NCI Protocol #: N/A

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TITLE: A Phase II Study of M6620 (VX-970) in Selected Solid Tumors

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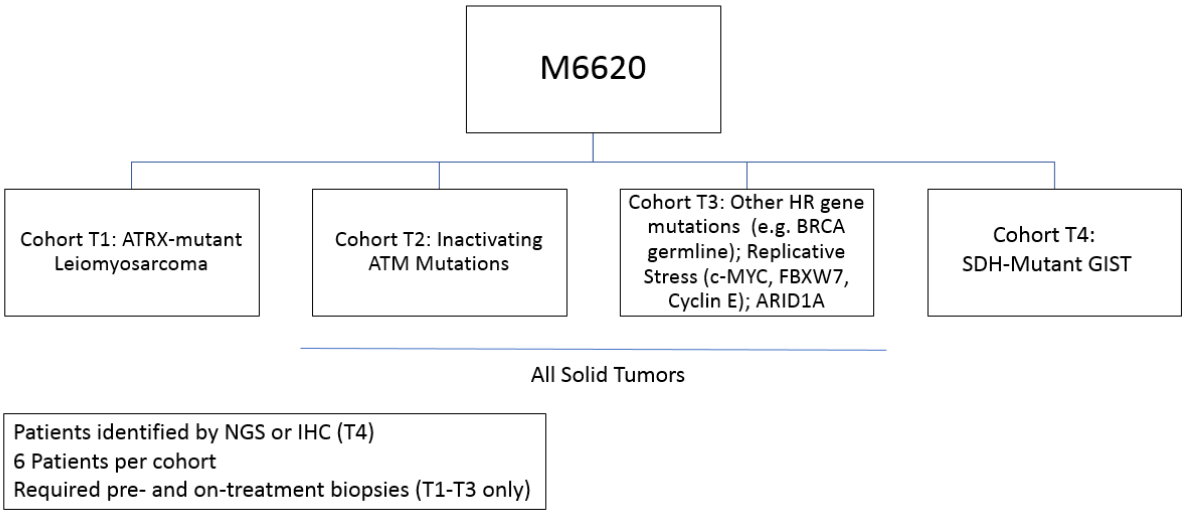
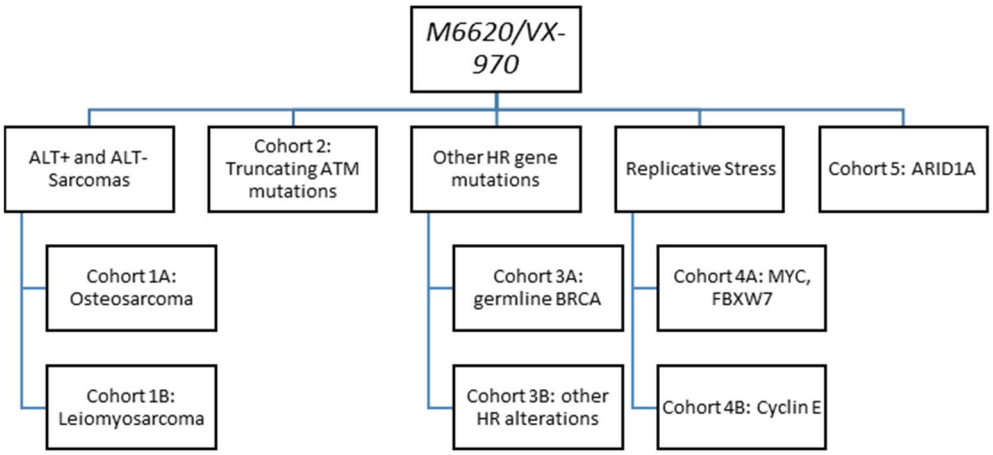
SCHEMA

Translational Lead-in Study:



Phase II Study:



Cohorts 1A and 2-5 will enroll 15 patients in stage 1.
Cohorts 1A and 2-5: If ≥3/15 patients meet the primary endpoint an additional 10 patients will be accrued (25 total)
Cohorts 1B: will enroll 30 patients in a single stage

Cohorts 2-5 will include all solid tumors with genetic alterations identified by next-generation sequencing (NGS)

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1. OBJECTIVES

1.1 Study Design

This is an open label, phase II basket trial of M6620 with a translational lead-in study in participants with select advanced solid tumors. Participants with tumors harboring the alterations listed in the tables below will be enrolled to the appropriate corresponding cohort. Enrollment to the translational study will initiate first. Following an analysis of the results from the lead-in study, enrollment to the phase II trial will commence. The single T3 patient with SDH-GIST will be moved to cohort T4 with the Amendment dated April 30, 2019. This will be counted towards T4 accrual numbers and not T3 accrual numbers.

Table 1: Translational Lead-in Study Design
Translational Cohort 1 (T1):
ATRX-mutant leiomyosarcoma (LMS)
Translational Cohort 2 (T2):
Truncating *ATM* mutations (any solid tumor)
Translational Cohort 3 (T3):
Germline *BRCA1/2* mutations, other homologous repair (HR) alterations (e.g. somatic *BRCA1/2, BARD1, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD51B, RAD51C* and *RAD51D*), *MYC* amplification, *FBXW7* truncating or missense mutations, *CCNE1* amplification, *ARID1A* mutations (any solid tumor)
Translational Cohort 4 (T4):
SDH-deficient Gastrointestinal Stromal Tumor (GIST)

*other ATM mutations may be considered see section 9.4

Table 2: Phase II Study Design		
Cohort 1		
	1A	Osteosarcoma
	1B	Leiomyosarcoma (LMS)
Cohort 2		
	2	Truncating *ATM* mutations
Cohort 3		
	3A	Germline *BRCA1/2* mutations
	3B	Other homologous repair (HR) alterations (e.g. somatic *BRCA1/2, BARD1, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD51B, RAD51C* and *RAD51D*)
Cohort 4		
	4A	*MYC* amplification, *FBXW7* truncating or missense mutations

	4B	*CCNE1* amplification
Cohort 5		
	5	*ARID1A* mutations

*other ATM mutations may be considered see section 9.4

1.2 Primary Objective – Translational Lead-In

- To determine the preliminary anti-cancer activity of M6620 monotherapy in selected cancer populations including *ATRX*-mutated LMS, *ATM*-mutated solid tumors, *BRCA1/2* and other HR-mutated cancers, malignancies with replicative stress, *ARID1A*-mutated cancers, and SDH-deficient GIST.
- To determine changes in the pharmacodynamic endpoints of phospho-Chk1 and γH2AX in biopsy specimens of patients treated with M6620 monotherapy.

1.3 Primary Objective – Phase II

- To determine the anti-cancer activity of M6620 monotherapy in selected cancer populations including osteosarcoma, LMS, *ATM*-mutated solid tumors, *BRCA1/2* and other HR-mutated cancers, malignancies with replicative stress, and *ARID1A*-mutated cancers.

1.4 Secondary Objectives

- To determine correlations between selected biomarkers and M6620 monotherapy anti-cancer activity, including mutations detected in targeted NextGen Sequencing (NGS), ALT status (telomeric repeat-containing RNA [TERRA] *in situ* hybridization [ISH], C-circle assay), ATM, RAD51, MYC, Cyclin E and ARID1A immunohistochemistry (IHC).
- To confirm the safety and overall tolerability of M6620 in these patient populations.

2. BACKGROUND

2.1 Study Diseases and Rationale

2.1.1 Cohorts T1, 1A and 1B: Sarcoma

The sarcomas represent a complex and heterogeneous group of orphan mesenchymal malignancies arising from bone or soft tissue. In metastatic soft tissue sarcomas, chemotherapy has led to a median survival of only 12 months across histologies[1,2]. In metastatic osteosarcoma 5-year survival rates are only in the range of 15-30%[3].

Cancers rely on either telomerase or the ALT pathway to extend telomeres and bypass senescence. ALT is a recombination-mediated mechanism that extends telomeres. Depending on

which assay is used, ALT is estimated to be activated in 10-15% of cancers[4]. Interestingly, the sarcomas have a high prevalence for ALT. For example, it has been reported that up to 50-60% of osteosarcomas and LMS are ALT+. ATRX-DAXX is a chromatin-remodeling complex that functions at genomic loci including telomeres. Loss of ATRX has been linked to activation of the ALT pathway in cancer cells. In work from the Zou laboratory[5], it was found that ATRX is a cell cycle-regulated repressor of the telomere non-coding RNA TERRA and ATRX loss compromises the cell-cycle regulation of TERRA leading to overexpression/persistent association throughout the cell cycle. This dysregulation ultimately generates a recombinogenic nucleoprotein structure (RPA-coated single-stranded DNA) at telomeres, which activates the ATR checkpoint kinase and promotes telomere recombination. Importantly, it was determined that ATR is a key regulator of ALT where ATR inhibitors disrupt the ALT pathway and selectively kill ALT+ cancer cells in a panel of osteosarcoma and glioblastoma cell lines.

In our clinical experience ATRX is mutated in ~39% of LMS[6]. In unpublished work, there is near 100% correlation with ATRX alterations and ALT+ status in LMS. However, we have also noted that there are sarcomas, including LMS, with intact ATRX that activate ALT through other pathways. To enrich for ALT in this translational aspect of the study we will focus on those with ATRX mutations and loss of expression by IHC. In the larger study, we will include all LMS or osteosarcomas, regardless of ATRX status, and retrospectively determine ALT status.

2.1.2 Cohort 2: Mutations in ATM

Complementation of an ATM patient derived cell line with wild-type ATM rescues M6620 hypersensitivity while cells complemented with a kinase-dead version of ATM remains hypersensitive (unpublished data, Center for DNA Damage and Repair, DFCI). These results are consistent with enhanced sensitivity of a variety of cell lines to ATR inhibitors after knock-down of ATM. Therefore, tumors with genomic deletion of *ATM* or lack of ATM expression due to epigenetic silencing are expected to respond to M6620. Since the kinase domain of ATM is located at the C-terminus of the protein, all truncating mutations resulting in deletion of the kinase domain would result in a non-functional protein. Among missense mutations, 23 are annotated as pathogenic in the ClinVar database, a freely accessible archive of the relationships among human variations and phenotypes supported by the National Center for Biotechnology Information (NCBI). Therefore, patients whose tumors harbor truncating mutations or one of the 23 missense mutations are likely to respond to M6620. Furthermore, the mutant allele may encode an unstable protein or may have a dominant-negative effect if the wild-type allele is retained. Both these scenarios are likely to result in enhanced sensitivity to M6620.

