NCI Protocol #: 10287
Version Date: June 13, 2024

SUMMARY OF CHANGES – Protocol

For Protocol Amendment # 8d:

NCI Protocol #: 10287
Local Protocol #: 201911082

NCI Version Date: June 13, 2024
Protocol Date: June 13, 2024

I. Disapproval Letter, 02/27/2024, P10287_A12PAMDREVW01 Amendment Review Letter*

*Of note, protocol version dated 11/28/2023 was titled Amendment 8c.

#	Section	Comments
1.	General	Due to the pharma collaborator strategic development plan for copanlisib, the decision is made to close the study. **PI Response: We acknowledge the notification. References to opening the Phase 2 portion of the study have been removed throughout the protocol. Section 2.7 was replaced with the study closure plan.**

II. Summary of Changes for Protocol Amendment 8d, dated June 13, 2024

#	Section	Comments
1.	Title Page	Added Protocol Amendment 8d, dated June 13, 2024
2.	Schema	The schema for Phase 2 was removed.
3.	1.1	The primary objectives (1.1.2 and 1.1.3) for Phase 2 were removed.
4.	1.2	The secondary objective (1.2.2) for Phase 2 was removed, and secondary objective 1.1.3 was modified to be applicable only to Phase 1 and moved to exploratory.
5.	1.3	The exploratory objective (1.3.10) for Phase 2 was removed and the remaining objectives modified to be applicable only to Phase 1.
6.	2.5.1.1	Removed language regarding comparing PFS between 2 arms given the removal of Phase 2.
7.	2.5.2.4	Replaced ULP-WGS with the TSO500 assay in the Approach section.
8.	2.7	Replaced Section 2.7, previously added as rationale for RP2D, with study closure information.

#	Section	Comments
9.	5.1	Removed footnote 6 reference to patients in the Phase 2 portion.
10.	5.7	Replaced references to the MoCha lab PI Dr. Mickey Williams with the new PI, Dr. Chris Karlovich, and updated the email address to chris.karlovich@nih.gov. Removed the Broad Institute from the ctDNA analysis and replaced with the MoCha lab. Removed footnote ** reference to patients in the Phase 2 portion.
11.	5.8	Replaced references to the MoCha lab PI Dr. Mickey Williams with the new PI, Dr. Chris Karlovich, and updated the email address to chris.karlovich@nih.gov.
12.	5.9.2.2	Removed Broad Institute and added that ctDNA will be performed at MoCha.
13.	5.9.2.3	Replaced Broad Institute shipping address with MoCha shipping address.
14.	5.9.2.4	Replaced Broad Institute shipping contact with MoCha shipping contact.
15.	6	Removed Phase 2 opening instructions.
16.	6.1.2	Removed Phase 2 randomization language for abemaciclib schedule.
17.	7.2	Removed FAC arm language.
18.	8.1.3.1	Removed treatment arm assignment language.
19.	9.1	Removed Phase 2 primary endpoint language.
20.	9.1.2	Added definition of PFS. Removed Phase 2 secondary endpoints.
21.	9.4	Removed Phase 2 accrual calculations.
22.	9.5 and 9.6	Removed endpoint analyses only applicable to Phase 2 and updated Phase 1 analyses. Clarified that differences between 2 patients groups instead of 2 Phase 2 arms will be compared. Removed Section 9.6.2. and 9.6.3 as they were applicable to Phase 2 only. In the new Section 9.6.2 Correlative Biomarker Analysis, removed language for Phase 2 arm comparisons. Added that descriptive statistics will be

#	Section	Comments
		provided for candidate gene mutations and PTEN loss. Removed statement that Phase 1 data will be analyzed separately from Phase 2 data.
23.	9.7	Removed per protocol analysis set as a population for analysis.
24.	9.8 and 9.9	Removed sections as they were applicable only to Phase 2.
25.	9.9 (renumbered)	Removed Phase 2 DSMB language.
26.	11	Removed Phase 2 language from footnotes 10 and 15.
27.	13.6 and 13.7	Removed sections as MoCha will not provide incidental findings.

III. CIRB Letter, 11/21/2023, CIRB Approval Pending Modification of Amendment Review

#	Section	Comments
28.	1.1.1	Section 1.1.1, Primary Objectives still lists RP2D. Please revise this section to reflect the current goal. **PI Response: This has been updated as requested.**
29.	2.8	Incorporate language regarding the recent copanlisib FDA approval withdrawal into the Protocol and Consent Form. **PI Response: This has been updated as requested.**

IV. Disapproval Letter, 09/27/2023, Review of Amendment #11 of Protocol #10287*

*Of note, protocol version dated 09/07/2023 was titled Amendment 8a.

#	Section	Comments
30.	4.1, Investigator and Research Associa	***Please revise the excerpt below as indicated.*** 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

#	Section	Comments
	te Registration with CTEP	individuals must obtain a Cancer Therapy Evaluation Program (CTEP) credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems ~~Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam~~. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr/. The RCR is a self-service online person registration application with electronic signature and document submission capability. **PI Response: This has been updated as requested.**
31.	4.2.3, Submitting Regulatory Documents	***Please revise the excerpt below as indicated.*** Submit required forms and documents to the CTSU Regulatory Office via 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 ~~at 1-866-651-2878 in order~~ to receive further instruction and support. **PI Response: This has been updated as requested.**
32.	4.3.1, OPEN/IWRS	***Please revise the excerpt below as indicated.*** • All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA)~~HIPAA~~ authorization form (if applicable). **PI Response: This has been updated as requested.**
33.	5.8.1.1	To clarify that the germline control for WES will come from the pre-treatment Steck cfDNA tube, revise the last paragraph in Section 5.8.1.1 as follows: 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 will be shipped to the central sequencing laboratory for analysis. Germline DNA will be extracted from blood collected in Streck cfDNA tubes at pre-treatment (baseline) following plasma processing. DNA will be quantitated. An aliquot of germline DNA will be shipped to the central sequencing laboratory for analysis. **PI Response: This has been updated as requested.**

#	Section	Comments
34.	8.1.X	**New Section – renumber subsequent section** Material Safety Data Sheets The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff. **PI Response: This has been updated as requested.**
35.	8.1.2.1, 8.1.3	Replace "establishment of a CTEP Identity and Access Management (IAM) account" and "establishment of a CTEP IAM account" with "establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems" **PI Response: This has been updated as requested.**
36.	8.1.2.2	Add the following as a second paragraph: Product Quality Complaint (PQC): A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff. **PI Response: This has been updated as requested.**

V. Disapproval Letter, 08/02/2023, Review of Amendment #10 of Protocol #10287*

*Of note, protocol version reviewed was Amendment #8 dated July 10, 2023

Comments Requiring a Response – Major Issues:

#	Section	Comments
2.	Throughout Section 5	Section 5.3.5.2 says that blood in EDTA should be processed to plasma and buffy coat; however, the protocol is not consistent about whether the buff coat samples should be sent to the EET Biobank. If buffy coat from the blood in EDTA should be submitted to the EET Biobank then please make the updates outlined in the comments below. **PI Response: Blood in EDTA will not be processed to buffy coat. Rather, the blood collected in the Streck cfDNA tube at pre-treatment time point will also be processed to buffy coat for germline DNA at EET Biobank.**

<table>
<tr><th>#</th><th>Section</th><th>Comments</th></tr>
<tr><td></td><td></td><td><u>This is now clarified throughout Section 5.</u></td></tr>
<tr><td>3.</td><td>5.1</td><td>cfDNA is not sent to the EET Biobank. To prevent confusion, it is suggested to revise the 2nd sentence above the Specimen Collection Table to the following:

Blood draws for EDTA (purple top), red top, and Streck cfDNA tubes could still be submitted on a day when treatment is held or delayed - do not discard samples.

<u>PI Response: This has been updated as requested.</u></td></tr>
<tr><td>4.</td><td>5.1</td><td>Archival and Pre-treatment should be listed as separate time points in the Specimen Collection Table. Please re-separate them as shown below.

Also, please add the blue shading to the header as shown and update “cfDNA Streck tubes” to “Streck cfDNA tubes” throughout the Table.
<table>
<tr><th>Time Point</th><th>Specimen</th><th>Send Specimens To:</th></tr>
<tr><td colspan="3">Archival</td></tr>
<tr><td></td><td>• Archival tumor sample[1]:
– 1 tumor-rich (more than 50% tumor cellularity) FFPE block (preferred)

If a block cannot be submitted, then submit:
– 1 H&E-stained slide at 3-5 microns
– 15-20 unstained, uncharged, air-dried slides cut at 10 microns*
– 2 unstained, charged slides cut at 4-5 microns*</td><td>EET Biobank</td></tr>
<tr><td colspan="3">Pre-treatment</td></tr>
</table>
</td></tr>
</table>

#	Section	Comments

		• Fresh tumor biopsy[2]: – 1st and 2nd tumor cores snap-frozen – 3rd and 4th tumor cores in formalin • 20 mL blood in Streck cfDNA tubes • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3] • 10 mL blood in red top tube, processed for serum and frozen[4]	EET Biobank

Section 5.3.5.2 says that blood in EDTA should be processed to plasma and buffy coat. If this is correct, please update the bullets for this collection throughout the Table as follows to make it clear the EET Biobank will be receiving both specimens:

- 10 mL blood in EDTA (purple top) tube, processed for plasma and buffy coat and frozen[3]

Section 5.3.5.4 indicates that blood collected for PK should be proceeded to plasma at the collection site. Update the bullets for this collection throughout the Table as follows to make this clear:

- [5 or 7] x 4 mL blood in Li-Heparin BD vacutainers (green top), processed to plasma, and frozen for each PK time point before and after copanlisib infusion

Also, is the 5 x 4 mL or 7 x 4 mL meant to indicate 20 or 28 mL per time point or 4 mL per time point for a total of 5 or 7 collections? If the latter remove the "5 x" or "7 x" from the respective bullets as this could lead to over collection.

Revise footnote #2 to remove the following sentence:

When completed, upload the corresponding pathology report to Rave and send a copy to the EET Biobank.

PI Response: The 5 x or 7 x have been removed to prevent over collection. Any mention of processing the EDTA tubes to buffy coat has been removed as well. Other updates were made as requested.

#	Section	Comments
5.	5.2.2	Add bullets to the last two paragraphs in Section 5.2.2 as shown so the formatting of this section is consistent. Also, will buffy coat from EDTA tubes will be submitted? If yes, then retain the text below in yellow highlight. If no, then remove the text in yellow highlight. • Frozen specimens, including processed serum, plasma, buffy coat, and frozen tissue, may be collected, processed, and shipped to the EET Biobank on Monday through Thursday, since the Biorepository does not need to perform additional processing. In the event that frozen samples cannot be shipped immediately, they must be stored in a -80 °C freezer. Fresh blood specimens may be collected and shipped Monday through Friday. **PI Response: The items were added to the bulleted list and buffy coat was removed from the collected specimens.**
6.	5.3.4	Replace the current language in Section 5.3.4 for the collection of frozen tissue with the following. The updated language is recommended to help prevent the tissue from sticking to the side of the cryovial. 5.3.4 Collection of Biopsy to Snap-Freeze 1. Tissue should be frozen as soon as possible. Optimally, freeze within 30 minutes from resection. 2. Prior to tissue collection: a. Label cryovial(s) according to instructions in Section 5.4.1. b. Place cryovial(s) on dry ice to freeze. The vials should appear frosty when ready. 3. Immediately place tissue in foil and allow to completely freeze (using either direct contact with dry ice, or liquid nitrogen vapor). 4. Gently remove the frozen tissue from the foil. If the tissue is sticking to the foil, then gently run a finger over the back of the foil to loosen the tissue. 5. Using clean forceps place each tissue core in a separate pre-chilled cryovial. Tissue should move freely in the vial. Place the tissue in a -70 to -80°C freezer. Keep frozen until shipment to the EET Biobank. **PI Response: This has been updated as requested.**
7.	5.3.5	Throughout this Section, revise ctDNA or cfDNA Streck tubes to Streck cfDNA tubes

#	Section	Comments
		PI Response: This has been updated as requested.
8.	5.4.1	Revise the third sentence in the third paragraph the following. **Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient study ID on each document** (either by adding a label or hand writing). **PI Response: This has been updated as requested.**
9.	5.4.2 & 5.4.3.2	Is collection time required on the specimen labels? It is not indicated on the Specimen Collection labels in 5.4.2, but is indicated in Step 2 of Section 5.4.3.2. If time is not required, remove "time" from the 1st bullet in Step 2 of Section 5.4.3.2. If time is required, then please update the labeling instructions in 5.4.2 to indicate for which samples it is needed (e.g., blood). If it is only required for a subset of samples (e.g., PK specimens only), please indicate that in parentheses both in the labeling instructions in Section 5.4.2 and in Step 2 of Section 5.4.3.3. For example, for 5.4.2.2, • Collection date and time (only for PK specimens) (to be added by hand) For example, for Step 2 in Section 5.4.3.2 Label specimen containers and write collection date and time (time only needed for PK samples) on each label. **PI Response: Time has been removed from the instructions in Step 2 of Section 5.4.3.2.**
10.	5.4.3.2	Revise the last sentence of the final bullet in Step 2 as follows: Do not redact SPID, block number or relevant dates (such as collection date), and include the UPID and the patient study ID on each document. **PI Response: No update has been made as the last sentence of Step 2 already stated the above.**
11.	5.5.1.1	Revise the Required Forms for Specimen Submissions Table as shown below

<table>
<tr><th>#</th><th>Section</th><th>Comments</th></tr>
<tr><td></td><td></td><td>to:
Update Tissue to Specimen in the header
Update Surgical to Operative in the new biopsy row
Update blood to Blood in Streck Tubes, Plasma, Buffy Coat, and Serum in the blood row
Note: only include Buffy Coat, if buffy coat from EDTA tubes should be shipped to the EET Biobank.

<table><tr><th>Specimen</th><th>Required Forms</th></tr><tr><td>Archival</td><td>1. Shipping List
2. Corresponding Pathology Report</td></tr><tr><td>New Biopsy</td><td>1. Shipping List
2. Tissue Biopsy Verification Form
3. Diagnostic Pathology Report
4. Operative and/or Radiology Report</td></tr><tr><td>Blood in Streck Tubes, Plasma, Buffy Coat and Serum</td><td>1. Shipping List</td></tr></table><u>PI Response: This has been updated as requested. Buffy coat was not included.</u></td></tr>
<tr><td>12.</td><td>5.5.2.3</td><td>If buffy coat from EDTA tubes is to be shipped to the EET Biobank, then revise the first sentence of Section 5.5.2.3 as follows:
Serum, plasma, and buffy coat collected at time points that do not include the collection of frozen tissue must be submitted using institutional supplies.
<u>PI Response: Buffy coat from EDTA is not collected; this was not updated.</u></td></tr>
<tr><td>13.</td><td>5.8</td><td>Replace the current Biomarker Table with revised Table below. This will bring the table up to date with current formatting SOPs and make some additional changes for clarification including:
Separate tissue-based and blood-based assays.
The integrated and exploratory entries for WES and RRPA have been combined to make it clear that each assay is only to be performed once. It is not necessary to separate out exploratory purposes.</td></tr>
</table>

<table>
<tr><th>#</th><th>Section</th><th>Comments</th></tr>
<tr><td></td><td></td><td>

The lab information has been updated to use the harmonized names for MoCha, the RRPA core, and the IHC lab.
The gene expression analysis biomarker name has been updated to RNAseq to match the harmonized name used across ETCTN Trials for this assay.
The PI and PI email information was added for the MoCha-based assays.
Collections for exploratory tissue-based biomarkers cannot be mandatory in the Biomarker Table. The pre-treatment collections for RNAseq and TILs were updated to optional.
Additional formatting changes the specimens tested, collection time point, and M/O information.
For the Plasma and serum proteomics and metabolomics biomarker, if buffy coat is not required from the EDTA tube, then please remove the yellow highlighted text.

<table>
<tr><th>Prio</th><th>Biomarker Name</th><th>Assay and CLIA: Y/N</th><th>Use in the Trial and Purpose</th><th>Specimens Tested</th><th>Collection Time Points</th><th>Mandatory or Optional</th><th>Assay Laboratory, Lab PI, and Lab PI Email</th></tr>
<tr><td colspan="7">Tissue-based</td><td></td></tr>
<tr><td>1</td><td>Whole exome sequencing (WES)</td><td>NGS
CLIA: N</td><td>Integrated
Mutations in PIK3CA or PTEN as predictive markers for response to PI3K inhibitors
Additional exploratory analyses: To assess mutations in in candidate</td><td>DNA from FFPE Tumor Tissue</td><td>Archival,
Pre-treatment (Baseline)</td><td>M
M</td><td>MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
Dr. Mickey Williams
mickey.williams@nih.gov</td></tr>
</table>
</td></tr>
</table>

#	Section	Comments							
					genes including *ESR1*, *Rb*, *TP53*, *AKT1*, *PIK3R1*, *RAS*, and others at baseline and progression and to correlate with response in each treatment arm.				
		2	PTEN expression	IHC CLIA: N	Integrated Loss of *PTEN* as predictive markers for response to PI3K inhibitors	Unstained slides from FFPE Tumor Tissue	Archival, Pre-treatment (Baseline)	M M	Clinical Immunohistochemistry Laboratory, MD Anderson Cancer Center (MDACC) Dr. Wei-Lien Wang wlwana@mdanderson.org
		3	Reverse Phase Prot	RPPA CLIA: N	Integrated Levels of phosphorylated Rb	Snap-frozen Tumor Tissue	Pre-treatment, C1D15,	M O	Functional Proteomics RPPA Core Facility,

#	Section	Comments								
			ein array (RPPA)		(pS807_S811), AKT (pS473), Cyclin D1: To test whether baseline AKT phosphorylation correlates with PFS benefit from the addition of copanlisib and whether the triplet therapy with copanlisib/fulvestrant/abemaciclib inhibits AKT phosphorylation, reduces cyclin D1 expression, and is more effective in reducing Rb phosphorylation than with the fulvestrant/abemaciclib doublet; Additional exploratory analyses:		Disease progression	O	MD Anderson Cancer Center (MDACC) Dr. Yiling Lu yilinglu@mdanderson.org	

#	Section	Comments							
					To assess baseline and treatment induced changes in in various cancer associated pathways, including but not limited to PI3K, MAPK, ER, cyclins, CDKs and CDK inhibitors,; to correlate with treatment response and progression				
		4	RNAseq	NGS	Exploratory Gene expression analysis: To correlate baseline and treatment induced changes in breast cancer intrinsic subtypes (PAM50),	RNA from snap-frozen tumor tissue	Pre-treatment, C1D15, Disease progression	O O O	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Mickey Williams mickey.williams@nih.gov

#	Section	Comments							
					and PI3K mRNA signature and expression of candidate genes with treatment response and benefit from adding copanlisib.				
		5	Tumor immune microenvironment (TIL)	Multi-plex IHC (CD4+, CD8+, CD3+ T cells)	Exploratory To assess tumor immune microenvironment to correlate changes in infiltrating lymphocytes with treatment response	Unstained Slides from FFPE Tumor Tissue	Pre-treatment, C1D15, Disease progression	O O O	Washington University Center for Human Immunology and Immunotherapy Programs (CHiiPs) Core Dr. Robert Schreiber rdschreiber@wustl.edu
		Blood-based							

#	Section	Comments							
		1	Whole exome sequencing (WES)	NGS CLIA: N	Integrated Germline control for WES	Germline DNA from blood in Streck cfDNA tubes	Pre-treatment (Baseline)	M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams mickey.williams@nih.gov
		2	ctDNA sequencing (*PIK3CA*, *PTEN*, *ESR1* and others)	NGS	Exploratory To correlate ctDNA mutation profiles with tumor sequencing, and correlate baseline ctDNA mutations, particularly in components of the PI3K pathway with treatment response, and correlate early changes in ctDNA	Plasma from blood in Streck cfDNA tubes	Pre-treatment C1D8, C1D15, C2D1, C4D1, C7D1 and then every 3 cycles after), Disease progression	M M M M M M	Broad Institute Dr. Gerburg Wulf gwulf@bidmc.harvard.edu

#	Section	Comments							
					VAFs with PFS, assess emergent resistant mutations at progression				
		3	Plasma and serum proteomics and metabolomics	Proteomic Analysis	Exploratory To characterize circulating markers before and after PI3K inhibitor therapy to predict treatment response and resistance mechanisms	Plasma from blood in EDTA tube & Serum from blood in red top tube	Pre-treatment C1D8, C1D15, C2D1, C4D1, C7D1 and then every 3 cycles after), Disease progression	M M M M M M	Beth Israel Deaconess Medical Center Proteomics Core/ Broad Institute Dr. Gerburg Wulf gwulf@bidmc.harvard.edu

#	Section	Comments							
		4	Copanlisib Pharmacokinetics **	LC/MS/MS	Exploratory To evaluate whether abemaciclib impacts the PK of copanlisib	Plasma from blood in Li-Heparin BD vacutainers	(i) C1D1 (a) Prior to copanlisib infusion and (b) After copanlisib infusion (10 min, 1 hr, 2 hr, 3 hr, 5 hr, 24 hr [before C1D2's abemaciclib and fulvestrant]) and (ii) C1D15(a) Prior to copanlisib infusion and (b) After copanlisib infusion (10 min, 1	M M	NorthEast Bioanalytical Laboratories Dr. Vipin Agarwal vipin.agarwal@nebiolab.com

#	Section	Comments							
							hr, 2 hr, 3 hr, 5 hr, 24 hr [before C1D16's abemaciclib and fulvestrant])		
		5	Abemaciclib Pharmacokinetics **	LC/MS/MS	Exploratory To evaluate whether copanlisib impacts the PK of abemaciclib	Plasma from blood in Li-Heparin BD vacutainers	(i) C1D15: Use the blood collected the same timepoints for the copanlisib PK. (ii) C1D22: Prior to, 1h, 2h, 4h, 23h post the	M M	NorthEast Bioanalytical Laboratories Dr. Vipin Agarwal vipin.agarwal@nebiolab.com

#	Section	Comments
		<table><tr><td></td><td></td><td></td><td></td><td></td><td>morning dose of abemaciclib</td><td></td><td></td></tr></table> ** PK samples are required for patients enrolled in the phase 1 portion and the first four patients enrolled to the FAC arm in the randomized phase 2 portion. **PI Response: This has been updated as requested. The contact for CHiiPs has also been updated from Dr. Schreiber to Diane Bender, PhD. Shipping of the buffy coat was removed from the table.**
14.	5.8.1 & 5.9.1	• Revise the title for 5.8.1 PIK3CA/PTEN mutation status to 5.8.1 Whole Exome Sequencing (WES). • In 5.8.1.3, revise the Attn: information to: Attn: Alyssa Chapman or Ruth Thornton Remove Section 5.9.1 as all WES-related biomarkers have been combined into one biomarker **PI Response: This has been updated as requested.**
15.	5.8.2	Add new subsections 5.8.2.3 and 5.8.2.4 as shown below: 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: Add the shipping information for the MD Anderson Clinical Immunohistochemical Laboratory 5.8.2.4 Contact Information for Notification of Specimen Shipment Add the contact information for notification of specimen shipments to the MD Anderson Clinical Immunohistochemical Laboratory **PI Response:**
16.	5.9.2	Move current Section 5.9.2 RPPA to the Section 5.8.3 since it is an integrated biomarker. Add new subsections 5.8.3.3 and 5.8.3.4 as shown below: 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:

#	Section	Comments
		Add the shipping information for the RPPA Core 5.8.3.4 Contact Information for Notification of Specimen Shipment Add the contact information for notification of specimen shipments to the RPPA Core **PI Response: This has been moved and updated as requested.**
17.	5.9.3	In 5.9.3.3, revise the Attn: information for RNAseq to: Attn: Alyssa Chapman or Ruth Thornton **PI Response: This has been updated as requested.**
18.	5.9.4	Add new subsections 5.9.4.3 and 5.9.4.4 as shown below: 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: Add the shipping information for the Broad Institute for ctDNA analysis 5.9.4.4 Contact Information for Notification of Specimen Shipment Add the contact information for notification of specimen shipments to the for the Broad Institute for ctDNA analysis **PI Response:**
19.	5.9.7	Add new subsections 5.9.7.3 and 5.9.7.4 as shown below: 5.9.7.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study Specimens will be shipped from the EET Biobank to: Add the shipping information for the Washington University CHiiP Core for TILs 5.9.7.4 Contact Information for Notification of Specimen Shipment Add the contact information for notification of specimen shipments to the Washington University CHiiP Core for TILs **PI Response: This has been updated as requested.**
20.	11.1 and 11.2	If buffy coat is to be sent to the EET Biobank, update the row in each study calendar for the EDTA tube to the following: Blood (EDTA purple top tube) for plasma and buff coat[6] **PI Response: Buffy coat is processed from the Streck cfDNA tube, so this change was not made.**

Comments Requiring a Response – Administrative and Editorial Issues:

#	Section	Comments
21.	ICD -Optional Studies for Phase 1 and Phase 2 ICD	Results will not be returned as stated under "Optional studies you can chose to take part in" Please delete the following language under "Samples for known future studies" I agree that my study doctor, or someone on the study team, may contact me or my doctor to see if I wish to learn about results from this study. YES NO **PI Response: This has been updated as requested.**
22.	9.4	Please insert a Domestic Planned Enrollment Table for Phase 1 & Phase 2 portions of the study. And also please add an "International Planned Enrollment Report" tables for Phase 1 & Phase 2 phases of the study since Princess Margaret Cancer Center is listed as a participant. **PI Response: This has been updated as requested.**
23.	Both consents & 9.4	Per 9.4 there will be 204 participants. Per the ICD "What is the purpose" there will be a total of 194 participants (Phase 1 n= 24; Phase 2 n=170). Please review and reconcile **PI Response: Section 9.4 has been updated to clarify that Phase 1 is complete as of the time of Amendment 8, and enrollment was 24 patients.**

Recommendations:

#	Section	Comments
24.	4.1, Investigator and Research Associate Registration with CTEP	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 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. Investigators and clinical site staff who are significant contributors to research must register in the Registration and

<table>
<tr><th>#</th><th>Section</th><th>Comments</th></tr>
<tr><td></td><td></td><td>
Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr/. The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes five person registration types.

