motif that occurs in numerous proteins that are important in DNA repair and tolerance mechanisms, cell cycle and apoptosis regulation, epigenetic control and transcription. ATX-101 was shown to directly interact with PCNA and to impair APIM-PCNA interactions in live cells.

In vitro, ATX-101 is able to induce apoptosis of cancer cell lines in a cell cycle independent manner. From a panel of 44 cancer cell lines, originating from a range of organs, ATX-101 was shown to exert a dose-dependent growth inhibitory effect as a single agent. ATX-101 was also shown to potentiate the growth inhibitory effect of different chemotherapeutic and targeted agents in cell lines. In vivo, the efficacy of ATX-101 in potentiating the effect of chemotherapeutic and targeted agents to reduce tumor growth was demonstrated using twelve different models. Administered in combination therapy, ATX-101 did not increase the toxicity observed above that of the anticancer agents alone. Please see the ATX-101 Investigator's Brochure for more details on these studies.

The efficacy of ATX-101 in sarcoma models is under evaluation in the laboratory of Dr. Gary Schwartz at Columbia University. In vitro studies in LPS and LMS cell lines show that ATX-101 monotherapy has a dose-dependent anti-proliferative effect on multiple dedifferentiated liposarcoma (LPS) and leiomyosarcoma (LMS) cell lines (Figure 1).

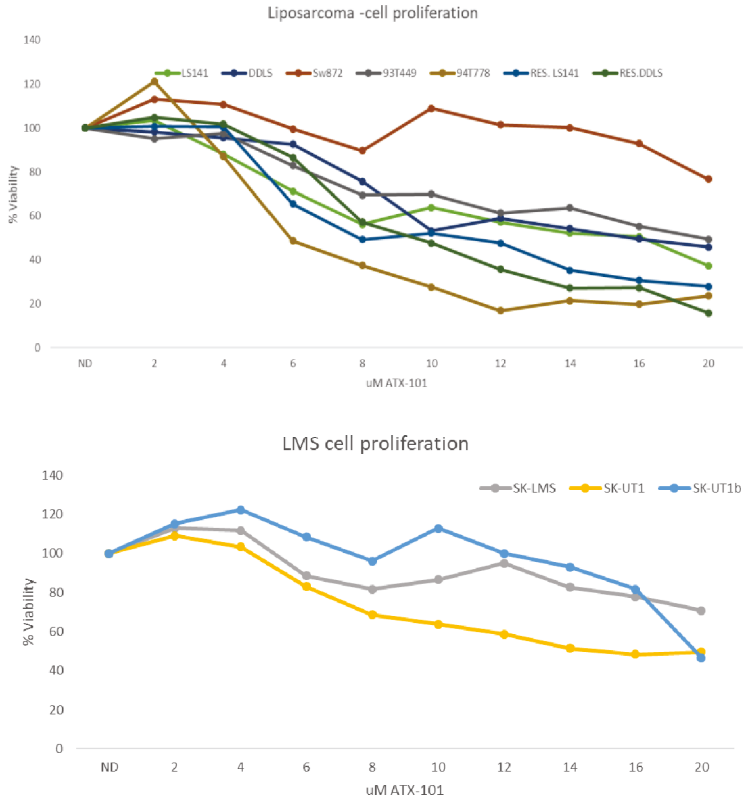


Figure 1. ATX-101 inhibits cell proliferation in LPS and LMS cell lines. Briefly, LMS cell lines (SK-LMS, SK-UT1 and SK-UT1b) and LPS cell lines (LS141, DDLS, SW872, 93T449, 94T778 and CDK4/6 resistant cell lines (Res. LS141 and Res. DDLS) were plated in 96-well plates and treated in six wells per condition with increasing doses of ATX-101 for 72 hours. A colorimetric cell proliferation assay (CCK-8 from Dojindo Molecular Technologies, Inc.) was used which quantifies the amount of formazan dye generated by the activity of dehydrogenases in the cells that is directly proportional to the number of living cells. The optical density at 450 nm was measured on an EMax Plus Microplate Reader (Molecular Devices Corp). Cell viability is expressed as a percentage of the no drug (ND) control.

Western blot studies were performed to evaluate the mechanism of the observed anti-tumor effect. We evaluated the effect of ATX-101 on cell signaling pathways by evaluating the levels of various proteins known to be involved in cell growth and proliferation in sarcoma by Western blotting (Figure 2). These studies demonstrated ATX-101 inhibits phosphorylation of ERK1/2 in LPS and LMS cell lines. Additional mechanistic studies are ongoing.

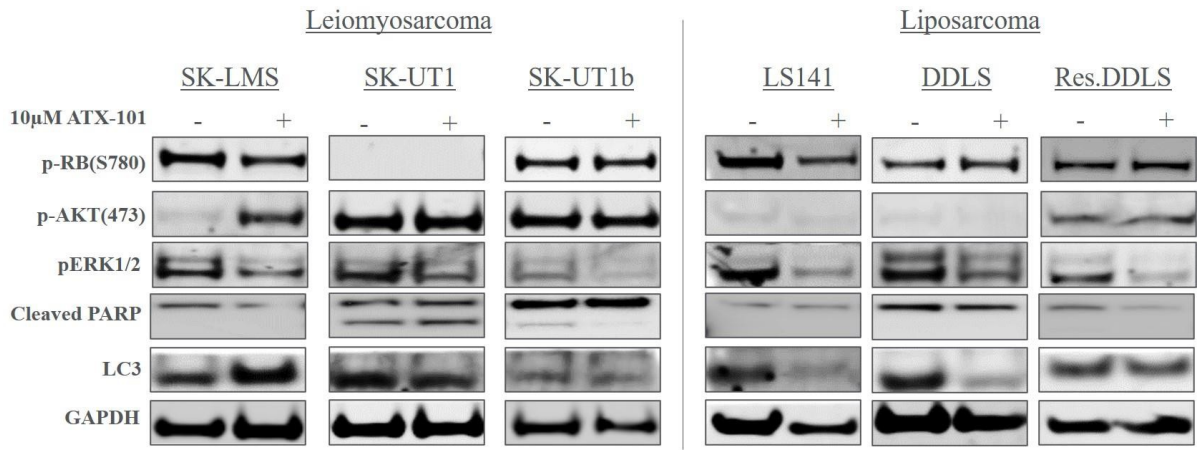


Figure 2. ATX-101 inhibits phosphorylation of ERK1/2 in liposarcoma and leiomyosarcoma. Western Blot of p-RB (Ser780), p-ERK1/2 (Thr202/Tyr204), p-AKT (Ser473) and LC3I/II and cleaved PARP is shown. GAPDH was used as a loading control. Cells were treated with 10μM ATX-101 for 72 hours. Briefly, leiomyosarcoma cell lines (SK-LMS, SK-UT1 and SK-UT1b) and liposarcoma cell lines (LS141, DDLS, CDK4/6 Res.DDLS) were treated and lysates were collected in RIPA buffer. Protein concentrations were determined and 20μg of protein were loaded onto an SDS/PAGE gradient gel and immunoblotted using indicated antibodies.

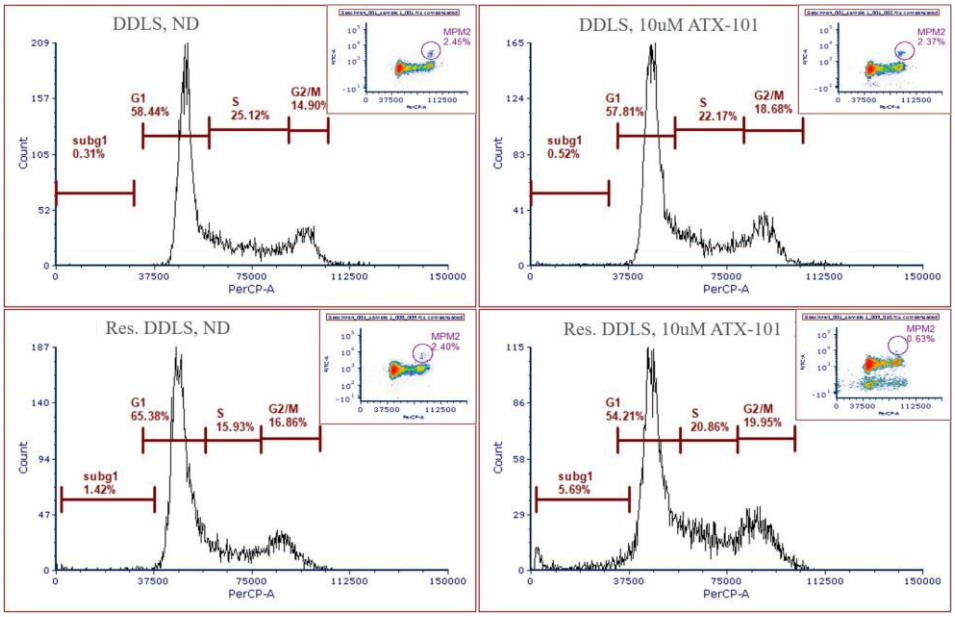


Figure 3. ATX-101 treatment affects the cell cycle in liposarcoma cell lines. Increase of G2 phase of the cell cycle in DDLS and induction of subG1 in Res. DDLS was observed with treatment. Briefly, propidium iodide DNA staining was performed on liposarcoma cell lines (DDLS and Res. DDLS) cells after 24 hours of 10μM ATX-101 treatment. Cells were fixed in 70% ethanol and stored at –20° C. Cells were then labeled with MPM-2 antibody (mitotic marker) and followed by a secondary antibody anti-mouse Alex-488. Bi-parameter flow cytometry was performed on a Becton Dickinson LSRII flow cytometer. Data analysis was done using FCS Express 7 software.

Cell cycle analysis was performed to study the effect of ATX-101 in LPS and LMS cell lines. Overall, we observed an increase in the sub-G1 fraction suggesting increasing cell death and apoptosis (Figures 3 and 4).