2.1.3 Cohort 3T, 3A, 3B, 4A, 4B, 5: Mutations in *BRCA1/2* and other genes implicated in HR, tumors with replication stress, and *ARID1A* alterations

Tumors with deficiencies in HR due to loss or deleterious mutations in HR pathway genes are expected to succumb to ATR inhibition. ATR is activated early in response to DNA double-strand breaks where ATR phosphorylates and activates multiple proteins that participate in HR and activation of cell cycle checkpoint[7,8]. Carriers of germline or somatic known deleterious mutations in *BRCA1* or *BRCA2* will be recruited to cohort 3A while cohort 3B will constitute patients whose tumors harbor somatic mutations in *BRCA* genes or mutations in other HR

pathway genes. Of specific interest are known deleterious mutations in *BARD1, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD51B, RAD51C* and *RAD51D*. Of note, among patients with *BRCA* mutations who have been treated with PARP inhibitors, reversal of PARP inhibitor resistance may be achieved by ATR inhibition. In cancer cells that have become PARP inhibitor-resistant via restoration of HR, ATR inhibition is expected to restore an HR-deficient state. In cancer cells that have become PARP inhibitor-resistant by stabilization of replication forks, ATR inhibition is expected to destabilize replication forks. Therefore, PARP inhibitor-resistant cancers may respond to ATR inhibitor monotherapy[9].

2.1.4 Cohort 4A and 4B: Tumors with replication stress

Unlicensed replication origin firing and compromised replication fork stability results in replication stress (RS). Single- and double-stranded DNA breaks and presence of extensive single-strand DNA are manifestations of RS. These conditions result in the activation of the ATR pathway, so that tumors with RS are dependent on ATR for survival[10]. In this cohort, we will test the efficacy of M6620 in tumors with amplifications in *MYC* (Cohort 4A) and *CCNE1* (Cohort 4B). In addition to patient tumors with *MYC* amplification, cohort 4A will also include subjects with truncating or missense mutations designated as deleterious (cBioPortal) in *FBXW7*. FBXW7 is a component of the SCF-like E3 ubiquitin ligase complex, of which, MYC is a major substrate.

The eligibility criteria for *CCNE1* amplification will be greater than 8-fold and that of *MYC* will be 6-fold. The Brigham and Women's Center for Advanced Molecular Diagnostics (CAMD) has performed preliminary analyses indicating that amplification identified by targeted NGS accurately reflects tumors in which these amplifications are identified by fluorescence *in situ* hybridization (FISH).

Although increased *CCNE1* copy number can lead to increased cyclin E protein expression, this is not a universal finding. Based on work done with our collaborators, it is expected that 50% of tumors will have high cyclin E protein levels in the presence of any degree of *CCNE1* amplification. Our collaborators have further evaluated the *CCNE1*-amplified group to see if a certain degree of gene amplification correlates with high cyclin E protein expression. This work is illustrated below. A minimum 6-fold *CCNE1* amplification will give the highest probability of treating tumors with high cyclin E protein expression.

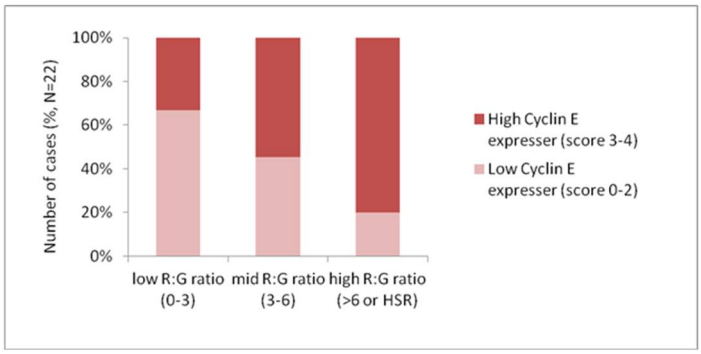

Figure: 6-fold *CCNE1* amplification is associated with high cyclin E expression within tumor cells. The graph above shows the degree of cyclin E protein expression for non-amplified, moderately amplified and highly amplified *CCNE1*. 80% of tumors with 6-fold or greater *CCNE1* gene amplification were highly expressed cyclin E protein, compared with 25% and 50% of tumors with low or moderate amplification respectively.

2.1.5 Cohort 5: ARID1A

ARID1A is among the most highly-mutated genes across various tumor types. Mutations are most prevalent in endometrial, colorectal, gastric and bladder cancer. The majority of mutations identified in the cancer genome atlas (TCGA) are truncating, and none of the missense mutations have been classified as deleterious by Mutation Assessor or annotated pathogenic in the ClinVar database. Although bi-allelic truncation mutations are less common, tumors with mono-allelic truncation mutation tend to have low levels of protein expression, and *in vitro* studies in cell lines suggest that ARID1A is a haplo-insufficient tumor suppressor. Lack of ARID1A protein determined by IHC is associated with poor prognosis in multiple tumor types. ARID1A is a large protein with a DNA binding domain while the C-terminus encodes multiple LXXLL protein interaction motifs. ARID1A interacts with several proteins to form the BAF complexes, a subfamily of mammalian SWI/SNF chromatin remodelers. ATM and ARID1A were the two top hits in an siRNA screen using HCC1143, a triple negative breast cancer cell line, to identify genes that exhibit a synthetic-lethal interaction with ATR inhibitor[11]. In an expanded set of tumor cell lines, ones with mutant *ARID1A* were found to be more sensitive to ATR inhibition compared to cells with wild-type *ARID1A*. *ARID1A* mutant/deficient cells are defective in TOPO2A and the G2/M checkpoint regulated by ATR. When ATR is inhibited, the cells enter mitosis without resolving replication defects resulting in genomic instability and apoptosis.

2.1.6 Cohort T4: SDH-deficient GIST

GIST are a subtype of sarcoma derived from the Interstitial Cells of Cajal in the GI tract. In nearly all GIST either the oncogene c-KIT or PDGFRA undergo activating mutations that drive cancer growth. In a subset of GIST, which lack KIT/PDGFRA mutations, there is deficiency in one of the SDHX gene subunits. Tyrosine kinase inhibitors have minimal activity in this subtype of GIST and there are no FDA-approved therapies for these patients. Notably, SDH-GIST also tend to be associated with the pediatric and adult population, while KIT/PDGFRA-GIST typically occurs in adults only.

It was recently recognized that tumors with mutations in Krebs-cycle proteins (e.g. SDH-deficient paraganglioma/pheochromocytoma) are deficient in HR dsDNA break repair.[12] The proposed mechanism is as follows. SDH deficiency in the tumor Kreb cycle leads to elevated fumarate and succinate. These in turn suppress HR activity through KDM4A/B, known regulators of DNA repair. Thus, there is a therapeutic potential for synthetic lethality in tumors with this alteration.

In the initial experience of this trial an adult with known SDH-deficient GIST refractory to sunitinib and regorafenib was treated with M6620. After 16 days of dosing the patient was observed to have a minor radiologic response with shrinkage of -13.84% and a marked improvement in clinical symptoms.

2.2 M6620 (VX-970)

M6620 is a potent inhibitor of ataxia telangiectasia mutated and Rad3-related kinase (ATR, inhibition constant [Ki] <300 pM) that blocks ATR activity in cells, with a concentration associated with 50% inhibition (IC_{50}) of 20 nM. Complete compound background information is available in the investigator's brochure (IB).

2.2.1 Pre-Clinical Summary

2.2.1.1 Pharmacology

M6620 (VX-970) is a potent inhibitor of ATR kinase (inhibition constant [Ki] <300 pM) with excellent selectivity over other kinases (>50-fold selectivity over 290/291 kinases tested). *In vitro*, M6620 sensitized many cancer cell lines and primary human tumor cells, but not normal cell lines, to the cytotoxic effects of a range of DNA-damaging drugs and ionizing radiation (IR). The reliance of many cancer cells on ATR for survival following treatment with DNA-damaging agents can be driven by a number of mechanisms including disruption of alternative DNA damage repair pathways in these cells. One such example is disruption of the signaling pathway mediated by ataxia telangiectasia mutated (ATM) kinase. This pathway is defective in many cancer cells. In contrast, normal cell tolerance to inhibition of ATR is attributed to the presence of alternative, compensatory DNA damage response (DDR) pathways.

In mouse xenograft models derived from human cancer cell lines and primary human tumors, M6620 dose-dependently enhanced the anti-tumor effects of multiple DNA-damaging drugs and IR. In many cases, marked regression was observed for combinations with M6620. Furthermore, the combination of M6620 and a DNA-damaging drug has been shown to be more effective than the DNA-damaging drug alone at its maximum tolerated dose (MTD). As a single agent, M6620 had no significant impact on tumor growth in the experimental models examined.

Dose schedule optimization studies in mouse models have demonstrated that administration of M6620 by IV injection at a dose of 20 mg/kg/week (either as a single injection or as two 10-mg/kg injections 3 days apart) was highly effective in combination with cisplatin or gemcitabine. Furthermore, timing of M6620 administration is critical, with maximal activity observed when M6620 was given 12 to 24 hours after cisplatin or gemcitabine treatment.

Therapeutic dose estimations were based on achieving an area under the concentration versus time curve (AUC) of 4080 ng·h/mL/week in whole blood (achieved with an IV dose of 20 mg/kg/week in mouse). Allometry predicts that a human dose of 2.5 mg/kg (corresponding to 100 mg/m2) will be sufficient to achieve this exposure. In murine models, M6620 was generally well tolerated at efficacious doses in combination with DNA-damaging agents. In some instances, some body weight loss was observed, and from intensive and sustained dosing of M6620 in combination with cisplatin, enhanced changes in specific peripheral blood cell population was observed versus cisplatin treatment alone. This enhanced effect was attributed to a synergistic increase in growth arrest in normal cells. *In vitro* studies have demonstrated that the enhanced growth arrest seen when ATR inhibitors are added to cisplatin treatment is reversed after ATR activity is restored.

2.2.1.2 Pharmacokinetics

In rats, mice, dogs, and monkeys, M6620 had a high volume of distribution; tissue exposure, including tumor, was high. In rats, no accumulation or retention was observed in tissues and the elimination half-lives were similar across all tissues and whole blood. Whole blood half-lives after IV administration in rats and dogs were 11.6 and 9.8 hours, respectively. M6620 had high plasma protein binding and the free fraction in human blood was 2.1%. A low potential for drug-drug interactions was predicted, based on minimal inhibition or induction of CYP isozymes by M6620; however, strong inducers or inhibitors of CYP3A4 may alter M6620 kinetics and blood levels. M6620 was primarily eliminated by oxidative metabolism, with CYP3A4 as the principle isozyme responsible. Metabolites were excreted in the urine and bile. All metabolites observed in human hepatocyte incubations were also observed in either rat or dog hepatocyte incubations and in the blood, bile, or urine from rats or dogs. Accordingly, rat and dog were recommended as the appropriate species for toxicology assessment. The systemic clearance values of M6620 following IV administration were determined to be 26 and 13 mL/min/kg in the rat and dog, respectively.

2.2.1.3 Safety Pharmacology

A battery of *in vitro* and *in vivo* safety pharmacology studies designed to evaluate effects of M6620 against multiple protein targets, and the cardiovascular system did not demonstrate any toxicologically significant effects at exposures or concentrations that exceed the clinically relevant exposures of M6620.

