NATIONAL CANCER INSTITUTE
Center for Cancer Research

TO: Cancer Therapy Evaluation Program

FROM: Anish Thomas, MBBS, M.D., Principal Investigator,
Developmental Therapeutics Branch, NCI, CCR

DATE: 10/21/2022

RE: **Amendment version date: 10/21/2022 for Protocol 20-C-0009 (#10268)**:
"Randomized Phase II Trial of Topotecan plus M6620 (VX-970) vs. Topotecan alone in Patients with Relapsed Small-Cell Lung Cancer"

The purpose of this amendment is to expand the assessment of circulating free DNA beyond exome and panel sequencing to include cfDNA fragmentomics and the application of spatial transcriptomics, both as exploratory studies.

Briefly, in the last 4-5 years since the clinical trial development started and patients were enrolled, the understanding of SCLC heterogeneity has undergone a major shift. Rather than mutations, heterogeneity of SCLC is now thought to be driven by the expression of distinct transcription factors [1-4]. SCLC consist of tumor cells with neuroendocrine (NE) and non-neuroendocrine (non-NE) features [1-6], further defined by differential expression of the three lineage-defining transcription factors (TFs) ASCL1, NEUROD1, and POU2F3. A fourth subgroup has been variously characterized by YAP1 expression [1-4] or low expression of all three transcription factors accompanied by an inflamed gene signature [5]. SCLC heterogeneity increases over the course of treatment, with expansion of the non-NE cell population associated with chemo-resistance [5-7]. These TFs cannot be inferred from cfDNA assays proposed in the current version of the protocol.

Dying cells in the human body release their content into the bloodstream. Genomic DNA that is bound by nucleosomes and TFs escapes endogenous nucleases and so remains protected in plasma [8]. Regular turnover of lymphoid and myeloid cells in the human body is the major contributor to the pool of cell-free DNA (cfDNA) in plasma. However, in cancer, a detectable fraction of cfDNA arises from tumors. This suggests that cfDNA has the potential to map the tumor transcriptome and therefore can help uncover the regulatory landscape of cancer from plasma. Fragmentomics seeks to uncover the tissue of origin of cfDNA using the information on cfDNA fragment length. We will apply a genome-wide approach for analysis of cfDNA fragmentation profiles called DELFI (DNA evaluation of fragments for early interception) [9]. This approach provides a view of cfDNA "fragmentomes", permitting evaluation in any individual of the size distribution and frequency of millions of occurring cfDNA fragments across the genome. In a proof of principle study, genome-wide fragmentation profiles could non-invasively distinguish between NSCLC and SCLC. Specifically, consistent decrease in aggregate fragment coverage at regions containing the ASCL1 binding sites (±200 bp) was observed in patients with SCLC compared to non-cancer individuals or those with other cancer types. In contrast, at distances further from ASCL1 binding sites (>2000 bp), the fragment coverage for patients with SCLC and other patients were similar. As cfDNA fragmentomes can comprehensively represent both genomic and chromatin characteristics, it has the potential to identify the determinants of response and resistance to topotecan and topotecan plus M6620.

Although our understanding of cell-extrinsic influences on SCLC plasticity is rudimentary, a key role of these non-genetic mechanisms is suggested by two important observations: (i) transcriptional subtypes of human SCLC are not strongly associated with specific mutational patterns [1] and (ii) differences in neuroendocrine gene expression programs between human tumors and patient-derived xenografts [6]. We hypothesize that the dynamic interactions between tumor microenvironment (TME) and the cancer cells shape SCLC cell states. The sequencing approaches proposed in the current protocol such as bulk RNA-seq does not provide a context of the spatial interactions between cells and TME in tissue. As seen in Figure 1, cancer associated fibroblast (CAF) and tumor associated macrophage (TAM) molecular signatures may be higher around non-NE SCLC when analyzed using bulk sequencing data. However, sequencing data from TME regions (spatial transcriptomic approach using Nanostring GeoMx digital spatial profiling) shows an opposite trend, proven by immunohistochemistry (IHC), showing higher enrichment of CAFs and TAMs around high NE tumors, highlighting the utility of the spatial transcriptomic approach.

Spatial transcriptomics is a novel approach allowing measurement and mapping of gene activity to the resolution of single to few cells keeping the position information intact [7,10-11]. Mapping the cell-intrinsic and -extrinsic factors that impact SCLC tumor cell states in their positional context could yield important insights into the biologic basis of response and resistance to topotecan and topotecan plus M6620.

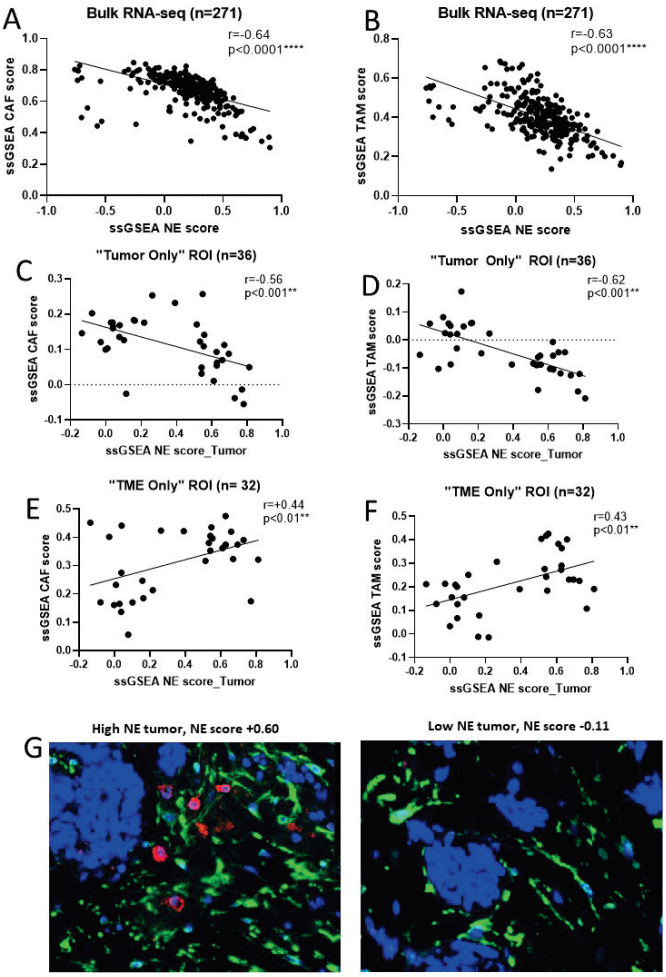

Figure 1. Discordance of stromal features derived from bulk and spatial transcriptome data. Correlation of **(A)** CAF **(B)** TAM gene expression signatures with tumor NE scores from bulk RNA-seq data. IMPOWER 133 trial data of treatment-naïve advanced SCLC patients Correlation of **(C)** CAF and **(D)** TAM gene expression signatures with tumor NE scores specifically in the tumor regions of interest. Preliminary data from 10 SCLC patient tumors profiled as transcriptome-wide spatial RNA profiling using GeoMX Whole Transcriptome Atlas platform Correlation of **(E)** CAF and **(F)** TAM gene expression signatures with tumor NE scores specifically in the TME regions of interest. Preliminary Data as above (**G**) Multiplex IHC showing high TAMs and CAFs in stroma around high NE tumor compared with low NE tumor (CAF marker, ACTA2 in green; TAM marker CD163 in red; Blue/DAPI labels DNA/nuclei showing SCLC tumor clusters).

References:

1. Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. Nat Rev Cancer. 2019;19(5):289-297.
2. Gazdar, A.F., et al., Characterization of Variant Subclasses of Cell-Lines Derived from Small Cell Lung-Cancer Having Distinctive Biochemical, Morphological, and Growth-Properties. Cancer Research, 1985. 45(6): p. 2924-2930.

3. Zhang, W., et al., Small cell lung cancer tumors and preclinical models display heterogeneity of neuroendocrine phenotypes. Translational Lung Cancer Research, 2018. 7(1): p. 32-+.
4. Calbo, J., et al., A Functional Role for Tumor Cell Heterogeneity in a Mouse Model of Small Cell Lung Cancer. Cancer Cell, 2011. 19(2): p. 244-256.
5. Lissa D, Takahashi N, Desai P, et al. Heterogeneity of neuroendocrine transcriptional states in metastatic small cell lung cancers and patient-derived models. Nat Commun. 2022;13(1):2023.
6. Thomas A, Takahashi N, Rajapakse VN, et al. Therapeutic targeting of ATR yields durable regressions in small cell lung cancers with high replication stress. Cancer Cell. 2021;39(4):566-579 e567.
7. Roper N, Velez MJ, Chiappori A, et al. Notch signaling and efficacy of PD-1/PD-L1 blockade in relapsed small cell lung cancer. Nat Commun. 2021;12(1):3880.
8. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin. Cell. 2016 Jan 14;164(1-2):57-68. doi: 10.1016/j.cell.2015.11.050. PMID: 26771485; PMCID: PMC4715266.
9. Mathios, D., Johansen, J.S., Cristiano, S. et al. Detection and characterization of lung cancer using cell-free DNA fragmentomes. Nat Commun 12, 5060 (2021). https://doi.org/10.1038/s41467-021-24994-w
10. Gavriel F, Nobuyoki T, Israa S, et al. Subtyping of Small Cell Lung Cancer using plasma cell-free nucleosomes. bioRxiv 2022; doi: https://doi.org/10.1101/2022.06.24.497386
11. Lissa D, Takahashi N, Desai P, et al. Integrated genomic and transcriptomic analysis of small cell lung cancer reveals inter- and intratumoral heterogeneity. Nat Communs. 2022: In press.

SUMMARY OF CHANGES

I. COMMENTS REQUIRING A RESPONSE (AMENDMENT 17) – ADMINISTRATIVE & EDITORIAL ISSUES:

#	Section	Comments
1.	5.7	1.1.1.1 In the Biomarker Plan Table, update the laboratory information in the Laboratory Performing Assay Column **for each** biomarker being performed by the MoCha laboratory to the following: NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov **PI Response:** Biomarker plan table has been update as requested.

<table>
<tr><th>#</th><th>Section</th><th>Comments</th></tr>
<tr><td>2.</td><td>5.7</td><td>In the Biomarker Plan Table, please separate cfDNA into two rows as two separate assays are being performed by two different laboratories. See below. Renumber the subsequent biomarkers accordingly.
<table>
<tr><td>3</td><td>cfDNA - Fragmentomics</td><td>Fragmentomics</td><td>Exploratory

To determine prognostic and predictive role, and their usefulness to track tumor evolution and heterogeneity in response to treatment</td><td>O</td><td>Blood
Baseline,
Cycle 1 Day 5 †,
Cycle 2 Day 1 †, Cycle 3 Day 1,
and at progression.</td><td>Delfi Diagnostics, Baltimore, MD
Lorenzo Rinaldi
rinaldi@delfidiagnostics.com</td></tr>
<tr><td>4</td><td>Circulating Tumor DNA (ctDNA)</td><td>TSO500</td><td>Exploratory

To determine prognostic and predictive role, and their usefulness to track tumor evolution and heterogeneity in response to treatment</td><td>O</td><td>Blood
Baseline,
Cycle 1 Day 5 †,
Cycle 2 Day 1 †, Cycle 3 Day 1,
and at progression.</td><td>NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
Dr. Mickey Williams
mickey.williams@nih.gov</td></tr>
</table>
<u>PI Response:</u> cfDNA have been separated into two rows as two separate assays are being performed by two different laboratories. subsequent biomarkers accordingly</td></tr>
<tr><td>3.</td><td>5.8.1.2</td><td>Replace current section 5.8.1.2 with the following:

1.1.1.2 5.8.1.2 Site Performing Correlative Study

RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).</td></tr>
</table>

#	Section	Comments
		5.8.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.8.1.2. 5.8.1.4 Contact Information for Notification of Specimen Shipment Thomas Forbes (NCLNGenomicsReceiving@nih.gov) **PI Response:** Section 5.8.1.2 has been updated as requested.
4.	5.8.2.2	Replace current section 5.8.2.2 with the following: 1.1.1.3 5.8.2.2 Site Performing Correlative Study RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR). 5.8.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.8.2.2. 5.8.2.4 Contact Information for Notification of Specimen Shipment Thomas Forbes (NCLNGenomicsReceiving@nih.gov) **PI Response:** Section 5.8.2.2 has been updated as requested.
5.	5.8.3.2	Replace current section 5.8.3.2 with the following: 1.1.1.4 5.8.3.2 Site Performing Correlative Study RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

#	Section	Comments
		5.8.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.8.3.2. 5.8.3.4 Contact Information for Notification of Specimen Shipment Thomas Forbes (NCLNGenomicsReceiving@nih.gov) **PI Response:** Section 5.8.3.2 has been updated as requested.
6.	**5.8.4**	Add the follow new Sections to 5.8.4 following current Section 5.8.4.2 5.8.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to: Please add shipping address for NCI DTB Laboratory 5.8.4.4 Contact Information for Notification of Specimen Shipment Please add contact email for notification of specimen shipment for this assay **PI Response:** Section 5.8.4 has been updated as requested.
7.	**5.9.2**	Please create separate sections for cfDNA - Fragmentomics and Circulating Tumor DNA as follows. Renumber subsequent sections accordingly : 1.1.2 5.9.2 cfDNA - Fragmentomics Characterization of plasma cfDNA can potentially provide rapid, noninvasive improved monitoring of disease burden, depth of responses to treatment, and timely warning of disease relapse in patients with SCLC [1]. Peripheral blood will be collected to assess cfDNA for genetic mutations specific to SCLC. 1.1.2.1 5.9.2.1 Specimen Receipt and Processing at the EET Biobank Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and will be stored in a -80°C freezer.

#	Section	Comments
		The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped for analysis. 1.1.2.2 5.9.2.2 Site Performing Correlative Study This study will be performed by Delfi Diagnostics. 5.9.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to: Please add shipping address for Delfi Diagnostics 5.9.2.4 Contact Information for Notification of Specimen Shipment Please add contact email for notification of specimen shipment for this assay 5.9.3 Circulating Tumor DNA (ctDNA) Characterization of plasma cfDNA can potentially provide rapid, noninvasive improved monitoring of disease burden, depth of responses to treatment, and timely warning of disease relapse in patients with SCLC [46]. Peripheral blood will be collected to assess cfDNA for genetic mutations specific to SCLC. 5.9.3.1 Specimen Receipt and Processing at the EET Biobank Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and will be stored in a -80°C freezer. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped for analysis. 1.1.2.3 5.9.3.2 Site Performing Correlative Study ctDNA will be performed at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

#	Section	Comments
		5.9.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.9.3.2. 5.9.3.4 Contact Information for Notification of Specimen Shipment Thomas Forbes (NCLNGenomicsReceiving@nih.gov) **PI Response:** Section 5.9.2 has been updated as requested.
8.	**5.9.4**	Add the following new Sections to current Section 5.9.3 SLFN11, cMYC, and ATM (now 5.9.4). 5.9.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to: Please add shipping address for NCI DTB Laboratory 5.9.4.4 Contact Information for Notification of Specimen Shipment Please add contact email for notification of specimen shipment for this assay **PI Response:** Section 5.9.4 has been updated as requested.
9.	**5.9.5.2**	In the Section for WES, replace current Section 5.9.4.2 (now 5.9.5.2) with the following: 1.1.2.4 5.9.5.2 Site Performing Correlative Study WES will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR). 5.9.5.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.9.5.2.

#	Section	Comments
		5.9.5.4 Contact Information for Notification of Specimen Shipment Thomas Forbes (NCLNGenomicsReceiving@nih.gov) **PI Response:** Section 5.9.5.2 has been updated as requested.

II. RECOMMENDATIONS (AMENDMENT 17):

#	Section	Comments
10.	**Title Page**	Please note that "Hillman" was omitted during the revision of LAO-PA015 / UPMC Cancer Center Institute LAO. Please update and add Hillman to this participating org. **PI Response:** "Hillman" has been added to the org name.
11.	**4.1**	Please replace the first paragraph with the updated language below: Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr. **PI Response:** First paragraph in section 4.1 has been updated as requested.

III. CTEP RECOMMENDATIONS FOLLOWING AMENDMENT 15 REVIEW

#	Section	Comments
1	**Cover Page**	***Please revise the following Participating Organization as indicated.*** LAO-PA015 / UPMC Hillman ~~University of Pittsburgh~~ Cancer Center ~~Institute~~ LAO **PI Response:** Change has been made.

2	**4.2**	***Please revise the excerpt below as specified.*** Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context ~~(SSW)~~ to the CIRB using IRBManager to indicate their intent to open the study locally. ***Please revise the excerpt below as specified.*** In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following ~~five~~ criteria to complete processing of the IRB/REB approval record to be completed: • Holds an active CTEP status, Active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization's roster, • If using NCI CIRB, active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record, ~~• Rostered at the site on the IRB/REB approval *(applies to US and Canadian sites only)* and on at least one participating roster,~~ ~~• If using NCI CIRB, rostered on the NCI CIRB Signatory record,~~ • Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, ~~and~~ • Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and • Holds the appropriate CTEP registration type for the protocol. **Additional Requirements** Additional site requirements to obtain an approved site registration status include: • An active Federalwide Assurance (FWA) number, • An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO), ~~and~~ • An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and • Compliance with all protocol-specific requirements (PSRs). **PI Response:** Change has been made.
3	**4.2.1**	***Please revise the excerpt below as specified.***

		Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted ~~based on person and site roster assignment. To participate, the institution~~ to institutions and its associated investigators and staff ~~must be associated with the LPO or a PO on the protocol. One way to search for a protocol is listed below.~~ on a participating roster. To view/download site registration forms: ***Please revise the excerpt below as specified.*** • Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select *Site Registration*, ~~and~~ to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.) **PI Response:** Change has been made.
4	**4.2.3**	***Please revise this subsection as indicated.*** Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' website. To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the Regulatory section, and select Regulatory Submission. Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately ~~at 1-866-651-2878~~ by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org in order to receive further instruction and support. **PI Response:** Change has been made.
5	**4.3.1**	***Please revise the excerpt below as specified.*** • To perform enrollments or request slot reservations: Must be ~~Be~~ on an LPO roster, ETCTN Corresponding roster, or PO ~~Participating Organization~~ roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) ~~AP~~ registration type.

		Please revise the excerpt below as specified. • All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) ~~HIPAA~~ authorization form (if applicable). **PI Response:** Change has been made.
6	**4.3.4**	***Please revise the Theradex Helpdesk number as indicated.*** This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 855-828-6113 ~~609-619-7862~~ or Theradex main number 609-799-7580; CTMSSupport@theradex.com. **PI Response:** Change has been made.
7	**10.3.1**	***Please delete the language within this subsection and replace with the following language.*** **10.3.1 Rave-CTEP-AERS Integration** The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of Adverse Events (AEs) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting. Sites must initiate all AEs for this study in Medidata Rave. ***Include the following (highlighted) paragraphs about pre-treatment AEs only if the study requires reporting of pre-treatment AEs. Pre-existing medical conditions are not considered adverse events and therefore should not be reported on an Adverse Event form.*** Pre-treatment AEs: AEs that occur after informed consent is signed and prior to start of treatment are collected in Medidata Rave using the Pre-treatment Adverse Event form. Pre-existing medical conditions (formerly referred to as baseline AEs) identified during baseline assessment are not considered AEs and therefore

should not be reported on the Pre-treatment Adverse Event form. If these pre-existing conditions worsen in severity, the investigator must reassess the event to determine if an expedited report is required. Whether or not an expedited report is required, the worsened event should be reported as a routine AE.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 Days after the Last Administration of the Investigational Agent/Intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free.

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence that internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link

		from Medidata Rave. Additional information about the CTEP-AERS integration is available on the CTSU members' website: • Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and • Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics*. NCI requirements for SAE reporting are available on the CTEP website: NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf. **PI Response:** Change has been made.
8	**13.2**	***Please replace the paragraph below with the provided language.*** Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management > Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name. ~~Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper~~

		~~right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.~~ **PI Response:** Change has been made.
9	**13.3**	***Please revise this subsection as indicated.*** **1.2** Data Quality Portal The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics. The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms. The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP. To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, ~~and~~ DQP Delinquent Forms, DQP Form Status, and DQP Reports modules. ~~Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol~~

		~~regarding questions about Rave Calendaring functionality.~~ ***Include the following paragraph only if study is not using the Calendaring functionality in Rave; otherwise, delete.*** This study does not use the Rave Calendaring functionality and therefore the DQP Delinquent Forms module will not include details for this study, and the DQP Summary table on the Rave Home page will display *N/A* for the Total Delinquencies summary count. **PI Response:** Change has been made.