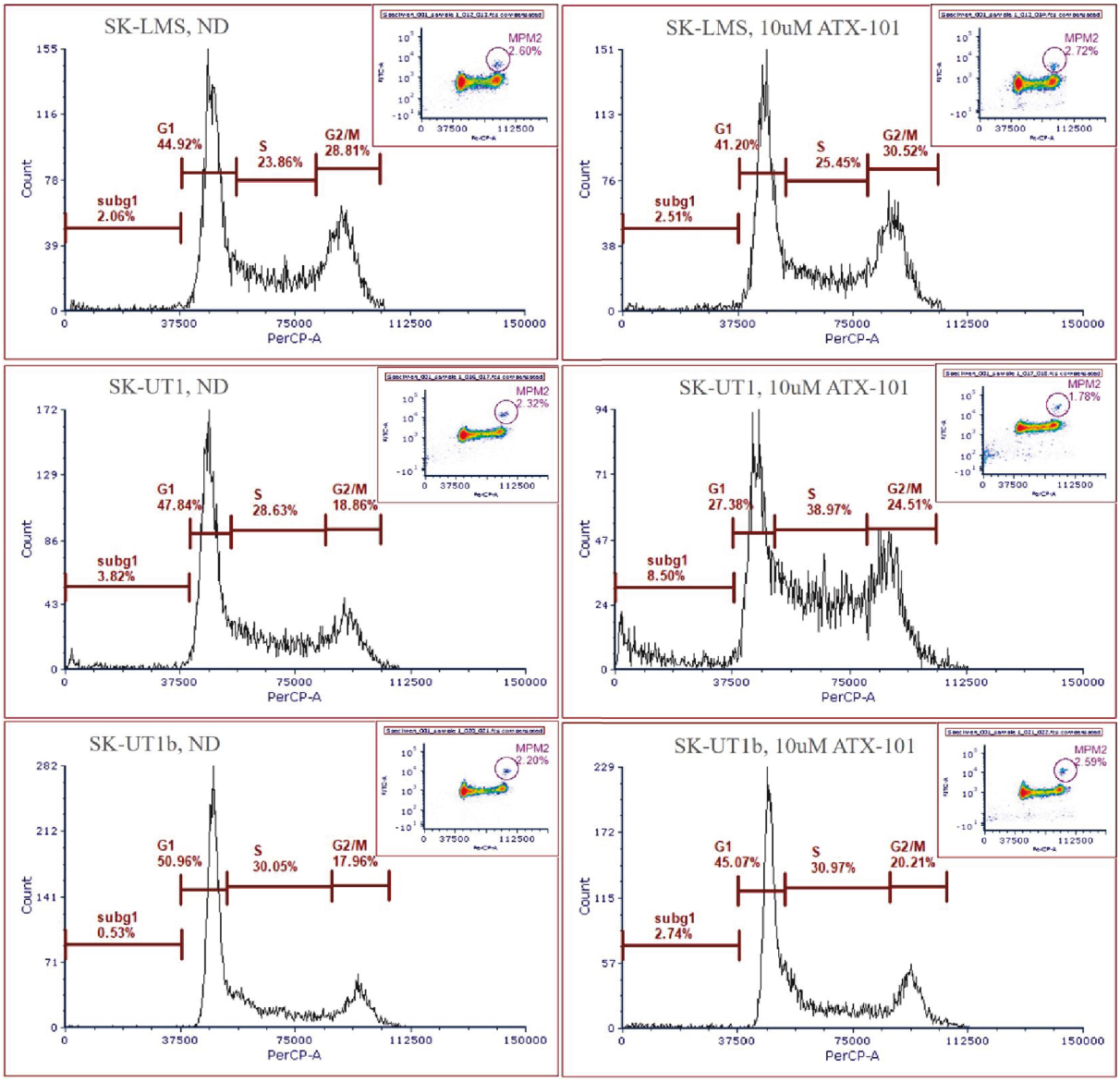


Figure 4. ATX-101 treatment affects the cell cycle in leiomyosarcoma cell lines. There is a slight increase of G2 phase of the cell cycle in SK-LMS and Sk-UT1b while increase of S phase and G2 phase in SK-UT1. Briefly, propidium iodide DNA staining was performed on leiomyosarcoma cell lines (SK-LMS, SK-UT1 and SK-UT1b) cells after 24 hours of treatment of 10 uM ATX-101. Cells were fixed in 70% ethanol and stored at –20° C. Cells were first labeled with MPM-2 antibody (mitotic marker) and followed by secondary antibody anti-mouse Alex-488. Bi-parameter flow cytometry was performed on a Becton Dickinson LSRII flow cytometer. Data analysis was done using FCS Express 7 software.

2. STUDY OBJECTIVES

This is an open-label, single-center, single-arm, phase II study of the peptide drug targeting PCNA, ATX-101 in adult patients with unresectable and metastatic LMS and dedifferentiated LPS who have received at least one prior line of systemic therapy.

2.1 Primary Objective

To assess the efficacy of ATX-101 in patients with advanced LMS and dedifferentiated LPS by evaluating the progression free rate at 12 weeks.

2.2 Secondary Objectives

2.2.1 To perform a safety lead-in among the first 10 patients enrolled and treated on the study to confirm the safety and tolerability of the drug in sarcoma patients.

2.2.2 To further evaluate the safety profile of ATX-101 by assessing adverse event rates (according to National Cancer Institute CTCAE version 4.0 criteria) in patients treated with this agent.

2.2.3 To assess the efficacy of ATX-101 in patients with advanced LMS and dedifferentiated LPS by evaluating the objective response rate (according to RECIST 1.1 criteria), duration of response, median progression free survival and median overall survival.

2.3 Correlative Science Objectives

2.3.1 To evaluate the mechanistic effects of ATX-101 in LMS and dedifferentiated LPS by evaluating ATX-101's effects on the immune microenvironment, intracellular signaling pathways and DNA damage response using paired tumor biopsies from a subset of 10 patients, and to use the results of these analyses to identify candidate biomarkers for sensitivity and resistance to ATX-101 in the sarcoma population.

3. INVESTIGATIONAL AGENT

ATX-101 is an intravenously administered therapeutic peptide comprised of a novel human PCNA interacting motif termed APIM, coupled to cellular and nuclear delivery domains. ATX-101 is currently being studied as a novel treatment for human cancer.

3.1 Preclinical Data

ATX-101 has been extensively studied in the preclinical setting. The following information is referenced from the Investigator's Brochure for ATX-101, as provided by APIM Therapeutics, unless otherwise noted. Please reference the ATX-101 Investigator's Brochure for further information.

3.1.1 Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion

The ATX-101 drug substance is a synthetic, N-terminally acetylated 25-mer peptide. The peptide is comprised of an APIM therapeutic domain, and two delivery domains: a nuclear localization signal (NLS) and a cell penetrating peptide (CPP). Its molecular weight is 4,320.9 g/mol (HCl salt) and it presents as a white to off-white powder. The ATX-101 drug substance is manufactured and controlled in compliance with Good Manufacturing Practices (GMP) by Bachem AG (Switzerland).

The investigational medicinal product (IMP), currently used in the phase 1 clinical study, is a sterile lyophilisate of ATX-101 for reconstitution for injection, supplied in single-use vials. It is available in 80 mg vials and the formulation does not include any excipient. The lyophilisate is intended to be reconstituted with sterile 0.9% NaCl. The IMP is manufactured and controlled in compliance with GMP by Bachem AG (Switzerland).

In vitro ATX-101 stability studies in whole blood and urine indicated half-lives of a few minutes in rat and human blood, and a few hours in human urine, as expected for such a peptide. In vivo tissue distribution studies in the rat showed that ATX-101 is rapidly distributed and internalized in tissues following IV administration.

ATX-101 administered IV to rats or dogs is rapidly cleared from the circulation with maximal plasma concentrations reached towards the end of infusion and no detectable plasma levels within approximately 20 minutes post-end of infusion. In terms of dose relationship, exposure to ATX-101 of rats treated for 6 cycles increased in a dose-dependent manner and was greater at the last day of treatment compared to the first. A similar systemic exposure to ATX-101 was observed between the first and last day of treatment in dogs treated for 4 to 6 weekly cycles. No significant differences were observed between genders.

3.1.2 Pharmacology

Non-clinical pharmacology studies have demonstrated ATX-101's efficacy in inhibiting the growth of cancer cells in a broad range of cancer cell lines in vitro, as well as in different in vivo models. The APIM consensus sequence is validated as a functional PCNA-binding motif that occurs in numerous proteins involved in DNA repair and damage tolerance. ATX-101 was shown to directly interact with PCNA and to be able to impair APIM-PCNA interaction in live cells. For example, peptides containing the APIM-motif such as ATX-101 inhibit the interaction between PCNA and the essential translesion synthesis DNA polymerase POLζ. ATX-101 was shown to reduce the function of POLζ in TLS. Thus, ATX-101 impairs the cells' ability to tolerate DNA lesions and thereby contributes to anti-cancer activity. Additionally, ATX-101 displayed anti-mutagenesis properties by attenuating spontaneous and ultraviolet (UV)-induced mutagenesis in E. Coli MG1655 cells.

In vitro, it has been shown that ATX-101 is able to induce apoptosis of cancer cell lines in a cell cycle-independent manner. From a panel of 44 cancer cell lines, originating from a range of organs, ATX-101 was shown to have a dose-dependent growth inhibitory effect as a single

agent. ATX-101 was also shown to potentiate the growth inhibitory effect of different chemotherapeutic and targeted agents in these cell lines.

In vivo, the efficacy of ATX-101 in potentiating the effect of chemotherapeutic and targeted agents to reduce tumor growth was demonstrated using tweleve different models. Administered in combination therapy, ATX-101 did not increase the toxicity observed above that of the anticancer agents alone.

3.1.3 Plasma protein binding

Not available

3.1.4 Metabolism

Not available

3.1.5 Enzyme Interactions

Not available

3.1.6 Toxicology

The toxicity of ATX-101 was assessed in the rat and in the dog. Preliminary studies were undertaken to compare several dose levels of ATX-101 after a single IV administration or over a 4-cycle treatment period, ranging from 12 to 80 mg/kg/dose in the rat and up to 25 mg/kg/dose in the dog, in order to set the dose range to be evaluated in the 6-cycle (35 days) repeated-dose studies. In the main rat study, ATX-101 treatment induced body weight losses for dose levels of 30 mg/kg and above, and the maximum tolerated dose (MTD) was considered to be 30 mg/kg (180 mg/m2) over 6 weekly administrations. In the main dog study, the MTD was considered to be at least 22.5 mg/kg (450 mg/m2) over 6 weekly IV administrations. Results in the rat, being the most sensitive species, and the corresponding MTD of 30 mg/kg were used for the determination of the phase 1 starting dose in human patients.

GLP-compliant safety pharmacology studies demonstrated that IV infusion of ATX-101 does not lead to any effect on the central and peripheral nervous systems of rats at dose levels up to 30 mg/kg. In the cardiovascular and respiratory study performed in dogs, increased heart rate was observed associated with higher arterial blood pressure, reduced pulse and respiratory frequency. However, all these effects were transient and returned to normal within approximately 1 hour post-end of infusion without any medication. These effects are currently attributed to histamine release induction by the peptide.

ATX-101 elicited an infusion reaction in the rat and in the dog, characterized by the appearance of edema localized mainly to the head during the treatment, as well as, in the dog, retching, emesis, and excessive struggling. This reaction was moderate and transient, resolving spontaneously within a few hours, without any medication. Symptoms of these reactions corresponded to a transient peak of histamine plasma levels in dogs.