2.2.1.4 Toxicology

The M6620 toxicology studies in rats and dogs identified the liver, testes, and peripheral blood cell populations as target organs following oral or IV administration. Importantly, liver and peripheral blood toxicities were reversed within 4 weeks of discontinuation of M6620. Following oral exposure, the MTD in rats was 150 mg/kg and M6620 was not tolerated at doses ≥30 mg/kg in dogs. IV administration in a regimen that more closely reflects the clinical regimen was tolerated in animals and allowed the determination of a severely toxic dose to 10% of animals (STD_{10}) in rats and a highest non-severely toxic dose (HNSTD) in dogs. The animal to human

exposure ratio on a weekly AUC basis at the rat STD_{10} following IV administration (30 mg/kg) was 5.3-fold the weekly human exposure at 210 mg/m2. The animal to human exposure ratio on a weekly AUC basis at the dog HNSTD following IV administration (20 mg/kg) was 7.7-fold the weekly human exposure at 210 mg/m2.

M6620 had no cardiovascular liabilities, was not genotoxic in a GLP mutagenicity assay, had no hemolytic potential in human blood or compatibility issues in human plasma, and was well tolerated in an acute rabbit parenteral injection study. M6620 absorbs in the UV-visible spectrum and has high tissue distribution in rats; therefore, appropriate precautions to safeguard patients from excessive UV-visible radiation should be taken in the clinic pending results from nonclinical phototoxicity assessment.

2.2.2 Clinical Summary

2.2.2.1 Pharmacokinetics

Noncompartmental analyses were conducted on preliminary clinical pharmacokinetic (PK) data obtained from Parts A and B of Study VX12-970-001 (Study 001) and Study VX13-970-002 (Study 002) for both whole blood and plasma matrices. The mean M6620 plasma concentrations versus time profiles were similar in shape to those of the corresponding whole blood profiles. Overall, the C_{max} were approximately 1.3-fold greater in whole blood than in the plasma matrix, suggesting that plasma is an appropriate matrix to characterize the PK of M6620. PK exposures tended to increase dose proportionally with increasing dose based upon C_{max} and $AUC_{0-\infty}$ after administration of M6620 as a single agent. The mean terminal elimination half-life was approximately 17 hours across all dose groups. Collectively, the data suggest that co-administration of gemcitabine, cisplatin, or carboplatin 24 hours before M6620 administration did not appear to affect the PK of M6620.

2.2.2.2 Single Agent Safety

M6620 was administered as a single agent in the lead-in periods of the phase 1 first-in-human study 001 Parts A and B (doses of 18 mg/m^2 to 210 mg/m^2, lead-in periods of 7 to 21 days); during the phase 1 study 002 Part A1 (once weekly, doses of 60 mg/m^2 to 480 mg/m^2) and Part A1 (twice weekly, doses of 240 mg/m^2); and during the single-agent phase of study 002 Part C (doses of 240 mg/m^2).

M6620 was generally well tolerated as a single agent. There were no dose-limiting toxicities (DLTs), few adverse events (AEs) leading to study drug discontinuation, and 1 AE leading to death. Most subjects receiving single-agent M6620 had AEs. The System Organ Classes (SOCs) in which AEs occurred most frequently were Gastrointestinal Disorders and General Disorders and Administration Site Conditions. The most commonly occurring AE was nausea. The only AEs that occurred in the single-agent phases of all 4 study parts were nausea and ALT increased.

The SOCs in which serious adverse events (SAEs) occurred most frequently were Neoplasms Benign, Malignant, and Unspecified; Respiratory, Thoracic, and Mediastinal Disorders; and Gastrointestinal Disorders. SAEs that occurred in more than 1 subject receiving single-agent

M6620 were metastases to central nervous system, malignant neoplasm progression, dyspnea, nausea, and blood creatinine increased. Most of the SAEs were assessed as not related to study drug.

Table 3: Adverse Events That Occurred in At Least 15% of Subjects and At Least 2 Subjects in Any Single-agent Study Part by SOC and PT for Subjects Receiving M6620 Single-agent Therapy, Safety Set

	Study 001		Study 002	
	Part A	**Part B**	**Part A**	**Part B**
System Organ Class Preferred Term	(All Doses) Lead-in Phase Lead-in Safety Set N=30 n (%)	140 mg/m^2 Lead-in Safety Set N = 4 n (%)	(All Doses) Safety Set N = 17 n (%)	240 mg/m^2 Single-agent Safety Set N = 15 n (%)
Subjects with any AEs	18 (60.0)	3 (75.0)	17 (100.0)	14 (93.3)
Gastrointestinal Disorders				
Nausea	6 (20.0)	1 (25.0)	6 (35.3)	8 (53.3)
Diarrhea	2 (6.7)	0	4 (23.5)	4 (26.7)
Vomiting	2 (6.7)	0	4 (23.5)	2 (13.3)
General Disorders and Administration Site Conditions				
Fatigue	3 (10.0)	0	6 (35.3)	4 (26.7)
Infections and Infestations				
Urinary tract infection	1 (3.3)	0	4 (23.5)	1 (6.7)
Investigations				
ALT increased	1 (3.3)	1 (25.0)	3 (17.6)	1 (6.7)
Blood creatinine increased	0	0	3 (17.6)	1 (6.7)
Metabolism and Nutrition Disorders				
Hyperglycemia	0	1 (25.0)	3 (17.6)	0
Neoplasms Benign, Malignant and Unspecified (Including Cysts and Polyps)				
Cancer pain	0	0	3 (17.6)	0
Malignant neoplasm progression	0	0	3 (17.6)	1 (6.7)
Blood and Lymphatic System Disorders				
Anemia	0	0	5 (29.4)	3 (20.0)
Vascular Disorders				
Flushing	0	0	4 (23.5)	2 (13.3)
Nervous System Disorders				
Headache	2 (6.7)	0	3 (17.6)	2 (13.3)
Dizziness	2 (6.7)	0	0	3 (20.0)
Injury, Poisoning and Procedural Complications				

Table 3: Adverse Events That Occurred in At Least 15% of Subjects and At Least 2 Subjects in Any Single-agent Study Part by SOC and PT for Subjects Receiving M6620 Single-agent Therapy, Safety Set				
	Study 001		Study 002	
	Part A	Part B	Part A	Part B
Infusion-related reaction	0	0	2 (11.8)	5 (33.3)
Abbreviations: AE: adverse event; ALT: alanine aminotransferase; N: number of subjects in each treatment group; n: number of subjects in category; PT: preferred term; SOC: system organ class **Notes: Every subject is counted once for each applicable specific AE category within same treatment group when the AE occurred.**				

2.2.2.3 Adverse Drug Reactions

After careful assessment, infusion-related reactions, nausea, and vomiting are considered adverse drug reactions (ADRs) for M6620, and myelosuppression events are considered ADRs for M6620 in combination with carboplatin.

Infusion-related reactions are common with IV administration of drugs used to treat cancer. These reactions occur during or shortly after administration of the drug, may be local and/or systemic, and are diverse.

Systemic infusion-related reactions to M6620 may include signs or symptoms such as pruritus, flushing, chills/rigors, urticaria/rash, headache, bronchospasm/dyspnea, and hypotension or hypertension, among others. Some systemic infusion-related reactions to M6620 have been serious, including those described as acute hypersensitivity reactions. Local infusion-related reactions to M6620, sometimes described as infusion site reactions, may include signs or symptoms such as infusion site erythema, swelling, or pain.

Nausea and vomiting have occurred commonly in patients receiving M6620 monotherapy. Many of the affected subjects experienced these events on the same day M6620 was administered, and there was some suggestion of a dose response.

Myelosuppression AEs for subjects who received M6620 in combination with carboplatin have included neutropenia, thrombocytopenia, platelet count decreased, and febrile neutropenia.

2.2.2.4 Pharmacodynamics

Paired M6620 tumor biopsies were obtained pre- and post-M6620 administration from a subset of subjects in study 002 Part B, and pS345 Chk1 levels assessed by IHC. A preliminary analysis of the data showed decreased Chk1 phosphorylation in 2/2 paired tumor biopsies (74% at M6620 90 mg/m^2 + carboplatin AUC5; 94% at M6620 120 mg/m^2 + carboplatin AUC5).

2.2.2.5 Single Agent Efficacy

Subjects in phase I study 002 Part A received single-agent M6620 therapy. One subject with colorectal cancer had a complete response (CR). The subject's tumor lesion diameter decreased from 18 mm at Screening to "too small to measure" at Cycle 31. This subject has completed 44 cycles of M6620 therapy and continues to be on treatment at the time of the data cut-off. Five of 17 subjects had stable disease (SD) as the best overall response.

2.2.2.6 Rationale for Translational Lead-in Study

The development of M6620 has largely been focused on optimizing dosing for chemotherapy combination studies (see **Section 2.2.2.1** VX12-970-001 and Study VX13-970-002). Patients included in the above studies were not pre-selected for biomarkers predicted to be sensitive to ATR inhibition. Moreover, it is unknown whether current dosing strategies (twice per week) are adequate to durably inhibit ATR without the stressor of concurrent chemotherapy. Interestingly, of note, the patient with a complete response described in **Section 2.2.2.5** was an ATM-mutant colorectal cancer patient treated at the first dose level (60 mg/m^2 once per week)[13]. With this background, we designed a Translational Lead-in Study to confirm target engagement, and potentially to observe clinical responses, in the indications where we anticipate the highest likelihood for response. This strategy will be critical for dosing confirmation prior to embarking on a larger basket-type study.

2.2.2.7 Rationale for ctDNA Sample Collection

C-circle assay (an assay of telomeric repeats, which proportionally reflects the average length of telomeres, is measured by quantitative PCR [qPCR])[19]) on T1 fresh biopsy samples and circulating tumor DNA (ctDNA), only.

2.3 Correlative Studies Background

It is anticipated that at the time of enrollment most participants on this study will have had their tumor genomically characterized by either the OncoPanel test at DFCI/BWH (a custom hybrid capture panel which via NGS will identify mutations and copy-number alterations in over 400 cancer associated genes) or via another CLIA-certified method (e.g. SNaPshot at MGH or Foundation Medicine). Gene expression profiling has been demonstrated to predict disease-free and overall survival in multiple cancers and guide future treatment decisions and biologic discovery[14-16].

Current methods for identifying ALT positive tumors by direct measurement of telomere length are best suited to testing cell lines or frozen tissue rather than paraffin embedded clinical samples. DNA testing for ATRX can be performed on clinical samples but this only identifies a subset of ALT cases associated with mutations in this gene[17]. The same limitation applies to tests based on the loss of ATRX staining by IHC. Though ATRX sequencing will be used for Translational Lead-in study entry, given the strong correlation to ALT status, we recognize that there are other ALT positive tumors that will be missed with this assay. Thus, there is interest in

developing other methods to detect ALT status. The laboratory of Miguel Rivera MD, PhD at MGH has extensive experience using RNA-ISH via a branched-DNA amplification to detect novel biomarkers in paraffin embedded clinical samples[18,19]. Moreover, as noted above, TERRA expression persists throughout the cell cycle in ALT positive cells. To this end, the Rivera laboratory is developing a RNA-ISH assay to detect high levels of TERRA and thereby identify ALT positive malignancies. For all T1, 1A and 1B FFPE samples on study, ALT status will be determined by TERRA ISH, with confirmatory testing by the C-circle assay (an assay of telomeric repeats, which proportionally reflects the average length of telomeres, is measured by quantitative PCR [qPCR])[20]) on T1 fresh biopsy samples, only.