IV. RESPONSE TO RA FROM CTEP (10/6/2022):

1. The following changes have been made to the participation organizations table:
    a. Added: Early Drug Development Opportunity Program (EDDOP)
    b. Deleted: Create Access to Targeted Cancer Therapy for Underserved Populations (CATCH-UP)

V. PI REQUESTED CHANGES:

1. **Title page:**
    a. Version date has been updated in all headers and in the title page.
    b. Data managers have been updated as previous ones are no longer part of the study.
2. **Section 2.5.4.1, cfDNA Fragmentomics and Spatial Transcriptomics:** Rationale for the expansion of circulating free DNA assessment to include fragmentomics and the application of spatial transcriptomics has been added.
3. **Section 5.7, Biomarker Plan: S**patial transcriptomics, fragmentomics and TSO500 have been added as assays used for the analysis of tumor tissue and/or blood samples. Corresponding labs performing the analysis have also been updated.
4. **Section 5.8.4, Spatial Transcriptomics**: Processing of samples for this new integrated analysis has been added.
5. **Section 5.9.2, cfDNA:** Delfi Diagnostics has been added as laboratory evaluating cfDNA in blood samples, per the added exploratory analysis.

NCI Protocol #: 10268

Local Protocol #: 20-C-0009

ClinicalTrials.gov Identifier: NCT03896503

TITLE: Randomized Phase II Trial of Topotecan plus M6620 (VX-970, Berzosertib) vs. Topotecan alone in Patients with Relapsed Small-Cell Lung Cancer

Corresponding Organization: LAO-NCI/ National Cancer Institute LAO

Principal Investigator: Anish Thomas, MBBS, M.D.
Developmental Therapeutics Branch (DTB)
Center for Cancer Research
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10 Center Drive, Room 4-5330
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Phone: 240-614-6467
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Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / UPMC Hillman Cancer Center LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO
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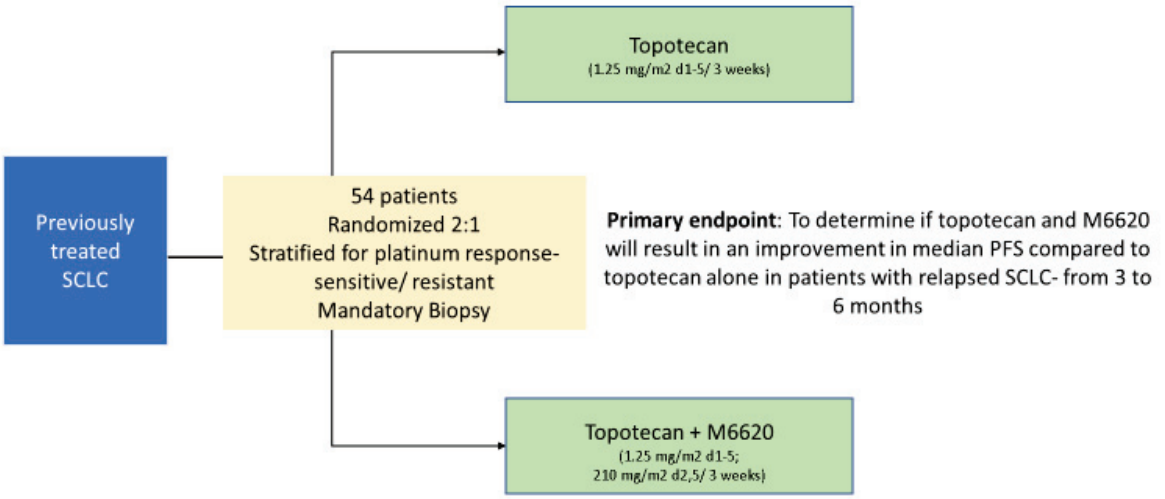


Patients on topotecan monotherapy arm may cross-over to receive the combination at progression

Up to 20 patients with extrapulmonary small cell cancers will be enrolled and treated with topotecan+M6620 in a separate exploratory cohort, while the SCLC arms are open

This study is a randomized Phase 2 study of patients with relapsed SCLC. SCLC patients who have failed prior therapy will be randomized 2:1 to receive either topotecan in combination with M6620 or topotecan alone. Patients will be stratified at the time of randomization for being sensitive or resistant/refractory to prior therapy. Patients who progress while on topotecan alone will be eligible to cross-over and receive topotecan with M6620 combination treatment. A separate cohort of patients with extrapulmonary small cell cancer will be accrued while the primary cohort is accruing and will receive the combination of M6620 and topotecan.

TABLE OF CONTENTS

1 OBJECTIVES

1.1 Primary Objective

To determine if the combination of M6620 with topotecan will result in an improvement in progression-free survival (PFS) compared to topotecan alone in patients with relapsed small cell lung cancer (SCLC).

1.2 Secondary Objectives

To determine the objective response rate (ORR) and overall survival (OS) with the combination of M6220 and topotecan in patients with relapsed SCLC and extrapulmonary small cell cancers.

1.3 Exploratory Objectives

- To perform molecular profiling assays on malignant and normal tissues, including, but not limited to RNA sequencing (RNA-Seq):
    - **To assess expression of genes Schlafen family member 11 (*SLFN11)*, *MYC,* and Ataxia-Telangiesctasia mutated (*ATM)* among others.**
    - To identify potential predictive biomarkers of response to a combination of M6620 with topotecan.
    - Identify mechanisms of drug sensitivity and resistance using DNA- and RNA-based assessment platforms.

- To contribute genetic analysis data from de-identified biospecimens to Genomic Data Commons (GDC), a well annotated cancer molecular and clinical data repository, for current and future research; specimens will be annotated with key clinical data, including presentation, diagnosis, staging, summary treatment, and if possible, outcome.

- To bank formalin-fixed, paraffin-embedded (FFPE) tissue, blood (for cell-free DNA analysis), and nucleic acids obtained from patients at the EET Biobank at Nationwide Children's Hospital.

- To characterize circulating cell-free DNA (cfDNA) and circulating tumor cells in patients with relapsed SCLC and extrapulmonary small cell cancers.

- To identify potential predictive biomarkers of response to a combination of M6620 with topotecan in patients with extrapulmonary small cell cancers.

2 BACKGROUND

2.1 Study Disease

SCLC is the most aggressive and lethal form of lung cancer. It represents 15% of all lung cancers, with an annual incidence of over 34,000 cases in the United States alone. SCLC is characterized by rapid doubling time, high growth fraction and early and widespread metastatic involvement [2]. Response rates to first-line chemotherapy are exceptionally high, in the order of 60% to 70%. Unfortunately, these responses are also disappointingly transient, with median PFS of <5 months [3, 4] and nearly all patients relapse within one year. Extensive-stage SCLC is generally unresponsive to chemotherapy at relapse, and fewer than 5% of patients survive for two years [2]. Treatment of SCLC as well as survival after a diagnosis of SCLC have not changed substantially in the past 30 years [5]. Due to an urgent need for effective interventions in this disease, SCLC was singled out by the NCI as a designated "recalcitrant" cancer based on incidence rate, exceptionally high lethality, and the lack of substantial therapeutic progress made over several decades [6, 7].

At the time of diagnosis with SCLC, most patients (60-70%) will have extensive-stage (ES) disease, defined as cancer that has spread beyond the ipsilateral lung and regional lymph nodes and cannot be included in a single radiation field [2]. The primary treatment modality for patients with ES-SCLC is systemic chemotherapy consisting of platinum and etoposide followed by prophylactic cranial irradiation in patients with a response [8]. Topotecan is Food and Drug Administration (FDA)-approved for patients with SCLC with chemotherapy-sensitive disease after failure of first-line chemotherapy (Hycamtin® Package Insert, 2015). Topotecan inhibits re-ligation of topoisomerase I-mediated single-strand DNA breaks leading to lethal double-strand DNA breaks (DSB) (Hycamtin® Package Insert, 2015). Sensitivity to first-line chemotherapy has been considered as the main driver of second-line therapy outcome in SCLC. For this reason, relapsed SCLC has been traditionally classified into sensitive and resistant disease according to the type of response to first-line therapy and to treatment-free interval (treatment-free interval longer or shorter than 60–90 days) [9]. Although this definition was designed many years ago and based on a small patient series [10], first-line chemotherapy sensitivity has since been confirmed to be a prognostic factor to predict response to second line and further therapy in meta-analyses [11] and randomized studies [12, 13].

Diagnosis of small cell cancers (including SCLC) is based on morphologic features and do not rely on immunohistochemical staining [14, 15]. Small cell cancer cells are defined by the following morphologic features: nuclear appearance, which includes finely granular chromatin, lack of prominent nucleoli; and marked nuclear fragility and malleability; scant cytoplasm and indistinct cell borders. High mitotic rate, apoptotic bodies, and large areas of geographic necrosis are typical. Tumor cells are usually positive for one or more of chromogranin or synaptophysin, although around 10 percent may be unreactive for neuroendocrine markers. Extrapulmonary small cell carcinoma is a distinct clinicopathologic entity that can arise in a wide range of extrapulmonary sites. These tumors have been described most frequently in the urinary bladder, prostate, esophagus, stomach, colon and rectum, gallbladder, larynx, salivary glands, cervix, and skin. They are extremely rare and management of systemic disease with chemotherapy is patterned after the approach used in SCLC [16].

2.2 Rationale

Because of frequent chromosomal alterations, dysregulation of genes that promote replication origin firing (*e.g.*, MYC) and the high expression of DNA damage repair proteins, we have postulated that SCLC has high levels of endogenous replicative stress- defined by collapse of DNA replication forks due to various perturbations that interfere with replication[17] (**Figure 1**). Cellular responses to replicative stress-referred to as the DNA damage response (DDR)-is critical for repair of the damage and normal cell cycle progression. Ataxia telangiectasia and Rad3-related (ATR) is a key DDR regulator. By using a synthetic lethal short interfering RNA screen, we identified depletion of ATR as a top candidate for topoisomerase 1 (TOP1) inhibitor synthetic lethality [18].

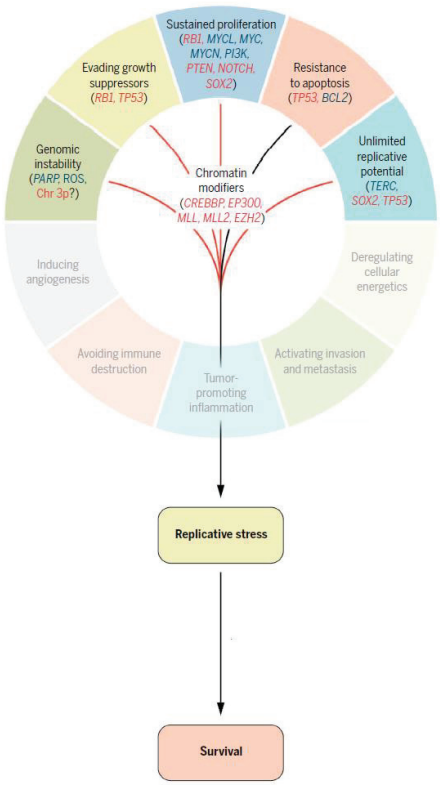


Figure 1: Replicative Stress in SCLC: Causes, Consequences and Therapeutic Opportunities: The hallmarks of SCLC include sustained proliferation, resistance to apoptosis, unlimited replicative potential, genomic instability, and evasion of growth suppressors (genes that are up-regulated are indicated in blue and those that are down-regulated in red). All of these hallmarks except resistance to apoptosis have been directly linked to replicative stress. Therapeutic approaches that further exacerbate replication stress by inhibiting the target proteins shown in the figure could selectively kill SCLC by replicative damage [**17**].

Inhibition of ATR may represent an effective strategy to overcome the rewiring of homologous recombination (HR) and related DNA repair pathways, which may underlie drug resistance in platinum-refractory SCLC cells. A recent study showed that ATR inhibition disrupted the rewired HR and replication fork protection pathways in breast cancer 1 gene (BRCA1)-deficient cancer cells that acquired poly (ADP-ribose) polymerase (PARP) inhibitor resistance [4]. Doerr

et al. (2017) have demonstrated an actionable dependence on ATR/checkpoint kinase 1 (CHK1)-mediated cell cycle checkpoints for SCLC [19]. In these studies, ATR inhibitors induced genotoxic damage and apoptosis in human and murine SCLC cell lines and murine SCLC tumors were highly sensitive to ATR inhibitors.

We showed that inhibition of ATR by short interfering RNA or VE-821 and its clinical derivative M6620 sensitizes tumor cells to TOP1 inhibitors. M6620, enhanced the *in vivo* tumor response to irinotecan without additional toxicity (**Figure 2**). ATR inhibition abrogated the S-phase replication elongation checkpoint and the replication origin-firing checkpoint induced by TOP1 cleavage complexes [18]. We found that a combination of an ATR inhibitor and topotecan can be safely combined and that heightening replicative stress in this manner can yield durable responses in small cell cancers and initiated a Phase 1/2 trial of the combination [20].

In this current study, we hypothesize that the combination of M6620 and topotecan can improve PFS compared to topotecan alone in patients with relapsed SCLC.

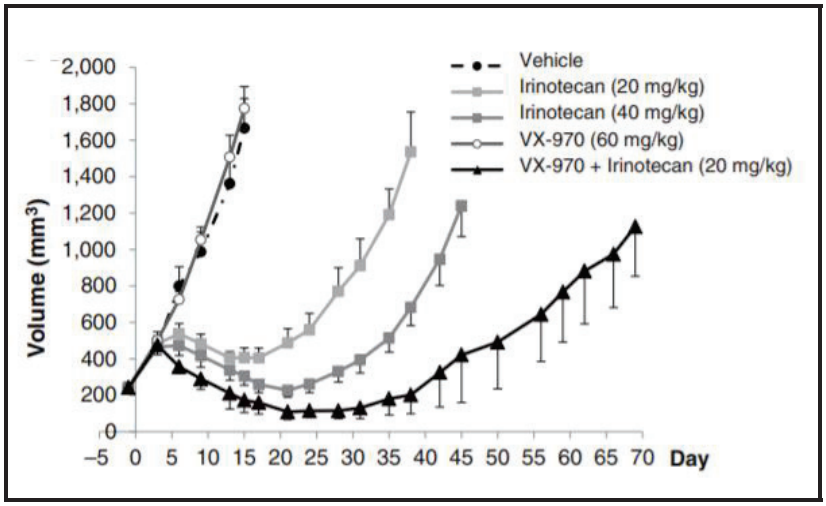


Figure 2: The clinical ATR inhibitor M6620 potentiates the efficacy of irinotecan in the colorectal cancer COLO205 mouse xenograft model. Mice bearing COLO205 tumors (volume ~250 mm^3) were treated with vehicle, irinotecan (20 mg/kg or 40 mg/kg administered by intraperitoneal bolus injection on day 0 of each 4-day cycle), M6620 (60 mg/kg administered by oral gavage on days 0, 1, and 2 of each 4-day cycle), or the combination of both drugs (using the schedule for administration of each compound alone). Treatment was continued for 4 cycles. Each group consisted of 8 mice. Animals receiving irinotecan or the combination of irinotecan and M6620 were allowed to recover after treatment to assess tumor regrowth. Tumor size was assessed twice a week [18].

Extrapulmonary small cell cancers of various tissues of origin may arise by different mechanisms. In case of prostate small cell, it arises in the setting of therapeutic resistance to highly potent AR-targeting therapy whereas in EGFR mutated cancers it arises in the setting of potent EGFR-targeting. But in general, extrapulmonary small cell cancers have in common signatures of RB1 loss [21-23] which in turn drives replication stress. A recent paper has shown that AR transcriptional activity was lower in small cell prostate cancers compared to adenocarcinoma [22]. Accordingly, in clinical practice small cell prostate cancer patients are continued on androgen suppression while they are on small cell-directed therapy. Taken together, it appears that although extrapulmonary small cell cancers (including prostate small

cell) can arise by different mechanisms but have in common high replication stress and may be susceptible to ATR inhibition, supporting their inclusion in this trial.

2.3 M6620 (VX-970, Berzosertib)

2.3.1 Mechanism of Action

M6620 (VX-970, berzosertib) is an ATP-competitive, highly potent, tightly binding inhibitor of ATR. M6620 blocks ATR with a concentration associated with 50% inhibition (IC_{50}) of 20 nM [24].

2.3.2 Nonclinical Pharmacokinetics

In all nonclinical species (the mouse, rat, dog, and monkey), M6620 exhibited a high volume of distribution (V_d); tissue exposure, including tumor, was high [24]. In rats, no accumulation or retention was observed in tissues, and the elimination half-lives ($t_{1/2}$) were similar across all tissues and whole blood. The whole blood $t_{1/2}$ was 11.6 h in rats and 9.8 h in dogs. M6620 was extensively bound to plasma proteins; the free fraction of M6620 was only 2.1% in human blood.

M6620 is primarily eliminated by oxidative metabolism, with the cytochrome 450 (CYP) 3A4 isoform being the principle isoform responsible [24]. Strong inducers or inhibitors of CYP3A4 may alter M6620 kinetics and blood levels. Based on its minimal inhibition or induction effects on CYPs, M6620 is expected to have a low potential for drug-drug interactions. M6620 metabolites are 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. The systemic clearance of M6620 following IV administration was 26 and 13 mL/min/kg in the rat and dog, respectively.

2.3.3 Clinical Pharmacokinetics

Clinical pharmacokinetics (PK) have been evaluated both in whole blood and plasma [24]. Mean exposure, area under the curve (AUC) profiles were similar in whole blood and plasma. Overall, the maximum observed concentration (C_{max}) was 1.3-fold greater in whole blood than in plasma. The results suggest that plasma is an appropriate matrix to characterize the M6620 PK. The terminal elimination half-life ($t_{1/2}$) was approximately 17 h across all doses. In the M6620 single dose studies, M6620 exposures (C_{max} and $AUC_{0-\infty}$) increased in a dose-proportional manner. 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.

2.3.4 Safety summary from studies with M6620 as single agent or in combination with cytotoxic therapy

Infusion-related reactions (local or systemic), nausea, and vomiting are considered adverse drug reactions (ADRs) for M6620, and myelosuppression events are considered ADRs for M6620 in combination with carboplatin.

- 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. In almost all cases, these reactions occurred within minutes of the second exposure to M6620 and they included hypotension and mental status changes. All subjects fully recovered with standard care procedures.
- 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. To minimize the possibility of phlebitis, M6620 should be administered through a large-bore catheter into large-caliber peripheral vein.
- Nausea and vomiting have occurred commonly in patients receiving M6620 monotherapy. Many of the affected subjects experienced these events on the same day as M6620 was administered, and there was some suggestion of a dose response.
- Hematologic adverse events (AEs) in subjects who received M6620 in combination with carboplatin have included anemia, neutropenia, thrombocytopenia, and febrile neutropenia.
- M6620 (VX-970, berzosertib) has not been assessed in developmental and reproductive toxicity studies at this stage of development. However, M6620 inhibits DNA-damage repair and it will be administered in conjunction with cytotoxic chemotherapy; thus, the potential for teratogenicity should be M6620 considered high. Patients on M6620 studies must take stringent measures to avoid fathering or bearing children while on study drug and for 6 months after discontinuation of M6620.

2.3.5 Clinical Efficacy

In a Phase 1 clinical trial of patients with advanced cancers, M6620 monotherapy was well tolerated with no dose-limiting toxicities (DLTs) at doses up to 480 mg/m^2 administered weekly [25]. We conducted a Phase 1/2 clinical trial of M6620 combined with topotecan, a highly selective inhibitor of Top1. The Phase 1 part of the trial enrolled patients with advanced solid tumors with progressive disease (PD) and was recently reported [20]. Phase 2 recently completed enrolment and the results are summarized below. The primary objective of Phase 1 was to determine the recommended Phase 2 dose (RP2D) by evaluating the feasibility, safety, AEs, DLT, and the maximum tolerated dose (MTD). Secondary objectives were to characterize the pharmacodynamics and to assess preliminary antitumor activity. Paired hair follicles and tumor biopsies obtained post-topotecan and post-M6620/topotecan at time points established for quantifying phosphorylation of the histone H2AX (γH2AX) were used to confirm DNA damage.