In the rat studies, one animal in each of the ATX-101 groups at 30 (study WIL-63506), 55 or 80 mg/kg (study WIL-63513) died just after the third infusion, and showed dark red areas in the lungs at necropsy. The cause of death for these three animals was undetermined. These macroscopic observations were reported in three additional animals that survived to the scheduled necropsy, including one untreated recovery animal. According to historical data from WIL Research (contract research organization in charge of these studies), this effect is seen in 1% of untreated rats. After re-analysis of the rat data by an independent anatomic pathologist, it was concluded that the death of these three animals could not clearly be attributed to ATX-101. No gender effect was evident in rats or in dogs in these studies.

3.1.7 Cardiovascular Effects

Not available

3.1.8 Genotoxicity

Not available

3.1.9 Carcinogenicity

None performed.

3.1.10 Developmental and Reproductive Toxicity

None performed.

3.2 Clinical Data

ATX-101 is currently being evaluated in the first in human study (ATX101-01 and -02), a phase 1 dose escalation study in solid tumors. In this study patients with solid tumors who have no further treatment options are enrolled at three active sites in Australia in a 3+3 study design. In the ATX-101-01 study ATX-101 is infused weekly over 6 weeks whereas the ATX-101-02 is a long term follow-up study in which ATX-101 is infused continuously with the same weekly regimen in patients who have not progressed after the 6 weeks of initial treatment. The ATX-101 dose used in this study ranged from 20 mg/m^2 IV once weekly to 60 mg/m^2 IV once weekly. Although no MTD was reached, after review of the available safety data, the RP2D was determined to be 60 mg/m2 IV weekly, with no plans for dose-escalate further.

Of the 21 patients enrolled in this study, 3 patients had sarcoma: one with uterine LMS, one with gastrointestinal stromal tumor (GIST) and 1 with undifferentiated pleomorphic sarcoma (UPS). While there were no responses according to RECIST, multiple patients experienced prolonged stabilization of disease, including the patient with uterine LMS who remained stable for more than 16 months of treatment.

As of October 31st, 2020, there have been no deaths with in 30 days of treatment with ATX-101. In general treatment has been well tolerated with patients discontinuing treatment with ATX-101 as a consequence of disease progression rather than toxicity. No ATX-101 related grade 3 or higher adverse events (AEs) or serious adverse events (SAEs) have been reported during the entire study duration. No dose limiting toxicity (DLT) has been observed. The most frequently observed ATX-101 related AEs were infusion related reaction. Grade 1 infusion reactions were reported in 13/21 patients and grade 2 infusion reactions were reported in 14/21 patients over the entire duration in all patients. Reported infusion reactions included itchiness, redness, urticaria, fever, rash, swelling, flushing, hives and confusion. Infusion reactions occurred during the first but also during later infusions and resolved completely after ATX-101 infusion interruption and/or treatment with corticosteroids and/or antihistamines. In the majority of patients, the infusion of ATX-101was safely restarted and treatment was completed. So far, there is no evidence for a dose relationship of infusion reactions. The mandatory premedications consisted of dexamethasone, histamine 1 and 2 receptor antagonists, acetaminophen and montelukast. Besides, infusion reactions, ATX-101 related AEs reported at least twice during the entire study duration were fatigue (6 times), diarrhea (3 times) and dysgeusia (2 times). All of these were grade 1 in severity with the exception of 2x fatigue which were grade 2. The table below reports all the adverse events observed in these 21 patients until October 31st, 2020.

Treatment related treatment emergent adverse events observed in study AM ATX101-01 (31 October 2020) – Number (%) of patients experiencing an adverse event, by dose level.

Event (Preferred Term)	Cohort 1 20 mg/m² (n=8)	Cohort 2 30 mg/m² (n=3)	Cohort 3 45 mg/m² (n=4)	Cohort 4 60 mg/m² (n=6)
Grade 1: mild	4 (50%)	3 (100%)	4 (100%)	4 (66.7%)
Grade 2: moderate	2 (25%)	1 (33.3%)	3 (75%)	5 (83.3%)
Grade 3: severe	0	0	0	0
Grade 4: life threatening	0	0	0	0

Treatment related treatment emergent adverse events observed at least twice in study AM ATX101-01 (31 October 2020) – Total number of events experienced by all patients on the study

Event (Preferred Term)	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction	13	14	0	0
Fatigue	4	2	0	0
Infusion site conditions*	3	2	0	0
Dysgeusia	3	0	0	0
Diarrhea	2	0	0	0

* Includes local infusion site -erythema; -extravasation; -pruritus; -urticaria; -reaction.

4. STUDY DESIGN

4.1 General Design

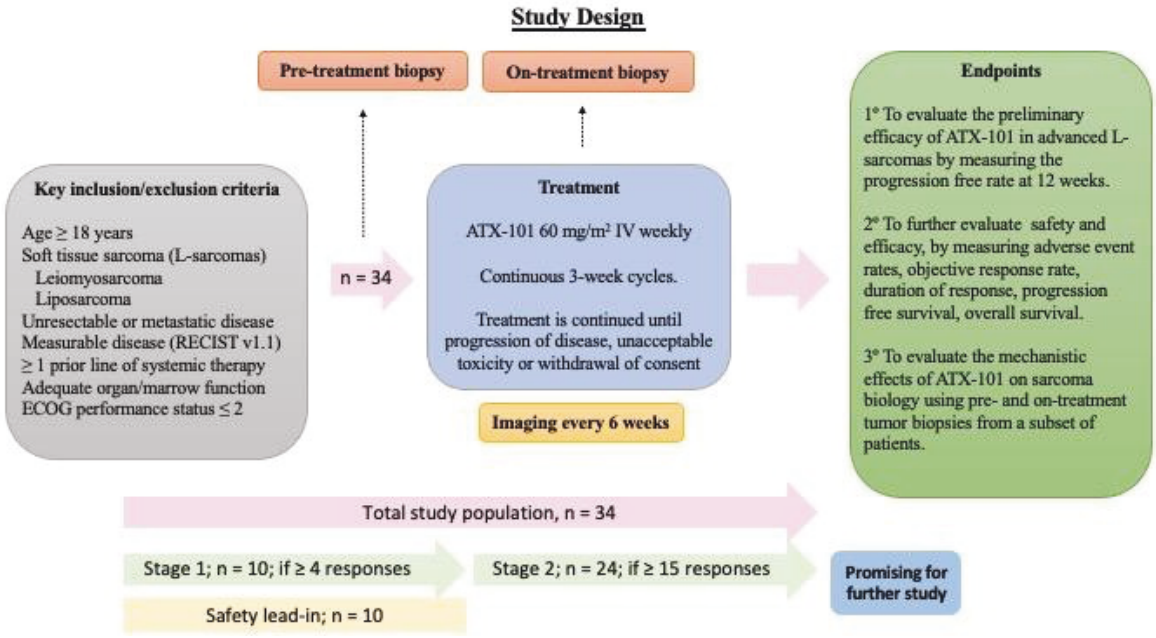
This is a phase II, single-arm, open-label, Simon 2-stage study evaluating the efficacy of ATX-101 in patients with unresectable or metastatic LMS or dedifferentiated LPS who have received treatment with at least one prior systemic therapy.

Because clinical data with ATX-101 is limited, a safety lead-in will be conducted among the first 10 patients enrolled and treated on the study. The safety lead-in is described in Section 4.2.

The study will be conducted at Columbia University Irving Medical Center. All subjects will be treated with the same agent and dose, ATX-101, at 60 mg/m^2 IV weekly in continuous 21 day cycles. Patients will receive treatment at the discretion of the principal investigator until disease progression, unacceptable toxicity or adverse event(s) or withdrawal of consent.

The primary endpoint is the progression free rate at 12 weeks (PFR_{12}). Secondary endpoints include overall survival, progression free survival, overall response rate and safety profile.

For further details on the statistical design, see Section 9.



4.2 Safety Lead-In

Recognizing the limited clinical experience with ATX-101, we will perform a safety lead-in among the first 10 patients enrolled and treated on the study to confirm the safety and tolerability of the proposed dose and schedule. The safety lead-in will apply to the first 10 patients treated for two cycles (6 weeks). These patients will undergo safety assessments as specified in the study calendar.

The study will not proceed to further accrual until these 10 patients have completed two cycles or 6 weeks of study treatment; however, patients who end study treatment during this period for reasons other than toxicity, i.e., disease progression, will not be replaced. After 10 patients complete 2 cycles of treatment, the study investigators will review the safety data. If 3 or more patients in the safety lead-in population experience DLTs as defined below, the study team will consider terminating the study or amending the study to evaluate an alternative dose and schedule. In addition, if 2 or more events of grade 4 infusion related reaction occur, the study team will also consider terminating the study. Formal documentation of the study team's review of toxicity data from the safety lead-in will be provided to the IRB and FDA prior to resuming accrual.

The following definitions of dose-limiting toxicity (DLT) apply to the safety lead-in. DLTs are defined as the following toxicities which are attributed as at least possibly related to the study drug and not to disease, and which occur (or first become evident) during a prespecified timeframe. For the purposes of the safety lead-in, this timeframe is the first two cycles of treatment.

1. Any grade ≥3 non-hematologic toxicity, except grade ≥3 fatigue, nausea, or vomiting which must persist >5 days despite maximal supportive care to be considered dose limiting. This includes any grade 3 or 4 infusion-related reaction.
2. Any grade ≥4 hematologic toxicity lasting >14 days, except grade 4 lymphopenia, which is not considered a DLT.
3. Grade ≥3 neutropenia with fever
4. Grade ≥3 thrombocytopenia with clinically significant bleeding.
5. Any laboratory abnormality not otherwise addressed above which is both associated with clinical sequelae and which fails to resolve to grade ≤1 within 5 days of occurrence, or any laboratory criteria meeting Hey's law for liver toxicity.

4.3 Number of Patients

The study size is 34 patients.

5. SUBJECT SELECTION AND WITHDRAWAL

This clinical trial can fulfill its objectives only if appropriate patients are enrolled. All clinically relevant considerations should be made when deciding whether this protocol is appropriate for a particular patient. Waivers to eligibility requirements cannot be granted by any party. In order to be considered eligible, a patient must meet all of the inclusion criteria and be free of all of the exclusion criteria as stated below.