• Investigator (IVR): MD, DO, or international equivalent,
• Non Physician Investigator (NPIVR): advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD),
• Associate Plus (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:

<table>
<tr><th>Documentation Required</th><th>IVR</th><th>NPIVR</th><th>AP</th><th>A</th><th>AB</th></tr>
<tr><td>FDA Form 1572</td><td>✓</td><td>✓</td><td></td><td></td><td></td></tr>
<tr><td>Financial Disclosure Form</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
<tr><td>NCI Biosketch (education, training, employment, license, and certification)</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
<tr><td>GCP training</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
<tr><td>Agent Shipment Form (if applicable)</td><td>✓</td><td></td><td></td><td></td><td></td></tr>
<tr><td>CV (optional)</td><td>✓</td><td>✓</td><td>✓</td><td></td><td></td></tr>
</table>

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,
• Selection as the treating, credit, or drug shipment investigator or consenting person in OPEN,
• Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval, and
• Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed
</td></tr>
</table>

#	Section	Comments
		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. Refer to the NCI RCR page on the CTEP website for additional information. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov. **PI Response: This has been updated as requested.**
25.	4.2, Site Registration	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 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 IRBManager 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 email or calling 1-888-651-CTSU (2878). In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record: • Have an active CTEP status, • Have an 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, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record, • Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, • List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and • Have the appropriate CTEP registration type for the protocol.

#	Section	Comments
		Additional Requirements Additional site requirements to obtain an approved site registration status include: • An active Federal Wide Assurance (FWA) number, • An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO), • An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and • Compliance with all applicable protocol-specific requirements (PSRs). **PI Response: This has been updated as requested.**
26.	4.2.1, Downloading Site Registration Documents	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 4.2.1 Downloading Site Registration 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 their associated investigators and staff on a participating roster. To view/download site registration forms: • Log in to the CTSU members' website (https://www.ctsu.org), • Click on *Protocols* in the upper left of the screen o Enter the protocol number in the search field at the top of the protocol tree, or o Click on the By Lead Organization folder to expand, then select *LAO-CT018*, and protocol number *10287*, • Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select *Site Registration* to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.) **PI Response: This has been updated as requested.**
27.	4.2.2 Requirements for 10287 Site Registration	***Please revise the excerpt below as indicated.*** o 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~~

#	Section	Comments
		~~Specimen Tracking System may require new training.~~ Users are strongly encouraged to take a refresher of the training if they have not entered specimen data for an extended period of time. ○ This training will need to be completed before first/further patient enrollment at a given site. ○ Please contact STS Support at Theradex for the training (STS.Support@theradex.com~~; Theradex phone: 609-799-7580~~). **PI Response: This has been updated as requested.**
28.	4.3.1, OPEN/IWRS	***Please revise the excerpt below as indicated.*** ~~• A valid CTEP-IAM account.~~ • Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems. • To perform enrollments or request slot reservations: ~~Be~~ Must be on an LPO roster, ETCTN Corresponding roster, or ~~Participating Organization~~ PO roster with the role of Registrar. Registrars must hold a minimum of an ~~AP~~ Associate Plus (AP) registration type. ***Please revise the excerpt below as indicated.*** • All patients have signed an appropriate consent form and ~~HIPAA~~ Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable). **PI Response: This has been updated as requested.**
29.	10.3.1, Rave-CTEP-AERS	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 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

#	Section	Comments
		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 and the CTSU standard Pre-Treatment AE form is used. Pre-existing medical conditions are not considered adverse events and therefore should not be reported on the Pre-Treatment 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 condition should be reported in Rave 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

#	Section	Comments
		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 (i.e., checking the box *Send All AEs for Evaluation* and save the 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: This has been updated as requested. The Pre-treatment AE page is not utilized for this study so the highlighted text was omitted from the update.**
30.	13.2, Data Reporting	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 13.2 Data Reporting Medidata Rave is the clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through

#	Section	Comments
		the CTEP-IAM system and role assignments. Requirements to access Rave via iMedidata: • Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems, 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: o Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type, o Rave Investigator role must be registered as a Non-Physician Investigator (NPIVR) or Investigator (IVR), and o Rave Read Only or Rave SLA role must have at a minimum an Associate (A) registration type. • Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL. Upon initial site registration approval for the study in the Regulatory application, 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 who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application 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*

#	Section	Comments
		Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com. **PI Response: This has been updated as requested.**
31.	13.3, Data Quality Portal	***Please delete the information within this subsection and replace with the following language to reflect the current CTEP language template.*** 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, 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. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms. The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available on the DQP modules. CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website. To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules. ***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

#	Section	Comments
		will display *N/A* for the Total Delinquencies summary count. **PI Response: This has been updated as requested. The calendaring function is not used so the language was kept.**
32.	8.1.2.1, 8.1.3	Please replace "Online Agent Order Processing (OAOP)" and "OAOP" with "AURORA" **PI Response: This has been updated as requested.**
33.	8.1.4	Please replace: PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/oaop With: PMB AURORA application: https://ctepcore.nci.nih.gov/aurora/login **PI Response: This has been updated as requested.**
34.	ICD Pg 19	Study Calendar – remove the X for copanlisib on Day 8 as Day 8 and day 22 are removed from cycles 2 and beyond. **PI Response: This has been updated as requested.**
35.	ICD Pg 19, 20 0f 19	Please correct page numbers **PI Response: This has been updated as requested.**

Company Comments – Requiring a Response:

#	Section	Comments
35.	Schema page 3	Schema (page 3): the old diagram is still in the protocol... it is still showing that the copa is dosed on D1, 8 and 15 in each cycle and abemaciclib as continuous dosing for 28 days. This figure should reflect the changes noted in the legend. **PI Response: This has been updated as requested.**

VI. Summary of Changes in Amendment #8 of Protocol #10287, dated July 10, 2023

#	Section	Comments
36.	Global	Updated version date and table of contents. Minor typographical and formatting changes were made throughout the document.

#	Section	Comments
37.	Title page	Updated University of Pittsburgh Cancer Institute to UPMC Hillman Cancer Center Added Amendment 8 / July 10, 2023
38.	Schema	Added RP2D for Phase 2 FAC arm as determined by the Phase 1 portion of the study. Abemaciclib will be dosed at 100 mg BID PO Days 1-5 of each week during each 28-day cycle (Days 1-5, 8-12, 15-19, and 22-26). Copanlisib will be dosed at 45 mg IV on Days 1 and 15. Clarified that the first 2 doses of abemaciclib on C1D1 will be skipped for patients participating in the PKs.
39.	Section 2.7	Added new Section 2.7 Rationale for Protocol Amendment #8 and RP2D.
40.	Section 4.3	Clarified that information for the 10287 Study Portal is found in LPO Documents for the study on CTSU.
41.	Section 5.3.2	Pre-treatment biopsies: Added that bone biopsies are acceptable and clarified that a minimum of 2 cores is required. C1D15 and Disease Progression biopsies: Corrected the biopsy collection time for patients on the FA regimen to at least 1 hour after *abemaciclib*, not copanlisib. Added that it is recommended to use the same site for both the pre-treatment and C1D15 biopsies, and clarified that a minimum of 2 cores is required.
42.	Section 6	Reworded the summary of the study design to clarify that the RP2D from the Phase 1 FAC regimen would be used for the randomized Phase 2 portion, and that the RP2D is Dose Level 1b for the FAC arm.
43.	Section 6.1	Added note to tables of potential dose levels for Part A and B stating: • Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is required that anti-histamine prophylaxis be initiated at least 3 days prior to Cycle 1 Day 1; however, if any issues arise that may necessitate a later start date for the anti-histamine prophylaxis, they must be discussed with the Principal Investigator.

#	Section	Comments
		Added the RP2D dose of abemaciclib 100 mg BID on a 5 days on 2 days off schedule and copanlisib 45 mg IV Days 1 and 15 of a 28-day cycle to both paragraph and the regimen table. Added statement to Randomized Phase 2 portion in both paragraph and in the regimen table stating "Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is required that anti-histamine prophylaxis be initiated at least 3 days prior to Cycle 1 Day 1; however, if any issues arise that may necessitate a later start date for the anti-histamine prophylaxis, they must be discussed with the Principal Investigator." Clarified that for the first 4 patients randomized to the FAC arm and undergoing PKs, the C1D1 dose of abemaciclib is skipped.
44.	Section 6.1.1	Added statement "Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is required that anti-histamine prophylaxis be initiated at least 3 days prior to Cycle 1 Day 1; however, if any issues arise that may necessitate a later start date for the anti-histamine prophylaxis, they must be discussed with the Principal Investigator." Clarified that fasting is only required prior to the first C1D1 glucose measurement. Added an allowance for patients to have a low carbohydrate meal on C1D1 is their infusions are scheduled at a later hour, due to their age, or when medically indicated.
45.	Section 6.1.2	Added the abemaciclib RP2D of 100 mg BID on Days 1-5, 8-12, 15-19, and 22-26 of each 28-day cycle. Clarified that for the first 4 patients enrolled to the FAC arm who are undergoing PKs, the first 2 doses of abemaciclib on C1D1 are skipped.
46.	Section 6.2.1	Clarified that the DLT window is the first 28 days of treatment. Added a note stating that "patients who have major protocol deviations (as determined by the study chair and the study statistician) in the dose and/or

#	Section	Comments
		schedule of the study drugs during Cycle 1 are not evaluable for DLT. These patients may be evaluable for overall AE and efficacy endpoints per statistical section."
47.	Section 6.3	Added requirement of daily oral antihistamine during copanlisib treatment.
48.	Section 7.1.2	Clarified that an occurrence of hypertension consists of either systolic or diastolic reaching the 150/90 threshold, and that the infusion can be resumed once both systolic and diastolic BPs are less than 150/90. Added statement that "In patients who have not had any episodes of post infusion blood pressures ≥150/90 mmHg for at least 2 consecutive cycles of treatment, the blood pressure monitoring at 1h and 2h could be omitted at the discretion of the treating physician."
49.	Section 7.1.6	Clarified that a new cycle of treatment with copanlisib can only start if both systolic and diastolic BPs are less than 150/90. Added a note stating that "If dose interruption for copanlisib is necessary, it is preferred that the dose of copanlisib be delayed rather than missed to maximize copanlisib dosing. However, copanlisib dosing schedule could be adjusted in subsequent cycles so to align with the fulvestrant administration, with the condition that no more than 2 consecutive weekly doses of copanlisib is administered." Added that cycle length should be 28 days +/- 7 days, and that a maximum duration of holding copanlisib is 3 consecutive weeks.
50.	Section 7.2	Added 50 mg BID *continuously* to Dose Level -1 to denote that the dosing schedule for the lowest dose reduction would be continuous irrespective of the initial dosing schedule.
51.	Section 9.1	Added an interim toxicity analysis performed by the DSMB after the first 10 patients are enrolled to the FAC arm in the Phase 2 portion of the study.
52.	Section 9.4.2	Added an interim toxicity analysis performed by the DSMB after the first 10 patients are enrolled to the FAC arm in the Phase 2 portion of the study.
53.	Section 11	Renamed Baseline visit as Screening visit. Due to the schedule changes from Phase I to Phase II, a Phase II calendar was added. Phase I Calendar Changes:

#	Section	Comments
		• Removed randomization row and subsequent cycles Day 22 column. • Added submission of genomic testing report at baseline if available. • Updated footnote 5 to include requirement for daily prophylactic antihistamine. • Updated footnote 7 to add a 14 day window for the tumor biopsy at baseline. • Updated footnote 15 to clarify that the first 2 doses of abemaciclib on C1D1 are skipped in patients undergoing PKs. • Updated footnote 17 with biopsy timing requirements relative to drug dosing. • Added footnote 22 clarifying where the genomic testing report should be uploaded if available. • Updated footnote ** to clarify that tumor imaging should be conducted at the end of cycle 3 and every 3 cycles after that, and removed the additional mention of every 12 weeks.

NCI Protocol #: 10287

Local Protocol #: 201911082

ClinicalTrials.gov Identifier: NCT03939897

TITLE: A randomized Phase I/II trial of fulvestrant and abemaciclib in combination with copanlisib (FAC) versus fulvestrant and abemaciclib alone (FA) for endocrine-resistant, hormone receptor positive, HER2 negative metastatic breast cancer (FAC vs FA)

Corresponding Organization: **LAO-CT018** / Yale University Cancer Center LAO.

Principal Investigator:
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St. Louis, MO 63110
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E-mail: cynthiaxma@wustl.edu

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
CATCHUP / Creating Access to Targeted Cancer Therapy for Underserved Populations

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Co-investigators:

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UNC Hospital
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NCI-Supplied Agent: Copanlisib ([BAY 80-6946] NSC 784727)
Other Agent(s): Fulvestrant (NSC 719276, Commercial)
Abemaciclib (NSC 783671, Commercial)

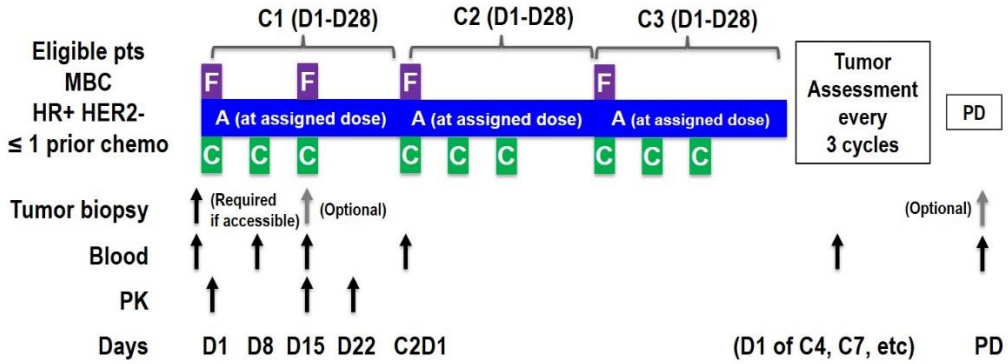
IND #:
IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date:

Original / December 6, 2018
Revision 1 / March 5, 2019
Revision 2 / April 3, 2019
Revision 3a / June 20, 2019
Revision 3b / July 18, 2019
Revision 4 / August 20, 2019
Amendment 1 / February 26, 2020 (Disapproved)
Amendment 2 / March 17, 2020
Amendment 3 / June 15, 2020
Amendment 4 / August 25, 2020
Amendment 5 / September 11, 2020
Amendment 6 / February 4, 2021 (Disapproved)
Amendment 6 / July 14, 2021 (Disapproved)
Amendment 6a / August 13, 2021
Amendment 6 / October 8, 2021
Amendment 7 / April 26, 2023
Amendment 8 / July 10, 2023 (Disapproved)
Amendment 8a /September 7, 2023 (Disapproved)
Amendment 8b / September 29, 2023 (Disapproved)
Amendment 8c / November 28, 2023 (Disapproved)
Amendment 8d / June 13, 2024

SCHEMA

Phase 1:



Due to study closure, Amendment 8d dated 07Jun2024 removes the Phase 2 portion of the trial as it will not open.

Note: Part A of Phase 1 tested abemaciclib at continuous dosing on Days 1-28 in combination with fulvestrant and copanlisib. With Amendment 6, Part B was added to test abemaciclib at 5 days on and 2 days off each week in combination with fulvestrant and copanlisib.

F: Fulvestrant at 500 mg intramuscular (IM) Days 1 and 15 of Cycle 1, then on Day 1 of C2+
A: Abemaciclib as follows:
Phase 1 Part A: at assigned dose BID orally (PO) Days 1-28 of each 28-day cycle.
Phase 1 Part B: at assigned dose BID PO Days 1-5 of each week during each 28-day cycle (Days 1-5, 8-12, 15-19, and 22-26).
C: Copanlisib as follows:
Phase 1: at assigned dose intravenously (IV) on an assigned schedule of Days 1, 8, 15 or Days 1 and 15 of each 28-day cycle, depending on the dose level.

Tumor biopsy: Refer to Section 5.3 for details.
Archival tumor specimens from prior diagnostic or therapeutic procedures are required.

Phase 1 Regimen Part A:

Potential Dose Level						
Possible Dose Level	**Copanlisib****		**Abemaciclib***		**Fulvestrant***	
	Dose (mg)	**Administration and Schedule**	**Dose (mg)**	**Administration and Schedule**	**Dose (mg)**	**Administration and Schedule**
Level 4	60	IV on D1, D8, and D15 every 28 days	150	PO BID	500	IM C1D2, C1D16, and D1 of every cycle[#] thereafter
Level 3	45		150		500	
Level 2	45		100		500	
Level 1 (Starting dose)	45	IV on D1 and D15 every 28 days	100		500	
Level -1	30		100		500	

IV = intravenous, PO = by mouth, BID = twice a day, IM = intramuscular, C1D2 = Cycle 1 Day 2, C1D16 = Cycle 1 Day 16, and D = Day.
Doses are stated as exact dose in units (*e.g.,* mg/m^2, mcg/kg, *etc.*) rather than as a percentage.
*The first dose (C1D1) of abemaciclib and fulvestrant is delayed to CID2 following the 24 h blood draw for copanlisib pharmacokinetics (PK). CID15 fulvestrant dose is delayed to C1D16 following the 24 h blood draw for copanlisib PK. Note that C1D16 dose of fulvestrant could be omitted in patients on stable doses of fulvestrant prior to enrolling on this study.
**Administered over 1 h (+/-10 minutes)
[#]Cycle length = 28 days

Phase 1 Regimen Part B:

Potential Dose Level						
Possible Dose Level	**Copanlisib****		**Abemaciclib***		**Fulvestrant***	
	Dose (mg)	**Route and Schedule**	**Dose (mg)**	**Route and Schedule**	**Dose (mg)**	**Route and Schedule**
Level 4b	60	IV on D1, D8, and D15 every 28 days	150	PO BID 5 days on and 2 days off each week	500	IM C1D2, C1D16, and D1 of every cycle[#] thereafter
Level 3b	45		150			
Level 2b	45		100			
Level 1b (Starting dose)	45	IV D1 and D15 every 28 days	100			

IV = intravenous, PO = by mouth, BID = twice a day, IM = intramuscular, C1D2 = Cycle 1 Day 2, C1D16 = Cycle 1 Day 16, and D = Day.
Doses are stated as exact dose in units (*e.g.,* mg/m^2, mcg/kg, *etc.*) rather than as a percentage.
*The first dose (C1D1) of abemaciclib and fulvestrant is delayed to C1D2 following the 24 h blood draw for copanlisib pharmacokinetics (PK). CID15 fulvestrant dose is delayed to C1D16 following the 24 h blood draw for copanlisib PK. Note that C1D16 dose of fulvestrant could be omitted in patients on stable doses of fulvestrant prior to enrolling on this study.
**Administered over 1 h (+/-10 minutes)
[#]Cycle length = 28 days

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1.1.1 To evaluate the safety profile of fulvestrant + abemaciclib + copanlisib (FAC) and determine the recommended Phase 2 dose (RP2D).

1.2 Secondary Objectives

1.2.1 To assess the objective response rate (ORR = partial response [PR] + complete response [CR]) and clinical benefit rate (CBR = PR + CR + stable disease [SD] ≥6 months) of FAC and median PFS with FAC

1.3 Exploratory Objectives

1.3.1 To assess whether triplet therapy with FAC inhibits AKT phosphorylation, reduces cyclin D1, and inhibits Rb phosphorylation.

1.3.2 To assess whether the combination of abemaciclib and fulvestrant affect the copanlisib pharmacokinetics (PK).

1.3.3 To assess the ORR, CBR, and median PFS in the following molecularly defined subgroups treated with FAC, such as mutations in genes in the PI3K pathway (PIK3CA, AKT, PTEN etc), ESR1, TP53, or PTEN IHC loss vs not.

1.3.4 To assess baseline and treatment induced changes in various cancer associated pathways, including but not limited to PI3K, MAPK, ER, cyclins, CDKs and CDK inhibitors; and to correlate with treatment response and progression.

1.3.5 To correlate baseline and treatment induced changes in breast cancer intrinsic subtypes (PAM50), and PI3K mRNA signature and expression of candidate genes with treatment response and benefit from adding copanlisib.

1.3.6 To evaluate ctDNA mutations at baseline and over time for response predictors at baseline, and clonal evolution associated with treatment.

1.3.7 To correlate ctDNA mutation profiles with tumor sequencing, and correlate baseline ctDNA mutations, particularly in components of the PI3K pathway with treatment response, and correlate early changes in ctDNA VAFs with PFS, assess emergent resistant mutations at progression

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1.3.9 To examine the molecular effects of FAC on tumor and circulating markers.

1.3.10 To analyze tumor infiltrating lymphocytes at baseline, during treatment, and at disease

progression.

2. BACKGROUND

2.1 Study Disease

2.1.1 Endocrine-resistant HR+ Breast Cancer and Efficacy of Cyclin Dependent Kinases 4 and 6 (CDK4/6) inhibitors

Hormone receptor positive (HR+) human epidermal growth factor receptor 2 (HER2) negative (HER2-) breast cancer represents over 70% of breast cancer diagnosis and is a leading cause of cancer death in women (Siegel *et al.,* 2017). Endocrine therapy includes the aromatase inhibitors (AI) and gonadotropin-releasing hormone (GnRH) agonists which reduce estrogen biosynthesis, the selective estrogen receptor modulator (SERM) - tamoxifen, and the selective estrogen receptor down regulator (SERD) - fulvestrant. The clinical use of these agents has led to significant improvements in survival outcomes of patients with estrogen receptor positive (ER+) breast cancer (Early Breast Cancer Trialists' Collaborative G, 2011). However, systemic relapse occurs in approximately 20% of patients who were initially diagnosed with early stage breast cancer during or after completion of adjuvant endocrine agents due to *de novo* or acquired endocrine resistance mechanisms (Early Breast Cancer Trialists' Collaborative G, 2011; Mauri *et al.,* 2006).

The cyclin D/CDK4/6 complex controls the G1 to S phase transition through phosphorylation of retinoblastoma susceptibility (RB1) gene product (Rb) and the subsequent release of E2F transcription factors to activate cell cycle progression genes (Sherr, 1996; Sherr and Roberts, 1999; van den Heuvel and Harlow, 1993; Weinberg, 1995). There is a significant association between CDK4/6 activation and ER signaling as cyclin D is a direct transcriptional target of ER and is the downstream effector of other mitogenic signals implicated in endocrine resistance (Thangavel *et al.,* 2011; Musgrove *et al.,* 2011; Finn *et al.,* 2009; Knudsen and Witkiewicz, 2017). In addition, the poor prognosis luminal B ER+ breast cancer type is enriched for cyclin D1 amplification (58%), CDK4 gain (25%), and loss of p16, the negative regulator of CDK4/6, which could lead to constitutive activation of CDK4/6 (Network TCGA, 2012). In preclinical studies, CDK4/6 inhibitors are particularly active in ER+ breast cancer and synergize with endocrine therapy in both endocrine sensitive and resistant cell lines (Finn *et al.,* 2009). Several CDK4/6 inhibitors, including palbociclib, ribociclib, and abemaciclib, are in clinical development and have shown efficacy in randomized Phase 3 trials with significantly improved PFS when added to standard endocrine therapy in the metastatic setting (Cristofanilli *et al.,* 2016; O'Leary *et al.,* 2016; Verma *et al.,* 2016; Turner *et al.,* 2015; Finn *et al.*, 2015; Hortobagyi *et al.,* 2016; Finn *et al.,* 2016a; George *et al.,* 2017; Goetz *et al.,* 2017; Slamon *et al.,* 2018). In the initial Phase 2 trial (PALOMA-1), palbociclib improved PFS when added to letrozole (20.2 months *vs*. 10.2 months) as first-line therapy for patients with HR+/HER2- metastatic breast cancer, leading to the Food and Drug administration (FDA) approval of palbociclib (Beaver *et al.,* 2015). The benefit of palbociclib in the first line setting was confirmed in the Phase 3 PALOMA-2 trial, in which the PFS was 24.8 (22.1-Normal Range [NR]) months in the

palbociclib/letrozole arm *vs.* 14.5 (12.9-17.1) months in the placebo/letrozole arm, HR 0.58; p< 0.000001 (Finn *et al.,* 2016b). Subsequently, data from the randomized Phase 3 trial of ribociclib plus letrozole as first line therapy for HR+ advanced breast cancer (MONALEESA 2 trial) again demonstrated a significantly improved PFS. After 18 months, PFS was 63.0% (95% confidence interval [CI], 54.6 to 70.3) in the ribociclib group and 42.2% (95% CI, 34.8 to 49.5) in the placebo group (Hortobagyi *et al.*, 2016). Similarly, MONARCH 3, a double-blind, randomized Phase 3 trial of abemaciclib or placebo plus a nonsteroidal AI in postmenopausal women with HR-positive, HER2-negative advanced breast cancer who had no prior systemic therapy in the advanced setting, demonstrated that the median PFS was significantly prolonged in the abemaciclib arm (hazard ratio, 0.54; 95% CI, 0.41 to 0.72; p = 0.000021; median: not reached in the abemaciclib arm, 14.7 months in the placebo arm (Goetz *et al.,* 2017).

The benefit of CDK4/6 inhibition for treating endocrine-resistant HR+, HER2- breast cancer was demonstrated in PALOMA-3 trial (Cristofanilli *et al.,* 2016) and more recently the MONARCH 2 trial (George *et al.,* 2017). In PALOMA-3, 521 patients with HR+, HER2 negative metastatic breast cancer whose disease progressed after prior endocrine therapy were randomly assigned 2:1 ratio to fulvestrant plus palbociclib (n = 347) and fulvestrant plus placebo (n = 174). The median PFS was 9.5 months (95% CI 9.2–11.0) in the fulvestrant plus palbociclib group and 4.6 months (3.5–5.6) in the fulvestrant plus placebo group (hazard ratio 0.46, 95% CI, 0.36–0.59, p< 0·0001). The combination was well tolerated with the most common grade 3 or 4 adverse events (AEs) being uncomplicated neutropenia (65%), anemia (3%) and leucopenia (28%). Results from the PALOMA-3 trial led to FDA approval for the combination of palbociclib and fulvestrant for the endocrine-resistant population, making this a new standard of care (Cristofanilli *et al.,* 2016). MONARCH 2 trial was a global, double-blind, Phase 3 study of women with HR+, HER2 negative advanced breast cancer who had progressed while receiving neoadjuvant or adjuvant endocrine therapy, ≤12 months from the end of adjuvant endocrine therapy, or while receiving first-line endocrine therapy for metastatic disease (George *et al.,* 2017). Six hundred and sixty-nine (669) patients were randomly assigned to receive abemaciclib plus fulvestrant (n = 446) or placebo plus fulvestrant (n = 223). Abemaciclib plus fulvestrant significantly extended PFS *vs* fulvestrant alone (median, 16.4 *vs*. 9.3 months; hazard ratio, 0.553; 95% CI, 0.449 to 0.681; p< 0.001). The most common adverse events in the abemaciclib *vs*. placebo arms were diarrhea (86.4% *vs.* 24.7%), neutropenia (46.0% *vs.* 4.0%), nausea (45.1% *vs.* 22.9%), and fatigue (39.9% *vs.* 26.9%). The longer PFS observed in the MONARCH 2 trial is explained by the requirement of no more than 1 prior endocrine therapy and no prior chemotherapy for advanced disease.