Between September 2016 and February 2017, 21 patients enrolled in the Phase 1 study. The combination was well tolerated, which allowed for dose escalation to the highest planned dose level (topotecan 1.25 mg/m^2, days 1 to 5; M6620 210 mg/m^2, days 2 and 5). One of six patients at this dose level experienced grade 4 thrombocytopenia that required transfusion, a DLT. Most common treatment-related grade 3 or 4 toxicities were anemia, leukopenia, and neutropenia (~40-50%% each); lymphopenia (14%); and thrombocytopenia (25%). The most common toxicities across all dose levels (DLs) (N = 21) were anemia and lymphopenia (100% each),

leukopenia (90%), neutropenia (81%), thrombocytopenia (76%), and nausea (62%). The most common grade 3 and 4 toxicities were anemia, leukopenia, and neutropenia (19% each); lymphopenia (14%); and thrombocytopenia (10%). Pegfilgrastim was not routinely administered during the first cycle but was used from cycle 2 onward in eight patients and cycle 4 onward in one patient.

Two partial responses (PR) (≥18 months, ≥7 months) and seven stable disease (SD) responses ≥3 months (median, 9 months; range, 3 to 12 months) were seen. Of five patients with SCLC in Phase 1, all of whom had platinum-refractory disease, three derived durable clinical benefit (one PR and two prolonged SD; ≥ 6, ≥ 7, and 10 months; **Figure 3**). All three were diagnosed with extensive-stage SCLC, had received four cycles of platinum and etoposide, and were found to have SD or near-complete responses (CR) to first-line therapy but had PD soon after first-line chemotherapy. The median PFS of patients with SCLC (n = 5) was 10.2 months (95% CI, 1.4 to 10.2 months), and 6-month PFS probability was 60.0% (95% CI, 12.6% to 88.2%).

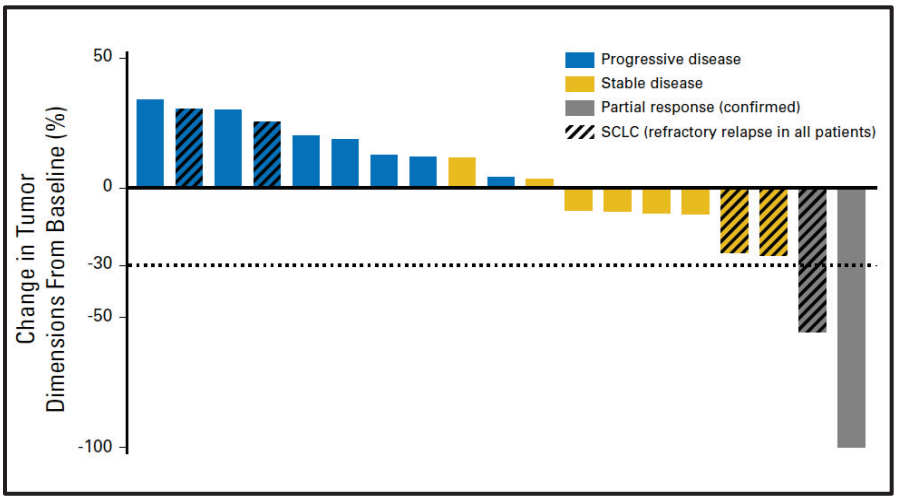


Figure 3: Response to topotecan plus M6620 in a Phase 1 trial. The waterfall plot shows responses in evaluable patients (n = 19) [**26**].

In summary, the Phase 1 trial established tolerable doses of M6620 in combination with topotecan and showed preliminary pharmacodynamic evidence of ATR inhibition and enhanced DSBs. The toxicity profile of the combination largely mirrored that of topotecan, which as monotherapy is associated with a high frequency of myelosuppression. The most common reason for dose reduction was neutropenia, which did not recur with pegfilgrastim prophylaxis. The lack of additive toxicities with ATR inhibition is possibly related to the differentiating features of rapidly dividing cancer cells, including higher replicative stress and increased DNA damage [27]. Although the number of patients with SCLC treated in the Phase 1 study was small, an important clinical activity signal was noted.
We recently completed enrolment on the Phase 2 trial evaluating the efficacy of topotecan and M6620 in previously treated subjects with SCLC. Both platinum-sensitive and refractory patients were eligible. The primary endpoint was ORR.. Response had to be confirmed by the investigator at least four weeks later. The trial was conducted using Simon two-stage Minimax design to rule out an unacceptably low 10% response rate (p0=0.10) in favor of targeted response

rate of 30% (p1=0.30), considered clinically meaningful based on outcomes with topotecan alone in relapsed SCLC [9].

Between June 2017 and April 2019, a total of 26 patients were enrolled, all of whom had evidence of disease progression before study participation. As of data cut-off on 3/18/2020, all patients completed at least one cycle (median: 5.5 cycles; range: 1–33) of treatment. Twenty-five patients were evaluable for the primary endpoint. Patient median age was 63 years (range: 51–78), and 17 patients (65.4%) were women. Most patients had extensive-stage at diagnosis (18/26, 69.2%). Notably, most patients had platinum-resistant disease (21/26, 80.8%). Seven of 16 (43.8%) patients had confirmed partial responses (PRs) in the first stage, allowing continued enrollment onto the second stage. In the overall study, nine of 25 patients (36.0%, 95% confidence interval, CI: 18.0–57.5) achieved a confirmed PR, meeting the primary endpoint for response (**Figure 4**, **Table 2**). One patient had an unconfirmed response at first imaging (39.7% tumor reduction) but disease progression soon after. Most patients (17/25 patients; 68.0%) experienced tumor regressions. After a median potential follow up of 20.7 months, the median progression-free survival (PFS) was 4.8 months (95% CI: 2.8–7.4). The PFS at 4 months and 6 months were 60.0% (38.4-76.1) and 36.0% (18.2–54.2), respectively. The median overall survival (OS) was 8.5 months (5.6-13.6). OS at 6 months and 12 months were 68.0% (46.1–82.5) and 32.0% (15.2–50.2) respectively.

Responses were observed in patients with both platinum-sensitive and platinum resistant disease (ORR 60.0% [3/5 patients; 95% CI: 14.7–94.7] and 30.0% [6/20; 95% CI: 11.9–54.3] respectively; p = 0.31 by Fisher's exact test) (**Figure 4**). The median (95% CI) duration of response (DOR) was 6.4 months (1.1–14.3). Durable responses among patients with platinum-resistant disease were notable given that these tumors rarely respond to topotecan monotherapy, and are typically fatal within weeks of relapse [28, 29]. Four of six (66.7%) responders with platinum-resistant SCLC had DOR more than 6 months. Two of the platinum-resistant responders (33.3%; patient # 33 and 50) remain without disease progression and on treatment at data cut-off, 24 and 13 months respectively after starting treatment. The DOR, PFS and OS were not significantly different between patients with platinum-sensitive and resistant disease, and patients who were previously treated with PD-1/PD-L1 inhibitors and those treated without.

Table 1. Treatment efficacy of M6620 and topotecan in SCLC patients

	Evaluable patients (n = 25) Ł
ORR (95% CI)	9 (36.0%, 18.0–57.5)
Best response	
PR (confirmed)	9 (36.0%)
PR (unconfirmed)[H]	1 (4.0%)
SD	11 (44.0%)
PD	4 (16.0%)
Median DOR (months, 95%CI)	6.4 (1.1–14.3)

Ongoing treatment	2 (8.0%)
PFS	
Median (months, 95%CI)	4.8 (2.8–7.4)
At 4 months (95%CI)	60.0% (38.4–76.1%)
At 6 months (95%CI)	36.0% (18.2–54.2%)
OS	
Median (months, 95%CI)	8.5 (5.6–13.6)
At 6 months (95%CI)	68.0% (46.1–82.5%)
At 12 months (95%CI)	32.0% (15.2–50.2%)

Data were presented as n (%). Tumor response was evaluated by RECIST version 1.1.
Ł: one patient was not evaluable due not to show 1st restaging scan.
H : One had PR initially but PD 19 days after. ORR: objective response rate; PR: partial response; SD: stable disease, PD: progressive disease; DOR: duration of response; CI: confidence interval; PFS: progression free survival; OS: overall survival.

Table 2. Duration of response among responding platinum-resistant patients

Platinum-resistant patient	Duration of response (months)
#26	7*
#27	6
#29	10
#33	24+
#42	4.1
#50	13+
Phase I	42+
Phase I	6
Phase I	10*

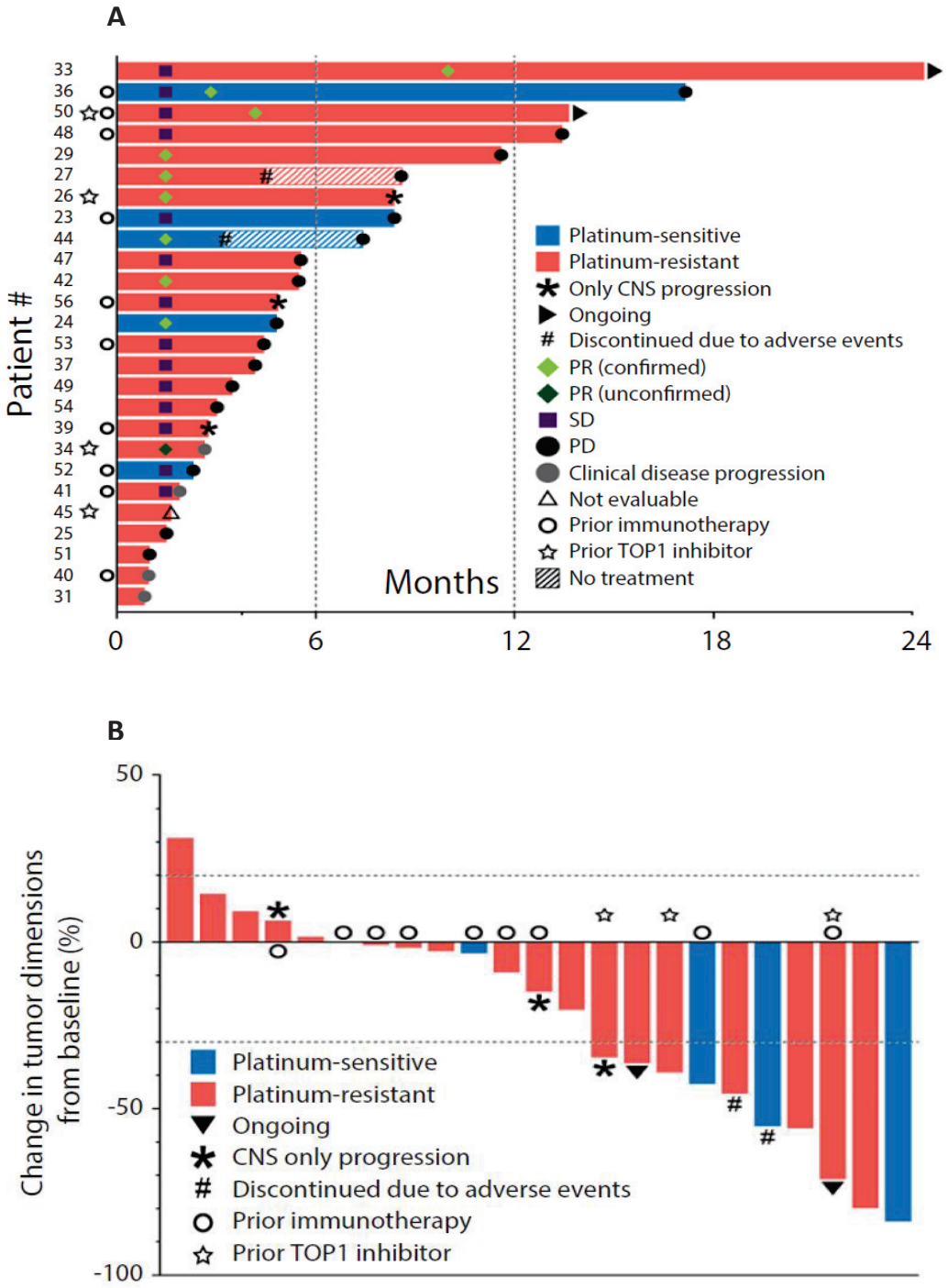


Figure 4: Efficacy of topotecan and M6620 in relapsed SCLC. A) Efficacy of the combination of M6620 and topotecan in SCLC patients based on duration of response. B) Tumor responses to the combination of M6620 and topotecan in SCLC patients based on maximum change in tumor dimensions from baseline. Each bar represents a patient's tumor response.

The toxicity profile of the combination in SCLC patients mirrored that of the phase I population of patients with solid tumors. The most common treatment-related AEs (n, %) were anemia (25, 96.2%), lymphopenia (25, 96.2%), thrombocytopenia (24, 92.3%), and neutropenia (13, 50.0%) followed by gastrointestinal symptoms such as nausea (13, 50.0%) and vomiting (11, 42.3%). Treatment-related serious AEs were infrequent and observed in 4/26 (15.4%) patients, and included grade 4 febrile neutropenia, grade 3 anemia, grade 4 thrombocytopenia, grade 3 vomiting, and grade 2 nausea. Most AEs were attributable to topotecan, which as monotherapy is associated with a high frequency of myelosuppression[9]. The differentiating features of rapidly dividing cancer cells, including higher replicative stress and increased DNA damage that render them more susceptible to ATR inhibition may explain the absence of major overlapping toxicities with this combination.

Table 3. Treatment-Related Adverse Events (maximum grade, all cycles)

Adverse Event (N=26)	All	≥Grade 3
Anemia	25 (96.2%)	14 (53.8%)
Lymphopenia	25 (96.2%)	18 (69.2%)
Thrombocytopenia	24(92.3%)	15 (57.7%)
White blood cell decreased	20 (76.9%)	8 (30.8%)
Neutropenia	13 (50.0%)	4 (15.4%)
Febrile neutropenia	1 (3.8%)	1 (3.8%)
Nausea	13 (50.0%)	1 (3.8%)
Fatigue	12 (46.2%)	0 (0%)
Vomiting	11 (42.3%)	1 (3.8%)
Anorexia	8 (30.8%)	2 (7.7%)
Diarrhea	3 (11.5%)	0 (0%)
Constipation	1 (3.8%)	0 (0%)
Total bilirubin increased	2 (7.7%)	0 (0%)
ALT increased	2 (7.7%)	0 (0%)
AST increased	1 (3.8%)	0 (0%)
Hypophosphatemia	11 (42.3%)	3 (11.5%)
Hypomagnesemia	2 (7.7%)	0 (0%)
Alopecia	4 (15.4%)	0 (0%)
Infusion related reaction	2 (7.7%)	0 (0%)
Abdominal pain	1 (3.8%)	1 (3.8%)
Bruising	1 (3.8%)	0 (0%)
Chills	1 (3.8%)	0 (0%)
Creatinine increased	1 (3.8%)	0 (0%)
Dyspepsia	1 (3.8%)	0 (0%)
Gastroesophageal reflux disease	1 (3.8%)	0 (0%)
Generalized muscle weakness	1 (3.8%)	0 (0%)
Hiccups	1 (3.8%)	0 (0%)
Malaise	1 (3.8%)	0 (0%)
Peripheral motor neuropathy	1 (3.8%)	0 (0%)

Adverse Event (N=26)	All	≥Grade 3
Peripheral sensory neuropathy	1 (3.8%)	0 (0%)
Sinus tachycardia	1 (3.8%)	0 (0%)
Skin and subcutaneous tissue disorders	1 (3.8%)	0 (0%)
Skin hypopigmentation	1 (3.8%)	0 (0%)

Adverse events were evaluated according to Common Terminology Criteria for Adverse Events version 4.0.
AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Given that SCLCs share a common molecular phenotype with extrapulmonary small cell cancers, we reasoned that these cancers – independent of their tissue of origin – may also be responsive to combined ATR and TOP1 inhibition. To address this hypothesis, we amended the clinical trial of M6620 and topotecan and enrolled 10 patients with extrapulmonary small cell cancers and tumor progression after at least one previous systemic chemotherapy. All ten patients were treated with at least one cycle (range: 1–10) and were evaluable for treatment efficacy. Treatment with M6620 and topotecan resulted in two confirmed PRs and an unconfirmed PR, yielding ORR of 20.0% (**Figure 5**). Notably, deep and durable responses were observed in tumors refractory to prior TOP1 inhibitor treatments. Two of five patients who achieved PR or SD had tumor progression previously on TOP1 inhibitors. This included a patient with treatment refractory breast SCNC who had a major tumor regression lasting 8.5 months.

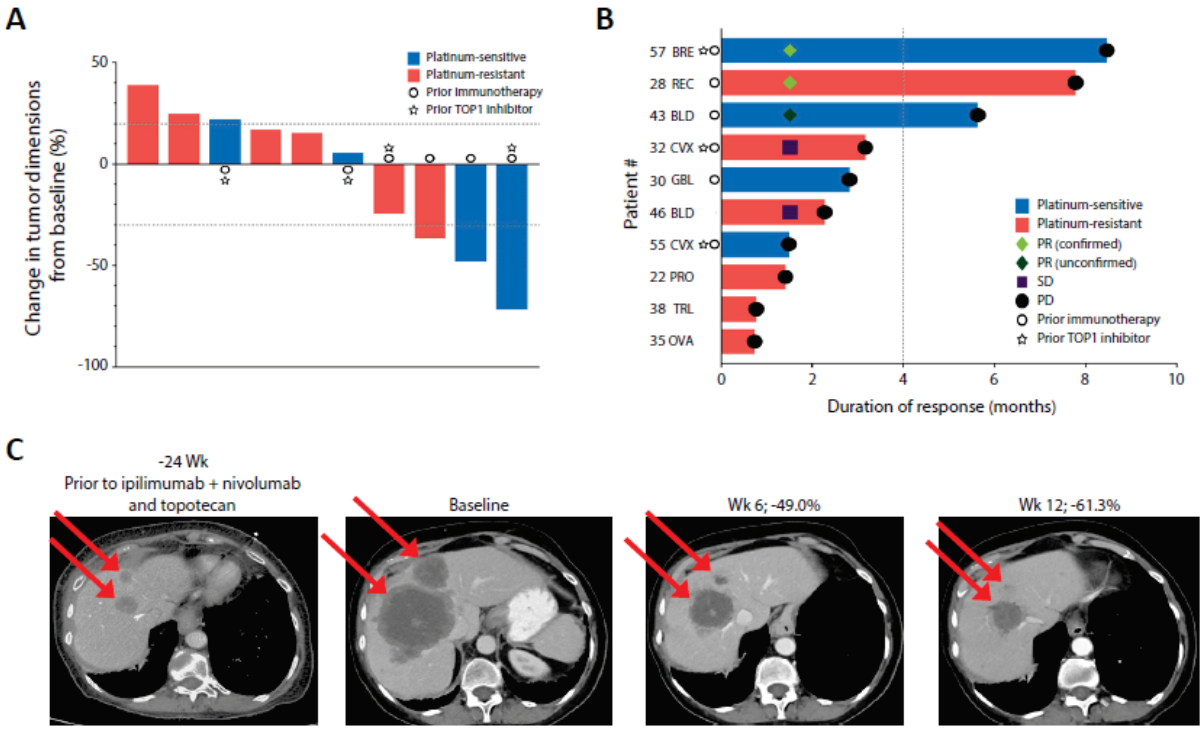


Figure 5: Efficacy of M6620 and topotecan in SCNCs regardless of tissue of origin. A) Responses to the combination of M6620 and topotecan in EP-SCNCs based on change in tumor dimensions from baseline. Each bar represents a patient's tumor response. B) Efficacy of the combination of M6620 and topotecan in EP-SCNCs based on duration of response. C) CT scans of the abdomen showing tumor regression in a patient with breast SCNC with

liver metastases (patient #57). The patient had undergone extensive previous treatments including topotecan. REC, rectal; BLD, bladder; TRL, transformed lung cancer; BRE: breast; CVX, cervix; GBL, gall bladder; PRO, prostate; OVA, ovarian.