To explore the determinants of response and resistance to M6620, biopsy tissue will be collected from all participants enrolling to the translational lead-in study at baseline and on treatment. IHC will be performed for selected biomarkers including γH2AX (a marker of dsDNA breaks), phosph-CHK1 (downstream of ATR), and possibly also ATM, RAD51, MYC, Cyclin E, and ARID1A as well as others as determined appropriate at the time of analysis. Results from tissue analysis will be used to confirm downstream target engagement (increased γH2AX), altered expression of proteins of interest by IHC and to confirm amplifications by FISH.

An optional biopsy at the time of disease progression will also be offered to all participants enrolling to the translational lead-in study.

The baseline fresh tissue obtained from participants will also undergo whole exome sequencing (WES) at an outside facility. WES enables a comprehensive analysis of DNA mutation in human tumor samples. Gene expression profiling has been demonstrated to predict disease-free and overall survival in multiple cancers and guide future treatment decisions and biologic discovery[14-16]. The analyses will involve up to 100 genes of interest in DNA repair. This will allow identification of further genomic variations that may occur outside of the pre-set enrollment genetic alterations of these patients. RNA-sequencing will also be performed on selected biopsy specimens based on the results of the WES and clinical outcome on trial. RNA-sequencing allows for monitoring of gene expression and transcriptome changes, giving the complete HR state of the tumor. WES will be performed again on any time of progression samples to assess genomic determinants of resistance. The data obtained via WES and RNA-sequencing will enable us to identify secondary alterations that may impact clinical response.

Additional specimens collected for clinical reasons (e.g. biopsies, effusion/ascites fluid) may be collected for correlative studies, if available.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants Enrolling to the Translational Lead-in Study:

3.1.1 For enrollment to cohort T1: participants must have metastatic or progressive LMS with a) treatment with at least one prior systemic therapy b) ATRX mutation by NGS.

3.1.2 For enrollment to cohorts T2 or T3: participants must have a histologically or cytologically confirmed advanced solid tumor for which no standard curative therapy is available.

3.1.3 For enrollment to cohort T2: participants must have a truncating *ATM* mutation. Other ATM mutations with sufficient evidence of gene inactivation may be considered eligible after review (see section 9.4). Testing may be completed via the DFCI/BWH OncoPanel, MGH SNaPshot, or any other CLIA-certified method.

3.1.4 For enrollment to cohort T3, participants must have one of the following (testing may be completed via the DFCI/BWH OncoPanel, MGH SNaPshot, or any CLIA-certified laboratory):

a) Germline mutation in *BRCA1* or *BRCA2* that is predicted to be deleterious or suspected deleterious (known or predicted to be detrimental/lead to loss of function).
b) A somatic mutation in *BRCA1* or *BRCA2*, or another mutation within a known HR gene including *BARD1, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD51B, RAD51C*, or *RAD51D*.
c) A *MYC* amplification of greater than 6-fold.
d) *FBXW7* truncating, missense, or other inactivating mutation designated as deleterious. Other deleterious mutations may be considered after discussion with the overall PI.
e) *CCNE1* amplification of greater than 8-fold.
f) An *ARID1A* mutation as determined by the DFCI/BWH OncoPanel or any other CLIA-certified method.
g) Other (unlisted) mutations within known HR genes may be considered with approval from the site principal investigator.

3.1.5 For enrollment to cohort T4: participants must have GIST with known mutation in SDHX genes or loss of expression of SDHX protein(s), as determined by standard pathology assays. Prior therapy is not required.

3.1.6 Age ≥ 18 years for T1, T2, and T3; Age ≥ 12 years for T4

3.1.7 ECOG performance status ≤ 2 (Karnofsky [KPS] ≥ 60%; KPS of 50 is not permitted) (See **Appendix A**)

3.1.8 For T1, T2, and T3, participants must have tumor amenable to biopsy, and be a candidate for tumor biopsy according to the treating investigator. The patient must be willing to undergo a fresh tumor biopsy for this study. If the biopsy is deemed unsafe, the patient may enroll with the permission of the overall PI (though the patient will not count towards accrual numbers, see section 9.2)

3.1.9 Participants must have archival tissue available for analysis. Participants without available archival tissue enrolling to the translational lead-in study may instead use tissue from the fresh pre-treatment biopsy.

Participants Enrolling to the Phase II:

3.1.10 For enrollment to cohort 1A: participants must have metastatic or progressive osteosarcoma treated with at least one prior systemic therapy.

3.1.11 For enrollment to cohort 1B: participants must have metastatic or progressive leiomyosarcoma treated with at least one prior systemic therapy.

3.1.12 For enrollment to cohorts 2 – 5: participants must have a histologically or cytologically confirmed advanced solid tumor for which no standard curative therapy is available.

3.1.13 For enrollment to cohort 2: participants must have a truncating *ATM* mutation. Other ATM mutations with sufficient evidence of gene inactivation may be considered eligible (see section 9.4). Testing may be completed via the DFCI/BWH OncoPanel, MGH SNaPshot, or any other CLIA-certified method.

3.1.14 For enrollment to cohort 3A: participants must have a germline mutation in *BRCA1* or *BRCA2* that is predicted to be deleterious or suspected deleterious (known or predicted to be detrimental/lead to loss of function). Testing may be completed via the DFCI/BWH OncoPanel, MGH SNaPshot, or any CLIA-certified laboratory.

3.1.15 For enrollment to cohort 3B: participants must have a somatic mutation in *BRCA1* or *BRCA2*, or another mutation within a known HR gene including *BARD1, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD51B, RAD51C*, or R*AD51D*. Other (unlisted) mutations within known HR genes may be considered with approval from the site principal investigator.

3.1.16 For enrollment to cohort 4A: participants must have a *MYC* amplification of greater than 6-fold or an *FBXW7* truncating or missense mutation designated as deleterious, as determined by the DFCI/BWH OncoPanel, MGH SNaPshot, or any other CLIA-certified method.

3.1.17 For enrollment to cohort 4B: participants must have a *CCNE1* amplification of greater than 8-fold as determined by the DFCI/BWH OncoPanel, MGH SNaPshot, or any other CLIA-certified method.

3.1.18 For enrollment to cohort 5: participants must have an *ARID1A* mutation as determined by the DFCI/BWH OncoPanel, MGH SNaPshot, or any other CLIA-certified method.

3.1.19 For enrollment to cohort 1A: Age > 12

3.1.20 For enrollment to cohorts 1B – 5: Age ≥ 18

3.1.21 ECOG performance status ≤ 2 (Karnofsky ≥ 60%; KPS of 50 is not permitted) for participants ≥ 16 years of age, Lansky ≥ 50% for participants < 16 years of age (see **Appendix A**)

3.1.22 Participants must have archival tissue available for analysis.

All Participants:

3.1.23 If any participant could enroll to more than one cohort based on mutational status, the cohort enrollment decision will be discussed with the overall principal investigator. For example, if a participant has both an *ATM* mutation and an *FBXW7* mutation and thus could enroll to either Cohort 2 or Cohort 4A, the decision of which cohort to enroll to will be made in conjunction with the overall principal investigator. The decision as to which cohort the participant should be enrolled on must be made prior to the initiation of study treatment.

3.1.24 Participants must have RECIST 1.1 measurable disease. See **Section 11** for the evaluation of measurable disease.

3.1.25 Participants must have normal organ and marrow function as defined below:

All Participants:

– Absolute neutrophil count	≥ 1,500/mcL
– Platelets	≥ 100,000/mcL
– Total bilirubin	≤ 1.5 × institutional upper limit of normal (ULN) for age

Adult Participants (Age ≥ 18 years):

– AST(SGOT)/ALT(SGPT)	≤ 2.5 × institutional ULN; **OR** ≤ 5 × institutional ULN if liver metastases are present
– Serum or plasma creatinine	≤ 1.5 × institutional ULN, **OR**
– Creatinine clearance	≥ 60 mL/min by Cockcroft-Gault equation for participants with creatinine levels above 1.5 × institutional ULN

Pediatric Participants (Age < 18 years):

– ALT (SGPT)	≤ 3 × institutional (ULN) Note: for the purposes of this study, the ULN for SGPT will be defined as 45 U/L.
– Serum or plasma creatinine	Participants 13 – 15 years: males ≤ 1.5 mg/dL, females ≤ 1.4 mg/dL, participants 16 – 17 years: males ≤ 1.7 mg/dL, females ≤ 1.4 mg/dL; **OR**
– Creatinine clearance	≥ 60 mL/min/1.73 m^2 by Schwartz formula for participants with creatinine levels above the limits described above

3.1.26 The effects of M6620 on the developing human fetus are unknown. For this reason and because anti-cancer agents are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 6 months after completion of M6620 administration.

3.1.27 Ability to understand and the willingness to sign a written informed consent document (or parent or legally authorized representative, if minor).

3.2 Exclusion Criteria (All Participants)

3.2.1 Participants who have had chemotherapy, immune therapy, other investigational therapy, or major surgery within 3 weeks (2 weeks for radiotherapy; 6 weeks for nitrosoureas or mitomycin C) prior to entering the study, or participants who have not recovered to ≤ Grade 1 or baseline from therapies administered more than 3 weeks prior (with the exceptions of 1. Alopecia and peripheral neuropathies which may be ≤ Grade 2 at study entry and 2. Laboratory abnormalities that are not listed in 3.1 and that are not considered clinically meaningful [e.g. decreased lymphocyte count, electrolyte abnormalities)]

3.2.2 Participants who have received oral tyrosine kinase inhibitors (TKIs) within 5 half-lives of study entry.

3.2.3 Participants who have previously received treatment with an ATR inhibitor, including but not limited to M6620 (VX-970).

3.2.4 Participants with known untreated brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Participants with a history of brain metastases that have been treated, no longer require corticosteroids, and have been stable on imaging for at least one month following the end of treatment are permitted.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to M6620.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Pregnant women are excluded from this study because M6620 is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with M6620, breastfeeding should be discontinued if the mother is treated with M6620.

3.2.8 Known HIV-positive participants are ineligible because of the increased risk of lethal infections when treated with potentially marrow-suppressive therapy.

3.2.9 Patients with a history of another malignancy are excluded with the exception of 1. patients who remain disease-free for 3 years and 2. adequately treated cervical carcinoma in situ, non-melanoma skin cancer (e.g. basal cell, squamous cell carcinomas), low-risk thyroid cancer.

3.2.10 Patients with Li Fraumeni syndrome or ataxia telangiectasia syndrome

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

Enrollment will begin with the translational lead-in study (cohorts T1, T2, T3, and T4, please refer to **Table 1**). Participants may be enrolled to any of the cohorts in parallel with no observation period or delay between the initiation of treatment for any of the patients. When enrollment to the translational study cohorts has been completed, a review of available safety, efficacy, pharmacodynamic, and pharmacokinetic data will occur prior to the initiation of the phase II trial. The review will involve the overall principal investigator in conjunction with the site principal investigators, any applicable study team members, as well as consultation with Merck KGaA (EMD Serono) as appropriate.