As discussed above, CDK4/6 inhibitors prolong the PFS in patients with endocrine-resistant breast cancers. However, metastatic HR+ HER2- breast cancer remains incurable due to inevitable disease progression (Cristofanilli *et al.,* 2016). Effective treatments that prevent or delay resistance to CDK4/6 inhibitors therefore remain an unmet clinical need.

2.1.2 Phosphatidylinositol 3-kinase (PI3K) in mediating resistance to endocrine therapy.

PI3K transmits signals from receptor tyrosine kinases (RTKs) to numerous cellular targets that are important for cell proliferation, survival, differentiation, and migration (Engelman, 2009; Lui *et al.,* 2013). PI3K/AKT signaling is commonly dysregulated in human cancers *via* various

mechanisms, *e.g.,* gene amplification, rearrangement, or activating and/or loss-of-function mutations of the pathway's molecular components (Engelman, 2009; Fruman and Rommel, 2014). Aberrant activation of class I PI3Ks has been associated with intrinsic and acquired resistance of tumors to targeted agents, chemotherapy, and radiotherapy (West *et al.,* 2002; Clark *et al.,* 2002).

Four PI3K isoforms (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ), all of which have a catalytic p110 subunit (p110α, β, γ, or δ), comprise the class I PI3K subfamily (Vanhaesebroeck *et al.,* 2010). Among the PI3K catalytic subunits, p110α and p110β are ubiquitously expressed and are implicated in promoting oncogenic growth, whereas the expression of p110δ and p110γ is mostly restricted to leukocytes (Engelman *et al.,* 2006; Chang *et al.,* 1997; Zhao *et al.,* 2005; Zhao *et al.,* 2003). Activating mutations have only been observed in *PIK3CA* and not in *PIK3CB* (Samuels *et al.,* 2004; Yuan and Cantley, 2008). p110α is required to mediate the signaling and tumorigenesis due to RTKs such as vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), or HER2 (Graupera *et al.,* 2008; Utermark *et al.,* 2012; Zhao *et al.,* 2006), as well as for Ras (Gupta *et al.,* 2007; Ramjaun and Downward, 2007). Tumor cells carrying *PIK3CA* mutations are highly dependent on p110α for survival (Crowder *et al.,* 2009; Juric *et al.,* 2012). In contrast, p110β does not directly bind to Ras proteins (Fritsch *et al.,* 2013). There is evidence that the background signaling state in the absence of ligands for PI3K might be p110β dependent (Dbouk *et al.,* 2010; Knight *et al.,* 2006; Vogt, 2011), therefore phosphatase and tensin homolog (PTEN)-deficient tumors would have deregulated p110β signaling. There is considerable evidence indicating that p110β is important in mediating PI3K signaling and AKT phosphorylation in PTEN-deficient cancers and p110β-selective inhibitors reduce pAKT and inhibit growth in both cell-based and *in vivo* studies of breast and other cancer cell lines (Jia *et al.,* 2008; Ni *et al.,* 2012; Torbett *et al.,* 2008; Wee *et al.,* 2008). In addition, PI3Kβ is involved in signaling downstream of certain G-protein coupled receptors (GPCRs) and RTK in PTEN null and mutant Rac lines (Kawazu *et al.,* 2013). However, there are clearly tumor cell lines featuring PTEN loss that are not dependent on p110β (Ni *et al.,* 2012; Wee *et al.,* 2008; Berenjeno *et al.,* 2012). Additionally, recent work has indicated that loss of PTEN in certain tissues may lead to p110α-dependency (Berenjeno *et al.,* 2012). Other reports show that some tumors may be equally dependent on p110α and p110β (Jia *et al.,* 2013; Wang *et al.,* 2013; Oda *et al.,* 2008). PI3Kδ-specific inhibitors have shown remarkable therapeutic efficacy in some human leukemias and lymphomas (Yang *et al.,* 2015). A major component of the mechanism of action of PI3Kδ inhibition in the B-cell malignancies is to attenuate the responsiveness of the tumor cells to supportive stimuli from the microenvironment (Okkenhaug and Burger, 2016). Inhibition of PI3Kδ has been shown to protect mice against a broad range of cancers, including non-hematological solid tumors (Ali *et al.,* 2014). Inactivation of PI3Kδ breaks regulatory T-cell (Treg)-mediated immune tolerance that unleashes a cytotoxic T-cell response and resulting in tumor regression. Copanlisib is a pan-class I PI3K small-molecule inhibitor exhibiting activity predominantly against the PI3Kα and PI3Kδ isoforms.

Components of the PI3K pathway are frequently mutated in ER+ breast cancer (Network TCGA, 2012; Ellis *et al.,* 2012; Gellert *et al.,* 2016; Ma *et al.,* 2011). *PIK3CA*, which encodes the alpha catalytic subunit of PI3K, is mutated in approximately 40% of ER+ breast cancer. Mutations have also been identified in *PIK3R1*, the regulatory subunit of PI3K (0.4% - 2%), *AKT1* (2% -

4%), and *PTEN* (4%). Although mutations in PTEN is at low frequency in ER+ breast cancer, loss of PTEN protein expression is more common. The incidence of combined PTEN mut/loss was reported to be 13% in luminal A, and 24% in luminal B subtypes in the TCGA breast cancer study (Network TCGA, 2012). Other studies reported loss of PTEN expression in 18% to 48% of ER+ breast cancers (Shoman *et al.,* 2005; Depowski *et al.,* 2001), and significant association between reduced PTEN expression in primary breast cancer and shorter relapse-free survival on adjuvant tamoxifen (Shoman *et al.,* 2005).

Up-regulation of the PI3K pathway signaling has also been observed in acquired endocrine-resistant, long-term estradiol deprived (LTED), ER+ breast cancer cells compared to the parental cell lines (Miller *et al.,* 2010). Similarly, in clinical samples, higher PI3K activity determined by the levels of phosphorylated forms of AKT, mammalian target of rapamycin (mTOR), glycogen synthase kinase 3 (GSK3), ribosomal protein S6 kinase (S6K), and PTEN by reverse phase protein array (RPPA) analysis, was associated with lower ER levels and luminal B status (Creighton *et al.,* 2010). Importantly, dual targeting of ER and the PI3K pathway results in synergistic anti-tumor effect in preclinical studies (Bosch *et al.,* 2015; Crowder *et al.,* 2009; Sanchez *et al.,* 2011), providing the rationale to combine endocrine therapy with PI3K pathway inhibitors in endocrine-resistant ER+ breast cancer. The combination strategy is also supported by the preclinical finding that PI3K pathway inhibition alone induces ER transcriptional activity, potentially mediated by increased FOXO3A-mediated transcription, while ER inhibition activates PI3K pathway activity (Bosch *et al.,* 2015; Miller *et al.,* 2010; Miller *et al.,* 2011). Figure 1 shows that treatment with the PI3K inhibitor copanlisib upregulates the mRNA expression of *ESR1* and ER target genes including *PgR*, *GREB1*, and *IGFBP4*, in the ER positive breast cancer cell line MCF7 (Bosch *et al.,* 2015). Similar results were identified in tumors from patients treated with PI3K inhibitors (Bosch *et al.,* 2015).

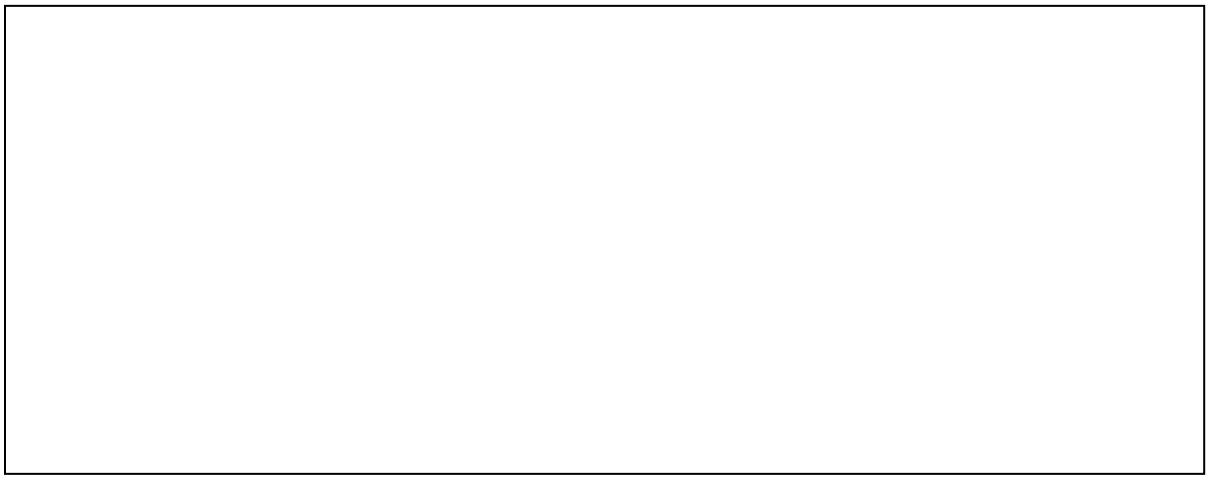


Figure 1: Copanlisib Stimulates ER-Dependent Gene Expression in ER+ MCF-7 Cells. (Bosch *et al.,* 2015). MCF7 cells were treated with vehicle (Ctrl) or BAY 80-6946 at 50 nM for 24 h. mRNA was isolated, and qPCR was performed to detect expression of β-ACTIN, ESR1, PGR, GREB1, and IGFBP4. Two-tailed Student's unpaired t test was performed to compare control *vs.* treated groups. Error bars represent the SEM of two independent experiments, each with three technical replicates.

Initial success in targeting the PI3K/mTOR pathway in the clinic was demonstrated by using rapalogs which indirectly inhibit the activity of mTOR complex 1 (mTOC1), a downstream

component of the PI3K pathway, through its interaction with FKBP12 (Guertin and Sabatini, 2009). Everolimus has received FDA approval for AI-resistant metastatic breast cancer based on the BOLERO-2 trial, which demonstrated an improvement in PFS from 3.2 months to 7.8 months with the addition of everolimus to exemestane (Hazard Ratio, 0.45; 95% CI, 0.38-0.54) (Piccart *et al.,* 2014). However, the median overall survival (OS) was not significantly different at 31 months (Hazard Ratio, 0.89; 95% CI, 0.73-1.10). The lack of OS benefit could potentially be explained by the feedback upregulation of AKT activity upon inhibition of mTORC1 by everolimus (O'Reilly *et al.,* 2006; Wan *et al.,* 2007). Direct kinase inhibitors against both mTORC1 and mTORC2, and inhibitors against AKT and PI3K are now in clinical trials with the hope for a more effective pathway inhibition (Ma, 2015).

Pan-PI3K inhibitors including buparlisib and pictilisib in combination with the selective estrogen receptor downregulator - fulvestrant were evaluated in randomized trials for the treatment of AI resistant breast cancer, but efficacy was limited due to dose limiting toxicities that include rash, hyperglycemia, diarrhea, and elevated transaminases (Krop *et al.,* 2014; Baselga *et al.,* 2015). In the Phase 3 BELLE-2 trial, 1,147 patients were randomly assigned in 1:1 ratio to receive either buparlisib 100 mg/day orally or placebo, in combination with fulvestrant (Baselga *et al.,* 2015). The study met its primary endpoint, demonstrating a PFS of 6.9 months *vs.* 5.0 months (Hazard Ratio, 0.78; $p< 0.001$) with the addition of buparlisib *vs*. placebo. However, up to 25% of patients in the combination arm experienced serious adverse events including hyperglycemia and increase in transaminases. Nonetheless, the study provided the proof of concept that PI3K inhibition could overcome endocrine resistance.

Alpha-specific PI3K inhibitors are likely with improved therapeutic window and are in Phase 3 trials, including alpelisib (SOLAR-1 [NCT02437318]), or taselisib (SANDPIPER [NCT02340221]) (Rugo *et al.,* 2016), but resistance mechanisms through acquiring mutations in *PTEN*, leading to growth dependence on PI3K beta, has been reported in clinical trials of alpha-specific inhibitors (Juric *et al.,* 2015). Although tumor cells carrying *PIK3CA* mutations are highly dependent on p110α for survival (Crowder *et al.,* 2009; Juric *et al.,* 2012), there is considerable evidence indicating that p110β is important in mediating PI3K signaling and AKT phosphorylation in PTEN-deficient cancers and p110β-selective inhibitors reduce pAKT and inhibit growth in both cell-based and *in vivo* studies of breast and other cancer cell lines (Jia *et al.,* 2008; Ni *et al.,* 2012; Torbett *et al,* 2008; Wee *et al.,* 2008). Recent data also indicates that combined targeting of both p110α and p110β most effectively induces apoptosis and tumor regression of PTEN-deficient ER+ breast cancer cells both *in vitro* and *in vivo* (Hosford *et al.,* 2017). Therefore, a pan-PI3K inhibitor may have therapeutic advantage if effective and safe administration could be achieved.

Recent data demonstrated that short-term, complete PI3K inhibition blocks cell growth more effectively than chronic, incomplete inhibition *in vitro* (Yang *et al.,* 2016; Toska *et al.,* 2016). Copanlisib, which is a potent pan-PI3K inhibitor administered weekly (3 of 4 weeks), has demonstrated a favorable safety profile and preliminary activity in ER+ breast cancer, making it an attractive agent (discussed below).

2.2 CTEP IND Agent

2.2.1 Copanlisib

Copanlisib (BAY 80-6946) was approved in the US on September 14, 2017 for the treatment of adult patients with relapsed follicular lymphoma who have received 2 prior systemic therapies. Copanlisib has an ongoing Phase 3 program in indolent non-Hodgkin's lymphoma (NHL) and is being clinically evaluated in multiple investigator-initiated Phase 1 and Phase 2 studies of solid tumors. However, as of November 2023, Bayer has decided to voluntarily withdraw the NDA for copanlisib. The FDA required clinical benefit to be confirmed by further studies when the original approval for copanlisib was issued in 2017, and the CHRONOS-4 study failed to meet the primary endpoint of progression-free survival benefit in comparison with the control arm in patients with relapsed follicular lymphoma.

Copanlisib is a novel small-molecule pan-class I PI3K inhibitor with exceptional inhibitory potency against δ and α PI3K isoforms (Investigator's Brochure, 2018). Copanlisib is an active ingredient (free base) of copanlisib dihydrochloride (BAY 84-1236), which is intended for an IV administration in humans. The copanlisib hydrochloride product for clinical use is formulated as a lyophilized product for reconstitution in saline to be administered *via* IV. Copanlisib dihydrochloride product is supplied in three formulation strengths: 20 mg, 60 mg, or 80 mg (free base) in a 6 mL injection vial for reconstitution with 2 mL, 4.4 mL, or 4 mL of saline, respectively, to produce copanlisib solution for injection at concentration of 10 mg/mL, 15 mg/ml, or 20 mg/mL, respectively.

2.2.1.1 Nonclinical Studies

A majority of the nonclinical data were produced using the copanlisib free-base.

Mechanism of Action

Copanlisib is a stronger inhibitor of PI3Kα and PI3Kδ than of PI3Kβ or PI3Kγ, as demonstrated by copanlisib half-maximal inhibitory concentrations (IC_{50}) that confer 50% of the maximum inhibition of PI3Kα PI3Kδ, PI3Kβ and PI3Kγ (IC_{50} of 0.5, 0.7, 3.7, 6.4 nmol/L, respectively) (Liu *et al.,* 2013). Compared to the PI3K isoforms, copanlisib was a much weaker inhibitor of mTOR (IC_{50} = 45 nmol/L). In a panel of ~220 kinases, copanlisib (1 mcmol/L) failed to achieve a 50% inhibition of any kinase other than PI3K isoforms and mTOR. In tumor cell lines with hyperactive PI3K signaling, copanlisib antitumor activity was paralleled by a robust decrease in basal levels of phosphorylated AKT, both at serine 473 (AKTpS473) and threonine 308 (AKTpT308), and by increases in caspase-9 levels, which is suggestive of induction of apoptosis.

Nonclinical *In vitro* Antitumor Activity

Copanlisib potently inhibited tumor cell proliferation (IC_{50} of 1-760 nmol/L) in human tumor cell lines of various histologies, including breast, ovary, endometrial, prostate, colon, lung, liver, brain, kidney, melanoma, pancreas, and hematological tumors (Figure 2), many of which exhibit constitutively activated PI3K signaling resulting from somatic mutations in PIK3CA and PTEN (Liu *et al.,* 2013).

To further analyze a relationship between molecular features and copanlisib activity, copanlisib was tested against 24 breast cancer cell lines with known *PI3KCA* gene mutation, *PTEN* gene

mutation or expression, and HER2 expression status (Liu *et al.,* 2013). Antiproliferation IC_{50}s of copanlisib were ~40-fold lower in cells with activating mutations in *PIK3CA* (IC_{50} = 19 nmol/L; n = 9) or HER2-positive cells (IC_{50} = 17 nmol/L; n = 7) than for cells with *PIK3CA* wild-type (WT) and HER2-negative status (average IC_{50} = 774 nmol/L; n = 11). However, no clear correlation has been found between sensitivity of cells to copanlisib and the loss of PTEN. The IC_{50}s for T47D (mutant *PIK3CA*), ZR-75-1 (PTEN null), or MCF7 (mutant *PIK3CA*) were 6, 24, and 27 nmol/L, respectively. Of note, copanlisib efficiently inhibited cell proliferation of breast cancer cell lines that are resistant to HER2 inhibitors (trastuzumab or lapatinib) such as BT20, BT474, and ZR-75-1 HER2 positive cell lines (Liu *et al.,* 2013).

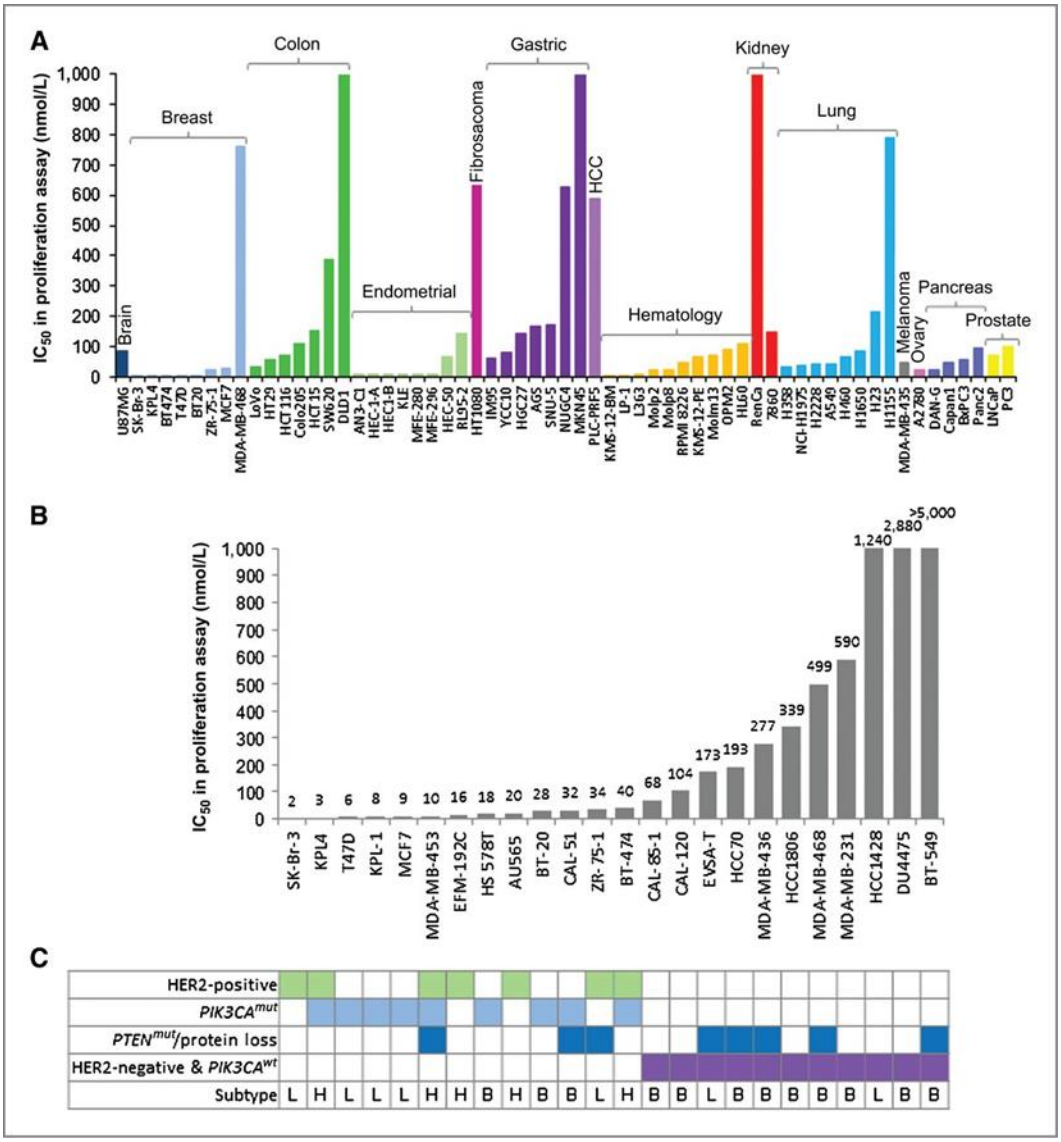


Figure 1: Antiproliferative activity against a panel of human tumor cell lines. (Liu *et al.,* 2013).
Panel A: Cell lines of various tumor histologies; Panel B: Breast cancer cell lines; Panel C: Molecular and histological characterization of breast cancer cell lines shown in panel B.
Legend: B: basal–like breast cancer cell lines; L: luminal-type breast cancer cell lines; H: HER2-positive breast cancer cell lines

Copanlisib was also tested against a panel of 32 human hematological cancer cell lines (Investigator's Brochure, 2018). Copanlisib was a more potent inhibitor than idelalisib, the PI3Kδ-selective inhibitor: idelalisib IC_{50}s were 1.4-fold to several thousand-fold higher than copanlisib IC_{50}s against these cell lines. Copanlisib IC_{50}s were <100 nmol/L for 14 cell lines, including acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), NHL, and myeloma. Some of the strongest responses to copanlisib (IC_{50} <10 nmol/L) occurred in AML (Kasumi-1, IC_{50} = 1.1 nmol/L), the Burkitt's lymphoma subtype of NHL (NAMALWA, IC_{50} = 1.7 nmol/L), the DLBCL subtype of NHL (Pfeiffer, IC_{50} = 0.8 nmol/L), and myeloma (MM-1R, IC_{50} = 1.0 nmol/L; and NCI-H929, IC_{50} = 2.7 and 2.2 nmol/L in 2 different experiments). Copanlisib was also more potent inhibitor against an aggressive NHL type such as diffuse large B-cell lymphoma (DLBCL) than idelalisib or a Bruton's tyrosine kinase (BTK) inhibitor - ibrutinib (Figure 3).

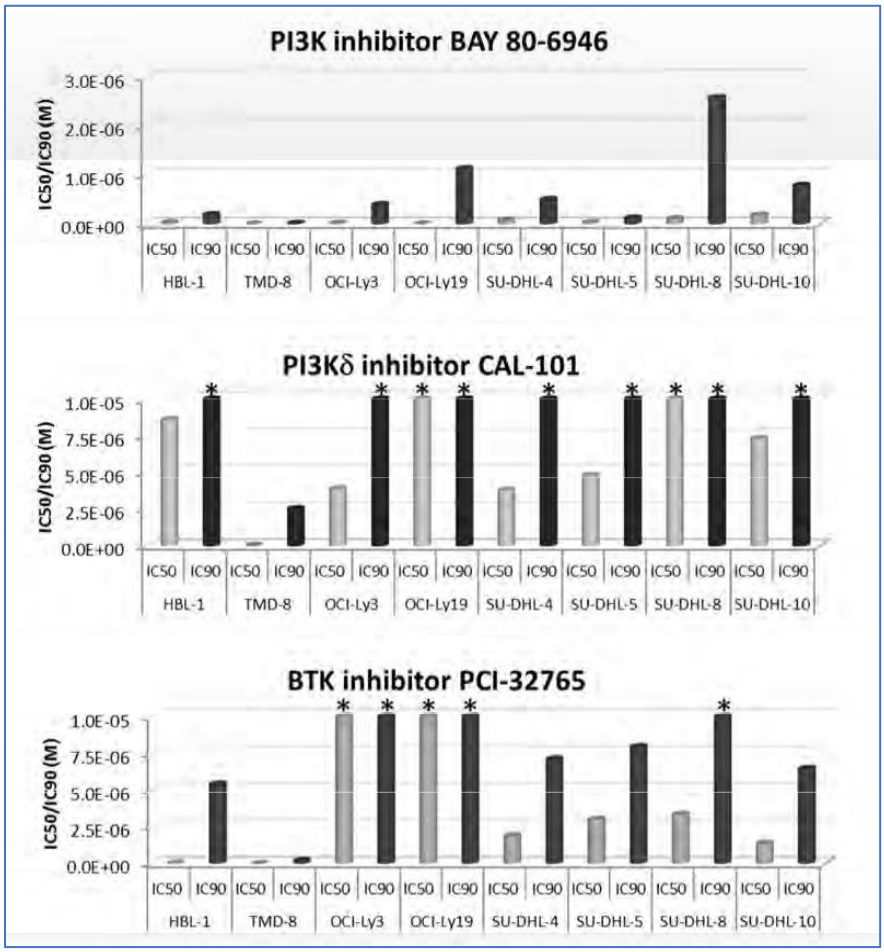


Figure 2: Antiproliferative effects of copanlisib (BAY 80-6946), idelalisib (CAL-101), and ibrutinib (PCI-32765) against DLBCL cell lines. (Investigator's Brochure, 2018). Legend: IC50: a drug concentration causing 50% inhibition of cell proliferation

Concurrent treatment with copanlisib and ibrutinib resulted in synergistic effects of the two inhibitors against ibrutinib-sensitive cell lines but antagonistic effects in ibrutinib-resistant cell lines.