2.4 Topotecan

Topotecan has been approved by the FDA for the treatment of 1) metastatic carcinoma of the ovary after disease progression on or after initial or subsequent chemotherapy, 2) SCLC platinum-sensitive disease in patients who progressed after first-line chemotherapy, and 3) as a combination therapy with cisplatin for Stage IV-B, recurrent, or persistent carcinoma of the cervix which is not amenable to curative treatment (Hycamtin® Package Insert, 2015). Topotecan is a semi-synthetic derivative of camptothecin and is an anti-tumor drug with topoisomerase I-inhibitory activity. Topotecan binds to the topoisomerase I-DNA complex and prevents religation of these single-strand breaks. The cytotoxicity of topotecan is thought to be due to double-strand DNA damage produced during DNA synthesis, when replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I, and DNA. Mammalian cells cannot efficiently repair these DSBs. Bone marrow suppression (primarily neutropenia) is the dose-limiting toxicity of topotecan at the approved doses (Hycamtin® Package Insert, 2015). The FDA approved dose of topotecan for treating SCLC is 1.5 mg/m^2. However, lower doses at 1.25 mg/m^2 appear to be as effective as the approved dose, but with reduced toxicity [30].

2.5 Correlative Studies Background

Several genomic alterations can drive responses to topotecan plus ATR inhibition [31, 32]. These include 1) low expression of *SLFN11*; 2) overexpression of replicative stress-inducing oncogenes such as *MYC*, Cyclin E1 (*CCNE1*), Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A (*APOBEC3A*), apolipoprotein B mRNA editing catalytic polypeptide-like 3B (*APOBEC3B*), others; 3) reduced expression of DDR/cell cycle checkpoint genes Excision Repair Cross-Complementation Group 1 (*ERCC1*), X-Ray Repair Cross Complementing 1 (*XRCC1*), Cell Division Cycle 25A (*CDC25A*), *ATM*, others; and 4) processes beyond DDR *e.g.*, loss of tumor suppressor proteins of the switch/sucrose nonfermentable chromatin remodeling complex including AT-rich interactive domain-containing protein 1A (ARID1A). Given the broad range of potential biomarkers to ATR inhibition, RNA-seq will be performed on fresh pre-treatment biopsies to allow simultaneous evaluation of multiple biomarkers, with a specific focus on expression of 3 genes – *SLFN11*, *MYC,* and *ATM* (see below). Additionally, archival tissue and tissue remaining after RNA-seq will be used for *SLFN11*, *MYC*, and *ATM* immunohistochemistry (IHC) and for whole exome sequencing (WES).

2.5.1 SLFN11 Expression

SLFN11 is frequently inactivated in cancer cell lines and has been shown to sensitize cancer cells to anticancer drugs. In particular, elevated SLFN11 expression is associated with sensitivity to DNA-damaging agents which concurrently promote replication fork stalling and cell cycle checkpoint activation, and induce replication stress [33].

SLFN11 is highly expressed in SCLC as compared with other histologies [34]. In contrast, SLFN11 expression is low to nearly absent in lung squamous cell carcinoma and

adenocarcinoma (**Figure 6A**) [35]. SLFN11 expression has a bimodal distribution in SCLC and may provide meaningful stratification as a predictive biomarker in clinical studies of SCLC. *SLFN11* gene expression by RNA-Seq is concordant with SLFN11 protein expression by quantitative western blot (**Figure 6B**).

SCLC cells with high SLFN11 expression are more sensitive to DNA-damaging chemotherapy, and SLFN11 inactivation confers resistance to these agents [34]. Patients with SLFN11-positive tumors treated with a combination of temozolomide and veliparib had a significantly prolonged PFS and OS compared with patients with SLFN11-negative tumors. Consistent with its role in determining chemosensitivity, SLFN11 expression is higher in tumors from SCLC patients who respond to DNA-damaging therapy versus those who do not and is higher in tumors from treatment-naive SCLC patients than in recurrent/relapsed SCLC [35]. Recent studies have shown that SLFN11 suppression by epigenetic silencing could be causal to acquired resistance to DNA-damaging chemotherapy in SCLC [35-37].

Studies from the National Cancer Institute (NCI) Developmental Therapeutics Branch (DTB) group demonstrated that SLFN11 blocks replication independently of ATR and that *SLFN11*-negative cells dominantly rely on ATR under replication stress [38]. Consistently, in terms of cytotoxicity, the effect of ATR inhibition under camptothecin is highly prominent in *SLFN11*-deleted cells but is limited in parental cells (**Figure 6C**). ATR inhibition overcomes resistance to topoisomerase 1 inhibitors in *SLFN11*-negative cancer cells.

Based on the above considerations, we hypothesize that relapsed SCLC tumors with low SLFN11 expression (and resistant to topotecan) would be rendered sensitive to topotecan by ATR inhibition.

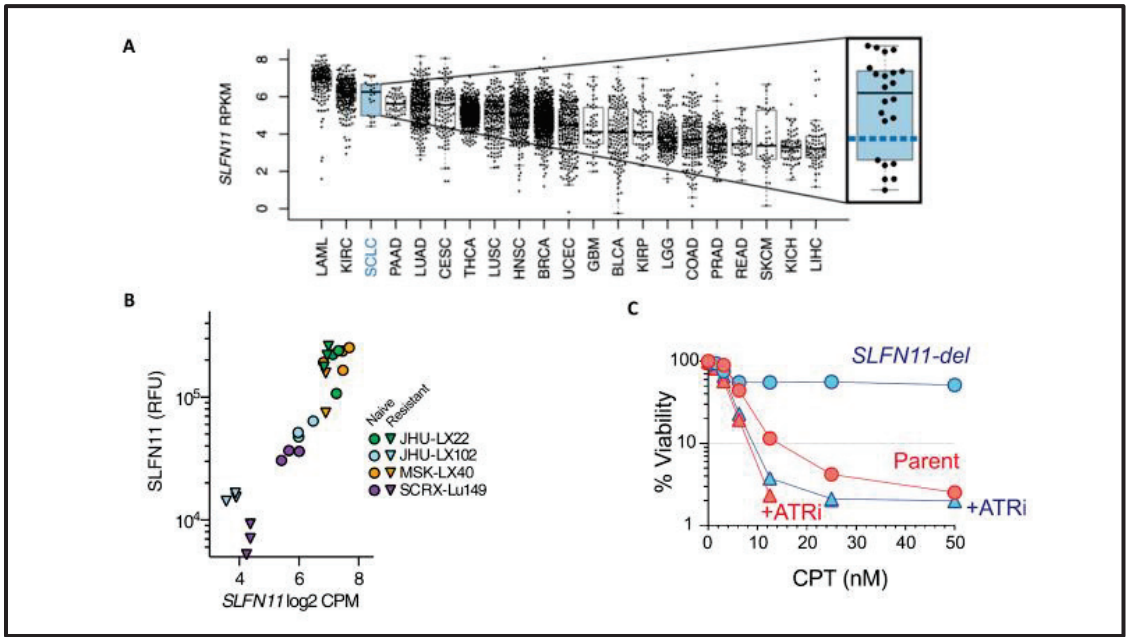


Figure 6: (A) SLFN11 expression in primary SCLC samples plotted with TCGA datasets [34]. (B) Concordance between SLFN11 gene expression by RNA-seq and SLFN11 protein expression by quantitative western blot [35]

(C) Viability curves for camptothecin alone (circles) and with ATRi (triangles) in isogenic DU145 prostate cancer cell lines [**39**]

2.5.2 ATM Expression

ATR inhibition could be synthetically lethal in ATM deficient tumors potentially due to the overlapping and cooperating roles of these pathways in coordinating cell cycle progression with DNA repair. One explanation for this is that the ATM-dependent DDR is necessary to cope with the DSBs that arise at stalled replication forks that collapse upon ATR inhibition. Accordingly, ATR inhibition has single-agent anti-tumor activity in ATM-deficient, but not ATM-proficient xenograft models [40]. Therefore, ATR inhibition could be more effective in ATM deficient SCLC.

2.5.3 MYC Amplification

Genomic amplifications in MYC have been identified in 6%-25% of primary human SCLC [41] and in 30%-50% of SCLC cell lines [42, 43] and has been associated with poor outcome, tumor progression, and treatment resistance [44]. Although the mechanisms underlying SCLC resistance are not very well elucidated, *in vivo* MYC-driven SCLC rapidly relapse following chemotherapy [45]. MYC amplification is thought to increase replicative stress and dependence on ATR signaling. In mice, reduced levels of ATR (found in a mouse model of the ATR-Seckel syndrome) completely prevented the development of MYC-induced lymphomas or pancreatic tumors, indicating a synthetic lethality between MYC and ATR *in vivo* [46].

Based on the above considerations, we hypothesize that MYC amplified SCLC tumors would respond better to topotecan plus M6620 compared to topotecan alone.

2.5.4 Circulating Cell-Free DNA and Circulating Tumor Cells (CTCs)

The molecular landscape of cancer is just beginning to be defined. However, we do not know enough about the genomic and molecular landscape of tumors from patients who enter early phase clinical trials. With this study, we will attempt to learn more about specific molecular features of cancers from this patient subgroup. It is particularly important to learn, as early as possible, if there are molecular features that can inform about potential response or resistance to treatments.

cfDNA is a promising biomarker that is being investigated in multiple tumor types. Changes in the levels of cfDNA can be used to monitor disease course, treatment responses, and recurrences. Lack of recurrent hotspot mutations- for example epidermal growth factor receptor (EGFR) exon 19 deletion or T790M, for which there are FDA approved tests, makes it challenging to use this approach in SCLC. Recent studies have shown feasibility of using a SCLC-specific panel to monitor disease burden, depth of response to treatment, and timely warning of disease relapse in patients with this disease [1].

Similar to cfDNA, molecular characterization of circulating tumor cells (CTCs) is emerging as an exciting opportunity in SCLC, given the limitations of tissue availability particularly at

relapse. A recent study demonstrated the high fraction of CTCs in SCLC and showed that CTCs reflect the tumor characteristics [47].

2.5.4.1 cfDNA Fragmentomics and Spatial Transcriptomics

In the last 4-5 years since the clinical trial development started and patients were enrolled, the understanding of SCLC heterogeneity has undergone a major shift. Rather than mutations, heterogeneity of SCLC is now thought to be driven by the expression of distinct transcription factors[48-51]. SCLC consist of tumor cells with neuroendocrine (NE) and non-neuroendocrine (non-NE) features[49, 50, 52-55], further defined by differential expression of the three lineage-defining transcription factors (TFs) ASCL1, NEUROD1, and POU2F3. A fourth subgroup has been variously characterized by YAP1 expression[49, 56-58] or low expression of all three transcription factors accompanied by an inflamed gene signature[59]. SCLC heterogeneity increases over the course of treatment, with expansion of the non-NE cell population associated with chemo-resistance[59-61]. These TFs cannot be inferred from cfDNA assays proposed in the version of the protocol prior to 10/21/2022.

Dying cells in the human body release their content into the bloodstream. Genomic DNA that is bound by nucleosomes and TFs escapes endogenous nucleases and so remains protected in plasma[62]. Regular turnover of lymphoid and myeloid cells in the human body is the major contributor to the pool of cell-free DNA (cfDNA) in plasma. However, in cancer, a detectable fraction of cfDNA arises from tumors. This suggests that cfDNA has the potential to map the tumor transcriptome and therefore can help uncover the regulatory landscape of cancer from plasma. Fragmentomics seeks to uncover the tissue of origin of cfDNA using the information on cfDNA fragment length. We will apply a genome-wide approach for analysis of cfDNA fragmentation profiles called DELFI (DNA evaluation of fragments for early interception) [63]. This approach provides a view of cfDNA "fragmentomes", permitting evaluation in any individual of the size distribution and frequency of millions of occurring cfDNA fragments across the genome. In a proof of principle study, genome-wide fragmentation profiles could non-invasively distinguish between NSCLC and SCLC. Specifically, consistent decrease in aggregate fragment coverage at regions containing the ASCL1 binding sites (±200 bp) was observed in patients with SCLC compared to non-cancer individuals or those with other cancer types. In contrast, at distances further from ASCL1 binding sites (>2000 bp), the fragment coverage for patients with SCLC and other patients were similar. As cfDNA fragmentomes can comprehensively represent both genomic and chromatin characteristics, it has the potential to identify the determinants of response and resistance to topotecan and topotecan plus M6620.

Although our understanding of cell-extrinsic influences on SCLC plasticity is rudimentary, a key role of these non-genetic mechanisms is suggested by two important observations: (i) transcriptional subtypes of human SCLC are not strongly associated with specific mutational patterns [48] and (ii) differences in neuroendocrine gene expression programs between human tumors and patient-derived xenografts [64]. We hypothesize that the dynamic interactions between tumor microenvironment (TME) and the cancer cells shape SCLC cell states. The sequencing approaches proposed in the current protocol such as bulk RNA-seq does not provide a context of the spatial interactions between cells and TME in tissue.

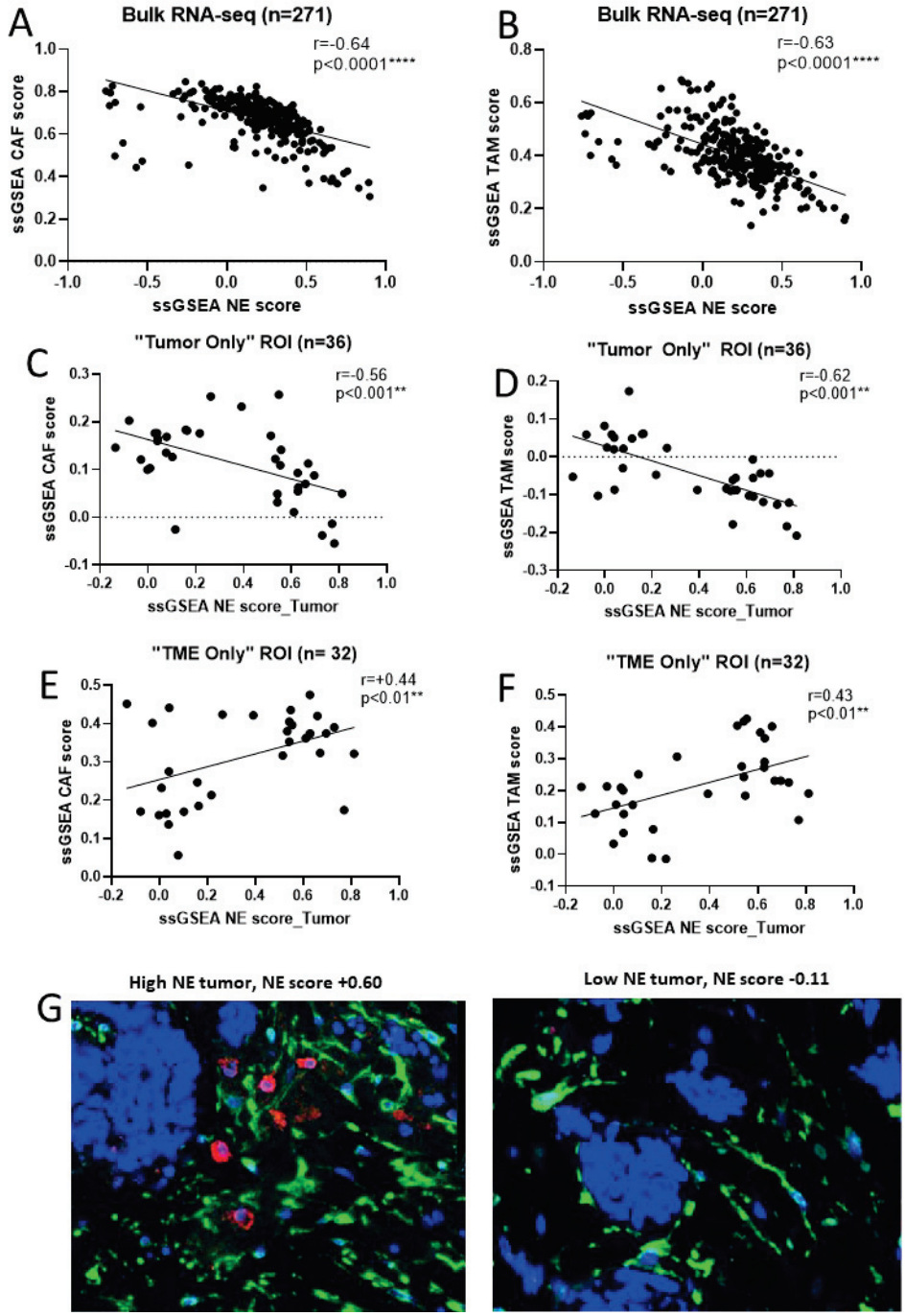


Figure 7: Discordance of stromal features derived from bulk and spatial transcriptome data. Correlation of (A) CAF (B) TAM gene expression signatures with tumor NE scores from bulk RNA-seq data. IMPOWER 133 trial data of treatment-naïve advanced SCLC patients Correlation of (C) CAF and (D) TAM gene expression signatures with tumor NE scores specifically in the tumor regions of interest. Preliminary data from 10 SCLC patient tumors profiled as transcriptome-wide spatial RNA profiling using GeoMX Whole Transcriptome Atlas platform Correlation of (E) CAF and (F) TAM gene expression signatures with tumor NE scores specifically in the TME regions of interest. Preliminary Data as above (G) Multiplex IHC showing high TAMs and CAFs in stroma around

high NE tumor compared with low NE tumor (CAF marker, ACTA2 in green; TAM marker CD163 in red; Blue/DAPI labels DNA/nuclei showing SCLC tumor clusters).

As seen in **Figure 7**, cancer associated fibroblast (CAF) and tumor associated macrophage (TAM) molecular signatures may be higher around non-NE SCLC when analyzed using bulk sequencing data. However, sequencing data from TME regions (spatial transcriptomic approach using Nanostring GeoMx digital spatial profiling) shows an opposite trend, proven by immunohistochemistry (IHC), showing higher enrichment of CAFs and TAMs around high NE tumors, highlighting the utility of the spatial transcriptomic approach.

Spatial transcriptomics is a novel approach allowing measurement and mapping of gene activity to the resolution of single to few cells keeping the position information intact [65-67]. Mapping the cell-intrinsic and -extrinsic factors that impact SCLC tumor cell states in their positional context could yield important insights into the biologic basis of response and resistance to topotecan and topotecan plus M6620.

2.5.5 Whole Exome Sequencing (WES)

Although a deadly and common cancer, SCLC was not included in The Cancer Genome Atlas (TCGA) due to challenges of tissue acquisition. Only a limited number of SCLC exomes- around 200 or so- have been published [41, 68]. The vast majority of published exomes of SCLC (>95% cases), are from primary tumors. The mutational landscape of relapsed SCLC as well as its relation to therapies is not known. Tissue collection on this trial provides an opportunity to explore the relapsed SCLC exome. Tissue remaining from studies outlined in Sections **2.5.1**, **2.5.2** and **2.5.3** will be archived for future WES. In addition, a tube of blood will be collected from each patient at baseline for germline DNA.

3 PATIENT SELECTION

In order to determine eligibility, subjects will be screened per the **STUDY CALENDAR** once the subject has signed the appropriate consent. In addition, the following minimal risk activities may be performed prior to obtaining subject consent:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

3.1 Eligibility Criteria

3.1.1 Inclusion Criteria

3.1.1.1 Patients enrolled to the primary cohort must have limited- or extensive-disease SCLC at diagnosis, with relapse at study entry with measurable disease at random assignment per RECIST 1.1. See Section **12** for the evaluation of measurable disease. Both platinum-sensitive and platinum-resistant patients will be included.

3.1.1.2 Patients with extrapulmonary small cell cancers (cancers with small cell morphology and arising outside the lung, such as small cell prostate, bladder, *etc.*; see Sections **2.1** and **2.2** for disease description and rationale.) will be eligible for the exploratory cohort.

3.1.1.3 Patients must be ≥18 years of age because no dosing or adverse event data are currently available on the use of M6620 in combination with topotecan in patients <18 years of age.