5.1 Inclusion Criteria

5.1.1	Histologically confirmed dedifferentiated liposarcoma (LPS) or leiomyosarcoma

	(LMS). Pathology review occurs at the center enrolling the patient on this trial.
5.1.2	Disease must be locally advanced and unresectable or metastatic. Disease which may be resected but with an associated level of morbidity deemed unacceptable by the treating clinician is considered eligible.
5.1.3	Patients must have measurable disease by RECIST criteria version 1.1. In addition, the first 10 patients enrolled on the study must have a site of disease amenable to image-guided biopsy at minimal risk or less, and must agree to undergo this biopsy.
5.1.4	Patients must evidence of disease progression, either clinically or radiographically, within the 12 weeks prior to study enrollment, as determined by the investigator enrolling the patient on the study.
5.1.5	Patients must have been treated with at least one prior systemic regimen for advanced sarcoma: LMS: Anthracycline-based chemotherapy, or gemcitabine/docetaxel. LPS: No specification as to the prior treatment received. Neoadjuvant or adjuvant systemic therapy does not qualify as prior treatment unless completed within 6 months of disease relapse.
5.1.6	Patients must be age 18 years or older. Because the safety of ATX-101 in patients less than 18 years of age has not been characterized, children are excluded from the present study.
5.1.7	Patients must demonstrate an ECOG performance status of 0-2.
5.1.8	Patients must demonstrate normal organ and marrow function as defined below: Absolute neutrophil count (ANC) $\geq 1{,}500/mm^3$ Platelet count $\geq 100{,}000/mm^3$ Creatinine $\leq$ 1.5 times upper limit of normal OR Calculated creatinine clearance > 45 mL/min* Total bilirubin $\leq$ 1.5 times upper limit of normal** AST/ALT $\leq$ 1.5 times upper limit of normal** *Notes:* Upper limit of normal is defined by the clinical laboratory performing the test. * Using the lean body mass formula only (Modified Cockcroft Gault) ** If transaminase elevation and/or bilirubin elevation is attributed to the presence of liver metastases, a total bilirubin $\leq$ 2.5 times the upper limit of normal and an AST and ALT $\leq$ 2.5 times the upper limit of normal are permissible. Patients with an elevated bilirubin level that is attributed to an inherited disorder, such as Gilbert's disease, may be enrolled at the discretion of the principal investigator.
5.1.9	The effects of ATX-101 on the developing human fetus are unknown. For this reason, women of child-bearing potential and all men must agree to use adequate contraception (for women: hormonal or barrier method of birth control, abstinence; for men: male condom, prior vasectomy, or abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should

	inform her treating physician immediately. All men and women of childbearing potential enrolled on this protocol must agree to use adequate contraception prior to the study, for the duration of study participation, and for 4 months after completion of ATX-101 administration. If patients do not agree to the above, they are not considered eligible.
5.1.10	Ability to understand and willingness to sign a written informed consent document.

5.2 Exclusion Criteria

5.2.1	Patients must not have received treatment with any chemotherapy, immunotherapy, radiotherapy or an investigational agent for malignancy within the 21 days of initiating treatment on this protocol. Patients may not have received treatment with a small molecule targeted agent (including off-label or investigational use) within 14 days of initiating treatment on this protocol, provided this represents at least 7 half-lives for that agent. Toxic effects from any prior therapy (except alopecia) must have resolved to grade 1 or less according to NCI CTCAE v4.0 or to the patient's baseline by the time of initiating treatment on this protocol.
5.2.2	Patients may not have uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, cerebrovascular accident within the last six months, uncontrolled diabetes mellitus, uncontrolled psychiatric illness or any other disease condition that would limit compliance with study requirements in the opinion of the principal investigator.
5.2.3	Patients may not be pregnant or nursing. Pregnant women are excluded from this study because the teratogenic effects of ATX-101 have not been adequately studied. A negative pregnancy test must be documented 7 days or less prior to initiating treatment on this protocol. Because there is an unknown but potential risk for adverse events to nursing infants secondary to treatment of the mother with ATX-101, breastfeeding must be discontinued prior to enrollment.
5.2.4	Patients may not have known active hepatitis B or C infection. In patients with a history of hepatitis B or C infection, resolution of infection must be demonstrated by negative serology for hepatitis B surface antigen (HBsAg) and/or negative hepatitis C virus (HCV) RNA.
5.2.5	Patients may not have uncontrolled HIV/AIDS infection. However, HIV positive patients on highly active retroviral therapy (HAART) with an undetectable viral load and CD4 T-cell count above 200 may participate.
5.2.6	Anticipated requirement for surgery during the study period or major surgery within 3 weeks of initiating treatment.
5.2.9	Active CNS metastases or leptomeningeal involvement. Patients with known CNS metastases must have received definitive radiotherapy or surgery at least 4 weeks

	prior to initiating treatment with imaging demonstrating no progression of disease over this interval.

5.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. In accordance with the NIH guidelines on the inclusion of women and minorities as subjects in clinical research, the Department of Health and Human Services (HHS) requires that all pilot, phase 1, phase 2, and phase 3 trials must include accrual targets for males, females, and minorities. The accrual targets reflect the expected accrual over the life of the study.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	5	+	5	=	10
Not Hispanic or Latino	12	+	12	=	24
Ethnic Category: Total of all subjects	17	+	17	=	34
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	2	+	2	=	4
Black or African American	3	+	3	=	6
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	12	+	12	=	24
Racial Category: Total of all subjects	17	+	17	=	34

5.4 Subject Recruitment

Patients will be recruited from investigator and co-investigator practices.

5.5 Early Withdrawal of Subjects

5.5.1 When and How to Withdraw Subjects

Patients will be removed from this study for failure to adhere to protocol requirements, withdrawal of consent, disease progression, unacceptable toxicity, global deterioration in health status, study termination or death. There is no evidence to suggest that abrupt withdrawal of this study agent would result in adverse clinical effects and therefore there is no taper of study drug in the event of stopping treatment.

5.5.2 Data Collection and Follow-up for Withdrawn Subjects

Although subjects may be withdrawn prematurely from the study, follow-up will continue for all such subjects. Subjects withdrawn because of unacceptable adverse events will be followed until resolution or stabilization of the adverse event. An attempt will be made to obtain survival information for all subjects for a period of at least 3 years following the time of registration or until death, whichever comes first. For more information, see section 11, study calendar. This will include attempts to contact subjects via telephone and by certified letter using information available to the investigators and the study team. When possible, at least two attempts will also be made to contact the subject's next of kin to obtain such information while observing relevant privacy laws, where applicable.

6. REGISTRATION PROCEDURES

6.1 CUMC Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures, along with applicable institutional policies and federal regulations.

Only Investigators/Research personnel properly trained and delegated to consent subjects for this protocol will participate in the consenting process. Furthermore, properly delegated/trained Physician Investigators (e.g., MD, MD PhD) are required to sign/verify a protocol specific Eligibility Checklist for each subject enrolled on the study, in addition to providing the relevant source documentation confirming subject eligibility.

All participants must be centrally registered through the Central Registration Office within Herbert Irving Comprehensive Cancer Center at CUMC prior to initiation of study treatment.

Registration hours are available Monday through Friday from 9:00am – 5:00pm EST (excluding holidays and weekends). Same day patient registrations (and after hour registrations) will be accommodated on a case by case basis provided that the study team has expressed all time sensitive registration concerns/cases in a timely manner to the Central Registration Office.

CPDM Central Registration Procedures: Within 48 hours of obtaining consent (excluding holidays and weekends), a completed/signed IRB approved informed consent HIPAA form, and demographics forms must be submitted to the CPDM Central Registration Office via an email to CPDMRegistration@columbia.edu or fax to 212.305.5292, with the subject line "*Protocol Number:* Pending Subject Registration Request (PHI)". Upon receipt, applicable subject information as well as a "pending eligibility" status will be entered into HICCC's institutional database. This status will remain until further source documentation is made available to

confirm overall patient eligibility. Required materials for all pending registration submissions are as follows:

- Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (e.g., tissue, DNA, etc.), as applicable.
- The completed/signed IRB approved HIPAA Authorization form
- Completed/signed CPDM ICF checklist
- Completed/signed HICCC personal census form
- Completed/signed CPDM Demographics Note to File

In order to confirm eligibility status, Investigators/designees (e.g., study specific Clinical Research Coordinator/Research Nurse, etc.) must submit the following documentation to the Central Registration Office via email or fax:

- The completed/signed study specific Eligibility Checklist (signed by a Physician level Investigator)
- Copies of source documentation necessary for each item to be verified on the CPDM specific Eligibility Checklist, including but not limited to:

  Copy of required laboratory test and procedure reports (e.g., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans, etc.)

  Copy of pathology and surgical reports

  Copy of clinic note(s) or other appropriate medical records capturing the consent process information, along with providing source documentation of any other items needed for screening/eligibility that are not captured in othe source document forms (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc.)

  Protocol deviation/waiver approvals (if applicable)

Please note: subject line of email or fax should include the following: "AAA@####: Complete Subject Registration Request (PHI)".

Upon receipt of the above mentioned documentation, participant eligibility information will be verified by a qualified Central Registration Registrar. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable study team personnel for clarification prior to enrollment. All applicable finalized registration/eligibility information will then be entered into HICCC's institutional CTMS database by the Central Registration Registrar. Upon completion, an official subject registration notification email will be sent to the

PI/research team which will include eligibility/enrollment status, as well as subject ID information. Protocol therapy may not be initiated prior to receipt of this notification from the Central Registration Office.

All screen fail/ineligible subjects, as well as subjects who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

7. TREATMENT PLAN

7.1 Agent Administration

REGIMEN DESCRIPTION					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
ATX-101	Dexamethasone Loratadine Famotidine, Acetaminophen (see below)	60 mg/m2 (maximum dose = 120 mg)	Intravenously	Weekly	21 days

ATX-101 will be dosed at 60 mg/m^2 and capped at a maximum of 120 mg total dose (i.e. for BSA > 2 mg/m^2, there will be no further increase in the dose of ATX-101). ATX-101 will be administered by IV infusion (peripheral or central vein) in the outpatient research infusion center using a stepwise increase in the infusion rate as outlined in the table below. Doses of ATX-101 will be administered by IV infusion starting with 5 mg/hr and increasing the infusion flow rate every 30 minutes as outlined in the infusion rate table below. The maximum infusion rate should not exceed 120 mg/hr. The infusion time can be extended in the event of an infusion reaction.

Infusion Rate Table

Infusion Rate (mg/hr)	**Infusion Time (min)**	**Cumulative Infusion Time (min)**	**Dose Infused for timepoint (mg)**	**Cumulative Dose infused**
5	30	30	2.5	2.5
20	30	60	10	12.5
60	30	90	30	42.5
90	30	120	45.0	87.5
120	-	-	-	-

All patients will receive the below listed mandated premedications prior to each ATX-101 infusion. Corticosteroids, H1 and H2 inhibitors and acetaminophen are to be administered within 60 to 90 minutes prior to infusion start. Montelukast is to be administered the night prior to each infusion.

- Dexamethasone 12 mg IV once (or pharmacologically alternative corticosteroid at an equivalent dose). The Investigator may reduce the dose in the case where corticosteroids have already been administered for other reasons or the patient is expected to have unacceptable toxicity from steroids.
- Loratadine 10 mg orally once.
- Famotidine 20 mg orally once, or equivalent dose of an alternative H2 antagonist.
- Acetaminophen (paracetamol) 1 g orally once.
- Montelukast (anti-leukotriene) 10 mg orally once, **on the night prior to infusion.**

For management of infusion reactions, please see Section 8.

All participants should be evaluated for at least 2 hours after administration of first dose.