In vivo Antitumor Activity

Copanlisib demonstrated antitumor activity *in vivo* in a variety of xenograft models of tumors exhibiting an activated PI3K pathway (Liu *et al.,* 2013). The drug displayed robust antitumor activity in the nude rat xenograft model of the KPL4 breast tumor cell line, which is an estrogen-independent HER2-positive breast carcinoma that carries a somatic *PIK3CA* mutation. Copanlisib was administered on Day 14 post-implant at doses ranging from 0.5-6 mg/kg IV every second day (Q2D) for a total of five doses. On Day 25, 3 days after the last dose, tumor growth inhibition (TGI) rates of 77%-100% were achieved (Figure 4, Panel A). Complete tumor regressions were observed in 100% of animals receiving dose of 3 or 6 mg/kg, and all rats remained tumor free at the termination of the study on Day 73. Delays in tumor growth of >25 days were observed in the 0.5- and 1- mg/kg groups. Copanlisib at 3 and 6 mg/kg IV Q2D x 5 doses resulted in the TGI of 75% and 88%, respectively, of the HCT-116 (colon tumor with mutant *PIK3CA* and mutant *KRAS*) xenograft rat model (Figure 4, Panel B). Copanlisib was also effective in the nude mouse patient-tumor xenografts of Lu7860 (erlotinib-resistant non-small cell lung cancer [NSCLC]) and MAXF1398 (luminal breast tumor). Copanlisib 14 mg/kg administered twice daily (BID) Q2D for 10 days led to an 88% TGI in the NSCLC model (Figure 4, Panel C) and a 71% TGI in the breast cancer model (Figure 4, Panel D).

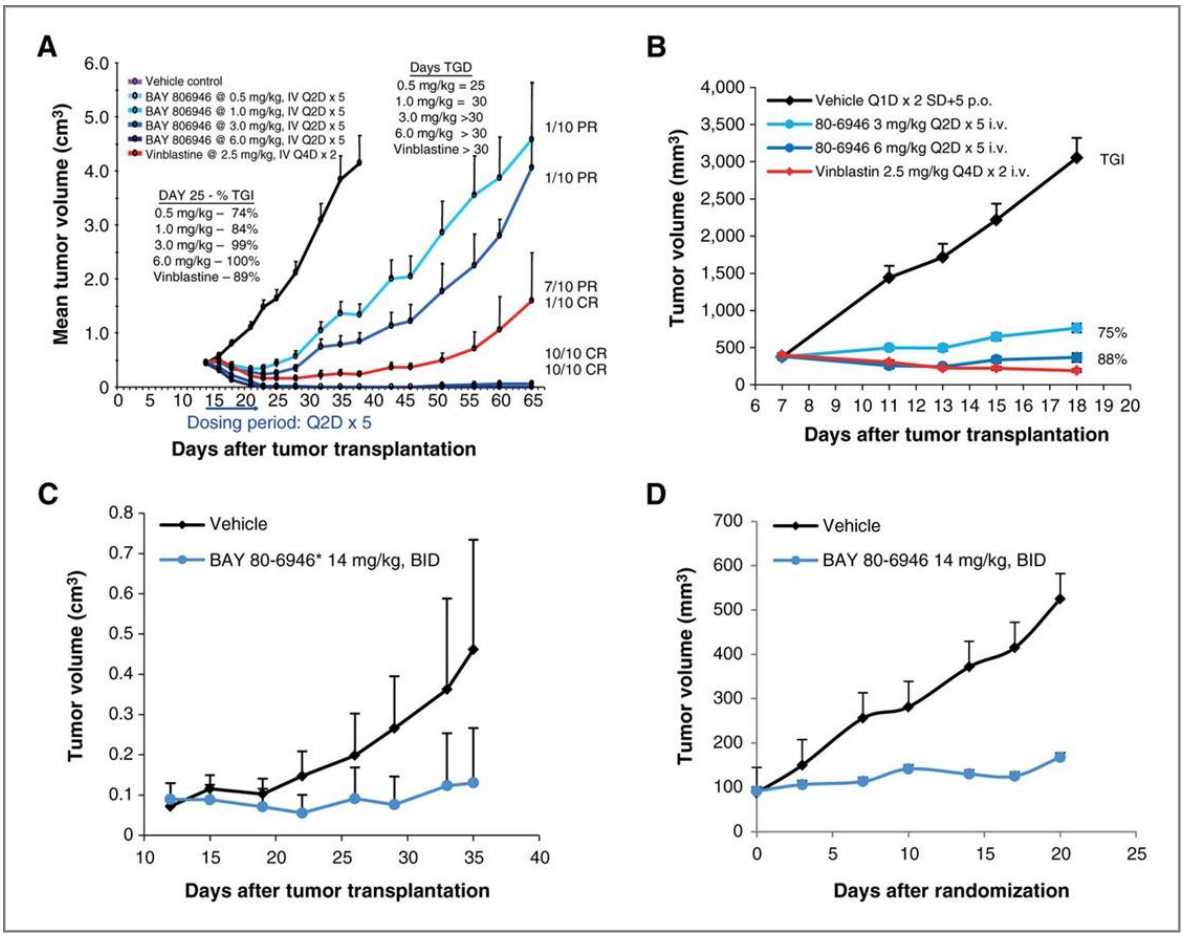


Figure 3: Activity of copanlisib (BAY 80-6946) in xenograft models using Q2D treatment schedule. (Liu *et al.,* 2013). Panel A: KPL4 breast cancer xenografts in nude rats (n=10/group). Panel B: HCT116 colon cancer xenografts in nude rats (n=10/group). Panel C: Lu7860 erlotinib-resistant, patient-derived NSCLC xenografts in

nude mice (n=5/group). Panel D: MAXF1398 patient-derived luminal breast cancer xenografts in nude mice (n=6/group). Legend: Q2D: every 2 days; BID: twice a day; IV: intravenously; TGD: tumor growth delay; PR: partial response; CR: complete response

Copanlisib was also evaluated on a weekly schedule. Two doses of 9 mg/kg on day 1/week caused 64% TGI in the HCT-116 xenograft model, which was equivalent to the effect of copanlisib given at 6 mg/kg Q2D for 10 doses (Figure 5A).

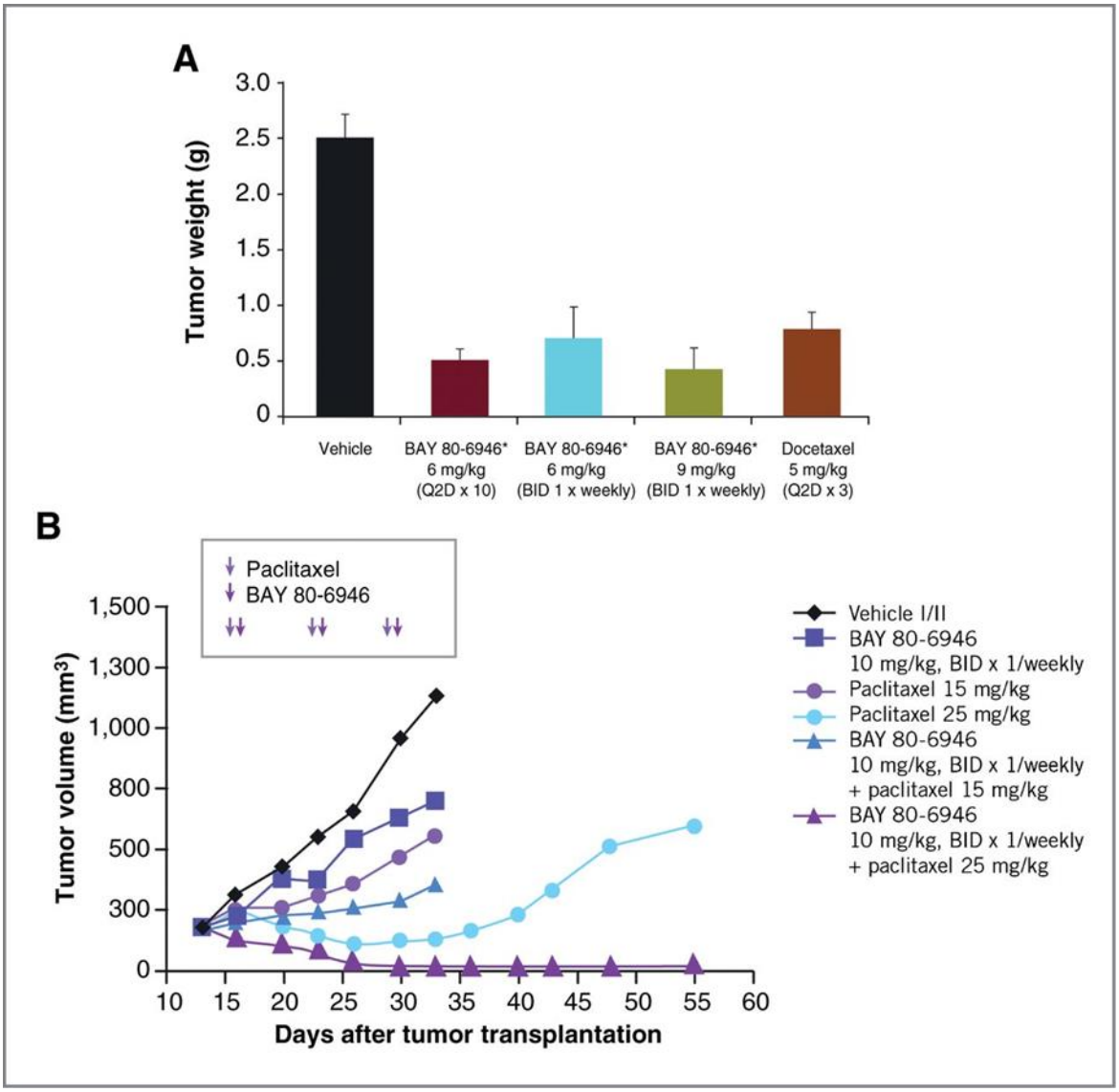


Figure 4: Activity of copanlisib (BAY 80-6946) in xenograft models using a BID once-weekly schedule. (Liu *et al.,* 2013). Copanlisib was formulated in 5% mannitol vehicle. Panel A: HCT-116 colon cancer xenografts in nude rats (8 rats/group). Panel B: Lu7343 patient-derived NSCLC xenografts in nude mice (10 mice/group). Legend: BID: twice a day; Q2D: every 2 days.

In vivo antitumor activity of copanlisib was also tested in combination with cytotoxic agent paclitaxel in the mutant *PIK3CA* squamous cell NSCLC patient-derived Lu7343 xenograft mouse model (Liu *et al.,* 2013). The drug combination was more potent in inhibiting tumor growth than either drug alone (Figure 5, Panel B). Copanlisib was well tolerated at all doses and

schedules tested in these studies without producing any lethality. The maximum tolerated dose (MTD) in rats was 6 mg/kg Q2D. A maximum mean body weight loss of 6%-10% occurred during the first few days at this dose and then consistently returned to the normal range by the end of the dosing period. The MTD in mice was more than 14 mg/kg Q2D.

Nonclinical Pharmacokinetics and Pharmacodynamics

Copanlisib plasma-free fraction across species was as follows: 35% in rats, 14% in mice, 33% in dogs, and 16% in humans (Liu *et al.,* 2013). The PK profile of copanlisib was evaluated following single and multiple IV doses in nude rats. Single-dosed copanlisib exhibited a very large volume of distribution (V_d = 32 L/kg), high plasma clearance (3.95 L/kg/h) and a long half-life ($t_{1/2}$ = 6.0 h). The copanlisib PK parameters at repeat dosing (Q2D x 5 doses), were similar those from single-dosing studies and suggested no drug accumulation in plasma. Copanlisib had a higher clearance (16 L/kg/h), shorter $t_{1/2}$ (0.7 h) and smaller V_d (12.9 L/kg) in mice than rats. A single bolus IV dose of copanlisib (6 mg/kg) in the H460 NSCLC xenograft rat model produced 100 times higher concentration of the drug in tumor tissue than in plasma at 48 h post-dosing; the drug clearance from the tumor was slower than from plasma. The pharmacodynamics analysis showed 90% inhibition of AKT pS473 at 24 h post-dosing compared to the control animals, and the AKT pS473 level remained suppressed up to 72 h. In addition, 65% and 75% reductions in Ki-67 and phospho-histone H3 levels, respectively, were observed at 24 h in the copanlisib group compared to the control group, suggesting copanlisib-induced G0 cell-cycle arrest. Copanlisib also demonstrated sustained inhibition (over 24 - 48 h) of ^{18}F-deoxyglucose (FDG) uptake in tumor. These preclinical data suggested that high and prolonged copanlisib tumor exposures can be reached *in vivo*, and there was a correlation between copanlisib exposure and inhibition of the PI3K pathway in the tumor.

In the rat tumor xenograft model studies, the efficacious exposure of copanlisib was estimated as the area under the concentration-time curve (AUC) for the unbound/free drug (AUC_u) in plasma of 370 mcg•h/L based on weekly dosing (Investigator's Brochure, 2018).

Summary of Nonclinical Pharmacology and Metabolism

Copanlisib is primarily metabolized by the cytochrome P450 (CYP)3A4 with a minor contribution of CYP1A1 (Investigator's Brochure, 2018). Copanlisib is a weak substrate of permeability glycoprotein (P gp) and of breast cancer resistance protein (BCRP). There is a low risk for clinically relevant PK drug drug interactions (DDI) through inhibition or induction of CYP enzymes, inhibition of uridine diphosphate glucuronosyltransferase (UGT) enzymes and inhibition of dihydropyrimidine dehydrogenase (DPD) by copanlisib. Copanlisib also inhibited P gp- and BCRP mediated transport *in vitro*. Furthermore, copanlisib was a strong inhibitor of the drug transporter multidrug and toxin extrusion protein 2 (MATE2K).

Summary of Nonclinical Safety

IV infusion of copanlisib caused vasoconstriction, enhanced insulin and glucose levels, impaired glucose tolerance, reduced gastrointestinal (GI) motility, increased renal volume and electrolyte excretion, and central nervous system (CNS) depressant effects in nonclinical species (Investigator's Brochure, 2018). A majority of these effects could be explained by inhibition of PI3K-dependent signaling, and they occurred at or slightly above the plasma concentrations shown to be efficacious in tumor xenograft rat models (maximum concentration [C_{max}]=30-80

mcg/L; C_{max} of unbound fraction [$C_{max,u}$] 11-28 mcg/L). The CNS depressant effects occurred at high plasma concentrations and are considered secondary to hyperglycemia. At pharmacodynamically relevant concentrations, copanlisib does not interfere with cardiac repolarization *in vitro* or *in vivo*.

Based on the findings from repeat-dose toxicity studies in nonclinical species, copanlisib is expected to adversely affect male and female reproduction. Developmental and reproductive toxicity of PI3K inhibitors is known. Maternal toxicity of increasing severity, severe post-implantation loss, and developmental toxicity, including teratogenicity, were seen in the rat starting at low doses. Copanlisib was not genotoxic *in vitro* or *in vivo*. There is no evidence that copanlisib has phototoxic potential. Significant toxicities were observed in animals at doses achieving plasma concentrations observed in humans.

2.2.1.2 Effects in humans

The First-in-Human Copanlisib Study

In the first-in human (FIH) Phase 1b trial in patients with advanced and/or refractory malignancies, of 57 patients (51 non-diabetic) treated, 17 took part in the dose-escalation phase with copanlisib (0.1-1.2 mg/kg) administered IV weekly for 3 weeks of a 4-week cycle (Patnaik *et al.,* 2016). The copanlisib MTD was 0.8 mg/kg IV (1 h) weekly for 3 weeks on a 28-day cycle. An additional 34 patients were treated in the MTD expansion cohorts: the solid tumor cohort (n = 25), NHL cohort (n = 9; 6 patients with follicular lymphoma [FL] and 3 patients with DLBCL). Finally, 6 patients with diabetes mellitus were treated with copanlisib at 0.4 mg/kg weekly x 3 weeks.

Clinical Safety

The most common (≥20%) copanlisib-related AEs (regardless of grade) included hyperglycemia (63%), nausea (37%), and hypertension (21%). The most common drug-related grade 3 AEs were hyperglycemia (30%), hypertension (14%), and rash (7%) (Patnaik *et al.,* 2016). Grade 3+ diarrhea occurred in one patient. Two patients (4%) experienced three drug-related grade 4 AEs: a dose-limiting hyperglycemia and increased aspartate aminotransferase (AST) in one patient and elevated serum amylase in another patient. Overall, serious AEs (SAEs) with positive association to copanlisib were observed in six patients (11%): grade 3 left ventricular systolic dysfunction (LVSD) which was a dose-limiting toxicity (DLT), chest pain, hypertension, and hyperglycemia (in one patient each), and pneumonitis (in two patients). None of seven grade 5 AEs (12%) were considered drug-related. Dose modifications (delays, interruptions, and reductions) caused by drug-related AEs occurred in 14 patients (25%). Four patients discontinued treatment due to toxicity. One drug-related AE (dose-limiting LVSD) led to permanent discontinuation of treatment. No patient discontinued the study because of hyperglycemia.

Hyperglycemia was transient, with a glucose level peaking at 5 – 8 h after copanlisib infusion on Cycle 1 Day 1 and declining to baseline by the time of the next infusion. Sixty-five percent of non-diabetic patients (33/51) received at least one dose of short-acting insulin to manage high blood glucose (>200 mg/dL). There was no trend for increased pre-dose glucose values over time, and no patients developed diabetic ketoacidosis during the study. Hemoglobin A1c

(HbA1c) levels changed only modestly over the course of copanlisib treatment. Post-infusion increases in blood pressure peaked at 1–2 h and resolved within 24 h post-infusion.

A similar transient pattern of elevated blood glucose post-infusion was seen for the cohort of six diabetic patients treated with 0.4 mg/kg copanlisib, all of whom received insulin following the first copanlisib infusion. The AE profile in the diabetic cohort of patients was similar to that in non-diabetic patients, with a total of four drug-related grade 3 AEs observed in three patients: hypertension in two patients, and hyperglycemia and rash/desquamation in one patient each.

Pharmacokinetics/Pharmacodynamics

Copanlisib plasma C_{max} was typically reached between 0.5 and 1 h (t_{max}) following the infusion [85]. Copanlisib exposure, expressed either as C_{max} or AUC between 0-25 h ($AUC_{0–25h}$), increased proportionally with dose between 0.1 and 1.2 mg/kg, and exhibited a moderate to high inter-patient variability. The terminal $t_{1/2}$ was 38.2 h and no accumulation was observed after once-weekly administration. The trough levels of copanlisib on Cycle 1 Day 8 were 4.92 mcg/L (range, 2.74–23.0 mcg/L).

A pharmacodynamic effect event was defined as an increase in plasma glucose level of ≥50 mg/dL from baseline within 2 h after the completion of the copanlisib infusion, and / or an increase in plasma insulin level to greater than two times the baseline value (Investigator's Brochure, 2018). At the MTD (0.8 mg/kg), 100% of patients showed the pre-defined increases in glucose and insulin. Overall, 53/57 (93%) patients experienced a predefined pharmacodynamics effect of an increased glucose plasma level following the first copanlisib infusion. Increases in glucose levels strongly correlated with copanlisib exposure (AUC_{0-25h}) (Patnaik *et al.,* 2016). A weak correlation between exposure and change in tumor FDG uptake (*via* FDG-PET) from baseline to Cycle 1 Day 3 or Day 4 was seen in 19/21 patients evaluated, with >25% reduction in the FDG uptake observed in the tumor of 7 patients (33%).

Antitumor Activity/Response

Among patients with solid tumors (n = 48), clinical responses were observed only in patients treated at the copanlisib MTD (Patnaik *et al.,* 2016). The responses were: 1 CR (2%) in a patient with endometrial cancer, 2 PRs (4%) in patients with breast cancer, 15 cases of SD (31%), and 15 cases of progressive disease (PD) (31%) according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. In comparison, by clinical assessment, 7 patients (15%) had PD, and 8 patients (17%) were not assessed. Overall CBR was 38%.

The CR responder had endometrial cancer with *PIK3CA* and *PTEN* mutations and complete PTEN loss. Of the two breast cancer patients who had PR, both had tumor positive for ER and progesterone receptor, one was negative and the other positive for HER2 and one had mutant and the other WT *PIK3CA* WT; *PTEN* status was unavailable in both. No clear relationship was found between *PI3KCA* mutational status and disease control (p =1.0).

2.2.1.3 Copanlisib Reference Safety Information

The recommended dose and administration of copanlisib

Based on the FIH company-sponsored study, the MTD of copanlisib in non-diabetic patients

with solid malignancies was 0.8 mg/kg administered IV over 1 h once weekly for 3 weeks (Days 1, 8, and 15) on a 28-day cycle (Investigator's Brochure, 2018).

A preliminary population PK analysis revealed no impact of either body weight, body surface area (BSA), or other body size-related factors on the clearance of copanlisib and thus a flat-dose regimen of copanlisib has been recommended. In addition, age, gender and race were shown to have no effect on copanlisib exposure.

Based on these data, the RP2D of copanlisib monotherapy is 60 mg given over a 1-h IV infusion once a week for 3 weeks (Days 1, 8, and 15) every 4 weeks. A dose reduction to 45 mg for toxicities have been allowed.

Drug-drug interactions

In vitro studies in human hepatocytes indicate that CYP3A4 is a major metabolizer (>90%) while CYP1A1 contributes <10% to metabolism of copanlisib (Investigator's Brochure, 2018). Therefore, the concomitant use of copanlisib with a strong CYP3A4 inhibitor (*e.g.,* ketoconazole, itraconazole, clarithromycin, ritonavir, indinavir, nelfinavir, and saquinavir) may markedly increase copanlisib plasma concentration and concomitant administration of copanlisib with a strong CYP3A4 inducer (*e.g.,* rifampin, phenytoin, carbamazepine, phenobarbital, and herbal preparations containing St. John's Wort) may result in a marked decrease in copanlisib plasma concentration.

Copanlisib metabolism is predominantly mediated by CYP3A4 (>90%) and to a minor extent by CYP1A1 (<10%). Itraconazole, a strong CYP3A4 inhibitor and a P gp and BCRP transporter inhibitor, increased copanlisib (60 mg) AUC by 1.53-fold with no effect on C_{max} (1.03–fold). If concomitant use with strong CYP3A inhibitors cannot be avoided, a dosage reduction to 45 mg is recommended. Rifampin, a strong CYP3A4 inhibitor and a P-gp transporter inhibitor, decreased the AUC of copanlisib (60 mg) by 63% with minimal effect on C_{max} (15%) and should be avoided.

Clinical pharmacology summary

Copanlisib plasma exposure (C_{max} and AUC) increased in a dose-proportional manner over an absolute dose range of 5 to 93 mg (0.08 to 1.55 times the approved recommended dose of 60 mg) (Investigator's Brochure, 2018). There is no time-dependency and no accumulation in the PK of copanlisib. The geometric mean terminal elimination half-life (CV%) of copanlisib was 39.1 h (40.8%) based on the pooled analysis of 3 Phase 1 studies (12871, 15205 and 16270). The geometric mean clearance (CV%) was 17.9 L/hr (45.6%). Copanlisib is eliminated predominantly *via* feces (64% of the administrative radioactive dose mean recovery with 30% unchanged copanlisib) compared to urine (22% mean recovery with 15% unchanged copanlisib). Copanlisib is excreted as unchanged compound and metabolites (about 50:50). Copanlisib metabolism is predominantly mediated by CYP3A4 (>90%) and to a minor extent by CYP1A1 (<10%). Itraconazole, a strong CYP3A4 inhibitor and a P gp and BCRP transporter inhibitor, increased copanlisib (60 mg) AUC by 1.53-fold with no effect on C_{max} (1.03–fold). If concomitant use with strong CYP3A inhibitors cannot be avoided, a dosage reduction to 45 mg is recommended. Rifampin, a strong CYP3A4 inhibitor and a P-gp transporter inhibitor, decreased the AUC of copanlisib (60 mg) by 63% with minimal effect on C_{max} (15%) and should

be avoided. Copanlisib has low potential for DDI as a perpetrator to influence the PK of other drugs by inhibition or induction of metabolizing enzymes (CYPs, UGT) and transport proteins, except MATE2-K transporter at clinically relevant concentrations. Copanlisib is a substrate of P-gp and BCRP and not a substrate for the efflux transporters MATE1 and MATE2K or the uptake transporters OCT, OAT and OATP. Population PK analyses suggest that body weight, age (20 to 90 years), gender, race (White, Asian, Hispanic and Black), smoking status, body weight (41 to 130 kg), mild hepatic impairment and mild to moderate renal impairment had no clinically significant effect on the PK of copanlisib. No dose adjustment is necessary based on these specific populations. Preliminary analysis of central tendency and exposure-response analyses suggest that copanlisib does not prolong QT/QTc interval.

Further details can be found in the latest available version of the investigator's brochure, which contains comprehensive information on the study drug and also the prescription drug labeling.

Pregnancy and lactation
Due to a mechanism of action as a PI3K inhibitor, adverse effects on development and reproduction are expected for copanlisib (Investigator's Brochure, 2018). Nonclinical repeat-dose toxicity studies demonstrated adverse effects of copanlisib on male and female reproduction. Maternal toxicity of increasing severity, severe post-implantation loss, and developmental toxicity, including teratogenicity were seen in a rat pilot developmental toxicity study beginning at a low dose. In the rat study with ^{14}C-labeled copanlisib, radioactivity was secreted into the milk of lactating animals although to a low extent (1.7% of dose). No data are available on the distribution of copanlisib to human milk. Therefore, unless potential benefits to patients outweigh unknown risks, women who are pregnant or nursing and children should be excluded from the clinical studies of copanlisib. In addition, women of child-bearing potential or female partners of male patients will be required to use an adequately effective barrier method of birth control.

Special safety warnings and precautions
Nonclinical studies suggest, and clinical studies confirm, blood glucose increases that persist for approximately 1-3 days after copanlisib administration (Investigator's Brochure, 2018). Blood or serum glucose, serum and urine ketones, and electrolytes should be monitored while on copanlisib treatment.

Standard cardiovascular parameters, including pulse and blood pressure (BP) should be monitored because of hypertension (during the first 3 h after start of infusion) that has been observed.

Respiratory infections (including pneumonia, *Pneumocystis jirovecii* pneumonia, cryptococcosis and bronchopulmonary aspergillosis) have been observed in studies with monotherapy and combination therapies. Some of these infections may have a life-threatening or a fatal outcome. Cases of pneumonitis observed in studies with monotherapy and in combination therapies were generally ≤grade 3 in severity and responded well to corticosteroid treatment; occasional events with life-threatening or fatal outcome have been observed. Since the early symptoms of pneumonitis overlap with those of a respiratory infection, monitoring of patients for typical clinical symptoms like cough, dyspnea or fever and further evaluation for respiratory infections

is recommended. Patients suspected of having a respiratory infection or noninfectious pneumonitis should be promptly treated with appropriate antimicrobial agents and/or corticosteroids as indicated.