3.1.1.4 ECOG performance status ≤2 (Karnofsky ≥60%, see **APPENDIX A: Performance Status Criteria**

3.1.1.5 Patients must have adequate organ and marrow function as defined below:

– Hemoglobin	≥9.0 g/dL - patients may receive transfusion to meet the Hb eligibility.
– Absolute Neutrophil Count (ANC)	≥1,500/mcL
– Platelets	≥100,000/mcL
– Total Bilirubin	≤2 mg/dL
– AST(SGOT)/ALT(SGPT)	≤3.0 × institutional ULN
– Creatinine	≤ institutional ULN
	OR
– Glomerular Filtration Rate (GFR)	≥60 mL/min/1.73 m^2 unless data exists supporting safe use at lower kidney function values, no lower than 30 mL/min/1.73m^2 (See **APPENDIX B**: **Formula to Estimate Renal Function Using Serum Creatinine** – Cockcroft-Gault Equation)

3.1.1.6 Human immunodeficiency virus (HIV)-positive subjects on combination antiretroviral therapy are eligible as long as they are on effective anti-retroviral therapy with undetectable viral load within 6 months and there is no drug-drug interaction with M6220.

3.1.1.7 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.1.8 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.1.1.9 The effects of M6620 on the developing human fetus are unknown. For this reason and because DNA-damage response (DDR) inhibitors as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 6 months after completion of M6620 administration. 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 for 6 months after completion of M6620 administration.

3.1.1.10 Patients with **treated brain metastases** are eligible if they are symptomatically stable while off steroid therapy for a minimum of 21 days.

3.1.1.11 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.1.12 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

3.1.1.13 Ability to understand and the willingness to sign a written informed consent document.

3.1.2 Exclusion Criteria

3.1.2.1 Patients with symptomatic brain metastasis are not eligible due to their extremely poor prognosis and since it is unclear whether the investigational agent penetrates the blood-brain barrier. However, subjects who have had treatment for their brain metastasis and are symptomatically stable while off steroid therapy for a minimum of 21 days may be enrolled.

3.1.2.2 Patients who have received prior topotecan therapy.

3.1.2.3 Patients who have had chemotherapy or radiotherapy within 3 weeks prior to enrolment. Note: Patients who have had palliative radiotherapy may be included as long as they have recovered from any radiotherapy related adverse events (allow at least a week between radiotherapy completion and study treatment).

3.1.2.4 Patients who have not recovered from adverse events due to prior anti-cancer therapy except hair loss and peripheral neuropathy (*i.e.*, have residual toxicities > Grade 1).

3.1.2.5 Patients who are receiving any other investigational agents.

3.1.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to M6620 or topotecan used in study.

3.1.2.7 M6620 is primarily metabolized by CYP3A4; therefore, concomitant administration with strong inhibitors of CYP3A4 (*e.g.*, ketoconazole, itraconazole, clarithromycin, HCV and HIV protease inhibitors, nefazodone, posaconazole, telithromycin, voriconazole) or inducers of CYP3A4 (*e.g.*, rifampin, phenytoin, carbamazepine, phenobarbital, St. John's Wort) should be avoided. Patients requiring any medications or substances that are strong inhibitors or inducers of CYP3A during the course of the study are ineligible. (Refer to **APPENDIX C: Patient Drug Interactions Handout and Wallet Card**). Because the lists of these agents are constantly changing, it is important to regularly consult a frequently updated medical reference for a list of drugs to avoid or of which to minimize use. The Patient Drug Interactions Handout and Wallet Card should be provided to patients (**APPENDIX C: Patient Drug Interactions Handout and Wallet Card**). As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.1.2.8 Patients with uncontrolled intercurrent illness.

3.1.2.9 Pregnant women are excluded from this study because M6620 as a DDR inhibitor may have 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. These potential risks may also apply to topotecan used in this study.

3.1.2.10 Patients with high grade neuroendocrine cancers, but with no small cell morphology will not be eligible.

3.1.2.11 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.1.2.12 Patients with Li-Fraumeni syndrome will not be eligible.

3.2 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate

with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4 REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		

Documentation Required	IVR	NPIVR	AP	A	AB
GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster,
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN,
- Act as the site-protocol Principal Investigator (PI) on the IRB approval, and

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context to the CIRB using IRB Manager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailing the email address above or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation,
- IRB-signed CTSU IRB Certification Form, and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following criteria to complete processing of the IRB/REB approval record to be completed:

- Holds an Active CTEP status,
- Active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization's roster,
- If using NCI CIRB, active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile,
- Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional site requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization,
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to institutions and its associated investigators and staff on a participating roster. To view/download site registration forms:

- Log on to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of your screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select LAO-NCI, and protocol number 10268,

- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter select *Site Registration*, to download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU.)

4.2.2 Protocol Specific Requirements for NCI Protocol 10268 Site Registration

- Specimen Tracking System Training Requirement:
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking system may require new training.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Please contact STS Support at Theradex for the training (STS.Support@theradex.com, Theradex phone: 609-799-7580).
- Requirement for LPO approval of participating site's local informed consent
  - Each participating site will need to provide the local informed consent prior to enrolling their first patient.
  - All amendments will require participating sites to receive approval of their local informed consent from LPO unless amendment specific PSRs are different.
  - The Local informed consent document will need to be submitted via CTSU per **4.2.3**.

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' website.

To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the Regulatory section, and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org in order to receive further instruction and support.

4.2.4 Checking Site Registration Status

Site's registration status may be verified on the CTSU website.

- Click on *Regulatory* at the top of the screen,
- Click on *Site Registration*, and
- Enter the site's 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN Corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
- Have an approved site registration for a protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes, and
- All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

4.3.2 Special Instructions for Patient Enrollment

Slot reservation:

Sites must submit the following documentation/information to the study team before their slot request will be approved and they are able to enroll the patient in OPEN:

Patient initials
Date of birth (month/year)
Diagnosis
Projected consent date/Screening start date
Projected date of enrollment
History and physical
Laboratory results
Pathology report

This Study will use the ETCTN Specimen Tracking System (STS).

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management section under the Rave Home tab and then under Rave Resource Materials.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found in Section **5.4**.

4.3.3 Two Step Registration Process

The study will utilize a two-step registration process in OPEN. Subjects who sign an informed consent form and meet the pre-registration eligibility criteria should be pre-registered to the study by completing the initial registration step, Step #1, in OPEN. This will generate a subject ID with which to label the pre-registration tissue specimen. The second registration step, Step #2, should be completed in order to register and randomize subjects to the treatment phase of the study or to record a screen failure.

4.3.4 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the PI. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen and Quantity	Send Specimens To:
Archival		
	• Formalin-fixed paraffin-embedded (FFPE) tumor tissue block (preferred)[1] If a block is not available, then submit: • 1 H&E stained slide • 30-50[2] unstained, charged, unbaked slides	EET Biobank
Baseline		

Time Point	Specimen and Quantity	Send Specimens To:

	• Tumor needle biopsy[3]
	  ○ 2 tumor cores frozen
	  ○ 3 tumor cores in formalin
	• 20 mL blood in cfDNA Streck tubes (2 x 10 mL)
	• 10 mL blood in EDTA tube (1 x 10 mL)
		EET Biobank

	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
Cycle 1, Day 2 (4 hours +/- 60 minutes after end of infusion)		
	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
Cycle 1, Day 4 (Pre-treatment)		
	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
Cycle 1, Day 5 (Pre-treatment)		
	• 20 mL blood in cfDNA Streck tubes (2 x 10 mL)	EET Biobank
Cycle 2, Day 1 (Pre-treatment)		
	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
	• 20 mL blood in cfDNA Streck tubes (2 x 10 mL)	EET Biobank
Cycle 3, Day 1 (Pre-treatment)		
	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
	• 20 mL blood in cfDNA Streck tubes (2 x 10 mL)	EET Biobank
Progression		
	• 10 mL blood in Streck tube (1 x 10 mL)	PADIS Laboratory
	• 20 mL blood in cfDNA Streck tubes (2 x 10 mL)	EET Biobank

Time Point	Specimen and Quantity	Send Specimens To:
[1]For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. [2] Should obtaining 30-50 slides not be feasible, collect as much as possible taking into account local policies. The reason the required quantity is not available should be noted. [3] For new biopsies, the Tissue Biopsy Verification Form (**APPENDIX E**), a copy of the radiology and/or operative reports from the tissue removal procedure and the diagnostic anatomic pathology report must be sent with the tissue to the EET Biobank. When completed, upload the corresponding pathology report to Rave and send a copy to the EET Biobank.		

5.2 Specimen Procurement Kits and Scheduling

5.2.1 EET Biobank

5.2.1.1 Specimen Shipping Kits

Kits for the collection and shipment of specimens to the EET Biobank can be ordered online via the Kit Management system: (https://ricapps.nationwidechildrens.org/KitManagement).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kit types per protocol per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

Note: Kits or supplies are only provided for specimens shipped to the Biorepository. Institutional supplies must be used for all other specimen collection and processing.

5.2.1.2 Scheduling of Specimen Collections

Please adhere to the following guidelines when scheduling procedures to collect tissue:

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.
- Tissue can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the EET Biobank at Nationwide Children's Hospital.

Specimens submitted frozen (*i.e.,* tumor biopsy tissue) can be collected on any day but must be stored frozen and shipped to the EET Biobank on Monday through Thursday. In the event that frozen specimens cannot be shipped immediately, they must be maintained at -80 °C.

Fresh blood specimens may be collected and shipped Monday through Friday.

5.2.2 PADIS Laboratory

5.2.2.1 Materials and Equipment Required

1. Blood shipment container provided by National Cancer Institute at Frederick (NCI-F) – store at room temperature
   - Controlled room temperature (CRT) gel packs
   - Styrofoam box
   - Shipping cardboard box (red/white)

2. Specimen collection kits provided by NCI-F – store at room temperature
   - Streck Cell-Free DNA Preservative Tubes, 10.0-mL draw capacity (Streck, Cat#: 218962). **Do not** use Streck tubes after expiration date.
   - 50-mL Falcon tube containing absorbent paper (secondary holding tube)
   - Zip lock bag – to protect Specimen Submission Form (**APPENDIX D**) upon return
   - FedEx Priority Overnight return label; pre-addressed with NCI account information

5.2.2.2 Ordering Shipping Kits and Blood tubes

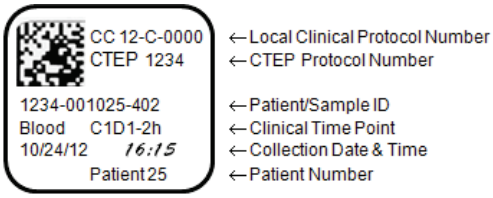
Important: Blood shipment containers and specimen collection kits are shipped separately.

1. Blood shipment containers can be requested by e-mail to NCI_PD_Support@mail.nih.gov. Specify the number of shipment containers you will need.
   - Allow at least six business days for receipt of the blood shipment containers.
   - A confirmation e-mail with the expected shipping date will be sent.

2. Specimen collection kits can be requested by e-mail to NCI_PD_Support@mail.nih.gov. Specify the number of specimen collection kits you will need.
   - Allow at least six business days for receipt of the specimen collection kits.
   - A confirmation e-mail with the expected shipping date will be sent.

5.2.2.3 Preparation

1. Store the blood shipment containers and the specimen collection kits at 18°C to 25°C (RT).
2. Do not use Streck tubes after their expiration date.
3. Print specimen labels for the Streck tubes to be collected including the Patient/Sample ID, CTEP/clinical protocol number, and collection date and time.
4. Patient/Sample IDs for blood samples collected at the NCI include the CTEP number followed by a unique patient identifier and a sequential specimen ID; NCI blood samples for CTC PD sampling are series 400 (see example label).



5.2.2.4 Scheduling of Specimen Collections

All samples MUST be shipped within 24 hours of collection to ensure quality results.

Blood for CTC analysis will be shipped at room temperature to the PADIS laboratory on the day it is collected. Note: the collected blood samples are stable for up to 48 hours at room temperature (15˚C to 30˚C) prior to processing (per PADIS laboratory standards). Avoid collecting specimens on Fridays, as PADIS laboratory is closed on Saturdays and Sundays. Also avoid shipping on the day before a federal holiday.

5.3 Specimen Collection

5.3.1 Biopsy Collection Procedure

Baseline tumor biopsy is mandatory for patients with small cell lung cancer. Pre-treatment biopsies will be optional for the cohort of extrapulmonary small cell patients. For the collection of biopsy specimens, core needle biopsy is the preferred technique. It is preferred that at least **five core biopsies** 16-18 gauge in diameter and at least 1 cm in length, are obtained; three in formalin and two frozen. Samples will be shipped on the same day as collection to the EET Biobank (see Section **5.5** below). Specimens should be placed immediately into buffered formalin (provided in the kit) in the provided specimen collection container.

Core needle biopsy (CNB) tumor tissues will be collected for molecular profiling. Core biopsies at least 1 cm in length will be obtained through Interventional Radiology by a percutaneous approach using a 16-18-gauge needle. Only percutaneous biopsies will be performed on patients with solid tumors. However, excisional biopsy or endoscopic biopsy is allowed if medically indicated and can be used for analysis. Biopsies will be sent for analyses as defined in the protocol. Two core biopsies will be immediately flash-frozen using a dry ice/alcohol slurry as flash-frozen tissue for biobanking, and **three** core biopsies will be fixed in formalin.

5.3.1.1 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in Section **5.4.2.2**.
2. Obtain three 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.

4. Secure the container lids and place the containers into the shipping kit. Keep tissue in formalin jars at room temperature until shipment to the EET Biobank.

5.3.1.2 Collection of Flash-Frozen Biopsy

1. Tissue should be frozen as soon as possible. Optimally, freeze within 30 minutes from resection.
2. Label two cryovials (with the core number) according to instructions in Section **5.4.2.2**.
3. Each core sample should be placed directly into a separate prelabeled and pre-chilled cryovial.

   Pre-chilling the cryovials helps to keep the tissue from sticking to the sides of the vial, making accessioning and banking by the EET Biobank easier.
4. Each specimen contained in its cryovial should be flash frozen using a dry ice/alcohol slurry.
5. Frozen specimens should be shipped (the day of collection) Priority Overnight on dry ice in an insulated shipper or a dual temperature-chambered kit (provided by Biorepository).

5.3.1.3 Archival Formalin-Fixed Paraffin-Embedded (FFPE) Tumor Specimen

The following criteria must be met for previously-collected FFPE tissue:

- Formalin-fixed paraffin-embedded tumor tissue block(s) must be submitted. The optimal block is at least 70% tumor. Specimen size requirement is as follows:
  - Surface area: 25 mm^2 is optimal. Minimum is 5 mm^2.
  - Volume: 1 mm^3 optimal. Minimum volume is 0.2 mm^3, however the success of DNA extraction decreases at suboptimal tissue volume.

Archival FFPE tissue from diagnosis or an earlier relapse will be collected. If an existing block cannot be submitted, the following are requested:

- One (1) H&E slide,
- Thirty to fifty (30 – 50)unstained, charged, unbaked slides. Note: fewer slides may be collected per section **5.1**.

See Section **5.4.2.2** for labeling instructions.

5.3.2 Blood Collection

5.3.2.1 Collection of Blood in Streck Tube

Important: If blood samples are collected by a second party (nursing staff, phlebotomist, etc.), remove all clinical and personal identifiers from tube when received and replace with the pre-printed specimen label. Do not just place specimen label on top of clinical label.

1. Place a pre-printed specimen label on the Streck tube.
2. Collect whole blood aseptically by venipuncture or from a venous port into a Streck tube.

3. Fill the Streck tube with a minimum of **8-mL volume for a 10-mL capacity tube** until the blood flow stops to ensure the correct ratio of sample to anticoagulant and preservative. If < 8-mL blood volume is collected, make a note on the Specimen Submission Form (**APPENDIX D**) for that sample so the processing laboratory is aware of the deviation.
4. Immediately mix the specimen tube by **gently inverting** the tube 8-10 times; tube inversion prevents clotting. **Do no shake** the tube, vigorous mixing can cause hemolysis. Inadequate or delayed mixing may result in inaccurate test results.
5. **Do not** refrigerate or freeze specimens; keep tubes at 18 °C to 25 °C (RT).
   - **Important**: Do not place sample(s) on ice. If the clinical staff placed the Streck tube on ice, make a notation on the Specimen Submission Form (**APPENDIX D**) and bring the tube to 18 °C to 25 °C (RT) before proceeding with sample processing.
6. Label each tube with Patient/Sample ID, collection date and time, and clinical trial time point (i.e., cycle, day, hour: C1D1-2h).
7. Blood specimens should be shipped **within 24 h of collection**, preferably on the day of collection to ensure quality results. Packaging and shipping instructions are in SOP Section 9.0.
   - Blood specimens may be stored or transported in Streck Tubes for up to 96 h at 18 °C to 25 °C (RT) prior to processing, but processing **must** begin within 96 h of collection.

Important:
- Processing limitations: a minimum volume of 8.0 mL of blood in the Streck tube is needed for processing.
- Blood specimens MUST be stored, transported, and processed at 18 °C to 25 °C.
- All samples MUST be shipped within 24 hours of collection to ensure quality results.

5.3.2.2 Collection of Blood in cfDNA Streck Tube

1. Label two 10 mL cfDNA Streck tubes according to the instructions in Section **5.4.2.1**.
2. Collect blood in two pre-labeled cfDNA Streck tubes (10 mL), Cat No. 218992 and gently invert to mix. **Note:** blood must be thoroughly mixed to ensure preservation of specimen.
3. **After collection, blood in cfDNA Streck tubes should never be refrigerated,** as this will compromise the specimen. Blood collected in cfDNA Streck tubes is stable at room temperature. These tubes will be shipped to the EET Biobank for further processing. Upon receipt at the EET Biobank, blood in cfDNA Streck tubes will be processed for plasma and DNA.

5.3.2.3 Collection of Blood in EDTA Tubes

1. Label EDTA tubes according to the instructions in section **5.4.2.1**.
2. Collect 10 mL blood in EDTA tube(s) and gently invert tube to mix.
3. Sample should be maintained and shipped at room temperature.
4. Ship on day of collection (whenever possible) according to instructions below.

5. If blood cannot be shipped on the day of collection (*e.g.*, a late scheduled collection), then refrigerate until shipment.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section **3**.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this or any other protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, the radiology and operative report(s) must also be uploaded into Rave. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System, please contact the STS Support at STS.Support@theradex.com.

The Shipping List report **must** be included with all sample submissions.

5.4.2 EET Biobank Specimen Labeling

5.4.2.1 Blood Specimen Labels

Include the following on blood specimens (including whole blood and frozen, processed blood products – like serum and plasma):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood)
- Collection date and time (to be added by hand)

5.4.2.2 Tissue Specimen Labels

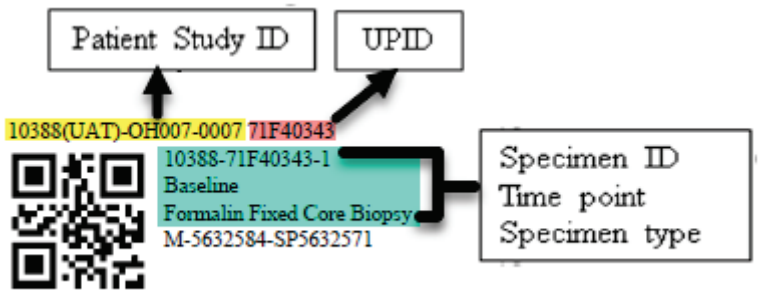
Include the following on all tissue specimens or containers (*e.g.*, formalin jar):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, FFPE Block, Formalin Fixed Tissue, Fresh Tissue in Media, *etc.*)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number
- Block number from the corresponding pathology report (archival only)
- Collection date and time (to be added by hand)

5.4.2.3 Example of Specimen Label Generated by STS

STS includes a label printing facility, accessed via the Print Label CRF in the All Specimens folder. A generated PDF is emailed to the user as a result of saving that form.

The following image is an example of a tissue specimen label printed on a label that is 0.5” high and 1.28” wide.



The QR code in the above example is for the Specimen ID shown on the second line.

Labels may be printed on a special purpose label printer, one label at a time, or on a standard laser printer, multiple labels per page. Theradex recommends the use of these low temperature waterproof labels for standard laser printers: https://www.labtag.com/shop/product/cryo-laser-labels-1-28-x-0-5-cl-23-colors-available/

The last line item on the label includes the following data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

Space is provided at the bottom of the label for the handwritten date and optional time. The last line on the example label is for the handwritten date and optional time.