7.2 General Concomitant Medication and Supportive Care Guidelines

ATX-101 has the potential to interact with certain other drugs which study patients may be taking which could result in unforeseen toxicities. The case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Patients should be asked at each study-related clinical visit about new medications, including herbal medications or supplements, which they are taking.

7.2.1 Excluded concomitant medications

Throughout the study drug dosing period, patients may not receive any of the following concomitant medications:

- Any investigational drug (including from within 21 days prior to initiating ATX-101); as per study exclusion criteria.
- Concurrent anti-cancer treatment (eg. cytoreductive therapy, radiotherapy except for palliative radiotherapy, immune therapy, or cytokine therapy) within 21 days or five half-lives (whichever is shorter) before the first dose of ATX-101.

All concomitant medications administered within 28 days before the first dose of trial treatment through the safety follow-up visit should be recorded.

7.2.2 Permitted concomitant medications

All treatments that the investigator considers necessary for a patient's welfare may be administered at the discretion of the investigator in keeping with the standard of care. All concomitant medication will be recorded including all prescription, over-the-counter (OTC),

herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date will also be recorded. Palliative treatment for disease signs and symptoms is permitted provided the treatment is keeping with the community standards of medical care and in the opinion of the investigator and sponsor does not constitute an additional risk to the patient's treatment compliance in the study.

Concomitant treatment with bisphosphonates or equivalent for the symptomatic control of bone metastases are permitted provided that treatment was initiated ≥ 14 days before first dose of study drug.

7.2.3 Medications affecting the QTc interval

The risk for QTc prolongation in patients receiving treatment with ATX-101 has not been adequately characterized in humans. Therefore, use of medications known to prolong the QTc interval and pose a risk of Torsades are prohibited during treatment with ATX-101. The use of medications known to prolong the QTc and pose a conditional risk of Torsades should be used with caution.

7.3 Duration of Therapy

Treatment with the study drug will continue until one of the following applies:

- Radiographic disease progression, per RECIST criteria
- Clinical disease progression, as assessed by the treating investigator
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient's decision to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

7.4 Duration of Follow Up

Patients who do not progress while on study treatment will be followed every 3 months after completion of study treatment for the first 12 months and then every 6 months thereafter for both survival and disease status until death or for a period of at least 3 years, whichever comes first.

For patients who end study treatment due to progression, information on survival will also be collected every 3 months after completion of study treatment for the first 12 months of follow up and then every 6 months until death or for a period of at least 3 years, whichever comes first.

Patients removed from study for unacceptable adverse events will also be followed for information on resolution or stabilization of the adverse event.

7.5 Approach to COVID-19 Related Treatment Interruptions

Patients whose protocol treatment is interrupted because of COVID-19 infection and/or COVID-19-related effects on the conduct of this study may resume treatment at the discretion of the treating investigator if the investigator believes the potential benefits outweigh risks. In this situation, the patient's clinical situation, previous benefit from study treatment, and other available treatment options should be considered.

This applies to subjects whose treatment is delayed beyond the protocol allowed timeframe of 28 days and also to subjects whose study-specified restaging imaging performed during the time of treatment interruption shows disease progression, and this progression can reasonably attributed to the period treatment was withheld.

In these situations, the treating investigator will document the specifics of the situation and consideration of risk/benefit in the electronic medical record.

8. DOSING DELAYS/DOSE MODIFICATIONS

The following guidelines should be observed:

- If a patient experiences multiple adverse events and there are conflicting recommendations as to management, the investigator should use the recommended dose adjustment that reduces the dose to the lowest level of the available options.

- Any patient who requires a delay in therapy exceeding 28 days for treatment-related adverse events should be removed from the study. Any patient who requires more than three dose reductions should also be removed from the study.

- Patients should receive all relevant supportive care during the study, including, but not limited to: blood product support, antibiotic treatment, and management of other newly diagnosed medical conditions. All blood products and concomitant medications (such as antiemetics, analgesics, antidiarrheals) received from the first day of study drug treatment until 30 days after the final dose of study drug treatment must be recorded.

- Patients who require radiation therapy while on study must be removed from the study.

- Patients who require surgery while on study may proceed with surgery provided resection or removal of sarcoma is not part of the surgery. However, if a dose delay of more than 28 days is required, the patient must be removed from the study.

- Blood products and growth factors should be used as clinically warranted and in accordance with institutional policy. The use of prophylactic filgrastim and related medications to avoid dose reductions or delays is discouraged. The use of these agents in the setting of complications from therapy, such as severe infection, is permitted and must be documented.

8.1 Dose Levels

Dose Level	ATX-101 Dose
-3	Off protocol therapy
-2	30 mg/m^2 intravenously once weekly
-1	45 mg/m^2 intravenously once weekly
0	60 mg/m^2 intravenously once weekly

8.2 Management Guidelines for Non-Hematologic Adverse Events

Management of Infusion-Related Reactions

If, despite premedication, a patient experiences an infusion reaction, the infusion rate should be modified, or the infusion should be stopped at the discretion of the Investigator and as discussed below. For patients who are observed to experience an infusion reaction for which, at the determination of the treating Investigator symptomatic treatment is required, another dose of an anti-histamine is to be administered. Implementation of other supportive measures for the management of an infusion reaction is at the discretion of the Investigator.

Grade	Modifications of AXT-101
1	Reduce the infusion rate or stop the infusion. If the IRR resolves, infusion can resume; resuming at a decreased infusion rate is recommended.
2	Stop the infusion. IRR must resolve to ≤ Grade 1 to resume infusion. If resuming infusion, the infusion rate must be decreased and should begin at no more than 50% of the rate at which the reaction occurred.
3	1st occurrence: Stop the infusion. Do not restart the infusion/complete the dose for that day; however, treatment may continue at the next scheduled visit at the investigator's discretion. If resuming infusion, infusion rate must begin at 5 mg/hr. 2nd occurrence: Permanently discontinue treatment.
4	Permanently discontinue ATX-101 treatment.

Increasing infusion rate above the rate listed in Infusion Rate Table above is not recommended without prior approval from the SMC. Decreasing or maintaining the suggested flow rate is allowed to effectively manage any infusion related reactions at the investigator's decision.

Management of Other Non-Hematologic Toxicity

All Other Non-Hematologic Events	**Management/Next Dose for ATX-101**	
	Treatment delay	**Dose Modification**
≤ Grade 1	No delay	No change in dose
Grade 2	In general, hold until ≤ grade 1 or resolved to baseline	Not required

All Other Non-Hematologic Events	**Management/Next Dose for ATX-101**	
Grade 3	Hold until ≤ grade 1, or resolved to baseline	Resume at one dose level lower
Grade 4		Discontinue protocol therapy

8.3 Management Options for Hematologic Adverse Events

Neutropenia	
Grade 1 (ANC < 1500 to < LLN)	Maintain dose level
Grade 2 (ANC 1000 to < 1500)	Maintain dose level
Grade 3 (ANC 500 to < 1000)	Hold therapy until resolved to ≤ grade 2, then: If resolved in ≤ 7 days, maintain dose level If resolved in > 7 days, reduce by 1 dose level
Grade 4 (ANC < 500 or febrile neutropenia)	Hold therapy until resolved to ≤ grade 2, then restart at one dose level lower.
Thrombocytopenia	
Grade 1 (PLT 75000 to < LLN)	Maintain dose level
Grade 2 (PLT 50000 to < 75000)	Hold therapy until resolved to ≤ grade 1, then: If resolved in ≤ 7 days, maintain dose level If resolved in > 7 days, reduce by 1 dose level
Grade 3 (PLT 25000 to < 50000)	Hold therapy until resolved to ≤ grade 1, then restart at one dose level lower.
Grade 4 (PLT < 25000)	

9. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

9.1 Adverse events

A summary list of reported treatment-related treatment-emergent adverse events for ATX-101 is provided below. For a detailed list of all treatment emergent adverse events reported in the phase 1 trial of ATX-101 in solid tumors, please see the Investigator's Brochure. Based on the most recently available information the phase 1 study (as of 10/31/2020 and representing 21 patients treated with the drug), the following treatment-related treatment-emergent adverse events were reported.

Treatment related treatment emergent adverse events observed in study AM ATX101-01 (31 October 2020)

Event (Preferred Term)	**Cohort 1 20 mg/m² (n=8)**	**Cohort 2 30 mg/m² (n=3)**	**Cohort 3 45 mg/m² (n=4)**	**Cohort 4 60 mg/m² (n=6)**
Grade 1: mild	4 (50%)	3 (100%)	4 (100%)	4 (66.7%)
Grade 2: moderate	2 (25%)	1 (33.3%)	3 (75%)	5 (83.3%)
Grade 3: severe	0	0	0	0

Grade 4: life threatening	0	0	0	0

Treatment related treatment emergent adverse events observed at least twice in study AM ATX101-01 (31 October 2020)

Event (Preferred Term)	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction	13	14	0	0
Fatigue	4	2	0	0
Infusion site conditions*	3	2	0	0
Dysgeusia	3	0	0	0
Diarrhea	2	0	0	0

* Includes local infusion site -erythema; -extravasation; -pruritus; -urticaria; -reaction.

9.2 Definitions

Adverse Event: An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including abnormal sign, symptom or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality, results in study withdrawal, is associated with a serious adverse event, is associated with clinical signs or symptoms, leads to additional treatment or to further diagnostic tests, is considered by the investigator to be of clinical significance

Serious Adverse Event: Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:

- Fatal
- life-threatening
- requires inpatient hospitalization/prolongation of existing hospitalization, unless: (1) routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control); (2) elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug; (3) treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above/below and not resulting in hospital administrations; (4) social reasons and respite care in the absence of any deterioration in the patient's general condition
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event. An important medical event is one that may not be

immediately life threatening but is clearly of major clinical significance. It may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in inpatient hospitalization, or intensive treatment of bronchospasm in an emergency department would all typically be considered serious.

Unanticipated Problem: An unanticipated problem is any incident, experience or outcome involving risks to subjects or others in any human subjects research that meets all of the following criteria:

- unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in such research (e.g., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

Adverse Event Reporting Period: The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures (after the first dose of study treatment) to the end of the study treatment (last dose of study treatment) and follow-up. For this study, the study treatment follow-up period is defined as 28 days following the last administration of study treatment.

Baseline/Preexisting Condition: A baseline/preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or if the character of the condition worsens during the study period.

General Physical Examination Findings: At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event: All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values: A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm

the abnormality

- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (e.g., change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.).

Hospitalization, Prolonged Hospitalization or Surgery: Any adverse event that results in hospitalization or prolongation of hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.3 Recording of Adverse Events

Signs or symptoms of the patient's cancer diagnosis and/or comorbidities at baseline will be recorded beginning on day 1. At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Assessment of adverse events will include type, incidence, severity (as graded by CTCAE version 4), timing, seriousness, and relatedness to study treatment. For each adverse event, the investigator will determine and document whether there exists a reasonable possibility that the study drug treatment caused or contributed to the adverse event. When the investigator does not know whether or not the study treatment is causally-related to the event, reporting for study purposes will be as "related" to study treatment.

Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.4 Reporting of Serious Adverse Events

9.4.1 IRB Notification by Sponsor-Investigator

Reports of all events (including follow-up information) that meet the definition of an unanticipated problem posing risk to subjects or others must be submitted to the IRB within one week (5 business days) following the occurrence of the unanticipated problem or the principal investigator's acquiring knowledge of the unanticipated problem in accordance with IRB policy. Additionally, the sponsor-investigator will submit a summary of all unanticipated problems that occurred since the beginning of the study at the time of continuing review. Copies of each report and documentation of IRB notification and receipt will be kept in the Regulatory binder.

9.4.2 FDA Notification by Sponsor-Investigator

Columbia University Medical Center will be responsible for all communication with the FDA. Columbia University Medical Center will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and for which there is evidence to suggest a causal relationship between the drug and the adverse event. These must be reported to the FDA and any affiliate sites as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. Columbia University Medical Center will also submit an IND annual report to the FDA in accordance with 21.CFR 312.33.

Columbia University Medical Center must report to the FDA and any affiliate site investigators as follows:

- Any unexpected fatal or life-threatening event must be reported as soon as possible, but no later than 7 calendar days after the sponsor investigator initial receipt of the information
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting
- Any findings from animal or in vitro testing whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator

determines that the information qualifies for reporting

- Any clinically important increase in the rate of a serious suspected adverse reactions over that listed in the protocol or Investigator Brochure
- Expected SAEs and AEs will be included in the IND Annual Reports.

Follow-up information to a safety report should be submitted as soon as the relevant information is available. However, if the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable are so reportable, the sponsor investigator must report such experience as soon as possible, but no later than 15 calendar days after the determination is made.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

9.4.3 DSMC Reporting by the Sponsor Investigator

Serious adverse events not constituting unanticipated problems are to be reported to the HICCC DSMC. Reporting should occur within 24 hours of knowledge of the SAE occurring at our institution or affiliate sites.

9.4.4 Reporting to Drug Manufacturer by Sponsor-Investigator

All Suspected Unexpected Serious Adverse Reactions (SUSARs) will be submitted to APIM Therapeutics on a MedWatch Form 3500A within the timelines as follows:

- Reports of fatal or life threatening Serious Adverse Drug Reactions will be forwarded within five (5) calendar days of Receipt Data

Reports of Serious Adverse Drug Reactions (other than fatal or life threatening) will be forwarded within twelve (12) calendar days of Receipts Date.

Please FAX IND Safety Reports (SUSARS) to 1-888-488-9697. For FAX problems call 1-800-201-8725 to report the event via phone.

In addition, all reports prepared for FDA submission will be submitted to APIM Therapeutics prior being submitted to the FDA. If no comment is received by APIM Therapeutics within 48 hours, the report will be submitted to the FDA in its original form, so as to ensure timely submission of documentation.

9.5 Reporting Process

Adverse events may be submitted on FDA Form 3500A, the HICCC DSMC Serious Adverse Event Reporting Form, or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 10. At the time of IRB

renewal or at the request of the manufacturer, the Sponsor-Investigator will submit a summary of all serious adverse events that have occurred during the study to the manufacturer.

10. PHARMACEUTICAL INFORMATION

10.1 Investigational Product

ATX-101 is a therapeutic peptide that has been designed to target PCNA and reduce the binding of APIM-containing proteins to PCNA. Evidence suggest that blocking of PCNA's interactions through APIM prevents interactions that are vital for the cellular response to genotoxic stress. This impairs the cell's ability to survive stress resulting in cell death by apoptosis. Please refer to the Study Protocol for further information on ATX-101.

ATX-101 is a synthetic, N-terminally acetylated 25-mer peptide, comprised of an APIM therapeutic domain, and two delivery domains: a nuclear localization signal (NLS) and cell penetrating peptide (CPP). The investigational product will be provided as a sterile lyophilisate for reconstitution and dilution prior to injection.

The lyophilisate is presented in colourless vials with rubber stoppers and aluminium flip-off seals with plastic discs, and is stored at -20°C ± 5°C protected from light. For any questions or any product related observations and/or complaints kindly contact your assigned CNS Clinical Research Associate and/or CNS Project Manager.

The Investigational Product (IP) for the Study will be available in 80mg vials:

- ATX-101 single use lyophilized powder 80mg vials. These vials have an assigned shelf life of 60 months if stored under the conditions mentioned below (extended stability studies on-going). A second batch of the same product has an assigned shelf life of 48 months under the same conditions (extended stability studies on-going).

The IP must be stored frozen at -20°C +/- 5°C and protected from light.

The investigational product (IP) will be shipped under controlled temperature (cold chain) conditions. The shipment will contain temperature loggers with instructions.

10.2 Receipt of Investigational Product

The IP will be sent to the site Pharmacy in a temperature-controlled shipment (-20°C +/- 5°C). The shipment will contain temperature loggers with instructions. The Research Pharmacy at Columbia will follow institutional practice/procedures when receiving the drug.

If ATX-101 is not deemed safe to use, maintain quarantine and organize return or destruction. All correspondence relating to temperature excursion, including decision not to use should be filed in the Pharmacy File.

10.3 Storage of Investigational Product and Management of Temperature Deviations

The IP must be stored at -20°C +/- 5°C and protected from light. Storage should be in a secure

location accessible only to study personnel. Prepared IV bags are required to be covered/ kept in the dark.

Temperature will be monitored during storage.

If there have been temperature deviations outside the range of -20°C +/- 5°C, the Sponsor should be notified, and copies of relevant documentation and temperature excursions should be provided. ATX-101 should be quarantined until further instructions on its use is provided. Temperature monitoring during quarantine is required.

When ATX-101 is cleared for use, all correspondence relating to temperature excursion, including clearance to use should be filed in the Pharmacy File.

If ATX-101 is not deemed safe to use, maintain quarantine and organize return to the Sponsor or destruction. All correspondence relating to temperature excursion, including decision not to use should be filed in the Pharmacy File.

Temperature logs will be reviewed during monitoring visits.

Once at the pharmacy, the vials will be kept in secondary packaging (cartons) as they arrived.

10.4 Dispensing and Accountability

All Pharmacy procedures will be conducted in accordance with site Pharmacy SOPs, the protocol, GCP guidelines, and all other applicable regulations and guidelines.

Each set of study product will include a dispensing label containing the protocol number, the participant number, the date of dispensing and the participant name.

The Pharmacist will record all dispensing information on accountability logs.

10.5 Preparation and Administration of Study Drug

The study drug is available as 80 mg vials to be reconstituted with 4 mL sterile water for injection. To aid with reconstitution the vial may be gently swirled, not shaken prior to dilution in normal saline (Baxter Viaflex) prior to IV infusion. It is advised that required number of vials should be removed from the freezer just prior to preparation, however if needed may be removed from the freezer and kept at 4 °C for **up to 2 hours** prior to reconstitution and preparation of infusion.

The subject dose will be calculated using BSA, to a maximum BSA of $2m^2$. The study drug will be diluted, and sufficient volume drawn up, to deliver the required ATX-101 dose of 60 mg/m2, with an absolute dosage cap of 120 mg. Site to ensure that a volume of saline, equal to that of reconstituted study drug, is removed from the infusion bag prior to preparation. Standard overage amount does not need to be removed from infusion bag prior to addition of study drug.

Baseline BSA may be used for subsequent dosing calculations only if weight at any particular visit does not differ by more or less than 10% from baseline.

Investigational product has proven to remain stable in 500 mL Baxter Viaflex bags at controlled room temperature ($20\text{-}25^0$C) for up to 24 hours after preparation of infusion. Total duration from reconstitution, preparation of infusion plus total infusion time should not exceed 24 hours.

For details on the premedication regimen and treatment schedule, see Section 7. ATX-101 is administered at a dose of 60 mg/m2 IV weekly with a maximum dose per infusion of 120 mg.

Infusion bag should be covered with a sleeve to protect Investigational Product against light, directly after preparation until end of infusion. An in-line filter should not be used during reconstitution process or for administration of study drug.

ATX-101 is to be administered under the observation of suitably qualified staff in the research infusion center. The site clinical team will maintain a Source Document file for each subject, with complete and accurate details of IP administration, including the time at which each infusion is started and stopped; any adverse event and action taken to manage these. The Source Document files will be made available during interim monitoring visits or upon request. Dosing errors must be immediately reported.

Doses of ATX-101 will be administered by IV infusion starting with 5 mg/hr and increasing the infusion flow rate every 30 minutes as outlined in the infusion rate table below. The maximum infusion rate should not exceed 120 mg/hr. Increasing infusion rate above the rate listed in Infusion Rate Table below is not recommended. Decreasing or maintaining the suggested flow rate is allowed to effectively manage any infusion related reactions at the investigator's decision.

An infusion rate calculation tool has been provided to calculate the suitable mL/h Infusion rate taking into account the total dose and infusion volume per administration. Please see Section 7.1.

At the end of dosing the used infusion bags and giving sets will be destroyed.

All participants should be evaluated for at least 2 hours after administration of first dose.

10.6 Subject Compliance Monitoring

The drug will be administered at an infusion center under the supervision of nursing staff. Patients who are non-complaint with the treatment regimen may be removed from the study at the discretion of the principal investigator.

10.7 Destruction and Return of Investigational Product

The pharmacy is required to keep any empty dispensing container and any unused study product until full drug accountability has been performed by the CRA. Used IP vials should be either labelled with or segretated by participant number and have date of infusion recorded on the vial or outer container. Used vials of ATX-101 to be retained by participating pharmacies, this is not a mandatory requirement for all sites/pharmacies. Volume of remaining amount of study product to be noted, and frozen for possible use as analytical controls and/or future non-clinical use.

At each agreed monitoring visit during the study and at the end of the study the ATX-101 vials will be reconciled, and a copy of the records given to the monitoring team who will review the inventory of study product and will perform final accountability.

Upon completion of the study, any surplus supplies will be either destroyed upon receipt of written approval of Sponsor and evidence of destruction supplied to the APIM; or will be returned to the supplier. If no supplies remain, this will be documented in the dispensing record.