5.1 Treatment Regimen

M6620 will be administered to all participants twice weekly on cycle days 1, 4, 8, 11, 15, 18, 22 and 25 via intravenous (IV) infusion at a dose of 240 mg/m^2, with 28 consecutive days defined as a treatment cycle. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 7**. Appropriate dose modifications are described in **Section 6**.

Table 4: Regimen Description					
Agent	**Precautions**	**Dose[1]**	**Route**	**Schedule[2]**	**Cycle Length**
M6620 (VX-970)	To be administered through a large bore catheter into a large caliber peripheral vein. Monitor for phlebitis or signs of inflammation. Alternatively, may be infused via a central line or implanted venous access system (i.e. Port-a-Cath®). M6620 should not come in contact with sodium chloride due to incompatability; 5% dextrose in water must be used for IV line priming and flushing.	240 mg/m^2	IV over 60 minutes (± 10-minute infusion window)	Days 1,4, 8, 11, 15, 18, 22, 25	28 days (4 weeks)
1. Body surface area (BSA) to be calculated per institutional standards of practice. In the event of changes in participant weight, institutional standards of practice should be followed for dose re-calculations. 2. After 16 weeks participants may consider a weekly dosing schedule on cycle days 1, 8, 15, and 22 after discussion with the site principal investigator.					

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

If screening assessments were completed ≤ 72 hours prior to cycle 1 day 1, laboratory tests do not need to be repeated on cycle 1 day 1 and the screening laboratory values can be used as the cycle 1 day 1 values. If cycle 1 day 1 laboratories are performed, the values must re-meet eligibility criteria.

5.2.2 Subsequent Cycles

Please refer to **Section 6** for toxicity management guidelines.

5.3 Agent Administration

5.3.1 M6620 (VX-970)

M6620 will be infused IV twice weekly on cycle days 1, 4, 8, 11, 15, 18, 22, and 25 via a large

bore catheter into a large caliber peripheral vein. Alternatively, M6620 may be infused via a central line or implanted venous access system (i.e. Port-a-Cath®). After 16 weeks participants may consider a weekly dosing schedule on cycle days 1, 8, 15, and 22 after discussion with the site principal investigator. The infusion should be delivered over 60 minutes, there is an allowable ± 10 minute infusion window. When the total volume of infusion exceeds 600 mL, the infusion time maybe be extended beyond 60 minutes (as tolerated), but no more than 90 minutes. Infuse using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micron filter. M6620 should not come in contact with sodium chloride, 5% dextrose in water must be used for IV line priming and flushing.

Body surface area (BSA) to be calculated per institutional standards of practice. In the event of changes in participant weight, institutional standards of practice should be followed for dose re-calculations.

5.3.2 Infusion-Related Reactions

M6620 is associated with infusion-related reactions. Systemic infusion-related reactions to M6620 may include signs or symptoms such as pruritus, flushing, chills/rigors, urticaria/rash, headache, bronchospasm/dyspnea, hypotension or hypertension, and mental status changes, among others. Some systemic infusion-related reactions to M6620 have been serious. In almost all cases, these reactions occurred within minutes of the second exposure to M6620. All subjects have fully recovered with standard treatment for the reactions, including immediate discontinuation of the inciting infusion and administration of IV corticosteroid and antihistamine, as well as IV fluids and oxygen when clinically indicated.

If any subject develops pruritus, flushing, or any other symptom suggestive of a systemic infusion reaction, standard measures may be employed to manage these symptoms (e.g., antihistamines and/or steroids).

In the event of the occurrence of any Grade 2 or greater infusion-related reaction, the M6620 infusion should be immediately stopped. Participants should be treated in accordance with institutional standards of practice. In the event of a Grade 2 infusion-related reaction, the infusion may be resumed at the treating investigator's discretion upon resolution of symptoms to baseline. If symptoms recur following re-initiation, the infusion should be discontinued. In the event of any Grade 3 or greater infusion-related reaction, protocol therapy should be permanently discontinued.

If a participant experienced a prior Grade 1 or Grade 2 infusion-related reaction, at the treating investigator's discretion the participant may be pre-medicated prior to subsequent infusions. Standard desensitizing measures should be followed (e.g. premedication with 200 mg hydrocortisone or equivalent approximately 60 minutes prior to the infusion, and 25-50 mg of diphenhydramine hydrochloride or equivalent approximately 30 minutes prior to the infusion). At the treating investigator's discretion, the rate of the infusion may be extended beyond 60 minutes, but may not exceed 90 minutes.

5.4 General Concomitant Medication and Supportive Care Guidelines

No investigational or commercial agents or therapies other than M6620 may be administered with the intent to treat the participant's malignancy, with the exception of palliative radiation therapy with the site principal investigator's agreement. M6620 should be held during palliative radiation therapy. Bisphosphonate or denosumab use is permitted. Participants with prostate cancer may continue androgen deprivation therapy, including luteinizing hormone-releasing hormone LHRH-analogues, as long as the therapy was initiated prior to study entry.

Patients should be monitored during M6620 (VX-970) infusion for acute hypersensitivity or severe infusion reactions, which may include loss of consciousness and/or hemodynamic instability, including hypotension. In the event of one of these reactions, the infusion should be stopped immediately, and standard supportive measures should be applied according to the presentation. This may include administration of fluids and epinephrine.

Investigators should use appropriate supportive medications to address toxicities that arise during the study, including but not limited to anti-emetics, anti-diarrheals, and blood product transfusion. Use of colony-stimulating factors (CSFs) should be avoided when possible. Investigators may use CSFs as medically necessary to address a current episode of neutropenia. Primary prophylactic use of CSFs is prohibited. Secondary prophylactic use of CSFs per ASCO guidelines following an instance of a neutropenic episode experienced while on trial will be allowed at the treating investigator's discretion.

M6620 absorbs in the UV-visible radiation spectrum and is widely distributed to tissues, including the skin, so subjects should be cautioned to minimize exposure to the sun and other sources of visible and UV radiation when possible. Participants should be advised to take protective measures when necessary, including wearing a broad-spectrum sunscreen with high SPF and protective clothing.

5.4.1 Drug-Drug Interactions

The potential for M6620 to induce CYP450 enzymes is low. M6620 is not anticipated to elicit clinically significant drug-drug interactions from reversible or time-dependent inhibition or induction pathways, however the drug interaction profile of M6620 has not been fully characterized. Caution should be used when co-administering medications with M6620. As M6620 is primarily metabolized by CYP3A4, concomitant administration with potent inhibitors or inducers of CYP3A4 should be avoided when medically feasible.

The following list of potent CYP3A4 inhibitors and inducers is not exhaustive:

- Potent CYP3A4 inhibitors: clarithromycin, itraconazole, ketoconazole, hepatitis C virus and human immunodeficiency virus protease inhibitors, nefazodone, posaconazole, telithromycin, voriconazole
- Potent CYP3A4 inducers: carbamazepine, rifampin, phenobarbital, phenytoin, St. John's wort

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression, unless the participant is exhibiting clinical benefit as determined by the treating investigator
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

The research team will update the relevant Off Treatment information in OnCore.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Gregory M. Cote MD, PhD at 617-724-4000.

5.6 Duration of Follow Up

Participants will be followed for 30 days after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death
- Completion of the 30 day follow up period after study drug discontinuation, or resolution or stabilization of any unacceptable adverse events (whichever is longer)

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

The research team will update the relevant Off Study information in OnCore.

Patients who require replacement and otherwise do not meet 5.5 Criteria for Taking a Participant Off Protocol Therapy and/or 5.7 Criteria for Taking a Participant Off Study may continue on study protocol.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Dose Delays

The study agent may be held for a maximum of 28 days to allow for recovery of toxicity. Participants requiring a longer delay should be removed from protocol therapy. Participants who are deriving clinical benefit who require a longer hold may be allowed to continue treatment following discussion and agreement with the overall principal investigator.

If a participant's dosing is held, the counting of cycle days and assessment schedule will continue without interruption. For example, a participant who does not receive their Cycle 1 Day 8 infusion due to toxicity may restart dosing on Cycle 1 Day 11 and proceed with their next scheduled visit as previously planned (Cycle 1 Day 11). Interim clinic visits may be conducted as clinically necessary to manage toxicity; however, the cycle will not restart due to adverse events and held infusions will not be administered or "made up" following toxicity resolution. Assessments during drug holds (laboratory values, vitals signs, exam, etc.) are only required as clinically indicated and will not be counted as protocol violations if not performed.

6.2 Dose Modifications

Dose reductions may be made in accordance with the tables below. Dose reductions below 120 mg/m^2 will not be permitted. Participants requiring dose reductions below 120 mg/m^2 should be discontinued from protocol therapy. Once a participant's dose has been reduced it may be re-escalated to a previous dose level after discussion with the site principal investigator.

Table 5: M6620 Dose Reductions (Twice Weekly Dosing)

Dose Level	M6620 Dose (IV on days 1, 4, 8, 11, 15, 18, 22, and 25)
-1	210 mg/m^2
-2	165 mg/m^2
-3	120 mg/m^2

Participants who have transitioned to a weekly dosing schedule will dose reduce based on the

table below:

Table 6: M6620 Dose Reductions (Once Weekly Dosing)	
Dose Level	**M6620 Dose (IV on days 1, 8, 15, and 22)**
-1	210 mg/m^2
-2	165 mg/m^2
-3	120 mg/m^2

6.3 Toxicity Management Guidelines

Management of toxicity considered at least possibly related to the study agent is detailed in the tables below.

Table 7: Management of Non-Hematological Toxicity		
Event Term	**CTCAE Grade**	**Management Guidelines**
Infusion Related Reaction	Grade 1	Monitor participant for worsening signs or symptoms. If symptoms worsen, follow the guidelines for the appropriate higher grade reaction. At the treating investigator's discretion, the infusion may be paused or discontinued. Consider pre-medication and/or slowing of the infusion rate for subsequent infusions if clinically appropriate as determined by the treating investigator (see **Section 5.3.2**).
Infusion Related Reaction	Grade 2	Stop infusion. Administer appropriate supportive care as per local standards of practice. At the treating investigator's discretion, the infusion may be re-started when symptoms resolve to baseline, or the infusion may be discontinued. If symptoms recur following restart, the infusion should be discontinued. Consider pre-medication and/or slowing of the infusion rate for subsequent infusions if clinically appropriate as determined by the treating investigator (see **Section 5.3.2**). At the treating investigator's discretion, study agent dose may be reduced for subsequent infusions by one dose level.
Infusion Related Reaction	≥ Grade 3	Permanently discontinue protocol therapy. Administer appropriate supportive care as per local standards of practice.
Any Other Non-Hematologic Toxicity	≥ 3[A]	Hold study medication dosing. Upon resolution to ≤ Grade 2 or baseline, resume dosing with one dose level reduction. Exceptions:

Table 7: Management of Non-Hematological Toxicity		
Event Term	**CTCAE Grade**	**Management Guidelines**
		• Grade 3 nausea, vomiting, diarrhea, or constipation that was not optimally managed. In this instance, hold study medication dosing and initiate appropriate medical management (e.g. anti-emetics, anti-diarrheals, laxatives). If symptoms resolve to ≤ Grade 1 or baseline within 48 hours of instituting appropriate care, participants may resume dosing at the same dose level or may be dose reduced by one dose level at the treating investigator's discretion. • Laboratory abnormalities that are judged by the treating investigator to be not clinically significant. Participants may continue dosing at the same dose level as judged appropriate by the treating investigator. • Asymptomatic laboratory abnormalities that resolve to ≤ Grade 1 or baseline within 48 hours of repletion. Study medication should be held until abnormalities resolve to ≤ Grade 1 or baseline and may be resumed at the same dose level as judged appropriate by the treating investigator. Repletion and resumption of study treatment may occur on the same day.
A. Participants experiencing intolerable Grade 2 events may have their study medication held and/or dose reduced at the treating investigator's discretion.		