5.4.3 Overview of Process at Treating Site

5.4.3.1 OPEN Registration

All registrations will be performed using the OPEN system. OPEN communicates automatically with the IWRS which handles identifier assignments, any study randomization and any prescribed slot assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

Any data entry errors made during enrollment should be corrected in Rave.

5.4.3.2 Rave Specimen Tracking Process Steps

Step 0: Log into Rave via your CTEP-IAM account, then navigate to the appropriate participant.

Step 1: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment** CRF**:** Enter Time Point, Specimen Category, Specimen Type, Block number, Tissue type, Surgical Path ID, number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using the **Print Labels** CRF located in the All Specimens folder, then collect specimen.

- Label specimen containers and write collection date and time on each label.
- After collection, store labeled specimens as described in Section **5.3.2**.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports and Tissue Biopsy Verification form (when applicable). Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen). Uploaded reports should have protected health information (PHI) data like name, date of birth, mailing address, medical record number or social security number (SSN) redacted. Do not redact SPID, block number, diagnosis or relevant dates, and include the UPID and patient Study ID on each document.

Step 3: Complete specimen data entry.

- **Specimen Transmittal** Form**:** Enter Collection date and time and other required specimen details.

Step 4: When ready to ship, enter shipment information.

- **Shipping Status** CRF**:** Enter tracking number, your contact information, recipient, number of sample containers and ship date once for the 1st specimen in a shipment.
- **Copy Shipping** CRF**:** In the specimen folders for additional specimens (if any) that will be shipped with the initial specimen, please use the **Copy Shipping** form to derive common data into additional **Shipping Status** forms. A few unique fields will still need to be entered in Shipping Status.

Step 5: Print shipping list report and prepare to ship.

- Shipping List report is available at the site level.
- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

Step 8: Monitor the Receiving Status form located in each specimen folder for acknowledgment of receipt and adequacy.

5.5 Shipping Specimens from Clinical Site to the EET Biobank

Core biopsies that are fixed in formalin and fresh blood should be shipped as one shipment at ambient temperature, whenever possible. The same box sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

For all archival tissue, the corresponding anatomical clinical pathology report is required both in the package and uploaded in the ETCTN specimen tracking system. If this is not available at the time of shipment, then it must be uploaded to the ETCTN specimen tracking system, or the specimen will not be processed. The pathology report must state the disease diagnosis made by the reviewing pathologist.

For formalin-fixed and frozen biopsies, if the corresponding anatomical pathology report is not available at the time of shipment, then the surgical and/or radiology report from the tissue removal procedure and the anatomic pathology report from diagnosis must be uploaded to the ETCTN specimen tracking system and included in the package, or the specimen will not be processed. When completed, upload the corresponding pathology reports in RAVE.

5.5.1 Specimen Shipping Instructions

Tissue in formalin must be shipped on the day of collection. Collect and ship on Monday through Wednesday.

Frozen specimens may be shipped on Monday through Thursday.

Fresh blood may be shipped on Monday through Friday. Please select “Saturday Delivery” when shipping fresh blood on a Friday.

5.5.1.1 Shipping Blood in an Ambient Shipper

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that the lids of all primary receptacles containing liquid are tightly sealed.
2. Prepare the SAF-T-TEMP Gel Pak for shipment. Note: If contents of the Pak are crunchy, place Pak in a warm water bath until gel is smooth. Do not refrigerate, freeze, or microwave.
3. Place the SAF-T-TEMP Pak in bottom of insulated chest. Note: The insulated chest must be shipped inside the provided cardboard box(es).
4. Place the blood collection tubes in zip-lock bags.
5. Next, place blood into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.

6. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
7. Place packaged blood collection tube(s) and a copy of the shipping manifest from the Sample Tracking System on top of the SAF-T-TEMP Pak.
8. Place the lid on the insulated chest.
9. Close the outer flaps of the shipping box and tape shut.
10. Attach a shipping label to the top of the shipping container.
11. Attach an Exempt Human Specimen sticker to the side of the box.
12. Ship specimens via overnight courier to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.1.2 Shipping Frozen and Ambient Specimens in a Dual-Chamber Kit

The Dual Chambered Specimen Procurement Kit is constructed to allow the shipment of frozen (on dry ice) and ambient (room temperature) specimens in the same container. **Dry ice may be placed in either compartment of the kit but should not be put in both.** The dual chambered kit is only used for shipments that contain both frozen and ambient specimens. If formalin-fixed tissue is shipped separately (not in the same shipment as frozen specimens), then it must be shipped using institutional shipping supplies.

- **Frozen specimens** may be shipped on Monday through Thursday. Ensure that sufficient dry ice is included to completely encase the specimens to maintain specimen integrity during shipment.
- **Formalin-fixed tissue** may only be shipped on Monday through Wednesday.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that lids of all primary receptacles containing liquid are tightly sealed.
2. Pre-fill one of the kit chambers about 1/3 with dry ice.
3. Prepare the frozen specimens for shipment:
   a. Place the specimens into zip-lock bags.
   b. Place the zip-lock bags into a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the biohazard envelope.
   c. Put each biohazard envelope into a Tyvek envelope. Expel as much air as possible and then seal the Tyvek envelope.
4. Quickly place the Tyvek envelope containing frozen specimens in the kit compartment that is pre-filled with dry ice. Place the Tyvek envelope on top of the dry ice. Cover the specimens with additional dry ice until the compartment is almost completely full.
5. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.

6. Prepare the ambient specimens for shipment:
    a. Seal the lids of the formalin jars with parafilm. Place absorbent material around the primary container of each liquid specimen. Place the specimens into zip-lock bags.
    b. Place specimens inside the secondary pressure vessel with bubble wrap.
    c. Secure the lid on the secondary pressure vessel and set it inside the kit chamber.
7. Insert a copy of the required forms in the kit chamber with the ambient specimens.
8. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
9. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
10. Complete a FedEx air bill and attach to top of shipping container.
11. Complete a dry ice label.
12. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
13. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2 Shipping Address

Ship to the address below. Ship formalin-fixed, frozen, and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank The Research Institute at Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions.

5.5.3 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Toll-free Phone: (800) 347-2486
E-mail: BPCBank@nationwidechildrens.org

5.6 Shipping of Specimens from Clinical Site to PADIS laboratory

5.6.1 Specimen Shipping Instructions

Samples will be shipped on the same day of blood draw directly to the PADIS Laboratory. This assay is currently being optimized.

Important:
All samples MUST be shipped within 24 h of collection to ensure quality results.

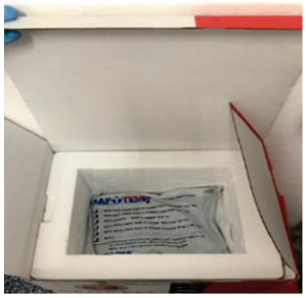
1. Complete a Specimen Submission Form (**APPENDIX D**) for all samples in the shipment.

2. For each sample record the Patient/Sample ID, Clinical Protocol/CTEP number, collection date and time, clinical trial time point, and clinical diagnosis if known. Inclusion of the clinical diagnosis allows the specimen processing laboratory to more efficiently enrich CTCs from the blood sample.

3. In the comments field, record any deviations during blood collection (e.g., < 8.0-mL blood).

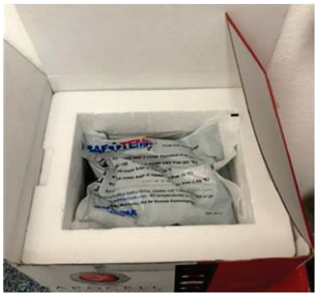
5.6.1.1 Packaging Instructions

1. Store CRT packs at 18 °C to 25 °C (RT). **DO NOT** refrigerate or freeze CRT packs.
2. Place an 18 °C to 25 °C (RT) CRT pack in the bottom of a Styrofoam shipping container.

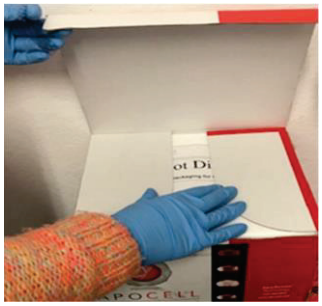


3. Wrap absorbent paper around the specimen-filled Streck tube and place in a 50-mL Falcon centrifuge tube or blood shipping tube.

4. Place the tube in the Styrofoam box on top of the CRT pack, put a second 18 °C to 25 °C (RT) CRT pack on top of the tube, and insert the completed Specimen Submission Form (**APPENDIX D**) into a Zip lock bag and place on top of the CRT gel pack.


5. Close the Styrofoam box and place it inside a cardboard shipping box.



6. Seal the cardboard box and attach the return shipping label to the outside of the box; do not cover the UN3373 (Biological substance, Category B) sticker with the shipping label.

5.6.2 Shipping Address

Specimens should be shipped to:

Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: 301 846-5748

Send an e-mail on the day of shipment to NCI_PD_Support @mail.nih.gov to ensure timely receipt and processing of the samples. The **subject line** should state: "PD Clinical Shipment Notification"

For each specimen being shipped include the following information:

- Patient/Sample ID (s)
- FedEx Tracking Number (s)

- Your Site Name

5.6.3 Contact Information for Assistance

For any other issues or questions regarding specimen collection and shipment for the PADIS laboratory, contact Amy Pantella at 301-846-6747 or Rachel Andrews at 301-846-1951 (Email: NCI_PD_Support@mail.nih.gov).

5.7 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Biomarker Assay	Biomarker Type and Purpose	M/O	Specimen(s) and Time Point(s)	Laboratory Performing Assay
1	SLFN11	RNA-Seq	Integrated Correlate level of expression with outcome	M*	Tumor needle biopsy** Baseline	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov
	Spatial transcriptomics	Spatial transcriptomics	Integrated Correlate level of expression with outcome	M*	Tumor needle biopsy** Baseline	NCI DTB Anish Thomas anish.thomas@nih.gov
	cMYC (amplification)	RNA-Seq	Integrated Correlate level of expression with outcome	M*	Tumor needle biopsy** Baseline	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov
	ATM	RNA-Seq	Integrated Correlate level of expression with outcome	M*	Tumor needle biopsy** Baseline	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov
2	CTC	phospho-ATR and γH2AX	Exploratory To determine prognostic and predictive role, and their usefulness to track tumor evolution and heterogeneity in response to treatment	O	Blood Baseline, Cycle 1 Day 2[#], Cycle 1 Day4[†], Cycle 2 Day 1[†], Cycle 3 Day 1, and at progression.	PADIS Dr. Ralph Parchment parchmentr@mail.nih.gov

Priority	Biomarker Name	Biomarker Assay	Biomarker Type and Purpose	M/O	Specimen(s) and Time Point(s)	Laboratory Performing Assay
3	cfDNA - Fragmentomics	Fragmentomics	Exploratory To determine prognostic and predictive role, and their usefulness to track tumor evolution and heterogeneity in response to treatment	O	Blood Baseline, Cycle 1 Day 5 †, Cycle 2 Day 1 †, Cycle 3 Day 1, and at progression.	Delfi Diagnostics, Baltimore, MD Lorenzo Rinaldi rinaldi@delfidiagnostics.com
4	Circulating Tumor DNA (ctDNA)	TSO500	Exploratory To determine prognostic and predictive role, and their usefulness to track tumor evolution and heterogeneity in response to treatment	O	Blood Baseline, Cycle 1 Day 5 †, Cycle 2 Day 1 †, Cycle 3 Day 1, and at progression.	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov
5	SLFN11, ATM, cMYC	Immunohistochemistry	Exploratory	O	Tumor needle biopsy** Baseline	NCI DTB Anish Thomas anish.thomas@nih.gov
6	WES	Whole Exome Sequencing	Exploratory	O	Tumor needle biopsy and blood (1x10mL, EDTA)** Baseline	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov

* Pre-treatment biopsies will be optional for the extrapulmonary cohort. The same panel of biomarker assays will be performed on samples from patients in the extrapulmonary cohort when biopsy is available.
** Archival tissue will be collected to use to supplement sampling from fresh biopsy.
ATM = Ataxia-Telangiectasia Mutated gene; cfDNA= circulating cell-free DNA; CTC = Circulating Tumor Cells; IHC = Immunohistochemistry; MoCha = Molecular Characterization Laboratory; MYC = family of regulator genes and proto-oncogenes that code for transcription factors; PADIS = Pharmacodynamic Assay Development; SLFN11 = Schlafen family member 11; TBD = To Be Determined; WES = Whole Exome Sequencing

[†] Blood samples should be collected prior to drug administration.
[#] Blood Samples for CTC at C1D2 should be collected 4 hours (+/- 60 minutes) after end of infusion.

5.8 Integrated Correlative Studies

5.8.1 SLFN11

Studies from the NCI, DTB group demonstrated that SLFN11 blocks replication independently of ATR and that *SLFN11*-negative cells dominantly rely on ATR under replication stress [38]. ATR inhibition overcomes resistance to topoisomerase 1 inhibitors in *SLFN11*-negative cancer cells.

SLFN11 expression characterized by RNA-seq of a baseline tumor biopsy in this study may demonstrate a correlation with topotecan sensitivity mediated by ATR inhibition.

5.8.1.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

5.8.1.2 Site Performing Correlative Study

5.8.1.3 RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section **5.8.1.2**.

5.8.1.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.8.2 ATM

ATR inhibition may be more effective in patients with ATM-deficient tumors [40]. ATM deficiency characterized by RNA-sequencing of a baseline tumor may demonstrate a correlation with topotecan sensitivity mediated by ATR inhibition.

5.8.2.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

5.8.2.2 Site Performing Correlative Study

RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

5.8.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section **5.8.2.2**.

5.8.2.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.8.3 cMYC amplification

Genomic amplifications in MYC may increase replicative stress and dependence on ATR signaling [46]. MYC amplification characterized by RNA-sequencing of a baseline tumor biopsy in this study may demonstrate a correlation with topotecan sensitivity mediated by ATR inhibition.

5.8.3.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

5.8.3.2 Site Performing Correlative Study

RNA-Seq will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

5.8.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section **5.8.3.2**.

5.8.3.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.8.4 Spatial Transcriptomics

Spatial transcriptomics is a novel approach allowing measurement and mapping of gene activity to the resolution of single to few cells keeping the position information intact [65-67]. Mapping the cell-intrinsic and -extrinsic factors that impact SCLC tumor cell states in their positional context could yield important insights into the biologic basis of response and resistance to topotecan and topotecan plus M6620.

5.8.4.1 Specimens Receipt and Processing at the EET Biobank

As this analysis is being added following completion of the study accrual, available tumor tissue will be used for this analysis. Tissue processing concerns will be addressed between the EET biobank and study team by various methods as needed

5.8.4.2 Site Performing Correlative Study

Analysis will be performed by the NCI Developmental Therapeutics Branch Laboratory.

5.8.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Anish Thomas
Center for Cancer Research
National Cancer Institute
Building 10 Room 4-5330
Bethesda, MD 20892

5.8.4.4 Contact Information for Notification of Specimen Shipment

Anish Thomas (anish.thomas@nih.gov)

5.9 Exploratory/Ancillary Correlative Studies

5.9.1 Circulating tumor cells

Patients with SCLC have demonstrated a high amount of CTC compared with patients with other solid tumor malignancies. CTCs ("liquid biopsies") may reflect the changing burden of disease over course of treatments. Peripheral blood will be collected to assess CTC and characterize disease [47].

5.9.1.1 Specimen Receipt and Processing at the PADIS Laboratory

Samples will be shipped on the same day of blood draw directly to the PADIS Laboratory. This assay is currently being optimized.

5.9.1.2 Site Performing Correlative Study

This study will be performed by the PADIS Laboratory.

5.9.2 cfDNA - Fragmentomics

Characterization of plasma cfDNA can potentially provide rapid, noninvasive improved monitoring of disease burden, depth of responses to treatment, and timely warning of disease relapse in patients with SCLC [1]. Peripheral blood will be collected to assess cfDNA for genetic mutations specific to SCLC.

5.9.2.1 Specimen Receipt and Processing at the EET Biobank

Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and will be stored in a -80°C freezer.

The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped for analysis.

5.9.2.2 Site Performing Correlative Study

This study will be performed by Delfi Diagnostics.

5.9.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Lorenzo Rinaldi
2809 Boston St, Suite 503
Baltimore MD 21224

5.9.2.4 Contact Information for Notification of Specimen Shipment

Lorenzo Rinaldi (rinaldi@delfidiagnostics.com)

5.9.3 Circulating Tumor DNA (ctDNA)

Characterization of plasma cfDNA can potentially provide rapid, noninvasive improved monitoring of disease burden, depth of responses to treatment, and timely warning of disease relapse in patients with SCLC [1]. Peripheral blood will be collected to assess cfDNA for genetic mutations specific to SCLC.

5.9.3.1 Specimen Receipt and Processing at the EET Biobank

Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and will be stored in a -80°C freezer.

The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped for analysis.

5.9.3.2 Site Performing Correlative Study

ctDNA will be performed at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

5.9.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section **5.9.3.2**.

5.9.3.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.9.4 SLFN11, cMYC, and ATM

5.9.4.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide.

5.9.4.2 Site Performing Correlative Study

Immunohistochemistry (IHC) will be performed on baseline tumor samples by NCI DTB.

5.9.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Anish Thomas, MD
Center for Cancer Research
National Cancer Institute
Building 10 Room 4-5330
Bethesda, MD 20892

5.9.4.4 Contact Information for Notification of Specimen Shipment

Anish Thomas (anish.thomas@nih.gov)

5.9.5 Whole Exome Sequencing

5.9.5.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

DNA and RNA will be co-extracted from tumor tissue. DNA will be extracted from the whole blood collected in the EDTA tube. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

5.9.5.2 Site Performing Correlative Study

WES will be performed on baseline tumor samples at The NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR).

5.9.5.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section **5.9.5.2**.

5.9.5.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

6 TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered in general on an outpatient basis unless a patient needs to be admitted for logistical reasons. Reported AEs and potential risks are described in Section **10**. Appropriate dose modifications are described in Section **7**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Participants meeting inclusion and exclusion criteria will receive either monotherapy with topotecan 1.25 mg/m^2 IV Days 1-5 or combination therapy consisting of topotecan 1.25 mg/m^2 IV Days 1-5 with M6620 210 mg/m^2 IV Days 2 and 5 administered each 21-day cycle until disease progression or development of intolerable side effects. Pegfilgrastim will be administered at least 24 hours after the last dose of topotecan, starting with the first cycle. Acceptable alternatives include filgrastim administered daily or pegfilgrastim administered via on-body injector. Concomitant administration of granulocyte stimulating factor (G-CSF) pegfilgrastim and topotecan can prolong the duration of neutropenia. Patients on the topotecan monotherapy arm will be eligible to receive the combination treatment at progression. On days 2 and 5, the drugs will be administered sequentially, with topotecan followed by M6620. M6620 should be administered within 15 minutes after topotecan administration is completed.

For cardiac safety measures, an EKG will be performed at pre-study and as clinically indicated.

Subjects will be monitored weekly during the first cycle by basic labs (complete blood count [CBC] with differential and chemistries [acute panel, hepatic panel, and mineral panel] on days 8 and 15) and if clinically indicated in subsequent cycles.

Regimen Description					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
M6620	Premedicate if subject develops infusion reactions (Section **6.1.1**); refer to Section **6.2** for restrictions	210 mg/m^2 in D5W to a final concentra-tion between 0.075 mg/mL to 1 mg/mL	IV over 60 minutes (+/- 10 minutes) (within 15 minutes after completion of topotecan administration)	Days 2 and 5	21 days
Topotecan	Antiemetic prophylaxis is recommended. For example, ondansetron 16 mg PO or IV 30 minutes prior to topotecan; Additionally, take home antiemetics are recommended as needed. For example, ondansetron 8 mg PO q6 prn.	1.25 mg/m^2	IV over 30 minutes	Days 1-5	
IV = intravenously; PO = by mouth; q6 prn = every 6 hours as needed					

6.1.1 M6620

M6620 will be supplied as a 20 mg/mL solution. M6620 must be diluted with 5% dextrose in water solution before intravenous infusion as detailed in Section **8.1.1**.