2.3 Other Agents

2.3.1 Abemaciclib

2.3.1.1 Mechanisms of action and approved indications for advanced hormone receptor positive breast cancer

Abemaciclib is a highly selective small-molecule inhibitor of CDK4 and CDK6 activity and is 14-fold more potent against CDK4/cyclin D1 than CDK6/cyclin D3 (Torres-Guzman *et al.,* 2017; Gelbert *et al.,* 2014). Abemaciclib is administered twice daily on a continuous schedule. In preclinical models, continuous *in vitro* exposure leads to senescence and apoptosis (Torres-Guzman *et al.,* 2017; Gelbert *et al.,* 2014). Abemaciclib is FDA approved in combination with fulvestrant for the treatment of women advanced or metastatic HR+ HER2- breast cancer. It is also approved as a monotherapy for the treatment of adult patients with advanced or metastatic HR+ HER2- breast cancer with disease progression following endocrine therapy and prior chemotherapy in the metastatic setting (Abemaciclib package insert, 2018).

2.3.1.2 Pharmacokinetics of Abemaciclib

The pharmacokinetics of abemaciclib were characterized in patients with solid tumors, including metastatic breast cancer, and in healthy subjects (Abemaciclib package insert, 2018). Following single and repeated twice daily dosing of 50 mg (0.3 times the approved recommended 150 mg dosage) to 200 mg of abemaciclib, the increase in AUC and C_{max} was approximately dose proportional. Steady state was achieved within 5 days following repeated twice daily dosing, and the estimated geometric mean accumulation ratio was 2.3 (50% CV) and 3.2 (59% CV) based on C_{max} and AUC, respectively.

- Absorption

The absolute bioavailability of abemaciclib after a single oral dose of 200 mg is 45% (19% CV). The median T_{max} of abemaciclib is 8.0 h (range: 4.1-24.0 h).

- Effect of Food

A high-fat, high-calorie meal (approximately 800 to 1000 calories with 150 calories from protein, 250 calories from carbohydrate, and 500 to 600 calories from fat) administered to healthy subjects increased the AUC of abemaciclib plus its active metabolites by 9% and increased C_{max} by 26%.

- Distribution

In vitro, abemaciclib was bound to human plasma proteins, serum albumin, and alpha-1-acid

glycoprotein in a concentration independent manner from 152 ng/mL to 5066 ng/mL. In a clinical study, the mean (standard deviation, SD) bound fraction was 96.3% (1.1) for abemaciclib, 93.4% (1.3) for M2, 96.8% (0.8) for M18, and 97.8% (0.6) for M20. The geometric mean systemic volume of distribution is approximately 690.3 L (49% CV).

In patients with advanced cancer, including breast cancer, concentrations of abemaciclib and its active metabolites N-desethylabemaciclib (M2) and hydroxyabemaciclib (M20) in cerebrospinal fluid are comparable to unbound plasma concentrations.

- Elimination

The geometric mean hepatic clearance (CL) of abemaciclib in patients was 26.0 L/h (51% CV), and the mean plasma elimination half-life for abemaciclib in patients was 18.3 h (72% CV).

- Metabolism

Hepatic metabolism is the main route of clearance for abemaciclib. Abemaciclib is metabolized to several metabolites primarily by CYP3A4, with formation of M2, representing the major metabolism pathway. Additional metabolites include M20, hydroxy-N-desethylabemaciclib (M18), and an oxidative metabolite (M1). M2, M18, and M20 are equipotent to abemaciclib and their AUCs accounted for 25%, 13%, and 26% of the total circulating analytes in plasma, respectively.

- Excretion

After a single 150 mg oral dose of radiolabeled abemaciclib, approximately 81% of the dose was recovered in feces and approximately 3% recovered in urine. The majority of the dose eliminated in feces was metabolites.

- Specific Populations

Age, Gender, and Body Weight
Based on a population PK analysis in patients with cancer, age (range 24-91 years), gender (134 males and 856 females), and body weight (range 36-175 kg) had no effect on the exposure of abemaciclib.

Patients with Renal Impairment
In a population PK analysis of 990 individuals, in which 381 individuals had mild renal impairment (60 mL/min ≤ Creatinine Clearance (CLcr) <90 mL/min) and 126 individuals had moderate renal impairment (30 mL/min ≤ CLcr <60 mL/min), mild and moderate renal impairment had no effect on the exposure of abemaciclib. The effect of severe renal impairment (CLcr <30 mL/min) on pharmacokinetics of abemaciclib is unknown.

Patients with Hepatic Impairment
Following a single 200 mg oral dose of abemaciclib, the relative potency adjusted unbound $AUC_{0\text{-}INF}$ of abemaciclib plus its active metabolites (M2, M18, M20) in plasma increased 1.2-

fold in subjects with mild hepatic impairment (Child-Pugh A, n=9), 1.1-fold in subjects with moderate hepatic impairment (Child-Pugh B, n=10), and 2.4-fold in subjects with severe hepatic impairment (Child-Pugh C, n=6) relative to subjects with normal hepatic function (n=10). In subjects with severe hepatic impairment, the mean plasma elimination half-life of abemaciclib increased to 55 h compared to 24 h in subjects with normal hepatic function.

- Drug Interaction Studies

Effects of Other Drugs on Abemaciclib

Strong CYP3A Inhibitors
Ketoconazole (a strong CYP3A inhibitor) is predicted to increase the AUC of abemaciclib by up to 16-fold.

Itraconazole (a strong CYP3A inhibitor) is predicted to increase the relative potency adjusted unbound AUC of abemaciclib plus its active metabolites (M2, M18 and M20) by 2.2-fold. Coadministration of 500 mg twice daily doses of clarithromycin (a strong CYP3A inhibitor) with a single 50 mg dose of abemaciclib (0.3 times the approved recommended 150 mg dosage) increased the relative potency adjusted unbound $AUC_{0\text{-}INF}$ of abemaciclib plus its active metabolites (M2, M18, and M20) by 1.7-fold *vs*. abemaciclib alone in cancer patients.

Moderate CYP3A Inhibitors
Diltiazem and verapamil (moderate CYP3A inhibitors) are predicted to increase the relative potency adjusted unbound AUC of abemaciclib plus its active metabolites (M2, M18, and M20) by 1.7-fold and 1.3-fold, respectively.

Strong CYP3A Inducers
Coadministration of 600 mg daily doses of rifampin (a strong CYP3A inducer) with a single 200 mg dose of VERZENIO decreased the relative potency adjusted unbound $AUC_{0\text{-}INF}$ of abemaciclib plus its active metabolites (M2, M18, and M20) by 67% in healthy subjects.

Moderate CYP3A Inducers
The effect of moderate CYP3A inducers on the PK of abemaciclib is unknown.

Loperamide: Co-administration of a single 8 mg dose of loperamide with a single 400 mg dose of abemaciclib in healthy subjects increased the relative potency adjusted unbound $AUC_{0\text{-}INF}$ of abemaciclib plus its active metabolites (M2 and M20) by 12%, which is not considered clinically relevant.

Fulvestrant: In clinical studies in patients with breast cancer, fulvestrant had no clinically relevant effect on the PK of abemaciclib or its active metabolites.

Effects of Abemaciclib on Other Drugs

Loperamide: In a clinical drug interaction study in healthy subjects, coadministration of a single 8 mg dose of loperamide with a single 400 mg abemaciclib (2.7 times the approved

recommended 150 mg dosage) increased loperamide $AUC_{0\text{-}INF}$ by 9% and C_{max} by 35% relative to loperamide alone. These increases in loperamide exposure are not considered clinically relevant.

Metformin: In a clinical drug interaction study in healthy subjects, coadministration of a single 1000 mg dose of metformin, a clinically relevant substrate of renal organic cation transporter 2 (OCT2), multidrug and toxin extrusion 1 (MATE1), and MATE2-K transporters, with a single 400 mg dose of abemaciclib (2.7 times the approved recommended 150 mg dosage) increased metformin $AUC_{0\text{-}INF}$ by 37% and C_{max} by 22% relative to metformin alone. Abemaciclib reduced the renal clearance and renal secretion of metformin by 45% and 62%, respectively, relative to metformin alone, without any effect on glomerular filtration rate (GFR) as measured by iohexol clearance and serum cystatin C.

Fulvestrant: In clinical studies in patients with breast cancer, abemaciclib had no clinically relevant effect on fulvestrant PK.

- *In Vitro* Studies

Transporter Systems: Abemaciclib and its major active metabolites inhibit the renal transporters OCT 2, MATE1, and MATE2-K at concentrations achievable at the approved recommended dosage. The observed serum creatinine increase in clinical studies with abemaciclib is likely due to inhibition of tubular secretion of creatinine *via* OCT2, MATE1, and MATE2-K. Abemaciclib and its major metabolites at clinically relevant concentrations do not inhibit the hepatic uptake transporters OCT1, organic anion transporting polypeptide (OATP) 1B1, and OATP1B3 or the renal uptake transporters organic anion transporter (OAT) 1 and OAT3. Abemaciclib is a substrate of P-gp and BCRP. Abemaciclib and its major active metabolites, M2 and M20, are not substrates of hepatic uptake transporters OCT1, OATP1B1, or OATP1B3. Abemaciclib inhibits P-gp and BCRP. The clinical consequences of this finding on sensitive P-gp and BCRP substrates are unknown.

- CYP Metabolic Pathways

Abemaciclib and its major active metabolites, M2 and M20, do not induce CYP1A2, CYP2B6, or CYP3A at clinically relevant concentrations. Abemaciclib and its major active metabolites, M2 and M20, down regulate mRNA of CYPs, including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4. The mechanism of this down regulation and its clinical relevance are not understood. However, abemaciclib is a substrate of CYP3A4, and time-dependent changes in PK of abemaciclib as a result of autoinhibition of its metabolism was not observed.

In vitro, abemaciclib is a substrate of P-gp and BCRP. The effect of P-gp or BCRP inhibitors on the PK of abemaciclib has not been studied.

2.3.2 Fulvestrant

2.3.2.1 Background

Fulvestrant (ICI-182,780) is a parenteral 7-alpha-alkylsulphinyl analog of estradiol. Fulvestrant is a pure ER antagonist without any known estrogen agonist effects. It is the first in a new class of antiestrogens that works by degrading the ER as opposed to blocking it as with tamoxifen. Fulvestrant has demonstrated potent inhibition of breast cancer cells *in vitro* and *in vivo*. In clinical trials, fulvestrant was as effective as anastrozole, an aromatase inhibitor, in postmenopausal women who had received previous hormonal therapy. The median time to progression (TTP) for fulvestrant (250 mg) and anastrozole was 5.4 and 3.4 months (North American trial) (Osborne *et al.,* 2002), respectively, and 5.5 and 5.1 months (European trial) (Howell *et al.,* 2002), respectively. The FDA approved fulvestrant for the second-line treatment of advanced breast cancer in postmenopausal women on April 25, 2002. In September 2010, the FDA changed the approved dosage regimen of fulvestrant to reflect data supporting increased efficacy with the administration of higher fulvestrant doses at 500 mg IM monthly. Prior to September 2010, the FDA approved dose of fulvestrant was 250 mg IM once monthly. In February 2016, it was approved in combination with palbociclib in women with HR-positive, HER2-negative advanced or metastatic breast cancer after disease progression following endocrine therapy. In August 2017, fulvestrant received FDA approval for expanded use for advanced HR+ HER2 negative breast cancer patients not previously treated with endocrine therapy based on the FALCON trial demonstrating a statistically-significant increase in median PFS, 16.6 months in the fulvestrant arm compared to 13.8 months in the anastrozole arm (Hazard Ratio: 0.797; 95% CI: 0.637-0.999; p = 0.049), representing a 20% reduction in the risk of disease progression or death (Robertson *et al.,* 2016).

2.3.2.2 Pharmacokinetics of Fulvestrant

Fulvestrant is given by IM injection. Fulvestrant undergoes rapid and extensive distribution. Fulvestrant is highly bound to plasma proteins, primarily very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions. The role of sex hormone-binding protein could not be determined. Metabolism of fulvestrant is similar to other endogenous corticosteroids and includes oxidation, aromatic hydroxylation, conjugation with glucuronic acid and/or phosphate at the 2, 3, and 17 positions of the steroid nucleus, and oxidation of the sulphoxide side chain. Identified metabolites are either less active or have similar activity as fulvestrant. The hepatic CYP3A4 isoenzyme is involved in the oxidation of fulvestrant; however, the importance of the CYP3A4 mechanism in the metabolism of fulvestrant *in vivo* is unknown. Fulvestrant is rapidly cleared by the hepatobiliary route with excretion primarily *via* the feces (approximately 90%). Renal elimination is negligible (<1%).

After an IM injection of fulvestrant, plasma concentrations are maximal at about 7 days and are maintained over a period of at least one month. Trough concentrations were about one-third of the C_{max}; steady state levels are reached within the first month of dosing.

2.4 Rationale

2.4.1 Preclinical Rationale

Preclinical studies shown that CDK4/6 inhibition induces adaptive activation of mitogenic signals which is prevented by co-treatment with PI3K pathway inhibitors (Herrera-Abreu *et al.,* 2016; Zhang *et al.,* 2016). Several studies indicated that treatment with CDK4/6 inhibitors rapidly increases the levels of cyclin D1 (Herrera-Abreu *et al.,* 2016; Dean *et al.,* 2010, Paternot *et al.,* 2014). In breast cancer models, this was accompanied by increased AKT phosphorylation and activation of CDK2, which led to failure to fully inhibit retinoblastoma (Rb) phosphorylation. However, concomitant treatment with PI3K pathway (PI3K, mTOR, AKT, PDK) inhibitors or insulin-like growth factor 1 receptor (IGF1R/InR) and CDK4/6 inhibitors reduced cyclin D1 accumulation, increased apoptosis and led to synergistic anti-tumor effects, therefore suggesting the role of receptor tyrosine kinase signaling/PI3K/AKT/mTOR signaling in the adaptive response (Herrera-Abreu *et al.,* 2016). Further studies suggested that upfront combination of PI3K inhibitors with CDK4/6 inhibitors could prevent resistance to CDK4/6 inhibitors. However, in the setting of acquired resistance to CDK4/6 inhibitors, although PI3K inhibition still modulates cyclin D1 expression, the combination was not able to restore the sensitivity to CDK4/6 inhibitors (Herrera-Abreu *et al.,* 2016). Zhang *et al.,* also provided evidence that inhibition of CDK4/6 could lead to mTORC2-mediated phosphorylation and activation of AKT(S473) and inhibitors against AKT and CDK4/6 were synergistic in reducing cell proliferation (Zhang *et al.,* 2016). In another study, CDK4/6 inhibition stimulated glycolytic and oxidative metabolism and was associated with an increase in mTORC1 activity, which is suppressed by mTOR inhibition, leading to synergistic anti-tumor effect (Franco *et al.,* 2016).

On the other hand, studies also indicate that CDK4/6 inhibition could overcome resistance to PI3K inhibitors in ER+ breast cancer. In a drug screen of 42 agents, the CDK4/6 inhibitor ribociclib was identified as a strong sensitizer to PI3K inhibition in 3 ER+ breast cancer cell lines with acquired resistance to PI3K inhibitors (Vora *et al.,* 2014). In this study, acquired resistance to PI3K inhibition was associated with maintained phosphorylation of S6 and Rb. Addition of ribociclib reduced Rb phosphorylation and induced synergist anti-tumor effect of the PI3K inhibitor, particularly in those with *PIK3CA* mutation and intact Rb. The synergism was also observed in the parental cell lines and those with *de novo* resistance to PI3K inhibitors.

The mutually beneficial effect of preventing adaptive resistance mechanisms from combining inhibitors against PI3K and CDK4/6 is translated to impressive anti-tumor activity observed in preclinical studies by several groups and provided the strong rationale for clinical investigations of triplet combinations of endocrine therapy, PI3K, and CDK4/6 inhibitors for the treatment of ER+ breast cancer (Knudsen *et al.,* 2017).

The synergistic anti-tumor effect of copanlisib, fulvestrant, and palbociclib observed in the ER positive breast cancer xenografts MCF7 (PIK3CA-E545K) and ZR751 (PI3K WT) (O'Brien *et al.,* 2016) (Figure 6). In this study, single agent copanlisib induced significant tumor growth inhibition relative to vehicle control in each of the xenograft models. Modest increases in anti-tumor activity were achieved when copanlisib was combined with either tamoxifen or fulvestrant. However, robust tumor regressions were observed with the triple combinations of copanlisib-palbociclib-tamoxifen and copanlisib-palbociclib-fulvestrant (Figure 6). Each of the single agent and treatment combinations tested were well tolerated in animals. These preclinical data provided the direct rational for investigating the triplet of fulvestrant, CDK4/6 inhibitor and copanlisib in HR+ breast cancer. We have chosen abemaciclib to combine with copanlisib in

this trial since copanlisib could induce cytopenia and abemaciclib has the least myelosuppressive effect compared to palbociclib and ribociclib.

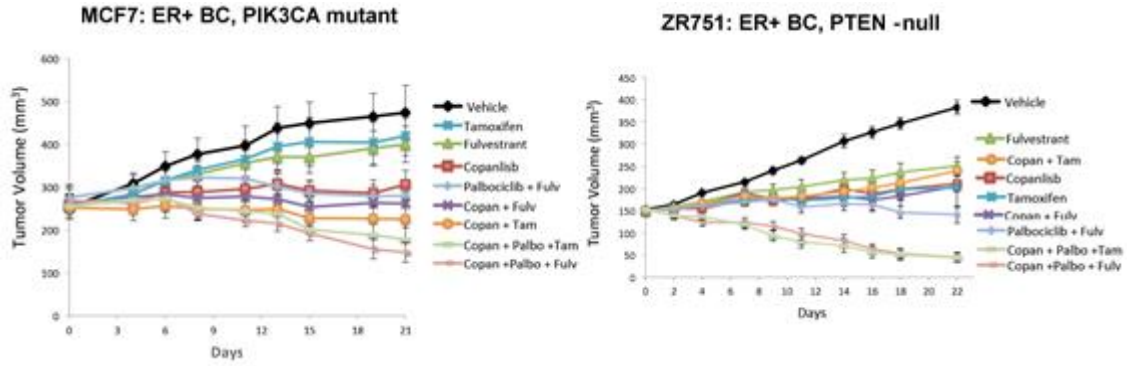


Figure 5: Robust Anti-tumor Activity of Copanlisib in Combination with Hormonal Blockade and Palbociclib in ER+ Breast Cancer (BC) Cell line Xenografts. (O'Brien *et al.,* 2016). Tumor bearing mice were treated once weekly (BID) by intravenous injection with clinically achievable doses of copanlisib (10 mg/kg) as a single agent or in combination with tamoxifen or fulvestrant with or without 75 mg/kg daily palbociclib for 21 days. Modest increases in anti-tumor activity were achieved when copanlisib was combined with hormonal blockade by either tamoxifen or fulvestrant. However, robust tumor regressions were observed with the triple combinations of copanlisib-palbociclib-tamoxifen and copanlisib-palbociclib-fulvestrant.

2.4.2 Potential Drug-Drug Interactions Between Copanlisib and Abemaciclib

We do not anticipate significant interactions between abemaciclib and copanlisib. Copanlisib is a CYP3A4 substrate, but not an inhibitor and inducer of CYP450s, uridine 5'-diphospho-glucuronosyltransferase (UGT), or dihydropyrimidine dehydrogenase (DPD) at clinically relevant concentrations (O'Brien *et al.,* 2016). Abemaciclib is a CYP3A4 substrate. Abemaciclib and its major active metabolites, M2 and M20, do not induce CYP1A2, CYP2B6, or CYP3A at clinically relevant concentrations. Since neither copanlisib or abemaciclib is an inhibitor and inducer of CYP450s, we do not expect any drug-drug interaction between these two drugs. We have built in PK studies during the Phase 1 portion of the trial.

In summary, CDK4/6 inhibitors in combination with endocrine therapy PFS compared to endocrine therapy alone in patients with HR+ HER2- metastatic breast cancer (MBC). However, *de novo* or acquired resistance exists, leading to treatment failure and disease progression. Preclinical evidence supports the incorporation of PI3K inhibitor to prevent and delay resistance to CDK4/6 inhibitor therapy, providing the rationale to evaluate the triplet combination of fulvestrant, abemaciclib, and copanlisib (FAC) in CDK4/6 inhibitor naïve endocrine-resistant HR+ HER2- MBC.

2.5 Correlative Studies Background

2.5.1 Integrated Studies

2.5.1.1 To assess tumor *PIK3CA* mutation, *PTEN* mutation/PTEN loss of expression

Hypothesis: Mutations in *PIK3CA* or *PTEN* or loss of PTEN protein expression activate PI3K

signaling, therefore are potential predictive markers for PI3K inhibitors. We hypothesize that FAC has superior antitumor activity than FA in the subset with *PIK3CA* mutation or *PTEN* mutation/loss. As a secondary objective of the study, we will compare the median PFS by *PIK3CA/PTEN* status.

Rationale: HR+ breast cancer is a heterogeneous group of disease. There is a significant diversity in genomic mutation and proteomic profiles (Network TCGA, 2012; Ellis *et al.,* 2012; Perou *et al.,* 2000; Mertins *et al.,* 2016). The advent of genome wide next generation sequencing studies allowed the identification of recurrent or significantly mutated genes which are potential driver mutations. As discussed in earlier sections, components of the PI3K pathway are frequently mutated in ER+ breast cancer (Network TCGA; Ellis *et al.,* 2012; Gellert *et al.,* 2016; Ma *et al.,* 2011). PIK3CA, which encodes the alpha catalytic subunit of PI3K, is mutated in approximately 40% of ER+ breast cancer. Mutations have also been identified in *PIK3R1*, the regulatory subunit of PI3K (0.4% - 2%), *AKT1* (2% -4%), and *PTEN* (4%). Although mutations in *PTEN* is at low frequency in ER+ breast cancer, loss of PTEN protein expression is more common (18% to 48%) (Shoman *et al.,* 2005; Depowski *et al.,* 2001), and significantly associated with relapse risk (Shoman *et al.,* 2005).

Majority of the PIK3CA mutations, including the 3 hotspot mutations E542K, E545K, and H1047R, are missense mutations that cluster in the evolutionarily conserved accessary domain and the kinase domain (Saal *et al.,* 2005). The oncogenic property of the common *PIK3CA* mutations was demonstrated by their ability to induce cellular transformation and tumor formation when overexpressed in mammary epithelial cells (Zhang *et al.,* 2008; Zhao *et al.,* 2005). Cancer cells carrying *PIK3CA* mutations are highly dependent on PIK3CA for cell survival and are sensitive to PI3K inhibitors in preclinical studies (Crowder *et al.,* 2009; Juric *et al.,* 2012), while PTEN-deficient cancers are sensitive to p110β-selective PI3K inhibitor (Jia *et al.,* 2008; Ni *et al.,* 2012; Torbett *et al.,* 2008; Wee *et al.,* 2008) or by combined targeting of both p110α and p110β (Hosford *et al.,* 2017).

However, the role of *PIK3CA* mutation or PTEN in predicting response to PI3K inhibitors in clinical trials have been inconclusive. The ctDNA sequencing analysis of the BELLE2 and BELLE3 trial indicated *PIK3CA* mutation in ctDNA was associated with buparlisib (pan PI3K inhibitor)-induced improvement in PFS (2-7). In the BELLE2 study of fulvestrant plus buparlisib/placebo, PFS was not significantly different between the two treatment arms in patients with PI3K activation (*PIK3CA* mutation and/or PTEN loss) in tumor (6.8 *vs*. 4 months). However, in an exploratory analysis, the improvement in PFS by buparlisib *vs*. placebo was found to be restricted to those with *PIK3CA* mutation identified in ctDNA (Baselga *et al.,* 2016). In the BELLE3 trial of 432 patients with AI and everolimus-resistant breast cancer (2:1 ratio to buparlisib *vs.* placebo plus fulvestrant), buparlisib significantly improved the PFS (3.9 *vs*. 1.8 months) and was associated with *PIK3CA* mutation in ctDNA or in the tumor (Angelo *et al.,* 2016). However, an improvement in PFS was also observed in the ctDNA PIK3CA WT cohort (Angelo *et al.,* 2016). The Phase 2 FERGI trial of the pan-PI3K inhibitor pictilisib (Krop *et al.,* 2016), however, show no difference in PFS with pictilisib in either *PIK3CA* mutant or WT patients (Krop *et al.,* 2016).

In a Phase 1b study of the PI3Kα inhibitor alpelisib, with letrozole, in patients with endocrine-

resistant metastatic ER+ breast cancer, efficacy was observed independently of *PIK3CA* mutation status, although a higher proportion of patients with *PIK3CA* mutated tumors experienced clinical benefit (Mayer *et al.,* 2017). Similarly, in a Phase 2 trial of another PI3Kα inhibitor taselisib (also had delta inhibitory effect) plus fulvestrant in HR+ breast cancer, activity was observed in both *PIK3CA* mutant and WT tumors although a numerically higher response was observed in the mutant population (Dickler *et al.,* 2016). However, in a Phase 1study of 34 patients with locally advanced or metastatic solid tumors treated with taselisib, responses were restricted to *PIK3CA* mutant tumors (ORR was 36% (5/14) for patients with *PIK3CA* mutant tumors compared to the 0% (0/15) in those without *PIK3CA* mutations).

In the 25 patients treated in the MTD cohort in the Phase 1 study of copanlisib, 2 PR, both with breast cancers, were observed, including 1 HR+/HER2-/*PIK3CA* WT and 1 HR+/HER2+/*PIK3CA* Mut (Patnaik *et al.,* 2016). One CR occurred in a patient with endometrial cancer with mutations in both *PIK3CA* and *PTEN*. Fifteen (15) SDs, with 6 lasting ≥6 cycles, including 1 with *PIK3CA* WT/*KRAS* Mut endometrial cancer, 1 PTEN loss endometrial cancer, 1 *PIK3CA* Mut breast cancer, were observed. Interestingly, of the 10 patients without complete PTEN loss, none had objective response or SD for ≥4 cycles. Among the 7 with complete PTEN loss, 3 (43%) had CR/PR/SD ≥6 cycles. Therefore, it appears that copanlisib may have enhanced activity among those with PTEN loss. No clear relationship was observed with *PIK3CA* status.

Therefore, it remains unclear whether *PIK3CA* mutation status and/or *PTEN* mutation status in the tumor is predictive of response to PI3K alpha selective or pan-PI3K inhibitors. Furthermore, there are no studies available to date that investigated whether *PIK3CA* and/or *PTEN* mutation status is predictive of benefit of a PI3K inhibitor when combined with a CDK4/6 inhibitor and endocrine therapy combination regimen.