Table 8: Management of Hematological Toxicity		
Event Term	**CTCAE Grade**	**Management Guidelines**
Neutrophil Count Decreased	≥ Grade 3 (not associated with fever/infection)	1st Occurrence: Hold study medication dosing until resolution to ≤ Grade 2 or baseline. Upon resolution, may resume at the same dose level at the treating investigator's discretion, or may reduce dose by one dose level. Repeat Occurrence: Hold study medication dosing until resolution to ≤ Grade 2. Upon resolution, reduce dose by one dose level.
Febrile Neutropenia	≥ Grade 3	Hold study medication dosing until resolution. Upon resolution, reduce dose by one dose level.
Platelet Count Decreased	Grade 3 (not associated with clinically significant	Hold study medication dosing until resolution to ≤ Grade 2 or baseline. Upon resolution, may resume at the same dose level at the treating investigator's discretion, or may reduce by one dose level.

Table 8: Management of Hematological Toxicity		
Event Term	**CTCAE Grade**	**Management Guidelines**
	bleeding)	
Platelet Count Decreased	Grade 4; Grade 3 (associated with clinically significant bleeding)	Hold study medication dosing until resolution to ≤ Grade 2 or baseline. Upon resolution, reduce dose by one dose level.
Anemia	Grade 4[A]	Hold study medication dosing until resolution to ≤ Grade 2 or baseline. Upon resolution, reduce dose by one dose level.
A. Participants experiencing Grade 3 anemia may have their study medication dosing held and/or dose reduced at the treating investigator's discretion.		

6.4 Overdose

There is currently no specific treatment in the event of overdose with M6620 and possible symptoms of overdose are not established. Adverse reactions associated with overdose should be treated symptomatically and should be managed appropriately.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event monitoring and reporting is a routine part of every clinical trial. The list of Expected Toxicities (**Section 7.2)** and the definitions and characteristics of an observed AE (**Section 7.1** and **Section 7.3**) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Definitions

7.1.1 Adverse Event ("AE")

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

7.1.2 Special Considerations

- Use of a medicinal product during pregnancy or breastfeeding: reports where embryo, fetus or child may have been exposed to medicinal products (either through maternal

exposure or transmission of a medicinal product via semen following paternal exposure),
- Reports of medication errors, uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Special Considerations will be described in the Final Study Report or any interim reports, as applicable.

7.1.3 **Serious Adverse Events** ("SAE" or "SAEs")

A SAE is any AE as defined above, which also fulfills at least one of the seriousness criteria below:
- results in death,
- is life-threatening[1)],
- requires in-patient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/ incapacity,
- is a congenital anomaly/ birth defect, or
- is otherwise considered as medically important[2)].

1) Life-threatening in this context refers to a reaction in which the patient was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might have caused death if more severe.

2) Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of dependency or abuse.

7.1.4 **Safety Issues/Concerns**

A safety issue/concern is defined as an important identified risk or important potential risk. Safety issues/concerns are events which may occur during the Study, not falling under the definition of SUSARs and consequently not being subject of the reporting requirements for SUSARs. Examples are events related to the conduct of the Study or the development of an investigational medicinal product likely to affect the safety of subjects, such as (but not limited to):
- a major safety finding from a newly completed animal study (such as carcinogenicity),
- recommendations of the DSMB, if any, where relevant for the safety of subjects,

7.1.5 Events not to be considered AEs

Medical conditions present in a patient and documented at the time of Study enrolment, and that do not worsen in severity or frequency during the Study and are defined as baseline medical conditions; are NOT to be considered AEs.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

7.1.6 Events not to be considered SAEs

Previously planned (prior to signing the informed consent form) surgeries, and non-disease related elective surgeries planned during the course of the study, should not be reported as SAEs unless the underlying medical condition has worsened or appeared during the course of the study.

Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (e.g., for the administration of study therapy or other protocol-required procedure) should not be considered SAEs.

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

Death due to disease progression should not be reported as an SAE unless the investigator deems it to be related to the use of study drug.

7.2 Expected Toxicities

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI, Funder or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form). All the adverse drug reactions (ADRs) described in this section are assessed as expected events for regulatory reporting purposes. Please refer to the investigator's brochure for M6620 for the most up-to-date information.

- Infusion related reactions (including flushing, pruritus, rash, rash erythematous, rash macular, rash pruritic, rhinitis allergic, erythema, eyelid edema, facial edema, and hypersensitivity)
- Infusion site reactions (including infusion site erythema, catheter site pruritus, catheter site related reaction, infusion site extravasation, catheter site pain, catheter site rash, infusion site discomfort, infusion site pruritus, infusion site rash, injection site rash, and injection site reaction, catheter site inflammation, infusion site swelling)

- Nausea
- Vomiting

AEs for M6620 that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided above or in the current Investigator's Drug Brochure.

7.3 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Attribution of the AE for Reporting Purposes:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.4 Expedited Reporting Requirements

7.4.1 Expedited Reporting Guidelines to the Overall Principal Investigator (PI)

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form. All events meeting SAE definition as defined in section 7.1.3 should be reported, regardless of attribution.

7.4.2 DF/HCC Expedited Reporting Guidelines to the DFCI IRB

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.4.3 Expedited Reporting to the Funder (Merck KGaA, Darmstadt, Germany (EMD Serono)

Institution shall ensure that information on any AE is recorded comprehensively and maintain high quality at all times. Institution shall perform a causality assessment of each recorded AE. For recording in the safety database, all SAEs (related and non-related, including *all* fatal outcomes), Special Considerations and Safety Issues/Concerns shall be sent to Funder in English language.

Participating Investigators must report SAE's that meet the SAE definition as defined in section 7.1.3 to the Funder. Only SAE's occurring after the initial dose of study treatment will be reported. The information shall be sent via facsimile or the email listed below within one (1) business day or three (3) calendar days, whatever comes first, after becoming aware of the event using Medwatch form 3500 A.

Fax No.: +49-6151-72 6914
Email address:ICSR_CT_GPS@merckgroup.com

A copy of this report must also be submitted to the Overall PI.

7.4.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.4.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.4.6 Expedited Reporting of Pregnancies

Investigators must report to the Overall PI any pregnancy occurring in a subject exposed to the Investigational Product (either through maternal exposure or transmission of the Product via semen following paternal exposure to the Product) during the course of the Study.

The Overall PI will inform the Funder of all reported pregnancies and will ensure that the case is followed up to the end of the pregnancy and provide all relevant documentation and a final report on the outcome to Funder.

7.5 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions to the Overall PI on the toxicity case report forms. AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.

7.6 Routine Reporting to the Funder (Merck KGaA, Darmstadt, Germany (EMD Serono)

7.6.1 Global Patient Safety Reporting

The Overall PI shall provide Funder's Global Patient Safety department on a quarterly basis with information on AEs, safety laboratory data, vital signs and any efficacy data necessary to assess and monitor the safety of the Investigational Product

7.6.2 **Reconciliation**

To ensure completeness and accuracy of the transfer of safety events to Funder, Overall PI shall perform a reconciliation of all safety events occurred during the Study versus Funder's Global Safety Database prior to closing the Study database. Funder shall provide the excerpt of its Global Safety Database to Institution in a timely manner.

7.6.3 **Periodic Reports**

The Overall PI shall provide Funder in a timely manner with a copy of any periodic report sent to health authorities or Ethics Committees.

7.6.4 **Coding of Safety Data**

Overall PI shall use accepted international standard definitions for severity, seriousness, the Medical Dictionary for Regulatory Activities (MedDRA) terminology (last version) for coding purposes. Furthermore, the related MedDRA coding conventions 'Points to Consider' published by the Maintenance and Support Services Organization shall be applied by Institution for coding of event data.

7.6.5 **Safety Data Monitoring Committee**

Overall PI shall evaluate the set-up of a safety data monitoring committee to ensure the safety of the participants and the validity and integrity of safety data generated from the study according to applicable Guidelines on Data Monitoring Committees.

7.6.6 **SUSARS**

Institution is responsible for assessment of Suspected Unexpected Serious Adverse Reactions ("SUSARs"). For expedited reporting only Institution's assessment of SUSARs shall be used.

7.6.7 **Signal Detection**

Funder will perform regular signal detection on the Investigational Product in its global safety database. In case of any action arising from such signal detection activities which is relevant for the conduct of the Study, Funder will inform the Overall PI in a timely manner.

Funder will perform a medical assessment for the purpose of signal detection and cumulative reporting of safety information and will also request follow-up information from the Overall Principal Investigator as needed. In case of any action arising from such signal detection activities which is relevant for the conduct of the Study, Funder will inform the Overall Principal Investigator in a timely manner.

7.6.8 Study Report

Complete data on AEs (including Special Considerations) shall be collected and provided to Funder at the end of the Study in the Final Study Report, and on special request from Funder during the course of the Study. The safety data sets shall be provided in an internationally accepted format which allows integration into the Global Safety Database of Funder.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational product administered in this study can be found in **Section 7.2**.

8.1 M6620 (VX-970)

8.1.1 Description

The active drug substance can be supplied as the hydrochloride salt or as the free base form.

Table 99: Hydrochloride Drug Substance

Code Name	M6620, VRT-0768079, VX-970
Molecular Formula	$C_{24}H_{26}ClN_5O_3S$
Molecular Weight	500.01 Da
Appearance	Yellow powder
Melting Point	295˚C
Solubility in Water	3.2 mg/mL @ pH 6.22
pKa	9.15 (base), 1.07 (base)

Table 1010: Free Base Drug Substance

Code Name	M6620, VRT-0768079, VX-970
Molecular Formula	$C_{24}H_{25}N_5O_3S$
Molecular Weight	463.55 Da
Appearance	Yellow powder
Melting Point	211˚C
Solubility in Water	0.1 mg/mL @ pH 6.71
Solubility in 1-butanol	0.01 mg/mL
Solubility in water saturated 1-butanol	2.0 mg/mL
pKa	9.15 (base), 1.07 (base)

8.1.2 Form

M6620 is a sterile solution intended for IV administration. It is presented at a concentration of 20mg/mL in a 10mL single use glass vial.

8.1.3 **Storage and Stability**

Single-use, sterile, light-protected vials of M6620 should be stored at room temperature (15°C to 30°C).