An appropriate volume of concentrated drug product solution is diluted before use, according to the dose indicated in the table above. Total dose of M6620 is not to exceed 800 mg/dose. The dose of M6620 in dextrose in water solution will be infused IV over 60 minutes (±10 minutes).

Infuse using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micron filter. 5% dextrose in water solution must be used for IV line priming and flushing. M6620 should not come in contact with 0.9% Sodium Chloride due to incompatibility.

If any subject develops phlebitis or signs or symptoms of inflammation that may progress to phlebitis or which the subject cannot tolerate, standard measures should be employed to ameliorate these symptoms, including removal of the infusion catheter and resumption of infusion through a different vein. If any subject develops pruritus or evidence of allergic reaction, standard measures may be employed to ameliorate these symptoms or to prevent recurrence of these symptoms (*e.g.*, premedication with acetaminophen 325 mg, PO approximately 30 minutes before the infusion, 200 mg hydrocortisone intravenously approximately 60 minutes before infusion, and 25 mg diphenhydramine intravenously approximately 30 minutes before infusion; alternative antihistamine and steroid combinations may be considered, as long as not prohibited by protocol). If standard procedures to limit symptoms of injection site reaction or pruritus are insufficient, then the infusion time may be extended beyond 60 minutes, but no more than 90 minutes.

6.1.2 Topotecan

Topotecan is a cytotoxic anticancer drug. Prepare topotecan under a vertical laminar flow hood while wearing gloves and protective clothing. If topotecan solution contacts the skin, wash the skin immediately and thoroughly with soap and water. If topotecan contacts mucous membranes, flush thoroughly with water. Use procedures for proper handling and disposal of anticancer drugs.

Each 4-mg powder for injection vial of topotecan is reconstituted with 4 mL Sterile Water for Injection. Then the appropriate volume of the reconstituted solution or solution for injection is diluted in 5% Dextrose or 0.9% Sodium Chloride IV Infusion prior to administration. Topotecan will be administered as an intravenous infusion over 30 minutes.

6.2 General Concomitant Medication and Supportive Care Guidelines

Patients should receive general concomitant and supportive care medications (including antibiotics, nutritional support, growth factor support, correction of metabolic disorders, optimal symptom control, and pain management, etc.) based on best medical practice. Supportive care can include palliative radiation therapy, including bone-directed radiation therapy for pain control in subjects deriving clinical benefit from treatment. Study medications can be held per investigator discretion during this period.

Because there is a potential for interaction of M6620 with other concomitantly administered drugs, the case report form (CRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The PI should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. For example, M6620 is metabolized by CYP 3A4 isoenzyme (CYP3A4); exposure to M6620 may be affected by concomitantly administered drugs that are strong inhibitors or inducers of CYP3A4. Sensitive substrates of CYP3A4 should be used with caution.

M6620 (VX-970, berzosertib) is a weak/moderate inhibitor of UGT1A1, UGT1A14, UGT1A9, UGT2B15, and UGT2B17. UGT2B7, UGT1A3, and UGT1A6 were weakly or not inhibited. M6620 (VX-970, berzosertib) is predicted to not inhibit significantly the metabolic clearance of

SN-38 (active metabolite of irinotecan) at therapeutic exposures. M6620 (VX-970, berzosertib) is a moderate inhibitor of P-gp and BCRP. It is a P-gp substrate but not BCRP. Based on in vitro data, there is low risk of drug-drug interaction with OATP1B3 and BCRP. Use caution when administered with sensitive substrates of OATP1B3 and BCRP.

The study team should check a frequently updated medical reference for a list of drugs to avoid or minimize use of. **APPENDIX C: Patient Drug Interactions Handout and Wallet Card** should be provided to patients if available.

Prior and concomitant medication/food restrictions are provided in the table below.

Restricted Medication/Food/Activity	**Study Period**	
	Screening Period	**Treatment Period**
Grapefruit/grapefruit juice Seville or blood oranges/marmalade	None allowed within 14 days before the first administration of study drug	None allowed
Strong CYP3A inhibitors or inducers	None allowed within 14 days before the first dose of study drug	None allowed
CYP: cytochrome P450		

Medications taken from 28 days before the first dose of study drug will be documented as a prior medication. Medications taken after the first dose of study drug through the end of the study will be documented as concomitant medications. All medications must be recorded with indication, route of administration, and start and stop dates of administration. All subjects will be questioned about concomitant medication at each clinic visit.

M6620 absorbs in the UV-visible radiation spectrum and is widely distributed including skin, so patients receiving M6620 should take protective measures to minimize sun exposure.

To minimize the possibility of phlebitis, M6620 should be administered through a large-bore catheter into a large-caliber peripheral vein or central venous access. The intravenous infusion site should be monitored closely for the development of erythema, induration, purulence, tenderness, or warmth.

6.3 Duration of Therapy

In the absence of treatment delays due to AE(s), treatment may continue until one of the following criteria applies:

- Disease progression*
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study

- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of childbearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

*For patients who initially received monotherapy and cross over to the combination arm at progression, treatment will be stopped when disease progression occurs after combination therapy.

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the CRF.

6.4 Duration of Follow-Up

Patients will be followed every three months after removal from study treatment until study closure or death, whichever occurs first. Patients without radiographic PD/death will also be followed for response at least every 12 weeks until PD/death or until 6 months after the last patient completes study treatment (whichever occurs first). Patients removed from study for unacceptable adverse event(s) will also be followed until resolution or stabilization of the adverse event. Study accrual will be completed approximately 2 years from study initiation.

7 DOSING DELAYS/DOSE MODIFICATIONS

7.1 M6620 dose modification

No dose modifications of M6620 may be made during Cycle 1.

The dose of M6620 may be reduced for the first occurrence of drug-related toxicity (except during cycle 1) using the following toxicity-dependent guidelines:

1. **For Grade 4 hematologic toxicity:** The dose of M6620 will be reduced by 25%.

2. **For Grade 3 non-hematologic toxicity** (except disease related hyponatremia for which dose reduction will only be made for grade 4): The dose of M6620 will be reduced by 25%.

3. **For Grade 4 non-hematologic toxicity:** The dose of M6620 will be reduced by 50%.

Based on the criteria above, a maximum of 2 dose reductions will be permitted. If a grade 4 non-hematologic toxicity were to recur, treatment will be discontinued.

Dose modifications for Grade 4 hematologic and Grade 3 non-hematologic drug-related AE	
Starting dose	210 mg/m^2
First dose reduction	158 mg/m^2
Second dose reduction	105 mg/m^2
Dose modifications for Grade 4 non-hematologic drug-related AE	
Starting dose	210 mg/m^2
First dose reduction	105 mg/m^2

In addition, in case of Grade 3 or higher toxicity during any cycle beyond Cycle 1, treatment may be interrupted and may be resumed when all toxicities have returned to Grade 2 or less, at the discretion of the investigator. Treatment interruptions may occur for a maximum of 21 days.

7.2 Topotecan dose modification

No dose modifications of topotecan may be made during cycle 1.

The dose of topotecan may be reduced for drug-related toxicity (except during cycle 1) using the toxicity-dependent guidelines in the table below. Topotecan dose reductions will be accomplished by decreasing the dose of topotecan for each of the 5 days.

To initiate subsequent cycles of topotecan, the day 1 ANC should be >1500/mm^3 and platelets >100,000/mm^3. Treatment interruptions may occur for a maximum of 21 days.

Based on the below criteria, a maximum of 2 dose reductions will be permitted.

A. Dose adjustments for renal functions (to be made regardless of whether the decrease in renal function is drug related or not)	
Creatinine clearance (Cockcroft-Gault formula)	Topotecan 1st Dose Reduction (mg/m^2)
>60	no adjustment
40-59	1
20-39	0.75
<20	Discontinue

B. Dose adjustments for non-hematologic toxicities		
Non-hematologic toxicity	Topotecan 1st Dose Reduction (mg/m^2)	Topotecan 2nd Dose Reduction (mg/m^2)
Grades 1 and 2	no adjustment	
Grades 3 and 4 (except grade 3 nausea)	1	0.75

C. Dose adjustments for hematologic toxicities		
Hematologic toxicity	Topotecan 1st Dose Reduction (mg/m^2)	Topotecan 2nd Dose Reduction (mg/m^2)
Grades 1 and 2	no adjustment	
Grade 3 neutropenia persisting after day 21	1	0.75
Grade 4 thrombocytopenia or Grade 4 neutropenia with fever or infection or of duration ≥ 7 days	1	0.75

In addition, in case of Grade 3 or higher toxicity during any cycle beyond Cycle 1, treatment may be interrupted and may be resumed when all toxicities have returned to Grade 2 or less, at the discretion of the investigator.

Subjects assigned to both agents who develop intolerance to topotecan, but who may be benefiting from therapy, may continue on single agent M6620, administered weekly (on days 2 and 5 of 21-day cycle) at the same dose they have received in combination therapy, until disease progression.

8 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and commercial agents administered in this study can be found in Section **10.1**.

8.1 CTEP IND Agent

8.1.1 M6620 (VX-970, Berzosertib) (NSC 780162)

Other Names: VRT-0768079, MSC2527093A, VX-970

Chemical Name: 5-(4-(isopropylsulfonyl)phenyl)-3-(3-(4-((methylamino)methyl)phenyl)isoxazol-5-yl)pyrazin-2- amine

Classification: ATR inhibitor **CAS Registry Number:** 1232416-25-9

Molecular Formula: $C_{24}H_{25}N_5O_3S$ **M.W.:** 463.55 Da

Mode of Action: Ataxia telangiectasia mutated and Rad3-related (ATR) kinase is an apical regulator of checkpoint pathways triggered by DNA damage. The DNA damage response (DDR) is regulated by ATR kinase and ataxia telangiectasia mutated (ATM) kinase, which are recruited to distinct DNA damage structures. M6620 (VX-970, berzosertib) disrupts ATR-mediated DNA damage response signaling and leads to sustained accumulation of DNA damage in cancer cells co-treated with DNA-damaging agents.

Description: The drug substance for M6620 (VX-970, berzosertib) is the free base.

How Supplied: M6620 (VX-970, berzosertib) is supplied by Merck KGaA/EMD Serono, Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as single-use 200 mg vials containing a sterile solution (20 mg/mL). M6620 (VX-970, berzosertib) solution for injection is a yellow liquid formulated in 20% betadex sulfobutyl ether sodium (w/v) and 86 mM acetate buffer, 10 mL total volume, supplied in clear glass vials in cardboard boxes with foam inserts.

Preparation: M6620 (VX-970, berzosertib) 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 (VX-970, berzosertib). To prepare the infusion solution add the dose volume of M6620 (VX-970, berzosertib) 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.

Storage: Store intact vials protected from light inside cardboard boxes at room temperature, below 25°C (77°F). Do not freeze.

> If a storage temperature excursion is identified, promptly return M6620 (VX-970, berzosertib) to below 25°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability testing of the intact vials is on-going**.** Prepared solutions must be protected from light and used within 4 hours from time of preparation if stored at room temperature or 24 hours if stored refrigerated (2-8°C).

Route of Administration: Intravenous (IV) infusion.

Method of Administration: Prior to administration the solution should be given one hour at ambient temperature to warm up if stored refrigerated following preparation. Infuse over 60 minutes using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micron filter. 5% dextrose in water solution must be used for IV line priming and flushing. M6620 (VX-970, berzosertib) should not come in contact with 0.9% Sodium Chloride due to incompatibility. The infusion time may be extended beyond 60 minutes (as tolerated) but no more than 90 minutes if standard procedures to limit symptoms of an infusion reaction are insufficient or if the total volume of the infusion exceeds 600 mL. To minimize the possibility of phlebitis, M6620 (VX-970, berzosertib) should be administered through a large bore catheter into a large caliber peripheral vein or central venous access.

Patient Care Implications: Monitor for infusion site reactions, irritation, and phlebitis. M6620 (VX 970, berzosertib) absorbs in the UV-visible radiation spectrum and is widely distributed including skin, so patients receiving M6620 (VX-970, berzosertib) should take protective measures to minimize sun exposure.

Women of childbearing potential and men should use appropriate contraception while on study drug and for 6 months after discontinuation of M6620 (VX-970, berzosertib).

Potential Drug Interactions: M6620 (VX-970, berzosertib) is primarily metabolized by CYP3A4. M6620 (VX-970, berzosertib) has a low potential to inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4, and a moderate potential to reversibly inhibit CYP2E1. The potential for M6620 (VX-970, berzosertib) to induce CYP450 enzymes CYP1A2, 2B6, and 3A4 at concentrations up to 6 µM is low. Concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided. Sensitive substrates of CYP3A4 should be used with caution.

M6620 (VX-970, berzosertib) is a weak/moderate inhibitor of UGT1A1, UGT1A14, UGT1A9, UGT2B15, and UGT2B17. UGT2B7, UGT1A3, and UGT1A6 were weakly or not inhibited. M6620 (VX-970, berzosertib) is predicted to not inhibit significantly the metabolic clearance of SN-38 (active metabolite of irinotecan) at therapeutic exposures.

M6620 (VX-970, berzosertib) is a moderate inhibitor of P-gp and BCRP. It is a P-gp substrate but not BCRP. Based on in vitro data, there is low risk of drug-drug interaction with OATP1B3 and BCRP. Use caution when administered with sensitive substrates of OATP1B3 and BCRP transporters.

Availability: M6620 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

M6620 is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section **13.5**).

8.1.2 Agent Ordering and Agent Accountability

NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

Drug orders of M6620 should be placed after enrollment onto the study and randomization. Provide the patient ID number in the comment box when submitting an order request.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status, a “current” password, and active person registration status. For questions about drug orders, transfers, returns, or accountability,

call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.2.1 Agent Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.1.3 Investigator Brochure Availability

The current versions of the IBs for the agent will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.4 Useful Links and Contacts

CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
PMB policies and guidelines:
http://ctep.cancer.gov/branches/pmb/agent_management.htm
PMB OAOP application: https://ctepcore.nci.nih.gov/OAOP
CTEP IAM account: https://ctepcore.nci.nih.gov/iam/
CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
IB Coordinator: IBCoordinator@mail.nih.gov
PMB email: PMBAfterHours@mail.nih.gov
PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial Agent

8.2.1 Topotecan

For additional information see the Topotecan Package Insert.

Chemical Name: (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1Hpyrano[3',4':6,7] indolizino [1,2-b]quinoline-3,14-(4H ,12H)-dione monohydrochloride

Other Names: HYCAMTIN®

Classification: Anti-tumor Topoisomerase Inhibitor

CAS Registry Number: 123948-87-8

Molecular Formula: $C_{23}H_{23}N_3O_5$ **MW:** 421.45 Da

Mode of Action: Topotecan is a semi-synthetic derivative of camptothecin and is an anti-tumor drug with topoisomerase I-inhibitory activity. Topoisomerase I relieves torsional strain in DNA by inducing reversible single-strand breaks. Topotecan binds to the topoisomerase I-DNA complex and prevents re-ligation of these single-strand breaks. The cytotoxicity of topotecan is thought to be due to double-strand DNA damage produced during DNA synthesis, when replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I, and DNA. Mammalian cells cannot efficiently repair these double-strand breaks.

How Supplied:

Powder for Injection (4 mg)
Topotecan powder for injection is supplied as a sterile, lyophilized, buffered, light yellow to greenish powder for reconstitution available in single-dose vials. Each vial contains topotecan hydrochloride equivalent to 4 mg of topotecan as free base. The reconstituted solution ranges in color from yellow to yellow-green and is intended for administration by intravenous infusion.

Solution for Injection (4 mg/4 mL)
Topotecan Injection is supplied as a clear yellow to yellow-green solution in single use vial for intravenous infusion only following dilution. Each mL contains topotecan hydrochloride equivalent to 1 mg of topotecan free base.

Preparation:
Topotecan Injection is a cytotoxic anticancer drug. Prepare topotecan hydrochloride injection under a vertical laminar flow hood while wearing gloves and protective clothing. If Topotecan Injection solution contacts the skin, wash the skin immediately and thoroughly with soap and water. If Topotecan Injection contacts mucous membranes, flush thoroughly with water. Handle and dispose of topotecan for injection consistent with recommendations for the handling and disposal of hazardous drugs.

Powder for Injection (4 mg)
Reconstitute each 4-mg vial of topotecan with 4 mL Sterile Water for Injection, USP.

Powder for Injection (4 mg) and Solution for injection (4 mg/mL)
Dilute the appropriate volume of the solution in a minimum of 50 mL of 5% Dextrose in Water Injection or 0.9% Sodium Chloride, USP prior to administration.

Storage:

Powder for Injection (4 mg)
Store at controlled room temperature between 20°C and 25°C (68°F and 77°F) [see USP]. Protect from light in original carton.

Solution for Injection (4 mg/mL)
Unopened vials of Topotecan Injection are stable until the date indicated on the package when stored at 2°C to 8°C (36°F and 46°F) and protected from light in the original package.

Stability: Unopened vials of topotecan powder for injection are stable until the date indicated on the package when stored between 20°C and 25°C (68°F and 77°F) [see USP] and protected from light in the original carton. Because the vials contain no preservative, contents should be used immediately after reconstitution. Topotecan diluted for infusion is stable at approximately 20°C to 25°C (68°F to 77°F) and ambient lighting conditions for 24 hours.

Route of Administration: Topotecan Injection is administered by intravenous infusion over 30 minutes.

Method of Administration: Verify dose using body surface area prior to dispensing. Recommended dosage should generally not exceed 4 mg intravenously.

Agent Ordering: Topotecan is commercially available.

9 STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This study is an open label, randomized Phase 2 study of patients with relapsed SCLC. SCLC patients who have failed prior therapy will be randomized 2:1 to receive either topotecan in combination with M6620 or topotecan alone. The rationale for 2:1 randomization is to obtain more clinical and molecular data from patients who receive combination of M6620 and topotecan. Patients will be stratified at the time of randomization for being sensitive or resistant/refractory to prior therapy. Patients on the topotecan monotherapy arm will be eligible to cross-over to receive the topotecan with M6620 combination treatment at progression. A separate cohort of patients with extrapulmonary small cell cancer will be accrued while the primary cohort is accruing and will only receive the combination therapy (see Section **9.3** for secondary endpoints).

Cohorts

Number	Name	Description
1	Primary Cohort	Patients with measurable SCLC.
2	Exploratory Cohort	Patients with extrapulmonary small cell cancer.

Arms

Number	Name	Description
1	Arm 1*-monotherapy	Topotecan
2	Arm 2 -combination	Topotecan + M6620

	treatment	

*Arm 1 patients (on the topotecan monotherapy) will be eligible to cross-over to Arm 2 at progression to receive the combination treatment.

Stratifications

Number	Name	Description
1	Platinum response-sensitive	First-line chemotherapy sensitivity response treatment-free interval ≥ 90 days
2	Platinum response-resistant	First-line chemotherapy sensitivity response treatment-free interval < 90 days

The primary objective of the trial is to determine if the combination of M6620 with topotecan will result in an improvement in PFS compared to topotecan alone in patients with relapsed SCLC.

Published results suggest that the median PFS for single agent therapy for previously treated patients which included a combination of sensitive and resistant /refractory patients was approximately 3 months (median 3.0-4.3 months for sensitive and 1.5-2.6 months for refractory; [9, 12, 13, 69, 70] (refer to the Selected randomized studies of cytotoxic agents for SCLC Table below). It would be desirable to improve the PFS to be as high as 6 months by use of a combination therapy. More recent data suggest that PFS of single-agent DNA targeted therapies in previously treated SCLC patients which included a combination of sensitive and resistant /refractory patients may be lower- closer to 2 months than 3 months [15].