11. STUDY CALENDAR

	Screening (1)	***Cycle 1(8)***			***Cycle 2 (8)***			***Cycle 3 + (8)***			***End of trtmnt (7)***
Procedure		1	8	15	1	8	15	1	8	15	
ATX-101 administration		X	X	X	X	X	X	X	X	X	
Informed consent	X										
Medical history	X										
Pathology confirmation	X										
Physical examination and vital signs*	X	X	X	X	X	X	X	X			X
Height and weight*	X	X	X	X	X			X			X
Vital signs*	X	X	X	X	X	X	X	X	X	X	X
ECOG performance status*	X	X	X	X	X			X			X
Adverse event assessment*	X	X	X	X	X	X	X	X			X
EKG (1)*	X										X

CBC w/differential (1,3)*	X	X	X	X	X			X			X
Comprehensive metabolic panel (1,3,4)*	X	X	X	X	X			X			X
Serum or urine pregnancy test (1, 3)	X										X
Urinalysis (1, 3)	X							X			X
CT or MRI of chest, abdomen, pelvis (2)	X							X			X
Tumor biopsy for correlative studies (5)	X				X						
Follow-up telephone contact (6)											X
*Clinical/laboratory safety assessments must be completed within 24 hours prior to treatment.											

(1) Screening procedures must be completed within 21 days of initiating study treatment, with the exception of the serum or urine pregnancy test, which must be completed within 7 days of initiating study treatment. Protocol-specified screening procedures that are performed as part of standard of care and are within the specified timeframes may be used for screening purposes.

(2) Baseline imaging can include either (a) CT, spiral CT or MRI, or (b) FDG-PET with diagnostic CT performed with IV and oral contrast and CT acquired with 5 mm or less slice thickness. Imaging studies used for baseline tumor measurements must be performed within 21 days of initiating study treatment. Imaging studies are performed every 2 cycles (6 weeks, 42 days) for the first 36 weeks of study treatment, and then every 3 cycles (9 weeks, 63 days) thereafter. Imaging studies may be performed +/- 5 days from the required date. In the event of dose delays/holds, imaging studies should still be obtained at the scheduled timepoints as measured from cycle 1 day 1, without delays to account for dose delays/holds. Confirmatory scans must be obtained at least 4 weeks after the documentation of an objective response. It is acceptable to perform confirmatory assessments at the next appointed evaluation per protocol. Response assessment should include assessment of all sites of disease and use the same imaging method as used at baseline whenever possible.

(3) All laboratory studies may be performed +/- 3 days from the required date. Local laboratory testing is permitted, except for the serum/urine pregnancy test.

(4) Comprehensive metabolic panel includes albumin, alkaline phosphatase, total bilirubin, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, total protein, AST, ALT and sodium.

(5) The first 10 patients enrolled on the study will undergo paired pre-treatment and on-treatment biopsies. The baseline biopsy specimen must be obtained within 21 days of cycle 1 day 1, provided consent is obtained and other eligibility criteria are met. The on-treatment biopsy specimen will be performed cycle 2, days 1 or 2. Biopsies will be obtained from the same anatomic site of disease whenever possible.

(6) A post-study follow-up contact via telephone by research staff will be conducted every 3 months during the first year after the end of study participation, and then every 6 months thereafter. Information is obtained on disease status, survival status, and new anti-cancer therapy received. Follow-up continues for at least 3 years as measured from the date of study registration or until death, whichever comes first.

(7) The end-of-study visit must be scheduled within 28 days after the last dose of study drug (+/- 7 days) and before starting any new anti-neoplastic therapy.

(8) Clinical assessments will occur on days 1, 8 and 15 of cycle 1, and then on day 1 of all cycles thereafter. Clinical assessments may occur +/- 3 days from the planned date but must occur within 24 hours of ATX-101 administration.

12. MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients will be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

12.1.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with ATX-101. The safety population will consist of all patients who received at least one dose of study drug.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.) Patients who end study treatment during cycle one of treatment may be replaced.

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

12.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as $\geq$ 20 mm by chest x-ray, as $\geq$ 10 mm with CT scan, or $\geq$ 10 mm with calipers

by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be

guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy/Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers will not be used in this study.

Cytology/Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

12.4 Response Criteria

12.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

12.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

12.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.6 Progression-Free Survival

Progression free survival is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12.7 Response Review

Not applicable.

12.8 Unblinding Procedures

Not applicable.

12.9 Stopping Rules

Adverse event stopping rule: Accrual will be temporarily suspended to this study if (a) at any time, 2 patients have experienced a grade 5 event considered at least possibly related to the study treatment and not related to disease progression or (b) more than 40% of all patients, when accrual is equal to or greater than 10 patients, have experienced a grade 3 or higher adverse event. In addition, each grade 5 event will be reviewed at the time of occurrence to determine whether study accrual should be suspended to protect the safety of patients. This adverse event stopping rule may be altered in the event of the study reopening after a temporary suspension or should new information become available related to the safety of the study drug.

13. CORRELATIVE STUDIES

13.1 Tissue procurement and Handling

The first 10 patients who are enrolled and initiate treatment will undergo paired pre-treatment and on-treatment tissue biopsies. The baseline biopsy specimen will be obtained within 21 days of cycle 1 day 1, provided consent is obtained and the other eligibility criteria are met. The on-treatment biopsy specimen will be obtained on cycle 1, days 1 or 2.

The same tumor lesion should be biopsied at both time points whenever possible. Specimens are obtained using a 16-18 gauge core needle under imaging guidance by an interventional radiologist at the treating institution. At least 4 cores are obtained, and up to 6-8 cores if feasible (with safety of the procedure prioritized in all cases). The first 4 cores are placed in FFPE and the subsequent 2-4 cores are cryopreserved and flash frozen. From the FFPE material, an H&E section should be cut for confirmation/diagnostic purposes, to be reviewed at the institution collecting the sample as per standard of care. These following specifications apply:

(1) A 16 to 18 gauge needle is satisfactory;

(2) Time from core needle biopsy (CNB) removal to initiation of fixation should be $\leq$ 30 min;

(3) CNB material should be immersed in sufficient volume of 10% neutral buffered formalin to

ensure adequate fixation (≥15 to 20 times greater than estimated volume of CNB);

(4) Formalin fixation to last between 6-72 hours at room temperature; and after fixation, proceed to paraffin embedding. Tissue blocks should be stored at ambient conditions.

(5) Material should be labeled, accessioned and stored consistent with standard of care policies and procedures at the participating institution.

The tissue will be used for correlative studies evaluating ATX-101's effects on the immune microenvironment, intracellular signaling pathways and DNA damage response pathways.

14. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements). The Data Safety Monitoring Plan is described in Section 12.2.

14.1 Data Collection

The Herbert Irving Comprehensive Cancer Center has an electronic clinical trials and data management system (CTMS) that will be used for data collection. CRFs for the study will be built into the CTMS for data entry. The system has full auditing capabilities which is web-based and housed on a server in a fully HIPAA compliant server room with restricted access and video camera monitoring. All users must login with their own application username and password. Users off campus must first access the Virtual Private Network with their assigned campus username and password and then use their application credentials. Users are only able to see study information if they are indicated as study personnel in our electronic IRB system. Users are limited to access based on the role assigned in their corresponding protocol. Subject data is entered directly into the system, which (in the case of Columbia subjects) confirms the correct identity of patients via an interface with the electronic medical patient index. Staff with the appropriate IRB defined roles can run reports within the system for reporting purposes.

14.2 Data Reporting

Case Report Forms will be completed for each subject enrolled into the clinical study through the CTMS. It is the investigator's responsibility for ensuring that all clinical and laboratory data entered on the corresponding CRFs are complete, accurate and authentic.

14.3 Data and Safety Monitoring Committee

The NCI-approved Data Safety and Monitoring Committee (DSMC) of the Herbert Irving Comprehensive Cancer Center (HICCC) will monitor every subject who receives treatment on this protocol for toxicity. This protocol will adhere to the policies of the currently approved HICCC Data and Safety Monitoring Plan (DSMP), which is in accordance with NCI and CUMC-IRB policy and guidelines. The committee is chair is appointed by the HICCC Director. The committee consists of HICCC faculty and staff with expertise in oncology, research

pharmacy, research nursing, and data management. The DSMC convenes twice a month to review patient safety and the conduct of the trial. The PI will submit data and safety monitoring reports to the DSMC at a frequency to be determined by the DSMC based on risk to the subjects.

At the time of renewal, the study team will submit the most recent DSMC approval letter for safety review to the CUMC IRB. Any modifications that are required by the DSMC to ensure patient safety will be submitted to the IRB. All protocol deviations, violations, and eligibility waivers will be submitted to and approved by the DSMC prior to being reported to the IRB. All study data reviewed and discussed during these meetings will be kept confidential.

For multicenter research, the principal investigator will assure that there is a mechanism in place to distribute the report to all participating investigators for submission to their local IRB. The report will document that a review of data and outcomes across all centers took place on a given date. It will summarize the DSMC's review of the cumulative toxicities reported from all participating sites without specific disclosure by treatment arm. It will also inform site investigators of the study the DSMC's conclusion with respect to progress or need for modification of the protocol.

14.4 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the CPDM Compliance Core on behalf of the HICCC DSMC. Additionally, the Compliance Oversight Committee of the IRB at Columbia University Medical Center may audit the study at any time per institutional policies and procedures. The investigator-sponsor and Columbia University Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

A risk-based approach will be used by the Compliance Core to determine the frequency, number of subject charts, and data elements to be monitored. The Compliance Coordinator will review the study status and summarize enrollment, toxicities, SAEs/UPs, dose escalation, statistical endpoints (e.g., stopping rules), etc. for the full DSMC membership at the regularly scheduled meetings.

Internal On-site Monitoring:

- Initial, recurrent, and close-out on-site monitoring visits will also be conducted at remote clinical sites, as appropriate/feasible. Other sites will have monitoring performed remotely (see below for further details).
- The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.
- The Compliance Coordinator will communicate with the site coordinator/Site Principle Investigator to schedule the monitoring visit and arrange for access to study materials and documentation.
- The assigned Compliance Coordinator will monitor IIT trials within 1 month after

the first subject is enrolled and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The Compliance Coordinator is responsible to notify the PI and CRNP/CRN/CRC of upcoming monitor visits and convey what information and documentation will be required for the visit(s). The Compliance Coordinator is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The Compliance Coordinator will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to local IRB and HICCC DSMC requirements. The Compliance Coordinator will issue queries and ensure resolution in a timely and efficient manner. The Compliance Coordinator will also monitor for applicable regulatory compliance and research pharmacy compliance (if applicable) and communicate any deficiencies as appropriate.

14.5 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (e.g., that the subject is alive) at the end of their scheduled study period.

The subject binders will be maintained with in the CPDM offices, a secured floor within the Herbert Irving Pavilion and only the investigator and study staff will have access to the file.

14.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

14.7 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A".

14.8 Records Retention

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). This minimum retention period applies whether or not any subjects were enrolled in the study. If the research is FDA regulated, records should be retained for at least two years after approval of the investigational agent by FDA; if it is not approved, records should be retained at least two years after the study is terminated and FDA is notified (note the additional requirement below for clinical research studies); Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy which is based on state law.

15. STATISTICAL PLAN

15.1 Primary Endpoint

The primary endpoint is the progression free rate at 12 weeks (PFR_{12}). This corresponds to the number of patients who are alive and without evidence of disease progression at 12 weeks out of all evaluable patients.