Diluted IV solution (IV bags) should be covered to protect from light and stored in the dark for a maximum of 24 hours at refrigerated conditions (2°C to 8ºC). Diluted solution is stable at ambient and refrigerated conditions for 24 hours.

8.1.4 **Compatibility**

No other medications should be administered via the same IV infusion line. M6620 is not compatible with sodium chloride, 5% dextrose in water (D5W) should be used to prime and flush the IV infusion line.

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

M6620 is an investigational product that will be supplied by Merck KGaA, Darmstadt, Germany (EMD Serono).

8.1.7 **Preparation**

M6620 solution for injection must be diluted with 5% dextrose in water solution prior to administration. Do not use 0.9% Sodium Chloride due to incompatibility with M6620.

To prepare the infusion solution add the dose volume of M6620 to a non-polyvinyl chloride (non-PVC), di(2-ethylhexyl) phthalate (DEHP)-free EVA infusion bag containing 5% dextrose in water. Gently invert the IV bag 5-10 times to mix the solution.

Confirm the solution is clear and free of precipitates and/or particulates. The final concentration must be between 0.075 mg/mL to 1 mg/mL. Place the IV bag into an opaque cover to protect from light.

8.1.8 **Administration**

M6620 will be infused IV twice weekly on cycle days 1, 4, 8, 11, 15, 18, 22, and 25 via a large bore catheter into a large caliber peripheral vein. Alternatively, M6620 may be infused via a central line or implanted venous access system (i.e. Port-a-Cath®). The infusion should be delivered over 60 minutes, there is an allowable ± 10 minute infusion

window. When the total volume of infusion exceeds 600 mL, the infusion time maybe be extended beyond 60 minutes (as tolerated), but no more than 90 minutes. Infuse using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micron filter. M6620 should not come in contact with sodium chloride, 5% dextrose in water must be used for IV line priming and flushing.

Of note, after 16 weeks participants may consider a weekly dosing schedule on cycle days 1, 8, 15, and 22 after discussion with the site principal investigator.

8.1.9 Ordering

M6620 will be ordered by site pharmacy personnel via contacting Merck KGaA, Darmstadt, Germany (EMD Serono) at: Lori Magnelli (lori.magnelli@emdserono.com; Ph 781-490-8556).

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

Expired or unused supplies of M6620 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Archival Tissue Collection (All Participants)

Archival tissue is required for enrollment to the trial. Archival tissue may be either a formalin-fixed paraffin-embedded (FFPE) block or unstained slides. Participants undergoing a baseline fresh tumor biopsy who do not have sufficient archival tissue at study entry may be allowed to use tissue from the fresh biopsy in lieu of archival tissue. Pediatric participants without sufficient archival tissue at study entry are not required to undergo fresh tumor biopsy.

Please refer to the protocol laboratory manual for specific handling and shipping instructions.

9.2 Fresh Tumor Tissue Biopsies (Translational Lead-in Cohorts T1, T2, T3 Only; Optional for T4)

Core needle, incisional, or excisional biopsies should be obtained for analysis. Please refer to the

protocol laboratory manual for specific handling and shipping instructions.

- Biopsy #1: Fresh tumor tissue biopsies will be obtained at baseline from all T1, T2, and T3 patients enrolling to the translational lead-in study. Biopsy is optional for T4.
    - The baseline fresh tumor biopsy may be obtained any time before the participant's Cycle 1 Day 1.
    - At the time the baseline biopsy sample is obtained, a germline blood sample should also be collected.
    - For cohorts T2 and T3, fresh biopsy is not required for patients with adequate archival biopsy available (15 slides; lower numbers may be allowed with permission from the overall PI) provided that the samples are not older than 6 months and the patient has not received systemic therapy between the time of biopsy and consent for study 18-274
- Biopsy #2: On-treatment biopsy will be obtained from all T1, T2, and T3 participants if medically feasible (optional for T4).
    - The biopsy should be scheduled to occur within 30 hours of the end of the infusion of the fifth (5^{th}) dose of study drug. Following agreement with the site principle investigator, the biopsy may alternately be obtained within 30 hours following the completion of the seventh (7^{th}) dose of study drug.
    - PT and PTT should be collected before Biopsy #2
    - The biopsy should ***not*** be performed if the patient did not receive the study drug infusion. If a participant does not receive their planned study drug infusion due to toxicity, every effort should be made to re-schedule the biopsy to occur within 30 hours of when they ***actually receive*** their fifth (or seventh) dose of study drug. In the event re-scheduling of the biopsy is not feasible it will not be considered a protocol violation, however the toxicity necessitating the dose hold and the reason the biopsy was not obtained must be clearly documented and discussed with the site principal investigator.
- Biopsy #3: An additional optional biopsy at the time of disease progression will be offered to all participants.
    - The time of progression biopsy should be obtained prior to the initiation of another anti-cancer therapy when possible. In the event this is not feasible, the time of progression biopsy may be obtained up to 30 days after the date that disease progression was determined. The date of progression may be considered either the date the radiological scan was performed, or the date the treating investigator judged the participant to have progressed.
- On the same day as the collection of any of the biopsies, a single PK sample will be obtained. Please refer to **Section 9.3** and the protocol laboratory manual for specific collection, handling, and shipping instructions.
- Any participant who is unable to complete both the baseline and on-treatment biopsies will be replaced to allow sufficient collection of pharmacodynamic data to evaluate the primary objective of the translational lead-in study.
- If the biopsy is deemed unsafe, the patient may enroll with the permission of the overall PI

9.3 Pharmacokinetic Sampling (Translational Lead-in Study Only)

Limited PK sampling will be collected on participants in the translational lead-in study at the time points indicated in the table below. Please refer to the protocol laboratory handbook for specific PK collection, handling, and shipping instructions.

Table 1111: PK Sample Collection

Visit Day	Time of Collection	Sample Number
Cycle 1 Day 15	Pre-dose (0 – 5 minutes prior to the initiation of the infusion)	PK-1
Cycle 1 Day 15	End of infusion (0-5 minutes after infusion is complete)	PK-2
Biopsy #1	On the same day as the biopsy collection	PK-B1
Biopsy #2	On the same day as the biopsy collection, within 30 hours of the end of the infusion of the 5th dose of study drug (or 7th if the biopsy is collected following the 7th infusion per **Section 9.2**); every effort should be made to obtain PK-B2 as close to the time of biopsy as is feasible (e.g. within 2 hours).	PK-B2
Biopsy #3	On the same day as the biopsy collection. Every effort should be made to obtain PK-B3 as close to the time of biopsy as is feasible (e.g. within 2 hours). If the participant does not undergo a time of progression biopsy, this PK sample will not be collected.	PK-B3

9.4 ATM Mutation Status and Immunohistochemistry

- For all ATM patients (T2 and Cohort 2) mutation status will be reviewed for eligibility by the overall PI (Cote), and if necessary, the DFCI site PI (Shapiro), other site PIs, and laboratory collaborators.
- For non-truncating ATM mutations, the NGS data will be evaluated for probability of ATM protein inactivation by the overall PI (Cote), the DFCI site PI (Shapiro), other site PI's, and laboratory collaborators. If the mutation is suggestive of ATM inactivation the eligibility will be confirmed by the overall PI (Cote), the DFCI site PI (Shapiro), and laboratory collaborators.
- If the patient and ATM mutation meet eligibility, the patient may be enrolled on study in

T2 and Cohort 2 and initiate study treatment as per protocol. In parallel, archival or fresh biopsy tumor will be analyzed for ATM status by IHC via a CLIA-approved assay at BWH.

- If ATM is expressed by IHC, the patient will be allowed to continue on treatment if clinically indicated; however, this patient will be replaced and not count towards accrual limits.
- If ATM is not expressed by IHC, the patient will continue study as procedures per protocol and the patient will count towards accrual limits.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy, with the exception of the informed consent and baseline imaging which must be done $\leq$ 28 days prior to the start of therapy.

Assessments must be performed prior to administration of any study agent.

Table 12: Study Calendar

	Pre-Study	Cycle 1 Day 1	Cycle 1 Day 4[a]	Cycle 1 Day 8[a]	Cycle 1 Day 11[a]	Cycle 1 Day 15[a]	Cycle 1 Day 18[a]	Cycle 1 Day 22[a]	Cycle 1 Day 25[a]	Cycle 2+ Day 1[b]	Cycle 2+ Day 4[a, c]	Cycle 2+ Day 8[a]	Cycle 2+ Day 11[a, c]	Cycle 2+ Day 15[a]	Cycle 2+ Day 18[a, c]	Cycle 2+ Day 22[a]	Cycle 2+ Day 25[a, c]	Off Treatment[d]
Informed consent	X																	
Demographics	X																	
Medical history	X																	
Physical exam	X	X		X		X		X		X				X				X
Vital signs[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																	
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Performance status	X	X				X				X								X
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X		X		X		X		X
Serum chemistry[f]	X	X	X	X	X	X	X	X	X	X		X		X		X		X
β-HCG[g]	X																	
ECG[h]	X	As clinically indicated																
Adverse event evaluation		X----------X																X
Radiologic evaluation	X	CT or MRI imaging of any disease-involved site. Imaging to be conducted every 8 weeks (± 7 day scheduling window).																X
Archival tissue collection[i]	X																	
Fresh Tumor Biopsy, Germline Blood Sample Collection[j]	X					X												X
M6620 administration[k]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PK Collection[l]	X					X												X
cfDNA Collection[m]	X																	

Table 12: Study Calendar

	Pre-Study	Cycle 1 Day 1	Cycle 1 Day 4[a]	Cycle 1 Day 8[a]	Cycle 1 Day 11[a]	Cycle 1 Day 15[a]	Cycle 1 Day 18[a]	Cycle 1 Day 22[a]	Cycle 1 Day 25[a]	Cycle 2+ Day 1[b]	Cycle 2+ Day 4[a, c]	Cycle 2+ Day 8[a]	Cycle 2+ Day 11[a, c]	Cycle 2+ Day 15[a]	Cycle 2+ Day 18[a, c]	Cycle 2+ Day 22[a]	Cycle 2+ Day 25[a, c]	Off Treatment[d]

a. A ± 3 day scheduling window to accommodate for adverse weather, vacations, holidays, or other scheduling issues may be permitted after discussion with the overall PI. There is a required minimum of 72 hours between doses in Cycle 1. After Cycle 1, if the patient is tolerating therapy without clinically significant AE's or SAE's, the investigator may shorten this window to up to 60 hours to allow for scheduling flexibility.
b. A -1 / +7 day scheduling window is allowable to accommodate adverse weather, vacations, holidays, or other scheduling issues.
c. Visit only required in participants receiving twice weekly infusions, not required in participants receiving once weekly infusions.
d. Off-treatment evaluation to occur 30 days following the end of study treatment, +/- 2 weeks. Note: for IND trials, follow up visits or other contact are required in order to identify SAEs during the 30 days following the last dose of study drug.
e. Heart rate, blood pressure, respiratory rate, and temperature. Assessments to be collected prior to study agent dosing when applicable.
f. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
g. Serum pregnancy test for women of childbearing potential. Childbearing potential defined as any female who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not post-menopausal (defined as amenorrhea >12 consecutive months; or women with a documented plasma follicle-stimulating hormone level >35µlU/mL).
h. Single ECG to be collected, QTc to be calculated per institutional standards.
i. Archival tumor tissue must be available for analysis for enrollment on the trial. Please refer to **Section 9.1** for details.
j. Translational lead-in study T1, T2, and T3 participants only, optional for T4, not applicable for phase II participants. Tumor tissue biopsy collection is required at baseline and again on-treatment, and optional at the time of disease progression as detailed in **Section 9.2**. A germline blood sample will be collected with the baseline tumor tissue biopsy. If the patient is not having a pre-treatment biopsy, the germline blood sample should still be collected during screening.
k. M6620 will be infused IV twice weekly on cycle days 1, 4, 8, 11, 15, 18, 22, and 25. After 16 weeks, participants may consider a weekly dosing schedule on cycle days 1, 8, 15, and 22 after discussion with the site principal investigator. Please refer to **Section 5.1** for more detail. Labs must result before dosing.
l. Translational lead-in study participants only, not applicable for phase II participants. PK to be collected as detailed in **Section 9.3**. Effort should be made to obtain the PK sample as close to the time as biopsy as feasible (e.g. within 2 hours)
m. 20 mL of peripheral blood will be collected in two 10 mL Streck cfDNA tubes at any time during screening before c1d1 for cohort T1 only.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks (± 7-day scheduling window). In addition to a baseline scan, confirmatory scans should also be obtained not less than 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 Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. 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.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants 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 target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants 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.