Selected randomized studies of cytotoxic agents for SCLC

No.	Patients	Treatments	Primary endpoint	Median PFS-topotecan	Median OS-topotecan	Reference
1	Sensitive SCLC	Topotecan *vs.* CAV	ORR	3.3 months (sensitive)	6.3 months (sensitive)	[9]
2	Refractory or sensitive SCLC; 2nd line	Topotecan *vs.* amrubicin	OS	4.3 months (sensitive) 2.6 months (refractory)	9.9 months (sensitive) 5.7 months (refractory)	[13]
3	Sensitive SCLC 2:1	Topotecan *vs.* amrubicin	ORR	3.3 months (sensitive)	7.6 months (sensitive)	[70]
4	Refractory or sensitive SCLC	Topotecan *vs.* amrubicin	ORR	3.0 months (sensitive) 1.5 months (refractory)	NA	[12]
5	Sensitive SCLC; 2nd line	PO *vs.* IV topotecan	ORR	3.7 months (sensitive)	8.7 months (sensitive)	[69]
CAV = combination therapy of cyclophosphamide, doxorubicin, and vincristine; ORR = objective response rate; OS = overall survival; PO = by mouth; IV = intravenously						

Kaplan-Meier curves and a one-tailed log-rank test will be the primary analysis methods. PFS assessment will be determined once 48 patients have progressed or died without progression. Assuming exponential PFS curves, the hazard rate for topotecan alone associated with a 3-month median PFS is 0.2310, or approximately a 23% hazard of progressing per month. If we assume that the addition of M6620 could increase the median PFS to 6 months, the corresponding hazard rate is 0.1155 and the resulting hazard ratio for the comparison of the two overall actuarial curves would be 2.00. Following the principles of a Phase 2 trial, to have 85% power to compare these curves and detect a difference with a one-tailed 0.10 significance level log-rank test, simulations demonstrate that a total of 18 evaluable patients randomized to the topotecan alone arm and 36 evaluable patients randomized to the topotecan and M6620 arm will need to be randomized over a 2-year period and followed for up to an additional 12 months; power will be reduced to 79% if follow-up ends after the 2-year enrollment period. If the comparison were between 2.5 and 5 months, the power remains at 85%. As indicated above, the final evaluation for PFS will take place after 48 patients have progressed or died without progression; thus, the actual time of the evaluation cannot be determined until these events have been noted.

An interim evaluation for futility will take place during accrual after 24 patients on both arms combined have progressed. If any unusual patterns are detected at this time or the efficacy signal strongly favors the topotecan arm, this may warrant an early study closure.

If patients are enrolled on the topotecan single agent and they have progressed, they will be eligible to receive the topotecan plus M6620 combination treatment at that time; they will be considered to have progressed at their initial time as their primary outcome. The small number of patients who could potentially cross-over (up to 18 patients) will be evaluated for their second PFS and response rate in an exploratory manner.

After the trial has completed accrual, the overall results will be reported as well as separately by sensitive vs. resistant/refractory patients. The overall results and results by stratum will be reported along with two-sided 80% and 95% confidence intervals at the median time.

9.2 Sample Size/Accrual Rate

It is expected that approximately 25-30 patients per year (about 2-3 patients per month) will be enrolled, and thus accrual will be completed in approximately 2 years.

We expect to enroll 18 evaluable patients randomized to the topotecan alone arm and 56 evaluable patients in the topotecan and M6620 arm (36 randomized evaluable SCLC patients and up to 20 patients with extrapulmonary small cell cancer).

To allow for inevaluable patients, the accrual ceiling will be set at 100 total patients.

DOMESTIC PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	3	3	2	2	**10**
Asian	4	3	2	2	**11**
Native Hawaiian or Other Pacific Islander	1	2	1	1	**5**
Black or African American	10	11	1	1	**23**
White	9	12	3	6	**30**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
More Than One Race	1	1	2	3	**7**
Total	**28**	**32**	**11**	**15**	**86**

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015) OMB No. 0925-0001/0002

INTERNATIONAL PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	0	1	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	0	1	0	0	**1**
White	2	3	2	1	**8**
More Than One Race	1	1	0	0	**2**
Total	**3**	**6**	**2**	**1**	**12**

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015) OMB No. 0925-0001/0002

9.3 Analysis of Secondary Endpoints

Secondary evaluations will include objective response rate and overall survival in both patients with relapsed SCLC and extrapulmonary small cell cancer.

In addition, a small cohort of patients with extrapulmonary small cell cancer patients will be included based on preliminary evidence of efficacy in these patients in the ongoing Phase 2 study. Up to 20 patients with extrapulmonary small cell cancer will receive the combination of

M6620 and topotecan; this cohort will only accrue while the SCLC patients are accruing, and accrual will be terminated at the end of the accrual to the randomized SCLC trial. There are currently no benchmarks of ORR for relapsed extra-pulmonary small cell cancers. In many types of relapsed cancers, an ORR of 30% or greater would be considered clinically meaningful. As such we would use the same threshold here for the extra-pulmonary small cell cancer cohort. These results will be considered hypothesis generating and an ORR of 30% or greater will be interesting and may lead to consideration of more definitive future trials in this population.

9.4 Reporting and Exclusions

9.4.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with M6620.

9.4.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

10 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

AE monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section **10.1**) and the characteristics of an observed AE (Sections **10.2** and **10.3**) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential AE associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

NOTE: Report AEs on the SPEER ONLY IF they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for M6620

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
M6620 (VX-970, berzosertib, NSC 780162)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 323 patients.* Below is the CAEPR for M6620 (VX-970, berzosertib).

> **NOTE:** Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.0, July 13, 2022[1]

Adverse Events with Possible Relationship to M6620 (VX-970, berzosertib) (CTCAE 5.0 Term) [n= 323]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Constipation		
Diarrhea			***Diarrhea (Gr 2)***
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
	Flu like symptoms		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		***Infusion related reaction (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		***Headache (Gr 2)***
VASCULAR DISORDERS			
	Flushing		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Adverse events reported on M6620 (VX-970, berzosertib) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that M6620 (VX-970, berzosertib) caused the adverse event:

CARDIAC DISORDERS - Cardiac arrest; Palpitations
EAR AND LABYRINTH DISORDERS - Tinnitus
GASTROINTESTINAL DISORDERS - Abdominal pain; Ascites; Colonic obstruction; Dyspepsia; Mucositis oral

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs
IMMUNE SYSTEM DISORDERS - Allergic reaction; Anaphylaxis
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (lower respiratory tract infection); Otitis externa; Sepsis; Soft tissue infection; Urinary tract infection
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; Creatinine increased; GGT increased; Hemoglobin increased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperglycemia; Hypokalemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Generalized muscle weakness; Myalgia
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (malignant neoplasm progression); Tumor pain
NERVOUS SYSTEM DISORDERS - Lethargy; Spinal cord compression; Syncope
PSYCHIATRIC DISORDERS - Confusion
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Atelectasis; Cough; Dyspnea; Respiratory, thoracic and mediastinal disorders - Other (hemotypsis)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Pruritus; Rash maculo-papular
VASCULAR DISORDERS - Hypertension; Hypotension; Thromboembolic event; Vascular disorders - Other (hypovolemic shock)

Note: M6620 (VX-970, berzosertib) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.2 Adverse Event List for Topotecan

The most common hematological grade 3/4 adverse reactions (≥5%) experienced by SCLC patients were neutropenia, anemia, thrombocytopenia and febrile neutropenia. The most common non-hematological grade 3/4 adverse reactions (≥5%) experienced by SCLC patients were sepsis, dyspnea, pneumonia, abdominal pain, nausea, fatigue, asthenia, and pain. See the topotecan package insert for more information

10.2 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI 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 website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

For expedited reporting purposes only:
AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section **10.1**) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

Attribution of the AE:
- 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.

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

Pre-treatment AEs: AEs that occur after informed consent is signed and prior to start of treatment are collected in Medidata Rave using the Pre-treatment Adverse Event form.

Pre-existing medical conditions (formerly referred to as baseline AEs) identified during baseline assessment are not considered AEs and therefore should not be reported on the Pre-treatment Adverse Event form. If these pre-existing conditions worsen in severity, the investigator must reassess the event to determine if an expedited report is required. Whether or not an expedited report is required, the worsened event should be reported as a routine AE.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 Days after the Last Administration of the Investigational Agent/Intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence that internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once internet connectivity is restored, the

24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU members' website:

- Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and
- Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics*.

NCI requirements for SAE reporting are available on the CTEP website:

NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

10.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: PI and AE Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

10.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires <u>both</u> routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

"24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
"10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

10.4 Routine Adverse Event Reporting

All AEs **must** be reported in routine study data submissions. **AEs reported expeditiously through** CTEP-AERS **must also be reported in routine study data submissions.**

AE data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via CTEP-AERS. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for AE Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 6 months after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: AE Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via Medidata Rave. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in the protocol.

11 STUDY CALENDAR

Patients may be hospitalized for convenience and logistical reasons for drug administration and/or research blood draws. After registration, patients should begin protocol therapy within 14 days.

Procedure	Pre-Study[#]	Baseline	Cycle = 21 days [a]													End of Treatment/ Disease Progression [b]	Post Therapy Follow-up [c]
			Cycle 1							Cycle 2+							
			Day 1[@]	Day 2	Day 3	Day 4	Day 5	Day 8*	Day 15*	Day 1[@]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	
M6620 (VX-970, berzosertib)				A			A				A			A				
Topotecan			B	B	B	B	B			B	B	B	B	B				
Informed consent	X																	
Demographics	X																	
Medical history	X																	
Concurrent Meds	X----------															----------X		
Physical exam [d]	X		X							X							X	
Vital signs [p]	X		X	X	X	X	X			X [p]	X	X	X	X			X	X
Height	X																	
Weight	X		X							X							X	X
Performance status	X		X							X							X	X
PT, PTT	X																X	
CBC w/diff, plts [e, f]	X		X					X	X	X							X	
Acute Care Panel [g, f]	X		X					X	X	X							X	
Hepatic Panel [h, f]	X		X					X	X	X							X	
Mineral Panel [i, f]	X		X					X	X	X							X	
EKG [m]	X																	
Serum Pregnancy Test [j]	X		X							X								
Clinical Disease Assessment	X									X							X	
Adverse Event Evaluation			X----------													----------X		
CT Scan	X		Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.														X	
Correlative Studies – Mandatory Biopsy [k]		X																
Correlative Study – CTC blood sample		X [n]		X [q]		X [n]				X [l, n]							X	
Correlative Study – cfDNA blood sample		X [n]					X [n]			X [l, n]							X	
Correlative Study – WES blood sample		X [n]																

Procedure	Pre-Study[#]	Baseline	Cycle = 21 days [a]													End of Treatment/ Disease Progression [b]	Post Therapy Follow-up [c]
			Cycle 1							Cycle 2+							
			Day 1[@]	Day 2	Day 3	Day 4	Day 5	Day 8*	Day 15*	Day 1[@]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	
Follow-up phone call																	X[o]

\# Pre-study assessments may be performed within 21 days prior to registration. After registration, patients should begin protocol therapy within 14 days. Patient may undergo the pre-study assessments while in the washout period from a previous treatment.

@ Exams, labs and pregnancy tests can be performed up to 2 business days prior to day 1 of each cycle.

* C1D8 and C1D15 laboratory assessments can be performed in a +/- 3 days window

A: M6620 (VX-970, berzosertib): 210 mg/m^2 IV (over 60 minutes (+/- 10 minutes) (within 15 minutes after completion of topotecan administration) day 2 and day 5, every 21 days

B: Topotecan: 1.25 mg/m^2 IV (over 30 minutes) day 1 to day 5, every 21 days

a. Number of cycles depends on disease progression and development of intolerable side effects. On-study assessments can be performed within ±5 days of the specified time, unless otherwise indicated. Next cycle treatment may be delayed up to 7 days to accommodate scheduling conflicts.

b. Approximately 4 weeks after treatment discontinuation

c. Follow-up for survival will be carried out every 3 months. Patients without radiographic PD/death will also be followed for response at least every 12 weeks until PD/death or until 6 months after the last patient completes study therapy (whichever occurs first).

d. Symptom-directed physical examinations will be performed as clinically indicated in the investigator's judgment

e. Includes neutrophils, lymphocytes, monocytes, eosinophils, basophils, WBC, RBC, hemoglobin, hematocrit, RBC indices, MCV, RDW, and platelets

f. Only done in cycle1 and if clinically indicated in subsequent cycles

g. Includes sodium (NA), potassium (K), chloride (CL) total CO_2 (bicarbonate), creatinine, glucose, urea nitrogen, eGFR

h. Includes alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin

i. Includes albumin, calcium, magnesium (Mg), phosphorus

j. Only for women of child-bearing potential

k. All biopsies will be optional for all patients with extrapulmonary small cell cancer. Archival tissue will be collected to supplement sampling from fresh biopsy. Biopsy needs to be done after registration and before C1D1 dosing. Biopsy can be done up to 7 days prior to C1D1 dosing.

l. Only done on Day 1 of Cycles 2 and 3 (and not in subsequent cycles) and at progression.

m. An EKG as clinically indicated at the after pre-study.

n. Blood draw may be collected on the day of biopsy at baseline and before administration of study drugs at other timepoints (Cycle 1 Day 4, 5; Cycle 2 Day 1; Cycle 3 Day 1; at progression).

o. Every 3 months.

p. Vital signs are to be performed on days 1 through 5 before drug administration on each day and as clinically indicated.

q. C1D2 CTC blood draw should be collected 4 hours (+/-60 min) after end of infusion.

r. Patients who cross over will get CTC and cfDNA sampling similar to the primary arm (CTC on cycle day 2 and day 4; cfDNA on day 5; cycle 2 day 1).

12 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

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

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*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.

12.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with M6620.

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

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

12.1.2 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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with a computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, 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 would be considered measurable if the lesion is increasing in size.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness

recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or magnetic resonance imaging (MRI)), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

12.1.3 Methods for Evaluation of Measurable Disease

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

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

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following 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 patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and Prostate-Specific Antigen (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 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.

12.1.4 Response Criteria

12.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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 (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

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

12.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] 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 PI).

12.1.4.3 Evaluation of Best Overall Response

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

For Patients 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	

CR = Complete Response; PR = Partial Response; PD = Progressive Disease; SD = Stable Disease

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

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

For Patients 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

CR = Complete Response; PD = Progressive Disease;

'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

12.1.5 Duration of Response

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

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

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

12.1.6 Progression-Free Survival

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

12.1.7 Response Review

Responses will not be reviewed independently in this study.

13 STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

AE lists, guidelines, and instructions for AE reporting can be found in Section **10** (Adverse Events: List and Reporting Requirements).

13.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol PI is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing

review of accrual, patient-specific clinical and laboratory data, and routine and serious AEs; reporting of AEs; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol PI and statistician have access to the data at all times through the CTMS web-based reporting portal.

The Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance. There will be an interim evaluation for futility after 24 patients on both arms combined have progressed.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

13.2 Data Reporting

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- A valid account, and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

  Rave role requirements:

  - Rave CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
  - Rave Investigator role, must be registered as a Non-Physician Investigator (NPIVR) or Investigator (IVR), and
  - Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.
- Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management* > *Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

13.2.1 Method

For studies assigned for **CTMS Routine** Monitoring:

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at:http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis.

For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

13.3 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

13.4 CTEP Multicenter Guidelines

N/A

13.5 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

14 REFERENCES

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15 APPENDICES

15.1 APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

15.2 APPENDIX B: Formula to Estimate Renal Function Using Serum Creatinine

Estimated creatinine clearance (Clcr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg\ /\ dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73m^2 with the patient's body surface area (BSA).

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15.3 APPENDIX C: Patient Drug Interactions Handout and Wallet Card

Information for Patients, Their Caregivers and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

Patient Name:	**Diagnosis:**	**Trial #:**
Study Doctor:	**Study Doctor Phone #:**	**Study Drug(s):**

Please show this paper to all your healthcare providers (doctors, physician assistants, nurse practitioners, pharmacists), and tell them you are taking part in a clinical trial sponsored by the National Cancer Institute.

These are the things that your healthcare providers need to know:
M6620 (VX-970, berzosertib) interacts with specific enzymes in the liver or other tissues like the gut and certain transport proteins that help move drugs in and out of the cell.

	Explanation
CYP isoenzymes	The enzyme in question is **CYP3A4**. M6620 (VX-970, berzosertib) is metabolized by CYP3A4 and may be affected by other drugs that inhibit or induce this enzyme.
Protein transporters	The proteins in questions are **OATP1B3 and BCRP**. M6620 (VX-970, berzosertib) is a moderate inhibitor of these proteins and may affect drugs that are moved in and out of cells/organs by these transport proteins.

These are the things that you need to know:
The study drug M6620 (VX-970, berzosertib), may interact with other drugs which can cause side effects. For this reason, it is very important to tell your doctors about all your medicines, including: (a) medicines you are taking before this clinical trial, (b) medicines you start or stop taking during this study, (c) medicines you buy without a prescription (over-the-counter remedy), (d) herbals or supplements (e.g. St. John's Wort). It is helpful to bring your medication bottles or an updated medication list with you.

Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inhibitors or inducers of CYP3A4 and sensitive substrates of CYP3A4, OATP1B3 and BCRP.

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- Make sure your doctor knows to avoid certain prescription medications.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine.

Version *Apr2021*

(Next page: Patient Drug Interaction Wallet Card)

Patient Drug Interaction Wallet Card

NIH NATIONAL CANCER INSTITUTE **EMERGENCY INFORMATION**	NIH NATIONAL CANCER INSTITUTE	NIH NATIONAL CANCER INSTITUTE **DRUG INTERACTIONS**	NIH NATIONAL CANCER INSTITUTE
Show this card to all of your healthcare providers. Keep it with you in case you go to the emergency room.	Tell your doctors **before** you **start** or **stop** any medicines. **Check with your doctor or pharmacist if you need to use an over-the-counter medicine or herbal supplement!**	**Carry this card with you at all times** M6620 (VX-970, berzosertib) interacts with specific enzymes in your liver or other tissues like the gut and transport proteins that help move drugs in and out of cells and must be used very carefully with other medicines.	
Patient Name: **Diagnosis:** **Study Doctor:** **Study Doctor Phone #:** **NCI Trial #:** **Study Drug(S):**	**Use caution and avoid the following drugs if possible:**	Your healthcare providers should be aware of any medicines that are strong inhibitors or inducers of CYP3A4, and sensitive substrates of CYP3A4, OATP1B3, and BCRP. • Strong inhibitors or inducers of CYP3A4 should be avoided. • Sensitive substrates of CYP3A4, OATP1B3, and BCRP should be used with caution. **Before prescribing new medicines**, your health care provider should check a **frequently updated medical reference** for a **list of drugs to avoid** or contact your study doctor. Version Apr/2021	
For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov

15.4 APPENDIX D: PD SPECIMEN SUBMISSION FORM AND CHAIN OF CUSTODY

If submitting more than 10 specimens, attach an additional copy of the Specimen Submission Form. Include a copy of the submission form and signed, chain of custody section with every shipment.

15.4.1 Specimen Submission Form

NOTE: Record times using **military time** (24-h designation); e.g., specify 16:15 to indicate 4:15 PM. If submitting more than 10 specimens, attach an additional Specimen Submission Form.

Total No. Specimens:		**Shipment Date:**	

Item No.	Patient/Sample ID	Protocol/ CTEP No.	Collection Date	Collection Time	Cycle/Day/ Hour	Clinical Diagnosis	Comments
Ex:	*1234-001025-402*	*1234*	*10/24/2012*	*16:15*	*C1D1-2h*	*sarcoma*	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

15.4.2 Chain of Custody Signatures

Prior to shipping the Clinical Center Specimen Handling personnel should verify contents of and sign and date on line 1 below to verify contents of container.

Task	Responsible Party	Signature	Date
1. Shipment of blood tubes (22°C ± 3°C controlled temperature gel packs)	Clinical Center		/ /
2. Receipt of specimen: log receipt, verify specimen(s), and verify shipping conditions.	NCI-F/FNLCR		/ /
3. Receipt of specimen for research use.	NCI-F/FNLCR		/ /

15.5 APPENDIX E: TISSUE BIOPSY VERIFICATION

A copy of the diagnostic pathology report must be shipped with all tissue specimens sent to the EET Biobank.

If the *corresponding* pathology report is not available for the biopsy, then a copy of the radiology report or operative report from the biopsy procedure and the diagnostic pathology report must be sent to the EET Biobank. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the EET Biobank.

Note: If this information is not provided with the biopsy specimen, then it will not be accepted by the EET Biobank.

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): Primary Metastatic

Time point (circle one): Baseline

Site Tissue Taken From: _____

Diagnosis: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

Clinician Signature _____ Date _____

Clinician Printed Name _____

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

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