Approach: *PIK3CA/PTEN* mutations will be determined by whole exome sequencing at the Molecular Characterization (MoCha) Laboratory. PTEN protein expression will be assessed by immunohistochemistry (IHC) under the CLIA CAP provision at Clinical IHC laboratory at the University of Texas MD Anderson Cancer Center.

2.5.1.2 To test whether baseline AKT phosphorylation level correlates with PFS benefit from the addition of copanlisib and whether triplet therapy with FAC inhibits AKT phosphorylation, reduces cyclin D1 expression, and is more effective than FA therapy in reducing Rb phosphorylation.

Rationale: Based on preclinical data (Folkes *et al.,* 2008; Muranen *et al.,* 2016; Kwei *et al.,* 2012), we hypothesize that tumors with increased PI3K pathway activity at baseline would benefit the most from adding copanlisib to the FA regimen. In addition, in ER+ breast cancer cell lines, treatment with a CDK4/6 inhibitor has been shown to rapidly induce PI3K pathway signaling activation and upregulate cyclin D expression, leading to recovery of Rb phosphorylation, which is prevented with combined therapy with PI3K inhibitors (Herrera-Abreu *et al.,* 2016). Therefore, we expect that treatment with FA would lead to up-regulation of PI3K pathway signaling on C1D15, while the triplet FAC treatment inhibits PI3K pathway signaling, decreases cyclin D level, leading to a more profound decrease in Rb phosphorylation.

Approach: Snap-frozen tumor biopsies will be analyzed by RPPA at MD Anderson.

2.5.2 Exploratory Studies

2.5.2.1 To examine archival or pre-treatment tumor tissue for somatic mutations

Rationale: As part of the exploratory objectives, we will examine somatic mutation profiles that may correlate with treatment response. Candidate genes include, but not limited to, other genes in the PI3K pathway such as *AKT1, PIK3R1*, as well as other recurrent mutations in HR+ breast cancer, including *ESR1, TP53, Rb1, RAS*, and others. Several studies found that *KRAS* or *BRAF* mutation is associated with resistance to PI3K pathway inhibitors (Engelman *et al.,* 2008; Ihle *et al.,* 2009; Garnett *et al.,* 2012; Di Nicolantonio *et al.,* 2010; Janku *et al.,* 2012). There is little data in the literature that examined the impact of mutations in other genes on sensitivity to PI3K inhibitors (Weigelt *et al.,* 2012). *Rb1* mutation has been implicated in resistance to CDK4/6 inhibitor (Finn *et al.,* 2009), but not all *Rb1* mutations are the same and CDK4/6 inhibitors are broadly effective regardless of the presence of mutations in *ESR1, TP53* and others (Cristofanilli *et al.,* 2016; Ma *et al.,* 2017a; Fribbens *et al.,* 2016). More data is needed.

Approach: Whole exome sequencing of tumor and germline DNA will be performed at the MoCha Laboratory.

2.5.2.2 To assess baseline and treatment-induced proteomic changes

Rationale: As an exploratory objective, we will examine fresh tumor biopsies collected at baseline (pre-treatment), C1D15, and at disease progression using reverse phase protein assay (RPPA) analysis to examine baseline and treatment-induced changes in various cancer associated pathways, including but not limited to PI3K, MAPK, ER, cyclins, CDKs and CDK inhibitors, to correlate with treatment response. Based on preclinical data (Folkes *et al.,* 2008; Muranen *et al.,* 2016; Kwei *et al.,* 2012), we hypothesize that tumors with increased PI3K pathway activity at baseline would benefit the most from adding copanlisib to the FA regimen, while increased ERK pathway signaling predicts resistance to the addition of copanalisib. In addition, in ER+ breast cancer cell lines, treatment with a CDK4/6 inhibitor has been shown to rapidly induce PI3K pathway signaling activation, upregulate cyclin D expression, leading to recovery of Rb phosphorylation, which is prevented with combined therapy with PI3K inhibitors (Herrera-Abreu *et al.,* 2016). Therefore, we expect that treatment with FA would lead to up-regulation of PI3K pathway signaling on C1D15, while the triplet FAC treatment inhibits PI3K pathway signaling, decreases cyclin D level, leading to a more profound decrease in Rb phosphorylation. Furthermore, baseline high levels of cyclin E and loss of Rb protein may be associated with resistance to FA combination (Herrera-Abreu *et al.,* 2016). We will also assess ER pathway signaling changes by examining ER phosphorylation, PgR levels, among others.

Approach: Snap-frozen tumor biopsies will be analyzed by RPPA at MD Anderson.

2.5.2.3 To assess baseline gene expression and treatment-induced gene expression changes

Rationale: As an exploratory objective, we will examine fresh tumor biopsies collected at baseline (pre-treatment), C1D15, and at disease progression by gene expression microarray to determine breast cancer intrinsic subtypes (PAM50), gene signatures of PI3K and MAPK activation, ER regulated genes, as well as other genes and gene signatures to correlate with treatment response. In NeoPalAna trial (neoadjuvant palbociclib and anastrozole), non-luminal subtype ER+ breast cancers were resistant to CDK4/6 inhibitors (Ma *et al.,* 2017a) which is consistent with results from several preclinical studies (Finn *et al.,* 2009). In addition, copanlisib appears to be actively predominantly in HR+ and HER2 positive breast cancers (O'Brien *et al.,* 2016), justifying the assessment of molecular subtypes. In preclinical studies, a gene expression signature of PI3K activation is associated with response, while MAPK or RAS signatures are associated with resistance to PI3K inhibitors (Folkes *et al.,* 2008; Kwei *et al.,* 2012). Candidate genes implicated in PI3K and CDK4/6 inhibitor activities will also be assessed. Examples include PIM1 (Le *et al.,* 2016), cyclin B (Ihle *et al.,* 2009), Myc and yes-associated protein (YAP) (Muranen *et al.,* 2016), which have been associated with PI3K inhibitor resistance.

Approach: Total RNA from tumor biopsies will be extracted and subjected to RNASeq analysis at the MoCha Laboratory.

2.5.2.4 To examine ctDNA mutation profiles

Rationale: Plasma in cancer patients often carries small amounts of fragmented cell-free DNA of 160-180 base pairs, which are originated from the necrosis or apoptotic process of cancer cells (Jahr *et al.,* 2001; Snyder *et al.,* 2016; Stroun *et al.,* 2001). Compared to a single tumor biopsy, ctDNA mutations may represent genomic alterations of different tumor clones or deposits and better reflect the inter- and intra-tumor heterogeneity (De Mattos-Arruda *et al.,* 2016; Murtaza *et al.,* 2015). In addition, with a half-life ranging from 16 minutes to a few hours (Yu *et al.,* 2013; Lo *et al.,* 1999), ctDNA provides real-time status of the tumor genome. Advances in the NGS technology and digital genomic techniques support the clinical validity of cell-free ctDNA sequencing analysis to non-invasively identify actionable genomic alterations, monitor treatment response, and investigate resistance mechanisms (De Mattos-Arruda *et al.,* 2016).

ctDNA sequencing is particularly helpful in cases that tumor DNA sequencing is not possible due to insufficient quality or quantity of the tumor tissue. In addition, changes in the variant allele frequencies (VAFs) of ctDNA mutations occur rapidly, prior to the detection of changes in tumor size, upon treatment with cancer therapies including those that target the PI3K pathway (Ma *et al.,* 2017b; Hyman *et al.,* 2017) and serial monitoring of ctDNA mutation profile allows the identification of treatment emergent resistant mechanisms (Ma *et al.,* 2017b; Hanker *et al.,* 2017; Ahronian *et al.,* 2017; Diaz *et al.,* 2012). There have been a plethora of studies that demonstrated the ability of ctDNA analysis in the detection of *PIK3CA* mutation, *PTEN* mutations, *ESR1*, *TP53*, and others, which may be better than tumor DNA sequencing (De Mattos-Arruda *et al.,* 2016; Juric *et al.,* 2017; Ma *et al.,* 2017c). In this trial, as part of correlative studies, we will examine baseline ctDNA mutations, including *PIK3CA*, *PTEN*, *ESR1*, and others, with treatment response, examine the dynamics of ctDNA mutation profile over time for early tumor response and treatment-emergent resistant mechanisms.

Approach: NGS of ctDNA will be performed with the plasma collected from the streck tubes

using the TSO500 assay (Illumina, San Diego, CA).

2.5.2.5 To assess plasma and serum proteomics and metabolomics

Rationale: As part of the exploratory objectives, serum and plasma samples at baseline, on therapy and at disease progression are collected for circulating markers that reflect changes due to treatment. In addition to regulating cell proliferation, PI3K pathway is important in regulating glucose and lipid metabolism (Engelman *et al.,* 2006), and circulating markers of metabolism before and after PI3K inhibitor therapy may predict treatment response and resistance mechanisms. Inhibition of PI3K pathway reduces glucose uptake in the cancer cells (Maynard *et al.,* 2017; Maynard *et al.,* 2016; Maynard *et al.,* 2013). PI3K inhibitors reduce glycolysis, affect the nonoxidative pentose phosphate pathway that delivers the Ribose-5-phosphate required for base ribosylation, therefore suppressing nucleotide synthesis and inducing replication stress and DNA damage (Juvekar *et al.,* 2016). In addition, there is evidence of reduction of enzymes involved in cholesterol biosynthesis upon PI3K inhibition, which leads to changes in levels of critical metabolites such as deoxyribonucleotide triphosphates (dNTPs), driving a cellular stress phenotype (Lynch *et al.,* 2017). Proteomic technology, such as mass spectrometry and SOMAscan technology, allows systematic profiling of cellular metabolites and metabolic enzymes in the serum or plasma in a parallel and multiplexed high-throughput manner (Gold *et al.,* 2010; Mehan *et al.,* 2014). We are particularly interested in members of the glycolytic pathway, both the enzymes as well as the metabolic intermediates, as these are expected to be sensitive to PI3K-inhibition (Juvekar *et al.,* 2016). Some of these studies will be performed at the Beth Isreal Deaconess Medical Center Proteomics Core, in collaboration with Dr. Gerburg Wulf.

Approach: Unbiased proteomic scan will be performed either using Mass Spectrometry or the SOMAscan, aptamer-based technology. In either case, a total of 1000-1500 proteins can be screened from a single plasma sample in an unbiased fashion. Unbiased metabolomics will be performed using Mass Spectrometry of Plasma-samples after metabolite extraction with either methanol or methyl-ether butyrate (MBTE).

2.5.2.6 To assess tumor immune microenvironment

Rationale: As part of the exploratory objectives, we will examine tumor biopsies collected at baseline, on-treatment, and at disease progression for treatment induced changes in tumor infiltrating lymphocytes and to correlate response. This is based on preclinical studies demonstrating that p110δ inhibition by copanlisib decreases both total Treg and CD4+ T cells, and increased IFNγ+ CD44 cells in tumors.

Approach: A multiplex IHC platform will be used to assess the immune cell populations in tumor biopsy FFPE sections before and after therapy.

2.5.2.7 Copanlisib and abemaciclib pharmacokinetics

Rationale: Copanlisib is predominantly metabolized by CYP3A4 (>90%) and to a minor extent by CYP1A1 (<10%). Copanlisib has low potential for drug-drug interaction as a perpetrator to

influence the PK of other drugs by inhibition or induction of metabolizing enzymes (CYPs, UGT) and transport proteins, except MATE2-K transporter at clinically relevant concentrations. Abemaciclib is a CYP3A4 substrate. Abemaciclib and its major active metabolites, M2 and M20, do not induce CYP1A2, CYP2B6, or CYP3A at clinically relevant concentrations. Based on this information, we do not expect any drug-drug interaction between these two drugs. However, we have built in PK studies during the Phase 1 portion of the trial to make sure there is no drug-drug interactions.

Approach: Peripheral blood will be collected at different time points before and after drug administration in the presence or absence of the other drug (please refer to protocol Section 5 for details). Serum/plasma will be analyzed by MS approach at Bayer with existing assays.

2.6 Rationale for Protocol Amendment #6

Between June 2020 and Dec 2020, 7 patients were enrolled to Phase 1, Dose Level 1 (copanlisib 45 mg IV D1 and D15; abemaciclib 100 mg PO Bid) in 2 sequential cohorts of 3 evaluable patients. One patient was not evaluable for DLT due to withdrawing consent during Cycle 1. The most common AE G3 or above experienced by the patients enrolled at Dose Level 1 was neutropenia. Among the 6 DLT evaluable patients enrolled to Dose Level 1, 4 experienced a G3 ANC during Cycle 1, leading to dose interruption of abemaciclib, although only one patient needed 2 weeks for ANC to recover sufficiently in order to resume abemaciclib (this is considered a DLT); the other 3 patients were able to resume abemaciclib after an interruption of 1 week. Since 1 of 6 patients experienced a DLT in Dose Level 1, dose escalation proceeded to Dose Level 2.

Between 2/4/2021 and 2/9/2021, 3 patients were enrolled to Dose Level 2 (copanlisib 45 mg IV D1, D8, and D15; abemaciclib 100 mg PO Bid). Two of these 3 patients experienced a DLT (one G3 rash, one G3 elevated bilirubin). Dose Level 2 therefore exceeded the maximum tolerated dose.

After reviewing the AEs observed in Dose Levels 1 and 2 with the CTEP monitor, the dosing schedule of abemaciclib has been modified to 5 days on and 2 days off each week (5-on/2-off) to improve tolerability. Therefore, the previous iteration of Phase 1 has been termed Phase 1 Part A, and the new Phase 1 dosing schedule has been termed Phase 1 Part B. In Phase 1 Part B, a maximum of 4 dose levels with abemaciclib administered at 5-on/2-off schedule will be tested. This is based on our prior experience using an alternative dosing schedule of palbociclib (5-on/2-off) in a phase II clinical trial in metastatic hormone receptor positive breast cancer (NCT03007979) (Krishnamurthy, J. *et al* 2020). The alternative dosing schedule of palbociclib showed improved tolerability with reduced incidences of G3+ ANC and did not impact the efficacy of the treatment.

The starting dose level to be tested in Phase 1 Part B (Dose Level 1b) is copanlisib 45mg IV D1 and D15 and abemaciclib 100 mg BID on Days 1-5 each week (Days 1-5, 8-12, 15-19, and 22-26 of each cycle).

2.7 Rationale for Protocol Amendment #8d and Study Closure

Bayer announced in November 2023 that they were voluntarily withdrawing their New Drug Application for copanlisib due to failure to meet their primary endpoint in ongoing clinical trials. This study is closing to any new patient enrollments. Patients currently on study who are incurring clinical and/or radiographic benefit may be allowed to continue on study treatment at the discretion of their treating physician, and if the patient consents.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Men and women must have histologically or cytologically confirmed ER and/or PR positive, HER2 negative or non-amplified breast cancer that is stage IV, with measurable or non-measurable disease. ER/PR positivity is defined as at least 1% positive or an Allred score of at least 3. HER2 status is defined per the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline.

3.1.2 All patients must agree to provide archival tumor material for research and must agree to undergo research tumor biopsy before treatment if presence of easily accessible lesions (judged by the treating physician). For patients with bone only disease, or patients without easily accessible lesions for the baseline research biopsy, availability of archival tumor material (2 x 4-5 micron section unstained slides, plus 15-20 x 10 micron section unstained slides or a tumor rich block) from previous breast cancer diagnosis or treatment is required for central PTEN and PIK3CA analysis.

3.1.3 No more than 1 prior chemotherapy in the metastatic setting. There is no limit on prior lines of endocrine therapy. (For patients enrolling to the Phase 1 portion of the study, prior fulvestrant, CDK4/6 inhibitor, and everolimus is allowed).

3.1.4 For patients enrolling to the randomized Phase 2 portion of this study, demonstrated resistance to prior endocrine therapy in the metastatic setting is required; this is defined as:

- Progressed on prior endocrine therapy in the metastatic setting or,
- Relapsed on adjuvant endocrine therapy or,
- Relapsed within 12 months of completing adjuvant endocrine therapy or,
- If received adjuvant CDK4/6 inhibitor, relapsed at least 2 years after completion of adjuvant CDK4/6 inhibitor.

3.1.5 Washout from prior systemic anti-cancer therapy of at least 3 weeks from chemotherapy or 5 half-lives from oral targeted drugs, and treatment related adverse events recovered to grade 1 (except for alopecia) before the start of study treatment. Washout from prior radiation therapy of at least 2 weeks before the start of the study treatment. Washout from prior endocrine therapy is not required.

3.1.6 Age ≥18 years. Because no dosing or AE data are currently available on the use of copanlisib in combination with abemaciclib and fulvestrant in patients <18 years of age, and because breast cancer is rare in children, children are excluded from this study.

3.1.7 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.8 Patients must have adequate organ and marrow function collected no more than 7 days before starting study treatment as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥ 8.0 g/dL
– total bilirubin	≤1.5 × institutional upper limit of normal (≤3 × institutional upper limit of normal for patients with Gilbert syndrome)
– AST(SGOT)/ALT(SGPT)	≤3 × institutional upper limit of normal (≤5 × institutional upper limit of normal for patients with liver involvement)
– Glomerular filtration rate	≥30 mL/min according to the Modification of Diet in Renal Disease (MDRD) abbreviated formula
– Lipase	≤1.5 x ULN
– INR and PTT	≤1.5 x ULN (except those on anti-coagulation therapy)
– HbA1c	≤8.5% or fasting glucose ≤ 120 mg/dL on at least 2 occasions within 14 days prior to registration if diabetic

3.1.9 Left ventricular ejection fraction (LVEF) ≥50%.

3.1.10 Patients may be postmenopausal or premenopausal women on or planned to receive GnRH agonist.

3.1.11 The effects of copanlisib on the developing human fetus are unknown. For this reason and because maternal toxicity, developmental toxicity and teratogenic effects have been observed in nonclinical studies and PI3K inhibitors as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 6 months after the last dose of copanlisib. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 6 months after completion of copanlisib administration.

3.1.12 Ability to understand and willing to sign a written informed consent document (or legally authorized representative, if applicable). Patient must agree to research team access to prior breast cancer diagnosis and treatment records, as well as reports of clinical tumor

and blood sequencing results.

3.1.13 Patients with a history of treated brain metastases are allowed in the Phase I portion of the trial provided there is no disease progression symptomatically and by imaging within 28 days prior to registration AND if the patient is off steroids.

3.1.14 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

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

3.1.16 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.

3.1.17 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.

3.2 Exclusion Criteria

3.2.1 For patients enrolling to the randomized Phase 2 portion of the study, prior treatment with a CDK4/6 inhibitor or fulvestrant, or a PI3K inhibitor in the metastatic setting is not allowed.

3.2.2 Patients who have had chemotherapy within 3 weeks or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study.

3.2.3 Patients who are receiving any other investigational agents.

3.2.4 Immunosuppressive therapy is not allowed while on study.

3.2.5 Receiving anti-arrhythmic therapy (beta blockers or digoxin are permitted).

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to copanlisib, PI3K inhibitors, or other agents used in study.

3.2.7 For the randomized Phase 2 portion of the study, patients with brain metastasis or a history of brain metastasis are not eligible.

For the Phase 1 portion of the study, patients with progressive brain metastases should be excluded because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other AEs.

3.2.8 Copanlisib is primarily metabolized by CYP3A4. Therefore, the concomitant use of

strong inhibitors of CYP3A4 (*e.g.*, ketoconazole, itraconazole, clarithromycin, ritonavir, indinavir, nelfinavir and saquinavir), and strong inducers of CYP3A4 (*e.g.*, rifampin, phenytoin, carbamazepine, phenobarbital, St. John's Wort) are not permitted from 14 days prior to enrollment until the end of the study.

For the list of specific medications prohibited while on copanlisib treatment refer to Appendix B. 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 minimize use of. Appendix C (Patient Wallet Card) should be provided to patients. 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.

3.2.9 Systemic corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not permitted while on study. Previous corticosteroid therapy must be stopped or reduced to the allowed dose at least 7 days prior to the CT/MRI screening. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose before the screening. Patients may be using topical or inhaled corticosteroids. Short-term (up to 7 days) systemic corticosteroids above 15 mg prednisolone or equivalent will be allowed for the management of acute conditions (*e.g.,* treatment non-infectious pneumonitis).

3.2.10 Major surgical procedure or significant traumatic injury (as judged by the investigator) within 28 days before start of treatment, or have not recovered from major side effects, open biopsy within 7 days before start of treatment.

3.2.11 Uncontrolled intercurrent illness, including but not limited to, symptomatic congestive heart failure (> NYHA class 2), unstable angina pectoris, new-onset angina, uncontrolled hypertension despite optimal medical management, seizure disorder requiring medication, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.12 Myocardial infarction <6 months before start of treatment

3.2.13 Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis or pulmonary embolism within 3 months before the start of study medication

3.2.14 The patient has a personal history of any of the following conditions: syncope of cardiovascular etiology, ventricular arrhythmia of pathological origin (including, but not limited to, ventricular tachycardia and ventricular fibrillation), or sudden cardiac arrest.

3.2.15 Proteinuria ≥grade 3 as assessed by a 24-h protein quantification or estimated by urine protein: creatinine ratio >3.5 on a random urine sample

3.2.16 History of bleeding diathesis. Any hemorrhage or bleeding event ≥grade 3 within 4 weeks prior to the start of study medication.

3.2.17 History or concurrent condition of interstitial lung disease of any severity and/or severely impaired lung function.

3.2.18 History of having received an allogeneic bone marrow or organ transplant

3.2.19 Patients with non-healing wound, ulcer, or bone fracture not due to breast cancer.

3.2.20 Patients with active, clinically serious infections >grade 2 (CTCAEv5.0)

3.2.21 Patients with HbA1c >8.5% at screening.

3.2.22 Concurrent diagnosis of pheochromocytoma.

3.2.23 Has undergone blood or platelet transfusion <7 days prior to start of treatment.

3.2.24 Pregnant women are excluded from this study because copanlisib is a PI3K inhibitor agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with copanlisib, breastfeeding should be discontinued if the mother is treated with copanlisib. These potential risks may also apply to other agents used in this study.

3.2.25 Hepatitis B (HBV) or hepatitis C (HCV). All patients must be screened for HBV and HCV up to 28 days prior to study drug start using the routine hepatitis virus lab panel. Patients positive for HBsAg and/or HBcAb will be eligible if they are negative for HBV DNA, these patients should receive prophylactic antiviral therapy. Patients positive for anti-HCV antibody will be eligible if they are negative for HCV RNA.

3.2.26 HIV positive patients on combination antiretroviral agents that are strong CYP3A4 inhibitors or inducers and who are unwilling or unable to change to antiretroviral therapies without such interactions are ineligible because of the potential for pharmacokinetic interactions with copanlisib, abemaciclib, and fulvestrant. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.27 Patients with history of, or current autoimmune disease are not eligible

3.2.28 History of major surgical resection involving the stomach or small bowel, or preexisting Crohn's disease or ulcerative colitis or a preexisting chronic condition resulting in baseline grade 2 or higher diarrhea

3.3 Inclusion of Women and Minorities

National Institute of Health (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)) credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr/. The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes five person registration types.

- Investigator (IVR): MD, DO, or international equivalent,
- Non Physician Investigator (NPIVR): advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- Associate Plus (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)	✓	✓	✓		
GCP training	✓	✓	✓		

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

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,
- Selection as the treating, credit, or drug shipment investigator or consenting person in OPEN,
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

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.

Refer to the NCI RCR page on the CTEP website for additional information. 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 IRBManager 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 email or calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active CTEP status,
- Have an 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, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,

- Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile,
- List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and

Have the appropriate CTEP registration type for the protocol.

Additional Requirements

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

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

4.2.1 Downloading Site Registration 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 their associated investigators and staff on a participating roster. To view/download site registration forms:

- Log in to the CTSU members' website (https://www.ctsu.org),
- Click on *Protocols* in the upper left of the 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-CT018*, and protocol number *10287*,
- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select *Site Registration* to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.2 Requirements For 10287 Site Registration

- Site Initiation Meeting (SIM) by the Study Chair/Lead Principal Investigator (Prior to the start of subject enrollment, participating sites must contact the Study Chair/Study Coordinator to schedule a SIM.)
- 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. Users are strongly encouraged to take a refresher of the training if they have not entered specimen data for an extended period of time.
- This training will need to be completed before first/further patient enrollment at a given site.
- Please contact STS Support at Theradex for the training (STS.Support@theradex.com).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via 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 Enrollment

In addition to systems provided through CTSU, Duke Cancer Institute LAO (corresponding organization) is utilizing an online 'study portal' for key study communication from participating sites to the Lead Principal Investigator and Study Coordinator. Communications *via* the study portal include notifications of subject consent, subject status updates (*e.g.*, screen failure, OPEN registered, withdrawal, *etc.*), lab shipments, serious adverse events, and protocol deviations. The study portal operates through REDCap, a secure, web-based application, managed by the Duke Cancer Institute LAO. Participating sites will not require REDCap user accounts or passwords to access the study portal for this study

Information about 10287 Study Portal use is available in LPO Documents for the study on www.ctsu.org.

Within 24 business hour of patient signing consent for this study, site staff must access the 10287 Study Portal to provide notification of consent. With this study portal notification, the Lead Principal Investigator and Study Coordinator will be alerted of subjects consented and in screening.

A stratified randomization will be conducted to assign patients at a 1:1 ratio to FA or FAC for the phase II portion of the study. The stratification factors are (1) the presence or absence of visceral metastasis, (2) menopausal status at study entry (postmenopausal *vs.* premenopausal or perimenopausal), and (3) primary *vs* secondary endocrine resistance.

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:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems.
- 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.

4.3.2 Special Instructions for Patient Enrollment

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 can be found in Section 5.4.

4.3.3 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.

4.3.4 Patient Registration/Randomization

Prior to accessing OPEN/IWRS to register/randomize patient, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

A stratified randomization will be conducted to assign patients at a 1:1 ratio to FA or FAC. The stratification factors are endocrine resistance in metastatic or adjuvant setting, bone-only or visceral metastases, and prior chemotherapy or not in the metastatic setting.

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

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 Principal Investigator. 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.

Within 24 business hours of patient enrollment in OPEN, withdrawal of consent, or determination ineligible for study, site staff must access the **10287 Study Portal** to provide update of subject status. With this study portal notification, the Lead Principal Investigator and Study Coordinator will be alerted of patient's status on study.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Patients who have treatment delays or holds should delay the PK blood draws until treatment resumes. If PK samples are collected on a day when treatment is held or delayed, these samples should be discarded and collected at the next scheduled dosing visit.

Blood draws for the EDTA (purple top), red top, and Streck cfDNA tubes could still be submitted on a day when treatment is held or delayed - do not discard samples.