11.1.2 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) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or 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 will not be considered measurable unless there has been demonstrated growth in the lesion following completion of the radiation treatment.

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/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered 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 participant, 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.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. 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 in 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 thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of 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.

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:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) 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.

(c) 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.

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.

MIBG (meta-iodobenzylguanidine).The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

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 data 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 from 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 alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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.

11.1.3.1 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.

11.1.3.2 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).

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 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.

Table 13: For Participants with Measurable Disease (*i.e.*, Target Disease)				
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: Participants 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.

Table 14: For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)		
Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.4 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, or

death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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.

11.1.5 Response Review

Evaluation of scans will be done centrally using the Tumor Imaging Metrics Core (TIMC).

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in **Section 7.0** (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date

participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

13.1.1 Translational Lead-in Study

A small translational lead-in study will be conducted prior to the initiation of the phase II trial. The translational lead-in will serve to determine changes in the pharmacodynamic endpoints of phospho-Chk1 and γH2AX in biopsy specimens of patients treated with M6620 monotherapy, and to evaluate the preliminary anti-cancer activity of M6620 monotherapy in selected cancer populations, including:

- Cohort T1: *ATRX*-mutated LMS
- Cohort T2: *ATM*-mutated solid tumors
- Cohort T3: *BRCA1/2* and other HR-mutated cancers, malignancies with replicative stress, and *ARID1A*-mutated cancers.
- Cohort T4: SDH-deficient GIST

Each cohort will enroll 6 participants. Mandatory baseline and on-treatment biopsies will be collected from all T1, T2, and T3 participants, and an optional time-of-progression biopsy will be offered to all participants. Biopsies are optional for T4. Any T1/T2/T3 patient unable to complete both the baseline and on-treatment biopsies will be replaced to allow for sufficient collection of pharmacodynamic data. Non-parametric paired analysis will be conducted to assess the significance of changes between baseline and on-treatment pharmacodynamics parameters. With 6 samples the study will be powered (80%) to detect an effect size of 1.067 in mean of paired difference between baseline and on-treatment phospho-Chk1 and γH2AX with a significance level of 0.05 using a two-sided Wilcoxon test and assuming that the actual distribution is double exponential.

The probability of rejecting the treatment in the lead-in phase for a true but unknown rate of 60% reduction in baseline phospho-CHK1 and a 50% increase in γH2AX of 50% is 0.65. If this rate is 20% the probability of rejection is 0.98 and if the rate is 80% the probability of rejection is 0.098

Following the completion of enrollment to the translational lead-in study, a review of all available safety, efficacy, pharmacodynamic, and pharmacokinetic data will occur prior to the initiation of the phase II trial. Review will be conducted by the overall principal investigator, site principal investigators, any applicable study team members, as well as consultation with Merck

KGaA (EMD Serono) as appropriate. Alteration of the phase II design based on findings from the translational lead-in study may occur with a protocol amendment following the review.

13.1.2 Phase II

After completion of the review of the initial translational lead-in study, a phase II, five-cohort basket trial with the primary endpoint of disease control rate at 16 weeks will open. The disease control rate is defined as achievement of confirmed objective response (complete or partial response), or stable disease that is confirmed at 16 weeks from the baseline disease evaluation. We will enroll 25 patients in cohorts 1A, 2-5 following a two-stage approach. We will enroll 30 patients in a single stage for cohort 1B.

Assuming the null and alternative hypothesis (null: 15%, alternative: 35%) is the same among cohorts 1A and 2-5, at the first stage analysis, we will need to observe at least 3 patients with controlled disease out of 15 patients to continue through the second stage. At the second stage, we will assess disease control rate again, and we will need to observe at least 7 instances of patients with controlled disease out of 25 patients to reject the null hypothesis and accept the treatment as having value for further study. The overall power for disease control rate at 16 weeks is 81%. The overall type I error, the chance of incorrectly rejecting the null hypothesis is 7%. The probability of stopping at the first stage under the null hypothesis is 60%. The operating characteristics of this design are based on the two-stage Minimax design. While cohort 1B is conducted as a single cohort, the hypothesis tested are identical to the ones in the other cohorts. With one-sided type 1 error of 7% (as in the other cohort) this arm will have 87% power to differentiate between the null of 15% and alternative of 35%. The null will be rejected if at least 8 patients have controlled disease.

It is estimated that ~50% of LMS are ALT+. Thus, for cohort 1B, study characteristics favor enrollment to a single stage of 30 patients. ALT status will be determined retrospectively after study completion and sub group analysis will be conducted on these patients. We expect approximately 15 patients to be included in this analysis. With 15 patients, the study will be sufficiently powered (at least 80%) to detect an increase of at least 25% from the null of poor disease control rate of 15% with one-sided significance level of 0.07. if at least 5 patients in this cohort are observed with controlled disease, the drug will be considered for further investigation in this study population.

Toxicity is an important secondary endpoint. With 25 patients in each cohort, there is at least 72% probability of observing one or more toxicities with a true rate as low as 5%. With 25-30 treated patients in each cohort, the maximum width of a 90% two-sided confidence interval for any estimated adverse event proportion ± 18%.

13.2 Sample Size and Study Duration

A total of 24 participants will be enrolled to the translational lead-in study among the three cohorts, and a maximum of 205 participants will be enrolled to the phase II trial among the five

cohorts. Total study duration (translational lead-in and phase II) is estimated to be approximately 60 months.

13.3 Reporting and Exclusions

13.3.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment.

13.3.2 Evaluation of the Primary Efficacy Endpoint

All eligible participants included in the study who receive at least one dose of study medication will be assessed for response, even if there are major protocol therapy deviations. Each participant should 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 because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

13.3.3 Evaluation of the Primary Pharmacodynamic Endpoint (Translational Lead-in Study Only)

All eligible participants who successfully underwent both the baseline and the on-treatment biopsies will be assessed for pharmacodynamic response as measured by changes in phospho-CHK1 and γH2AX. Any participant who was unable to undergo both the baseline and on-treatment biopsy will be replaced. In the event the biopsy tissue obtained from either the baseline or on-treatment biopsy was not sufficient for analysis, the participant will be replaced.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

REFERENCES

1. Italiano A, Mathoulin-Pelissier S, Cesne AL, et al. Trends in survival for patients with metastatic soft-tissue sarcoma. *Cancer.* 2011;117(5):1049-1054.
2. Karavasilis V, Seddon BM, Ashley S, Al-Muderis O, Fisher C, Judson I. Significant clinical benefit of first-line palliative chemotherapy in advanced soft-tissue sarcoma: retrospective analysis and identification of prognostic factors in 488 patients. *Cancer.* 2008;112(7):1585-1591.
3. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer.* 2009;115(7):1531-1543.
4. Shay JW, Reddel RR, Wright WE. Cancer. Cancer and telomeres--an ALTernative to telomerase. *Science.* 2012;336(6087):1388-1390.
5. Flynn RL, Cox KE, Jeitany M, et al. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. *Science.* 2015;347(6219):273-277.
6. Cote GM, He J, Choy E. Next-Generation Sequencing for Patients with Sarcoma: A Single Center Experience. *Oncologist.* 2017.
7. Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. *Pharmacol Ther.* 2015;149:124-138.
8. Krajewska M, Fehrmann RS, Schoonen PM, et al. ATR inhibition preferentially targets homologous recombination-deficient tumor cells. *Oncogene.* 2015;34(26):3474-3481.
9. Yazinski SA, Comaills V, Buisson R, et al. ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant BRCA-deficient cancer cells. *Genes Dev.* 2017;31(3):318-332.
10. Hsieh HJ, Peng G. Cellular responses to replication stress: Implications in cancer biology and therapy. *DNA Repair (Amst).* 2017;49:9-20.
11. Williamson CT, Miller R, Pemberton HN, et al. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. *Nat Commun.* 2016;7:13837.
12. Sulkowski PL, Sundaram RK, Oeck S, et al. Krebs-cycle-deficient hereditary cancer syndromes are defined by defects in homologous-recombination DNA repair. *Nat Genet.* 2018;50(8):1086-1092.
13. O'Carrigan B, de Miguel Luken MJ, Papadatos-Pastos D, et al. Phase I trial of a first-in-class ATR inhibitor VX-970 as monotherapy (mono) or in combination (combo) with carboplatin (CP) incorporating pharmacodynamics (PD) studies. *Journal of Clinical Oncology.* 2016;34(15_suppl):2504-2504.
14. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature.* 2002;415(6871):530-536.
15. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med.* 2002;347(25):1999-2009.
16. Van Allen EM, Wagle N, Stojanov P, et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. *Nat Med.* 2014;20(6):682-688.
17. Heaphy CM, Subhawong AP, Hong SM, et al. Prevalence of the alternative lengthening of telomeres telomere maintenance mechanism in human cancer subtypes. *Am J Pathol.* 2011;179(4):1608-1615.
18. Ting DT, Lipson D, Paul S, et al. Aberrant overexpression of satellite repeats in

pancreatic and other epithelial cancers. *Science.* 2011;331(6017):593-596.
19. Ferrone CR, Ting DT, Shahid M, et al. The Ability to Diagnose Intrahepatic Cholangiocarcinoma Definitively Using Novel Branched DNA-Enhanced Albumin RNA In Situ Hybridization Technology. *Annals of surgical oncology.* 2016;23(1):290-296.
20. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 2002;30(10):e47.

APPENDIX A PERFORMANCE STATUS CRITERIA

Performance Status Criteria

Karnofsky and Lansky performance scores are intended to be multiples of 10

ECOG (Zubrod)		Karnofsky		Lansky*	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.