Two recent randomized phase 3 studies evaluated novel agents (trabectedin, eribulin) in a population of patients with LPS and LMS. In both studies, the cytotoxic agent dacarbazine was used as the active comparator/control arm. Dacarbazine is a chemotherapy agent often used for later-line treatment of soft tissue sarcoma, including LPS and LMS.

In the randomized study of trabectedin versus dacarbazine in patients with LPS and LMS (n=518), median PFS was 4.2 months versus 1.5 months, and the progression-free rate at 3 months (12 weeks) was 56% versus 34%, respectively[1]. In a subset analysis, outcomes were similar in LPS and LMS. Similarly, in the randomized study of eribulin versus dacarbazine (n=452), the median PFS was 2.6 months versus 2.6 months, and the progression-free rate at 3 months (12 weeks) was 33% versus 29%.[2]

Based upon the results of these studies, a $PFR_{12} \leq 30\%$ would be considered inactive and unworthy of further study, whereas a $PFR_{12} \geq 55\%$ would suggest clinically meaningful activity, worthy of further evaluation in subsequent studies.

A Simon optimal 2-stage design is used. The study will enroll 10 patients with LMS and LPS in the first stage. If 4 or more patients meet the PFR_{12} endpoint in the first stage (4/10), the study will proceed to the second stage, enrolling an additional 24 patients for a total sample size of 34 patients. If 15 or more total patients meet the PFR_{12} endpoint in the overall study population (15/34), the agent will be considered promising and worthy of further study.

An interim analysis will be performed after the first stage is completed. In the event that the study meets the stage 1 endpoint, but all patients who meet the PFR_{12} endpoint are of the same histology (either LMS or LPS), then the study will proceed to full accrual in that respective histology only. In this event, no further patients will be enrolled from the histology in which no patients met the PFR_{12} endpoint during stage 1.

This design provides 85% power and type 1 error (1-sided) of 0.05 to evaluate for an improvement in PFR_{12} from $\leq 30\%$ to $\geq 55\%$.

For the first stage of enrollment, among the 10 patients enrolled, 5 patients will have LMS, and 5 patients will have LPS. If the study proceeds to the second stage, among the 12 patients subsequently enrolled, 12 patients will have LMS and 12 patients will have LPS.

15.2 Size/Accrual Rate

The sample size is 34 patients. The planned accrual rate is 2-3 patients per month. Therefore, accrual is expected to be complete in no more than 17 months. Assuming patients continue treatment for up to 1 year (12 months) and allowing 6 months for quality assurance, the anticipated duration of the study is approximately 35 months.

15.3 Stratification Factors

There are no prespecified stratification factors for the primary endpoint.

15.4 Analysis of Secondary Endpoints

Safety Lead-In: A safety lead-in will be conducted as described in Section 4.2. A summary of the adverse events and any DLT observed during the safety lead-in phase will be provided, as well as the study team's assessment of whether the protocol-specified dose and schedule is tolerable.

Adverse event rates: Adverse events will be recorded at each clinical visit and will be categorized according to NCI CTCAE version 4.0. Adverse event rates will be reported as counts and percentages per adverse event basis by grade.

Overall survival and progression free survival: Overall survival is defined as the time from the date of first treatment with study drug to the time of death from any cause or last follow-up if alive. Progression free survival is defined as the time from first treatment with the study drug to the earliest of either disease progression or death from any cause. Patients who are alive and progression free will be censored at the time of their last follow-up. The Kaplan-Meir method

will be used to evaluate all time to event endpoints. Median overall survival and median progression free survival will be reported with 95% confidence intervals.

Response rate: Patient responses will be evaluated according to RECIST version 1.1 as described in section 12. The response rate is defined as the number of patients having a best objective tumor status of complete response or partial response lasting at least four weeks divided by the number of evaluable patients. The response rate will be reported with 95% confidence intervals.

15.5 Analysis of Correlative Science Endpoints

Analysis of correlative endpoints will be considered exploratory and hypothesis generating.

15.6 Reporting and Exclusions

15.6.1 Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first treatment with ATX-101. The safety population will consist of all patients who received at least one dose of study drug.

15.6.2 Evaluation of response

All patients included in the study who receive at least one treatment with the study drug will be assessed for response to treatment, even if there are major protocol deviations or if they are ultimately deemed ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death from other cause, or 9) unknown (not assessable or insufficient data).

16. PROTECTION OF HUMAN SUBJECTS

This study is to be conducted in accordance with applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be obtained before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, as outlined in the IRB approved protocol, and the investigator-designated research professional obtaining the consent.

17. STUDY FINANCES

17.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the Columbia University Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved prior to participation in this study. All CUMC investigators will follow the University conflict of interest policy.

17.2 Subject Stipends or Payments

No stipends or payments will be given to subjects.

18. PUBLICATION PLAN

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

19. CITED REFERENCES

1. Demetri, G.D., et al., *Efficacy and Safety of Trabectedin or Dacarbazine for Metastatic Liposarcoma or Leiomyosarcoma After Failure of Conventional Chemotherapy: Results of a Phase III Randomized Multicenter Clinical Trial.* J Clin Oncol, 2016. **34**(8): p. 786-93.
2. Schoffski, P., et al., *Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial.* Lancet, 2016. **387**(10028): p. 1629-37.
3. *SEER Cancer Stat Facts: Soft Tissue Cancer.* . [cited 2021 1/3/2021]; Available from: https://seer.cancer.gov/statfacts/html/soft.html.
4. Maki, R.G., et al., *Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected].* J Clin Oncol, 2007. **25**(19): p. 2755-63.
5. van der Graaf, W.T.A., et al., *Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind, placebo-controlled phase 3 trial.* The Lancet, 2012. **379**(9829): p. 1879-1886.
6. Schöffski, P., et al., *Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial.* The Lancet, 2016. **387**(10028): p. 1629-1637.
7. Krishna, T.S.R., et al., *Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA.* Cell, 1994. **79**(7): p. 1233-1243.
8. Wyman, C. and M. Botchan, *DNA Replication: A familiar ring to DNA polymerase processivity.* Current Biology, 1995. **5**(4): p. 334-337.
9. Mailand, N., I. Gibbs-Seymour, and S. Bekker-Jensen, *Regulation of PCNA-protein interactions for genome stability.* Nat Rev Mol Cell Biol, 2013. **14**(5): p. 269-82.
10. Moldovan, G.L., B. Pfander, and S. Jentsch, *PCNA, the maestro of the replication fork.* Cell, 2007. **129**(4): p. 665-79.
11. Naryzhny, S.N. and H. Lee, *Proliferating cell nuclear antigen in the cytoplasm interacts with components of glycolysis and cancer.* FEBS Lett, 2010. **584**(20): p. 4292-8.
12. Olaisen, C., et al., *PCNA-interacting peptides reduce Akt phosphorylation and TLR-mediated cytokine secretion suggesting a role of PCNA in cellular signaling.* Cell Signal, 2015. **27**(7): p. 1478-87.
13. Rosental, B., et al., *Proliferating cell nuclear antigen is a novel inhibitory ligand for the natural cytotoxicity receptor NKp44.* J Immunol, 2011. **187**(11): p. 5693-702.
14. Witko-Sarsat, V., et al., *Proliferating cell nuclear antigen acts as a cytoplasmic platform controlling human neutrophil survival.* J Exp Med, 2010. **207**(12): p. 2631-45.
15. Warbrick, E., *PCNA binding through a conserved motif.* BioEssays, 1998. **20**(3): p. 195-199.
16. Gilljam, K.M., et al., *Identification of a novel, widespread, and functionally important PCNA-binding motif.* J Cell Biol, 2009. **186**(5): p. 645-54.
17. Bacquin, A., et al., *The helicase FBH1 is tightly regulated by PCNA via CRL4(Cdt2)-mediated proteolysis in human cells.* Nucleic Acids Res, 2013. **41**(13): p. 6501-13.

18. Ciccia, A., et al., *Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress.* Mol Cell, 2012. **47**(3): p. 396-409.
19. Fattah, F.J., et al., *The transcription factor TFII-I promotes DNA translesion synthesis and genomic stability.* PLoS Genet, 2014. **10**(6): p. e1004419.
20. Gilljam, K.M., et al., *Nucleotide excision repair is associated with the replisome and its efficiency depends on a direct interaction between XPA and PCNA.* PLoS One, 2012. **7**(11): p. e49199.
21. Aas, P.A., et al., *Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA.* Nature, 2003. **421**(6925): p. 859-63.
22. Gederaas, O.A., et al., *Increased Anticancer Efficacy of Intravesical Mitomycin C Therapy when Combined with a PCNA Targeting Peptide.* Transl Oncol, 2014. **7**(6): p. 812-23.
23. Sogaard, C.K., et al., *APIM-peptide targeting PCNA improves the efficacy of docetaxel treatment in the TRAMP mouse model of prostate cancer.* Oncotarget, 2018. **9**(14): p. 11752-11766.
24. Sogaard, C.K., et al., *Targeting the non-canonical roles of PCNA modifies and increases the response to targeted anti-cancer therapy.* Oncotarget, 2019. **10**(68): p. 7185-7197.
25. Raeder, S.B., et al., *APIM-Mediated REV3L(-)PCNA Interaction Important for Error Free TLS Over UV-Induced DNA Lesions in Human Cells.* Int J Mol Sci, 2018. **20**(1).
26. Muller, R., et al., *Targeting proliferating cell nuclear antigen and its protein interactions induces apoptosis in multiple myeloma cells.* PLoS One, 2013. **8**(7): p. e70430.
27. Oza, J., et al., *Homologous recombination repair deficiency as a therapeutic target in sarcoma.* Semin Oncol, 2020. **47**(6): p. 380-389.
28. Van Glabbeke, M., et al., *Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas.* European Journal of Cancer, 2002. **38**(4): p. 543-549.
29. Dickson, M.A., et al., *Phase 2 study of the CDK4 inhibitor abemaciclib in dedifferentiated liposarcoma.* Journal of Clinical Oncology, 2019. **37**(15_suppl): p. 11004-11004.
30. Dickson, M.A., et al., *Progression-Free Survival Among Patients With Well-Differentiated or Dedifferentiated Liposarcoma Treated With CDK4 Inhibitor Palbociclib: A Phase 2 Clinical Trial.* JAMA Oncol, 2016. **2**(7): p. 937-40.
31. Dickson, M.A., et al., *Phase II trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma.* J Clin Oncol, 2013. **31**(16): p. 2024-8.
32. Sleijfer, S., et al., *Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European organisation for research and treatment of cancer-soft tissue and bone sarcoma group (EORTC study 62043).* J Clin Oncol, 2009. **27**(19): p. 3126-32.
33. Simon, R., *Optimal two-stage designs for phase II clinical trials.* Control Clin Trials, 1989. **10**(1): p. 1-10.