Time Point	Specimen	Send Specimens To:
Archival		
	• Archival tumor sample[1]: – 1 tumor-rich (more than 50% tumor cellularity) FFPE block (preferred) If a block cannot be submitted, then submit: – 1 H&E-stained slide at 3-5 microns – 15-20 unstained, uncharged, air-dried slides cut at 10 microns* – 2 unstained, charged slides cut at 4-5 microns*	EET Biobank
Pre-treatment		
	• Fresh tumor biopsy[2]: – 1st and 2nd tumor cores snap-frozen – 3rd and 4th tumor cores in formalin • 20 mL blood in Streck cfDNA tubes • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3] • 10 mL blood in red top tube, processed for serum and frozen[4]	EET Biobank
Cycle 1 Day 1 (C1D1)		

Time Point	Specimen	Send Specimens To:
Baseline 10 min 1 h 2 h 3 h 5 h 24 h	• 4 mL blood in Li-Heparin BD vacutainers (green top) for each PK time point before (baseline) and after copanlisib infusion at indicated time[6].	NorthEast Bioanalytical Laboratories
Cycle 1 Day 8 (C1D8)		
	• 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3] • 10 ml blood in red top tube, processed for serum and frozen[4] • 20 mL blood in Streck cfDNA tubes	EET Biobank
Cycle 1 Day 15 (C1D15)		
	• Fresh tumor biopsy[2] (optional) – 1st and 2nd tumor cores snap-frozen – 3rd and 4th tumor cores in formalin • 20 mL blood in Streck cfDNA tubes • 10 mL blood in red top tube, processed for serum and frozen[4] • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3] Note that for patients on copanlisib, the tumor biopsy schedule should follow that of copanlisib, in case if C1D15 copanlisib is delayed.	EET Biobank
Baseline 10 min 1 h 2 h 3 h 5 h 24 h	• 4 mL blood in Li-Heparin BD vacutainers (green top) for each PK time point before and after copanlisib infusion[6]. **Note: Abemaciclib should be taken right after the 1 h blood draw.** Note that the PK sampling schedule should follow that of copanlisib, in case if C1D15 copanlisib is delayed.	NorthEast Bioanalytical Laboratories
Cycle 1 Day 22 (C1D22)		
Baseline 1 h 2 h 4 h 23 h	• 4 mL blood in Li-Heparin BD vacutainers (green top) for each PK time point before (baseline) and after taking the morning dose of abemaciclib[6]. Note that the PK sampling schedule should follow that of copanlisib, in case if delays of copanlisib occurs in Cycle 1.	NorthEast Bioanalytical Laboratories
Cycle 2 Day 1 (C2D1)		

Time Point	Specimen	Send Specimens To:
	• 20 mL blood in Streck cfDNA tubes • 10 mL blood in red top tube, processed for serum and frozen[4] • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3]	EET Biobank
Cycle 4 Day 1 (C4D1), Cycle 7 Day 1 (C7D1) and every 3 cycles after		
	• 20 mL blood in Streck cfDNA tubes • 10 mL blood in red top tube, processed for serum and frozen[4] • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3]	EET Biobank
Progression[5]		
	• Fresh tumor biopsy[2] (optional) – 1st and 2nd tumor cores snap-frozen – 3rd and 4th tumor cores in formalin • 20 mL blood in Streck cfDNA tubes • 10 mL blood in red top tube, processed for serum and frozen[4] • 10 mL blood in EDTA (purple top) tube, processed for plasma and frozen[3]	EET Biobank

[1]For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. If submitting slides, then slides must be processed in order, and numbered sequentially (*e.g.*, H&E stained slide is created first and labeled 1, unstained slides are then created and numbered 2 – 23).

[2]For new biopsies, **the Tissue Biopsy Verification Form (Appendix J), 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. Kits are provided by the EET Biobank. Please refer to Section 5.3.2, 5.3.3 and 5.3.4 for details.

[3]Process samples at Site according to Section 5.3.5.2.
[4]Process samples at Site according to Section 5.3.5.1.
[5]Progression specimens should be collected prior to beginning any additional therapies.
[6]PK samples are required for patients enrolled in the phase 1 portion. Kits are provided by NorthEast Bioanalytical Laboratories.

*Clearly label the section thickness on slides.

5.2 Specimen Procurement Kits and Scheduling

5.2.1 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://kits.bpc-apps.nchri.org/).

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. NorthEast Bioanalytical Laboratories will provide kits for collection and shipping of PK samples. Refer to the PK Sample Manual for instructions on how to order kits for PK samples.

5.2.2 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.

Frozen specimens, including processed serum, plasma, and frozen tissue, may be collected, processed, and shipped to the EET Biobank on Monday through Thursday, since the Biorepository does not need to perform additional processing. In the event that frozen samples cannot be shipped immediately, they must be stored in a -80 °C freezer.

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

5.3 Specimen Collection and Processing

5.3.1 Archival tumor (Mandatory)

Tissue from previous diagnostic or therapeutic procedures of the primary breast cancer or metastatic site is required for all patients. (There is no time limit for this specimen; any previously collected specimen is accepted.) A tumor rich (more than 50% tumor cellularity) block is preferred. Alternatively, 1 H&E-stained slide (3-5 microns), 15-20 unstained slides (10 microns thick) and 2 unstained slides (4-5 microns) may be submitted in place of the FFPE block. If submitting slides, then slides must be processed in order and numbered sequentially.

5.3.2 Biopsy Collection Procedure - Tumor Specimen Allocation and Prioritization

Pre-treatment (mandatory)
Tumor biopsy is required for all patients with accessible tumors that could be safely biopsied as determined by the site investigator. Bone biopsy is acceptable. This will serve as additional tumor material to analyze in cases of insufficient quantity, or quality, archival tumor material for sequencing studies.

- Biopsy Core Collection: Either skin punches (in cases of skin metastasis) or core biopsies (in the clinic or by radiology guidance) will be performed according to the sequence of priority listed below. If feasible, up to 4 cores or punches (with a minimum

of at least 2 cores) will be taken at each time point. Punches could be divided to smaller pieces to make up the 4 cores.

- Core (punch) #1: Snap-freeze according to instructions in Section 5.3.4 (for RPPA).
- Core (punch) #2: Snap-freeze according to instructions in Section 5.3.4 (for RPPA).
- Core (punch) #3: Formalin fix following instructions in Section 5.3.3 (for NGS and IHC).
- Core (punch) #4: Formalin fix following instructions in Section 5.3.3 (for NGS and IHC).

For cores 1 and 2, if immediate bedside freezing is not possible, place samples on ice for a maximum of 2 h before freezing procedure to avoid protein and RNA degradation.

C1D15 and Disease Progression (optional)

For patients on the FAC regimen, collect biopsy within 2-8 h post the administration of copanlisib, for scheduling issues, up to 24 hours following the copanlisib is acceptable. Note that for patients on copanlisib, the tumor biopsy schedule should follow that of copanlisib in C1D15, in case copanlisib is delayed. For patients on FA regimen, collect biopsy on C1D15 at least 1h after the first dose of abemaciclib that day.

Biopsy core collection: It is recommended the biopsy site at C1D15 the same as that at pre-treatment time point. Either skin punches (in cases of skin metastasis) or core biopsies (in the clinic or by radiology guidance) will be performed according to the sequence of priority listed below. If feasible, up to 4 cores or punches (with a minimum of at least 2 cores) will be taken at each time point. Punches could be divided to smaller pieces to make up the 4 cores.

- Core (punch) #1: Snap freeze following instructions in Section 5.3.4 (for RPPA).
- Core (punch) #2: Snap freeze following instructions in Section 5.3.4 (for RPPA).
- Core (punch) #3: Formalin fix following instructions in Section 5.3.3 (for NGS and IHC).
- Core (punch) #4: Formalin fix following instructions in Section 5.3.3 (for NGS and IHC).

For cores 1 and 2, if immediate bedside freezing is not possible, place samples on ice for a maximum of 2 h before freezing procedure to avoid protein and RNA degradation.

5.3.3 Collection of formalin tissue specimen using kits

1. Label formalin-filled containers according to instructions in Section 5.4.1.
2. Obtain two 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. Perform fixation at room temperature (20-25°C). Record the time of fixation and enter into the Sample Tracking System (Rave) for all submitted specimens. The optimal duration of fixation should be 16-24 h by the time it is received at the EET Biobank.
5. Secure the container lids and package containers into the shipping kit according to instructions in Section 5.5. Keep tissue in formalin jars at room temperature until shipment to the ETCTN Biorepository.

5.3.4 Collection of Biopsy to Snap-Freeze

1. Tissue should be frozen as soon as possible. Optimally, freeze within 30 minutes from resection.
2. Prior to tissue collection:
    a. Label cryovial(s) according to instructions in Section 5.4.1.
    b. Place cryovial(s) on dry ice to freeze. The vials should appear frosty when ready.
3. Immediately place tissue in foil and allow to completely freeze (using either direct contact with dry ice, or liquid nitrogen vapor).
4. Gently remove the frozen tissue from the foil. If the tissue is sticking to the foil, then gently run a finger over the back of the foil to loosen the tissue.
5. Using clean forceps place each tissue core in a separate pre-chilled cryovial. Tissue should move freely in the vial.

Place the tissue in a -70 to -80°C freezer. Keep frozen until shipment to the EET Biobank.

5.3.5 Blood Samples

Blood samples for serum, plasma, and Streck cfDNA tubes are collection at the following time points:

- Pre-treatment
- C1D8, if applicable for cohort
- C1D15
- C2D1
- C4D1
- C7D1 then every 3 cycles
- Progression

5.3.5.1 Whole blood collection in red-top tubes for serum processing

1) Label one **10 mL red-top tube** according to the instructions in Section 5.4.1.
2) Collect 10 mL of whole blood in the red-top tube.
3) Allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 hours. Process serum from red top tubes by centrifuging at 1,200 x g at 4°C for 10 minutes.
4) **Using a clean transfer pipette**, aliquot serum into pre-labeled cryovials (using the label printed from the ETCTN Specimen Tracking System or following instructions

in Section 5.4.1) at an aliquot volume of 1 mL per tube. Do not disturb the red blood cells when aliquoting by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube. Tightly secure the cap of the vials before storage. Aliquoting and freezing of serum specimens should be completed within 1 h of centrifugation.

5) Store serum cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to shipping. Do not allow specimens to thaw after freezing.

See Section 5.5 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.3.5.2 Whole blood collection in purple-top (EDTA) tubes for plasma processing

1) Label 10 mL purple-top (EDTA) tube(s) according to the instructions in Section 5.4.1.
2) Collect 10 mL of whole blood in purple-top (EDTA) tube(s).
3) After collection, gently invert tube(s) 5-10 times to ensure adequate mixing of anticoagulant.
4) Process plasma from EDTA tube at **all time points** by centrifuging at 1,200 x g at 4°C for 10 minutes.
5) Using a clean transfer pipette, aliquot plasma into pre-labeled cryovials (using the label printed from the ETCTN Specimen Tracking System or following the instructions in Section 5.4.1) at a volume of 1 mL per tube. Do not disturb the buffy coat or red blood cells when aliquoting by keeping the pipet above these layers and leaving a small amount of plasma in the tube.
6) Tightly secure the caps of the vials before storage. Aliquoting and freezing of plasma specimens should be completed within 1 h of centrifugation.
7) Store plasma cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to shipping. Do not allow specimens to thaw after freezing.

See Section 5.5 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.3.5.3 Whole blood collection in Streck cfDNA tubes

1) Label two Streck cfDNA tubes according to the instructions in Section 5.4.1.
2) Collect 18 mL of blood into the pre-labeled tube and invert to mix. **Note: blood must be thoroughly mixed to ensure preservation of specimen.**
3) After collection, blood in Streck cfDNA tubes **should never be refrigerated**, as this may compromise the specimen**.** Blood collected in Streck cfDNA tubes is stable at room temperature.

Streck cfDNA tubes must be shipped within a week of collection. See Section 5.5 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.3.5.4 Whole blood collection for PK

(Kits are provided by NorthEast Bioanalytical Laboratories, See PK sampling Manual [Appendix I]). Note that the PK sampling schedule should follow that of copanlisib, in cases of treatment delays.

1) Blood will be collected in 1 x 4 ml pre-labeled Li-Heparin BD vacutainers provided in the kit at each of the following time points:
   - C1D1
     For Copanlisib PK Only:
     - Prior to copanlisib infusion
     - 10 minutes after copanlisib infusion
     - 1 h after copanlisib infusion
     - 2 h after copanlisib infusion
     - 3 h after copanlisib infusion
     - 5 h after copanlisib infusion
     - 24 h after copanlisib infusion (before C1D2's abemaciclib and fulvestrant)
   - C1D15
     For Copanlisib and Abemaciclib PK:
     - Prior to copanlisib infusion
     - 10 minutes after copanlisib infusion
     - 1 h after copanlisib infusion (take abemaciclib right after this blood draw)
     - 2 h after copanlisib infusion
     - 3 h after copanlisib infusion
     - 5 h after copanlisib infusion
     - 24 h after copanlisib infusion (before C1D16's abemaciclib and fulvestrant dose)

     Note: Instruct patient to take abemaciclib right after the 1 h blood draw

   - C1D22
     For Abemaciclib PK
     - Prior to morning dose of abemaciclib
     - 1 h after taking abemacicliclib
     - 2 h after taking abemacicliclib
     - 4 h after taking abemacicliclib
     - 23 h (1h before C1D16's abemaciclib and fulvestrant dose)

     Note: Instruct patient to take the evening dose of abemaciclib 12 hours post the morning dose of abemaciclib.

2) Gently invert tube several times to ensure adequate anticoagulation and place on wet ice. Deliver tube to laboratory within 30 minutes of draw.
3) Centrifuge blood at 1000 x g for 10 minutes at 4°C.
4) Remove plasma using a pipette and distribute evenly between three screw-top tubes (*e.g.,* Corning 2 mL externally-threaded cryo-vial, provided in the kit) – roughly 1 mL of plasma per tube. Avoid disturbing the buffy coat layer.

5) Store plasma in a -80°C freezer within three hours of venipuncture.

See PK sampling manual and Section 5.6 for instructions for shipping to NorthEast Bioanalytical laboratories.

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: Do not redact SPID, block number, diagnosis, or relevant dates (such as collection date), and include the UPID and patient study ID on each document** (either by adding a label or hand writing)**.**

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 STS Support at STS.Support@theradex.com.

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

5.4.2 Specimen Labeling

5.4.2.1 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 (to be added by hand)
- Core number (for snap-frozen tissue and tissue cores in formalin)
- Section number (archival slides only)

5.4.2.2 Blood Specimen Labels

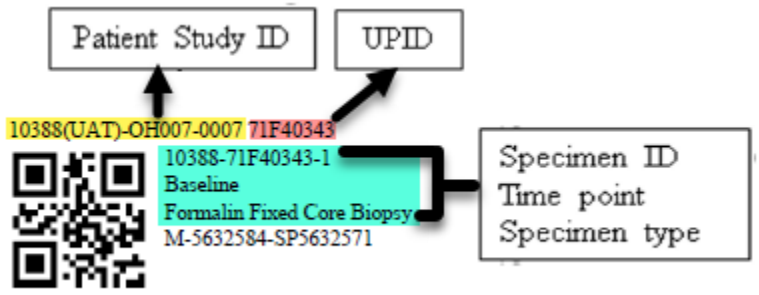
Include the following on blood specimens (including whole blood and frozen, processed blood products – like serum and plasma):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)
- Collection date (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 on each label.
- After collection, store labeled specimens as described in Section 5.3
- 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. 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) and/or the Tissue Biopsy Verification Form (Appendix J). 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 or relevant dates (such as collection date), and include the UPID and the 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 first 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 Sites to the EET Biobank

5.5.1 General Shipping Information

When kits are provided, the shipping container 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.

5.5.1.1 Required Forms for Specimen Submissions

Each document submitted with the specimen must be labeled with a label printed from the STS, or the Universal ID and Patient Study ID.

Specimen	Required Forms
Archival	1. Shipping List 2. Corresponding Pathology Report
New Biopsy	1. Shipping List 2. Tissue Biopsy Verification Form 3. Diagnostic Pathology Report 4. Operative and/or Radiology Report
Blood in Streck cfDNA tubes, plasma, and serum	1. Shipping List

5.5.2 Specimen Shipping Instructions

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

Frozen specimens and archival (FFPE) tissue 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.

5.5.2.1 Shipping of FFPE Blocks and Glass Slides

1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.1.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimens. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.

6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2.2 Shipping Blood in cfDNA Streck Tubes 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 SAFT-TEMP Gel Pack 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. Pace the SAF-T-TEMP Pak in bottom of insulated chest. **Note:** The insulated chest must be shipped inside the provided cardboard box.
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 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 below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2.3 Shipping Frozen Plasma and Serum Specimens in an Insulated Shipping Container

Serum and plasma collected at time points that do not include the collection of frozen tissue must be submitted using institutional supplies.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and matches the data on the paperwork to be included in the shipment and that lids of all primary receptacles containing liquid are tightly sealed.
2. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type and time point.
3. Place the zip-lock bags in a biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
4. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place frozen specimens in an insulated container with dry ice. Layer the bottom of the container with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with the dry ice until the container is almost completely full.
6. Insert a copy of the required forms into a plastic bag and place in the shipping container.

7. Close the shipping container and tape it shut with durable sealing tape. Do not completely seal the container.
8. Complete a FedEx air bill and attach to top of shipping container.
9. Complete a dry ice label.
10. Attach a dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
11. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2.4 Shipping Frozen and Ambient Specimens in a Dual Chambered Specimen Procurement 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 provided for time points when both ambient and frozen tissue are shipped (Pretreatment, C1D15, and Progression) and should only be 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 enough 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. If included in the shipment, formalin jar lids should be wrapped in parafilm.
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 into a plastic bag and place in the kit chamber with the ambient samples.
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 filament or other 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

5.5.2.5 Shipping Address

Ship to the address below. Ship formalin-fixed 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.

There is no central Courier account for this study. Sites are responsible for all costs for overnight shipments to the EET Biobank, utilizing the site screening and base intervention payments.

5.5.2.6 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 Specimens from Clinical Site to Other Laboratories

Please refer to the PK Sample Manual for shipment of PK samples to NorthEast Bioanalytical Laboratories. Ship to the address below.

Vipin Agarwal, PhD
925 Sherman Avenue
Hamden, CT 06514
USA
Tel: +1-203-361-3768
Fax: +1-203-407-0703
E-mail: vipin.agarwal@nebiolab.com or jonathan.quick@nebiolab.com

5.7 Biomarker Plan

List of Biomarker Assays in Order of Priority

Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing.

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
Tissue-based							
1	Whole exome sequencing (WES)	NGS CLIA: N	Integrated Mutations in *PIK3CA* or *PTEN* as predictive markers for response to PI3K inhibitors Additional exploratory analyses: To assess mutations in in candidate genes including *ESR1*, *Rb*, *TP53*, *AKT1*, *PIK3R1*, *RAS*, and others at baseline and progression and to correlate with response in each treatment arm.	DNA from FFPE Tumor Tissue	Archival, Pre-treatment (Baseline)	M M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Chris Karlovich chris.karlovich@nih.gov
2	PTEN expression	IHC CLIA: N	Integrated Loss of *PTEN* as predictive markers for response to PI3K inhibitors	Unstained slides from FFPE Tumor Tissue	Archival, Pre-treatment (Baseline)	M M	Clinical Immunohistochemistry Laboratory, MD Anderson Cancer Center (MDACC) Dr. Wei-Lien Wang wlwana@mdanderson.org
3	Reverse Phase Protein array (RPPA)	RPPA CLIA: N	Integrated Levels of phosphorylated Rb (pS807_S811), AKT (pS473), Cyclin D1: To test whether baseline AKT phosphorylation	Snap-frozen Tumor Tissue	Pre-treatment, C1D15,	M O O	Functional Proteomics RPPA Core Facility, MD Anderson Cancer Center (MDACC) Dr. Yiling Lu

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
			correlates with PFS benefit from the addition of copanlisib and whether the triplet therapy with copanlisib/fulvestrant/abemaciclib inhibits AKT phosphorylation, reduces cyclin D1 expression, and is more effective in reducing Rb phosphorylation than with the fulvestrant/abemaciclib doublet; Additional exploratory analyses: To assess baseline and treatment induced changes in in various cancer associated pathways, including but not limited to PI3K, MAPK, ER, cyclins, CDKs and CDK inhibitors,; to correlate with treatment response and progression		Disease progression		yilinglu@mdanderson.org
4	RNAseq	NGS	Exploratory Gene expression analysis: To correlate baseline and treatment induced changes in breast cancer intrinsic subtypes (PAM50), and PI3K mRNA signature and expression of candidate genes with treatment response and benefit from adding copanlisib.	RNA from snap-frozen tumor tissue	Pre-treatment, C1D15, Disease progression	O O O	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Chris Karlovich chris.karlovich@nih.gov
5	Tumor immune microenvironment (TIL)	Multi-plex IHC (CD4+, CD8+,	Exploratory To assess tumor immune microenvironment to correlate changes in infiltrating	Unstained Slides from FFPE Tumor Tissue	Pre-treatment, C1D15,	O O	Washington University Center for Human Immunology and

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
		CD3+ T cells)	lymphocytes with treatment response		Disease progression	O	Immunotherapy Programs (CHiiPs) Core Diane Bender, PhD and MT (ASCP) dianebender@wustl.edu
Blood-based							
1	Whole exome sequencing (WES)	NGS CLIA: N	Integrated Germline control for WES	Germline DNA from buffy coat in Streck cfDNA tubes	Pre-treatment (Baseline)	M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Chris Karlovich chris.karlovich@nih.gov

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
2	ctDNA sequencing (*PIK3CA*, *PTEN*, *ESR1* and others)	NGS	Exploratory To correlate ctDNA mutation profiles with tumor sequencing, and correlate baseline ctDNA mutations, particularly in components of the PI3K pathway with treatment response, and correlate early changes in ctDNA VAFs with PFS, assess emergent resistant mutations at progression	Plasma from blood in Streck cfDNA tubes	Pre-treatment C1D8, C1D15, C2D1, C4D1, C7D1 and then every 3 cycles after), Disease progression	M M M M M M M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Dr. Chris Karlovich chris.karlovich@nih.gov

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
3	Plasma and serum proteomics and metabolomics	Proteomic Analysis	Exploratory To characterize circulating markers before and after PI3K inhibitor therapy to predict treatment response and resistance mechanisms	Plasma from blood in EDTA tube & Serum from blood in red top tube	Pre-treatment C1D8, C1D15, C2D1, C4D1, C7D1 and then every 3 cycles after), Disease progression	M M M M M M M	Beth Israel Deaconess Medical Center Proteomics Core/ Broad Institute Dr. Gerburg Wulf gwulf@bidmc.harvard.edu

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
4	Copanlisib Pharmacokinetics **	LC/MS/MS	Exploratory To evaluate whether abemaciclib impacts the PK of copanlisib	Plasma from blood in Li-Heparin BD vacutainers	(i) C1D1 (a) Prior to copanlisib infusion and (b) After copanlisib infusion (10 min, 1 hr, 2 hr, 3 hr, 5 hr, 24 hr [before C1D2's abemaciclib and fulvestrant]) (ii) C1D15 (a) Prior to copanlisib infusion and (b) After copanlisib infusion (10 min, 1 hr, 2 hr, 3 hr, 5 hr, 24 hr [before C1D16's abemaciclib and fulvestrant])	M M	NorthEast Bioanalytical Laboratories Dr. Vipin Agarwal vipin.agarwal@nebiolab.com
5	Abemaciclib Pharmacokinetics **	LC/ MS/ MS	Exploratory To evaluate whether copanlisib impacts the PK of abemaciclib	Plasma from blood in Li-Heparin BD vacutainers	(i) C1D15: Use the blood collected the same time points for the	M	NorthEast Bioanalytical Laboratories Dr. Vipin Agarwal

Priority	Biomarker Name	Assay and CLIA: Y/N	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory, Lab PI, and Lab PI Email
					copanlisib PK. (ii) C1D22: Prior to, 1h, 2h, 4h, 23h post the morning dose of abemaciclib	M	vipin.agarwal@nebiolab.com

** PK samples are required for patients enrolled in the phase 1 portion.

5.8 Integrated Correlative Studies

5.8.1 Whole Exome Sequencing (WES)

Please refer to Section 2.5.1 for the testing of *PIK3CA* mutation, *PTEN* mutation as integrated biomarkers in this trial. Mutation status of *PIK3CA* and *PTEN* will be based whole exome sequencing and mutations will be called based on established methodology by the MoCha Laboratory. Redacted reports of prior clinical tumor and blood sequencing results will also be collected.

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 will be shipped to the central sequencing laboratory for analysis.

Germline DNA will be extracted from blood collected in Streck cfDNA tubes at pre-treatment (baseline) following plasma processing. DNA will be quantitated. An aliquot of germline DNA will be shipped to the central sequencing laboratory for analysis.

5.8.1.2 Sites Performing Correlative Study

MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the direction of Dr. Chris Karlovich (chris.karlovich@nih.gov).

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:

MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
Attn: Alyssa Chapman or Ruth Thornton
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702

5.8.1.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov.

5.8.2 PTEN Immunohistochemistry

5.8.2.1 Specimens Receipt and Processing at the EET Biobank

Upon receipt, archival FFPE tissue blocks and slides will be accessioned, barcoded, and stored at room temperature. Tissue received in formalin will be processed and embedded in paraffin, and stored as an FFPE tissue block. Prior to distribution, the EET Biobank will prepare unstained slides for this assay. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide to be provided, either digitally or physically, with unstained slides. Unstained, charged, air-dried 4-micron slides will be labeled appropriately and shipped under ambient conditions to the MD Anderson Clinical Immunohistochemical Laboratory. When possible, slides should be prepared on the planned day of shipping.

5.8.2.2 Sites Performing Correlative Study

PTEN IHC will be performed under the CLIA CAP provision at MD Anderson Clinical Immunohistochemistry Laboratory.

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:
Wei-Lien Wang, M.D.
Department of Pathology
The University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd Unit 085
Room B3.4611
Houston, TX 77030

5.8.2.4 Contact Information for Notification of Specimen Shipment

Wei-Lien Wang, M.D., wlwang@mdanderson.org

5.8.3 RPPA

5.8.3.1 Specimens Receipt and Processing at the EET Biobank

Upon receipt at the Biorepository, the snap-frozen tissue will be accessioned, barcoded, and banked in a liquid nitrogen vapor phase freezer until distribution for testing.

Snap-frozen tissue will be shipped from the EET Biobank to the RPPA Core lab at MD Anderson.

5.8.3.2 Site Performing Correlative Study

This correlative study will be performed at MD Anderson RPPA Core.

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:
MD Anderson Cancer Center

6565 MD Anderson Blvd, Rm Z4.2040
Houston, TX 77030

5.8.3.4 Contact Information for Notification of Specimen Shipment

Doris Siwak, PhD, dsiwak@mdanderson.org

5.9 Exploratory/Ancillary Correlative Studies

5.9.1 Gene expression by RNA Seq

5.9.1.1 Specimens Receipt and Processing at the EET Biobank

Snap frozen tissue or fresh tumor in formalin (if insufficient material from snap frozen tissue) will be embedded and sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

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

5.9.1.2 Site Performing Correlative Study

RNASeq will be performed at MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the direction of Dr. Chris Karlovich (chris.karlovich@nih.gov).

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

Specimens will be shipped from the EET Biobank to:

MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
Attn: Alyssa Chapman or Ruth Thornton
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702

5.9.1.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov.

5.9.2 ctDNA sequencing

5.9.2.1 Specimens Receipt and Processing at the EET Biobank

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

Note that buffy coat will be saved from the baseline Streck cfDNA tube for germline DNA.

5.9.2.2 Site Performing Correlative Study

At the end of the study, plasma aliquots from cfDNA Streck tubes will be distributed for testing. ctDNA will be performed at MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the direction of Dr. Chris Karlovich (chris.karlovich@nih.gov).

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:

MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
Attn: Alyssa Chapman or Ruth Thornton
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702

5.9.2.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov.

5.9.3 Plasma and serum proteomics and metabolomics

5.9.3.1 Specimens Receipt and Processing at the EET Biobank

Upon receipt at the Biorepository, serum and plasma will be accessioned, barcoded, and banked in a -80°C freezer.

5.9.3.2 Site Performing Correlative Study

At the end of the study, the EET Biobank will distribute serum and plasma processed from EDTA. This correlative study will be performed at the Broad Institute and other laboratories if needed.

5.9.4 Copanlisib and abemaciclib pharmacokinetics

5.9.4.1 Specimens Receipt and Processing at Northeast Bioanalytical Laboratories

Blood samples for PK analysis will be further processed and analyzed using MS using established protocol at the Northeast Bioanalytical Laboratories.

5.9.4.2 Site Performing Correlative Study

This correlative study will be performed at Northeast Bioanalytical Laboratories.

5.9.5 Tumor immune microenvironment (TILs)

5.9.5.1 Specimens Receipt and Processing at the EET Biobank

Upon receipt, archival FFPE tissue blocks and slides will be accessioned, barcoded, and stored at room temperature. Tissue received in formalin will be processed and embedded in paraffin, and stored as an FFPE tissue block. Prior to distribution, the EET Biobank will prepare unstained slides for this assay. All FFPE blocks will be sectioned to generate an initial H&E-stained slide to be provided, either digitally or physically, with unstained slides. Unstained, charged, air-dried 4-micron slides will be labeled appropriately and shipped under ambient conditions to the Washington University CHiiP Core or other performing laboratories When possible, slides should be prepared on the planned day of shipping.

5.9.5.2 Site Performing Correlative Study

IHC analysis will be performed to identify CD4+, CD8+, CD3+ T cells according to SOP established at the Washington University CHiiP Core. This correlative study will be performed at Washington University CHiiP Core or other laboratories if needed.

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:
Diane Bender, PhD and MT (ASCP)
Washington University School of Medicine
425 S. Euclid Ave, BJC-IH Room 7105
St. Louis, MO 63110

5.9.5.4 Contact Information for Notification of Specimen Shipment

Diane Bender, PhD and MT (ASCP), dianebender@wustl.edu

6. TREATMENT PLAN

This trial will start with the Phase 1 portion. At the start of study, this was intended to be followed by the randomized Phase 2 portion; however, due to Bayer's withdrawal of the NDA for copanlisib, the study is closing with Amendment 8d and will not continue to Phase 2.

Note that premenopausal women need to receive GnRH agonist goserelin 3.6 mg SC Q28 days on trial. Patients on Q3 month dosing of goserelin prior to study entry should switch to monthly dosing with their next scheduled dose.

6.1 Agent Administration

Phase 1

Treatment will be administered on an outpatient basis. 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.

Potential dose levels for Part A:

Dose Level	Dose*		
	Copanlisib	**Abemaciclib#**	**Fulvestrant#**
Level 4	60 mg IV on Days 1, 8 and 15	150 mg PO BID	500 mg IM on C1D2, C1D16, and D1 of every cycle** thereafter
Level 3	45 mg IV on Days 1, 8 and 15	150 mg PO BID	
Level 2	45 mg IV on Days 1, 8 and 15	100 mg PO BID	
Level 1 (Starting dose)	45 mg IV on Days 1 and 15	100 mg PO BID	
Level -1	30 mg IV on Days 1 and 15	100 mg PO BID	

IV = intravenous, PO = by mouth, BID = twice a day, IM = intramuscular

*Doses are stated as exact dose in units (*e.g.,* mg/m^2, mcg/kg, *etc.*) rather than as a percentage. Additional dose levels may be tested after discussion with NCI and Bayer.
**Cycle length is 28 days (4 weeks)
#**The first dose (C1D1) of abemaciclib and fulvestrant is delayed to C1D2 following the 24-h blood draw for copanlisib PK. C1D15 fulvestrant dose is delayed to C1D16 following the 24-h blood draw for copanlisib PK.**

Notes:

- C1D16 dose of fulvestrant could be omitted in patients on stable doses of fulvestrant prior to enrolling on this study.
- Copanlisib will be administered IV over 1 h (+/- 10 min).
- Copanlisib infusion should be scheduled early in the morning since patients should take the morning dose of abemaciclib at least 1 h after completion of the copanlisib infusion. This will limit any potential pharmacokinetics interactions between the copanlisib and abemaciclib. The evening dose of abemaciclib should be taken 12 h after the morning dose. If it is not possible to schedule copanlisib early in the morning, it should be scheduled in such a way that the abemaciclib doses may be taken 12 h apart, and that at least 1 h elapses after the end of the copanlisib infusion before a dose of abemaciclib is taken (This is only applicable on days that PKs are drawn. On non-PK days the patient may take their abemaciclib at home.).
- Premenopausal women will receive GnRH agonist goserelin 3.6 mg SC Q28 days on trial.

Potential dose levels for Part B:

Dose Level	Dose*		
	Copanlisib	**Abemaciclib[#]**	**Fulvestrant[#]**
Level 4b	60 mg IV Days 1, 8 and 15	150 mg PO BID 5 days on and 2 days off each week	500 mg IM on C1D2, C1D16, and D1 of every cycle** thereafter
Level 3b	45 mg IV Days 1, 8 and 15		
Level 2b	45 mg IV Days 1, 8 and 15	100 mg PO BID 5 days on and 2 days off each week	
Level 1b (starting dose)	45 mg IV Days 1 and 15		

IV = intravenous, PO = by mouth, BID = twice a day, IM = intramuscular

*Doses are stated as exact dose in units (*e.g.,* mg/m^2, mcg/kg, *etc.*) rather than as a percentage. Cycle length is 28 days (4 weeks).

[#]**The first dose (C1D1) of abemaciclib and fulvestrant is delayed to C1D2 following the 24-h blood draw for copanlisib PK. C1D15 fulvestrant dose is delayed to C1D16 following the 24-h blood draw for copanlisib PK.**

Notes:

- C1D16 dose of fulvestrant could be omitted in patients on stable doses of fulvestrant prior to enrolling on this study.
- Copanlisib will be administered IV over 1 h (+/- 10 min).
- Copanlisib infusion should be scheduled early in the morning, since patients should take the morning dose of abemaciclib at least 1 h after completion of the copanlisib infusion. This will limit any potential pharmacokinetics interactions between the copanlisib and abemaciclib. The evening dose of abemaciclib should be taken 12 h after the morning dose. If it is not possible to schedule copanlisib early in the morning, it should be scheduled in such a way that the abemaciclib doses may be taken 12 h apart, and that at least 1 h elapses after the end of the copanlisib infusion before a dose of abemaciclib is taken. (This is only applicable on days that PKs are drawn. On non-PK days the patient may take their abemaciclib at home.)
- Premenopausal women will receive GnRH agonist goserelin 3.6 mg SC Q28 days on trial.

Patients will be requested to maintain a medication diary (Appendix H) of each dose of abemaciclib. The medication diary will be returned to clinic staff at the end of each course.

6.1.1 Copanlisib

Based on the company-sponsored studies with copanlisib in patients with oncologic malignancies, the RP2D of copanlisib monotherapy is 60 mg administered by 1 h (+/- 10 min) IV infusion weekly for 3 weeks (Days 1, 8, and 15) every 4 weeks. The starting dose for copanlisib

is 45 mg administered IV on Days 1 and 15 every 4 weeks. The use of corticosteroids as antiemetics prior to copanlisib administration is not allowed.

Administer copanlisib as an IV infusion over one hour (+/- 10 min). After administration, flush the line with 0.9 % sodium chloride to ensure complete dose is given. No IV glucose preparations should be administered on the days of infusion.

Patients who do not meet the pre-dose glucose level may be treated the same day or the next day with copanlisib if hyperglycemia is treated and the glucose level comes down to within the acceptable pre-dose level. Patients whose glucose level does not come down to within the acceptable pre-dose level by the next day will not receive that dose of copanlisib. Modification of doses of copanlisib due to hyperglycemia is listed in Section 7.

Recommendations on meal timing on copanlisib infusion days

Because of an inhibitory effect on PI3Kα-isoform, which is implicated in insulin metabolism, copanlisib infusions could be associated with temporarily increase in blood glucose. Consuming meal in close proximity to copanlisib infusion may exacerbate a glucose level increase. It is recommended that timing and content of caloric intake on infusion days is monitored by the investigators. Consultation with diabetologist or endocrinologist is advised**.**

The investigator may manage the timing of post-infusion meals based on the glucose profile during prior infusion days to minimize glucose increases. This is in addition to glucose lowering medication. On infusion days, a low carbohydrate diet is recommended. The timing and content of meal intake, and additional glucose testing (if clinically indicated), should be managed and monitored by the investigators based on glucose response patterns during prior treatment days. However, caloric restriction is not intended for the population under study. Refer to Appendix D for glycemic indices of common foods.

NOTE: If patient needs to take a low glycemic meal, then glucose test should be taken prior to meal intake and at 1 and/or 2 h after the meal. All glucose measurements, oral glucose lowering medication and/or insulin administration, if applicable, and meal timing will be collected as part of the clinical source documentation.

NOTE: Caloric intake and timing recommendations for diabetic patients who require insulin treatment prior to the infusion at any cycle visit should be managed by the investigator based on consultation with treating physician or diabetes/endocrinologist physician.

Pre-dose glucose levels

Period	Pre-dose glucose levels (first glucose measurement)
Day 1 of cycle 1	<160 mg/dL (fasting*) < 200 mg/dL (non-fasting**)
Subsequent infusions after Cycle 1 Day 1	<160 mg/dL (fasting*) < 200 mg/dL (non-fasting**)

*Fasting refers to a ≥ 8 h fast.
**Non-fasting status includes any caloric intake such as meals and also juice, snacks, and other caloric intake not consistently called a meal.

Cycle 1 Day 1:
On infusion days a low carbohydrate diet is recommended, the timing and content of meal intake and additional glucose testing (if clinically indicated) is managed and monitored by the investigators based on glucose response patterns during prior treatment days.

Day 1 of Cycle 2+:
Fasting is not required before the start of the infusion. A low carbohydrate meal may be taken at least 4 h before the start of the study drug infusion for patients who have their infusions scheduled at a later hour, or due to their age or medical condition when fasting prior to infusion is not viable.

Day 8 and Day 15 of each cycle:
Fasting is not required before the start of infusion. A low carbohydrate meal may be taken at least 4 h before the start of the study drug infusion for patients who have their infusions scheduled at a later hour or due to their age or medical condition when fasting prior to infusion is not viable.

Glucose monitoring is required before and after each copanlisib infusion. The glucose testing is scheduled as follows:

- On Cycle 1 Day 1: Glucose test is performed before starting copanlisib IV infusion at time 0 h, at the end of the infusion (1 h after starting infusion), 1 h after completing the infusion, and 2 h after completing the infusion. For fasting requirements refer to the table above.
- On Cycle 1 Days 8 and 15 and all subsequent cycles: Glucose test is performed before starting copanlisib IV infusion at time 0 h. Post-dose glucose monitoring after C1D1 is performed as clinically indicated at the investigator's discretion.

6.1.2 Abemaciclib

Abemaciclib will be taken by mouth at approximately the same time twice a day continuously (Days 1-28 of each 28-day cycle) or on a 5 days on and 2 days off each week schedule (Days 1-5, 8-12, 15-19, and 22-26 of each 28-day cycle). Schedule will be dictated in Phase 1 by enrollment to Part A or Part B.
Note that in Phase 1 Regimen, abemaciclib is delayed to Day 2 of Cycle 1.

Abemaciclib will be taken at the assigned dose (for patients randomized to FAC) or at 150 mg BID (for patients randomized to FA).

If a patient misses a day's dose entirely, he/she must be instructed not to make it up at the next dose but just take her regular dose at the next assigned time. If a patient vomits any time after taking a dose, she must be instructed not to retake the dose but resume subsequent dosing at the next assigned time. If a patient inadvertently takes an extra dose during the day, she must be instructed to not take the next day's dose. Patients are to swallow tablets whole (do not chew, crush, or spit them prior to swallowing).

Instruct patients at the first sign of loose stools to initiate antidiarrheal therapy such as loperamide (4mg PO x 1, then 2mg after each loose stool, maximum 16 mg/day; loperamide prophylaxis per physician discretion is allowed), increase oral fluids, and notify their healthcare provider.

6.1.3 Fulvestrant

Fulvestrant will be administered at a dose of 500 mg as two IM injections (250 mg each into each buttock) on Days 1 and 15 of Cycle 1, and then on Day 1 of every cycle thereafter.
Note that in the Phase 1 Regimen and in the first 4 patients randomized to FAC in the Phase 2 Regimen, fulvestrant administration is delayed to Days 2 and 16 of Cycle 1. Note that C1D16 dose of fulvestrant could be omitted in patients on stable dose of fulvestrant prior to enrolling to the study.

6.2 Definition of Dose-Limiting Toxicity

6.2.1 Dose Limiting Toxicities (DLTs)

Dose-limiting toxicity is defined as adverse events occurring during the first 28 days of treatment that are determined to be at least possible related to treatment using NCI-CTCAE v5.0 and that meet the following criteria:

- Any death not clearly due to the underlying disease or extraneous causes
- Hematology:
  - Febrile neutropenia
  - Grade ≥4 thrombocytopenia (regardless of duration)
  - Grade ≥3 thrombocytopenia with bleeding
  - Grade 4 neutropenia lasting more than 7 days
- Hyperglycemia: persistent occurrence of blood glucose >250 mg/dL (Grade ≥3) for 72 hours despite optimal glucose lowering therapy.
- Grade 3 or above non-hematologic toxicity with the following exceptions:

  - Transient infusion-related hyperglycemia lasting <7 days
  - Transient infusion-related hypertension responsive to intervention.
  - Alopecia of any grade
  - Grade ≥3 fatigue < 1 week
  - Grade ≥3 amylase or lipase elevation NOT associated with symptoms or clinical manifestations of pancreatitis
- Grade ≥3 nausea/vomiting or diarrhea >72 hours with adequate antiemetics and other supportive care.
- Hy's law
- Grade ≥3 electrolyte abnormality that lasts >72 hours, unless the patient has clinical symptoms, in which case all grade 3+ electrolyte abnormality regardless of duration should count as a DLT
- For patients with hepatic metastases, AST or ALT >8x ULN or AST or ALT >5x ULN for ≥14 days.
- Failure to receive 2 copanlisib infusions during the first 28 days
- Failure to receive at least 3 of the 4 weeks of abemaciclib scheduled doses for the first 28 days
- All AEs of the specified grades should count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes.

Note that patients who have major protocol deviations (as determined by the study chair and the study statistician) in the dose and/or schedule of the study drugs during Cycle 1 are not evaluable for DLT. These patients may be evaluable for overall AE and efficacy endpoints per statistical section.

6.2.2 Dose Escalation Criteria

Dose escalations/de-escalations will proceed continuously based on the CRM estimated DLT rate at each dose level (see Section 9).

Management and dose modifications associated with the above adverse events are outlined in Section 7.

6.3 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of copanlisib with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The known potential targets for drug interaction are CYP3A4 inducers or inhibitors, as well as drugs modulating MATE2K function. Concomitant use of medications listed in Appendix B is prohibited while on copanlisib. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix C (Patient Wallet Card) should be provided to patients if available.

- **Antihistamine administration, such as daily oral cetirizine, is required during the**

entire course of treatment with copanlisib to decrease the incidence and severity of rash.

- Sensitive substrates of the renal drug transporter MATE2K (*e.g.*, metformin) need to be used with caution. Metformin should be interrupted for 48 h after receiving iodinated contrast media. Please see prescribing information for further information.

- Patients taking medications with narrow therapeutic indices should be proactively monitored if these medications cannot be avoided. These medications may include, but are not limited to quinidine, cyclosporine, and digoxin.

- Systemic corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not permitted while on study. Previous corticosteroid therapy must be stopped or reduced to the allowed dose at least 7 days prior to the CT/MRI screening. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose before the screening. Patients may be using topical or inhaled corticosteroids. Short-term (up to 7 days) systemic corticosteroids above 15 mg prednisolone or equivalent will be allowed for the management of acute conditions (*e.g.*, treatment non-infectious pneumonitis). The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

- Patients should stop using herbal medications at least 7 days prior to the first dose of copanlisib. Herbal medications include, but are not limited to St. John's Wort, Kava, ephedra, gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, black cohosh, and ginseng.

- Prophylactic antiemetics may be administered according to standard practice. The routine use of standard antiemetics, including 5-HT3 blockers such as granisetron, ondansetron, or equivalent agents, is allowed as needed.

- Palliative radiation or other measures to non-target lesions for pain control is allowed while on study after consult with study PI. Patients should hold abemaciclib and copanlisib during radiation course.

While taking abemaciclib, patients should be instructed to avoid food or drugs that are known strong CYP3A4 inhibitors or inducers, including grapefruit and grapefruit juice. Please refer to Appendix B for a list of prohibited medications.

Patients should also not take any medications with the potential to prolong the QT interval.

6.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue indefinitely 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 render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing 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

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

6.5 Duration of Follow-Up

Patients will be followed every 3 months for 5 years after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 Copanlisib

Dose Levels for Dose Modification	Copanlisib
1	60 mg IV days 1, 8, 15
-1	45 mg IV days 1, 8, 15
-2	45 mg IV days 1, 15
-3	30 mg IV days 1, 15

If a dose reduction beyond 30 mg on D1 and 15 of each cycle is required, the patient will discontinue copanlisib.

7.1.1 Dose Modification rules for transient post-infusion hyperglycemia

Patients who develop transient post-infusion glucose >250 mg/dL after study drug administration may continue treatment. However, the next infusion must be delayed until the patient's pre-infusion glucose levels return to <160 mg/dL (fasting) or <200 mg/dL (non-fasting). Guidelines for the management of transient glucose increases are given in Appendix E and Appendix F. Continuing occurrence of post-infusion blood glucose >400 mg/dL, based on repeated laboratory

analysis despite optimal glucose lowering therapy after 2 infusions of copanlisib, will require dose reduction by one dose level.

- Further dose reduction (where appropriate per study design/population) is allowed as long as discontinuation criteria was not met.
- Dose re-escalation is allowed when a patient has achieved controlled glucose levels per investigator's judgment.
- Persistent occurrence of post-infusion blood glucose >400 mg/dL based on laboratory analysis which occurred at the lowest dose level despite optimal glucose lowering therapy (after at least one cycle of treatment) with consultation of a diabetes specialist requires permanent discontinuation of the study drug. Grade 4 hyperglycemia for any duration requires discontinuation of copanlisib.

7.1.2 Treatment of blood pressure increases associated with copanlisib

It is important that patients with pre-existing arterial hypertension adhere to their regular medication schedule and take their usual doses on the days of study drug infusion.

The management of acute blood pressure (BP) increases following copanlisib will need to be individualized for each patient, but experience from a Bayer-sponsored Phase 1 study with copanlisib has suggested the benefit of dihydropyridine calcium channel blockers (*i.e.,* amlodipine, felodipine). Topical nitrates should also be considered. In general, it is advisable for sites to be prepared, so that anti-hypertensive medication is readily available in case of need.

In the event of the occurrence of arterial hypertension ≥150/90 mmHg (either systolic or diastolic) during infusion of copanlisib at any cycle, antihypertensive treatment is suggested as indicated in Appendix G. In the event of the occurrence of grade 3 arterial hypertension (≥160/100 mmHg) during infusion of copanlisib, the infusion should be interrupted, and anti-hypertensive treatment as suggested above is administered. Infusion can be resumed when BP has returned to <150/90 mmHg (both systolic and diastolic).

Blood pressure measurement on treatment days

Blood pressure will be measured every 5-10 min prior to each copanlisib dose (no more than 4 measurements) until there are two consecutive results <150/90 mmHg (both systolic and diastolic). If blood pressure is ≥150/90 mmHg, the investigator can consider a medical intervention or delaying the infusion of study drug. The patient should rest for 5-10 min before blood pressure is recorded.

On infusion days, blood pressure will be measured at 0 h (pre-dose), 30 min (mid-infusion), 60 min (end of infusion), and 1 h and 2 h after the end of infusion. In patients who have not had any episodes of post infusion blood pressures ≥150/90 mmHg for at least 2 consecutive cycles of treatment, the blood pressure monitoring at 1h and 2h could be omitted at the discretion of the treating physician.

NOTE: A window of ±10 min is allowed for all BP measurements, except for pre-dose (0 h)

measurement.

7.1.3 Non-infectious pneumonitis

The investigator is requested to differentiate between non-infectious pneumonitis, and infectious pneumonitis (viral, bacterial, or fungal), aspiration pneumonitis, or other pneumonitis clearly not due to a potential hypersensitivity reaction to the copanlisib infusion; and provide the basis for his/her assessment that it is infectious or other, as appropriate. The investigator is requested to report with the most specific clinical terms to describe the condition, not simple "pneumonitis".

In the event of suspected non-infectious pneumonitis, modify copanlisib treatment as per table below.

Dose adjustment for non-infectious pneumonitis

Suspected or confirmed NIP per CTCAE	**Action Taken**	**Re-treatment dose after recovery**
Grade 1	No Change	NA
Grade 2	Dose Interruption Until recovery to ≤grade 1	Decrease dose to the next lowest dose level[a]
Grade 2 second re-occurrence	Permanent Discontinuation	NA
Grade 3	Permanent Discontinuation	NA
Grade 4	Permanent Discontinuation	NA

NA = Not applicable; NIP = Non-infectious pneumonitis; CTCAE = Common Terminology Criteria for Adverse Events.
a: Not applicable for 45 mg dose level. No re-escalation is allowed after the dose reduction.
The lowest dose level is 45 mg; if a patient is already on the 45 mg dose level and cannot tolerate treatment study treatment will be discontinued permanently

7.1.4 Hematologic Toxicities

Toxicities	**Adverse Reaction Grade**	**Management**
Neutropenia	ANC Grade 3	Maintain or hold copanlisib at physician discretion, monitor ANC weekly.
	ANC Grade 4	Hold copanlisib, monitor ANC weekly until ANC recovers to grade 3 or lower. If ANC grade 4 recurs, reduce copanlisib by 1 dose level.
Thrombocytopenia	Less than 25k	Hold copanlisib; resume when platelet levels return to 75k or greater. If recovery occurs within 21 days, reduce one dose level. If recovery does not occur within 21

		days, discontinue copanlisib.

7.1.5 Other severe and non-life-threatening toxicities

Toxicities	**Adverse Reaction Grade**	**Management**
Other severe and non-life-threatening toxicities	Grade 3	Hold copanlisib until toxicity is resolved to at least grade 1, then reduce by 1 dose level.

7.1.6 Retreatment Criteria for Copanlisib

A new cycle of treatment with copanlisib may start only if:

- ANC ≥500/mcL
- Platelet count ≥75,000/mcL
- Glucose less than 160 mg/dL fasting or 200 mg/dL non-fasting.
- Arterial blood pressure <150/90 mmHg (both systolic and diastolic)
- Copanlisib related non-hematologic toxicities have returned to baseline or grade ≤1 severity (or, at the investigator's discretion, grade ≤2 if not considered a safety risk for the patient)

Criteria for dose interruption for copanlisib within cycle:

- Glucose >160, may start if controlled on the day or the day after.
- BP >150/90, may start if controlled with antihypertensives.
- Copanlisib related non-hematologic toxicities have not returned to baseline or grade ≤1 severity (or, at the investigator's discretion, grade ≤2 if not considered a safety risk for the patient)

Note: If dose interruption for copanlisib is necessary, it is preferred that the dose of copanlisib be delayed rather than missed to maximize copanlisib dosing. However, copanlisib dosing schedule could be adjusted in subsequent cycles so to align with the fulvestrant administration, with the condition that no more than 2 consecutive weekly doses of copanlisib are administered. Cycle length is 28+/- 7 days. A maximum duration to hold copanlisib is 3 consecutive weeks including the scheduled weeks off despite dose reduction to the lowest dose level.

7.2 Abemaciclib

Patients will be monitored for toxicity and the dose of abemaciclib may be adjusted as indicated in the dose modification table below.

Dose Levels for Dose Modification	**Abemaciclib Dose**
2	150 mg BID[1]
1	100 mg BID[1]
-1	50 mg BID continuously

[1] patients who start on a 5 days on and 2 days off schedule will continue the same intermittent schedule of abemaciclib at 100 mg or 150 mg dosing.

Recommended dose reductions for abemaciclib are detailed in the table above. Doses may be held as needed for toxicity resolution during a cycle. Cycle length is dictated by that of copanlisib.

7.2.1 Abemaciclib Dose Modifications – Hematologic Toxicities

Adverse Reaction Grade	**Management**
Grade 1 or 2	No dose modification
Grade 3	Hold dose until toxicity resolves to ≤grade 2. Dose reduction is not required.
Grade 3 recurrent, or grade 4	Hold dose until toxicity resolves to ≤grade 2. Resume at next lower dose.

7.2.2 Abemaciclib Dose Modification - Diarrhea

At the first sign of loose stools, start treatment with antidiarrheal agents and increase intake of oral fluids.	
Adverse Reaction Grade	**Management**
Grade 1	No dose modification
Grade 2	If toxicity does not resolve within 24 h to ≤grade 1, hold dose until resolution. No dose reduction is required.
Grade 2 that persists or recurs after resuming the same dose despite maximal supportive measures	Hold dose until toxicity resolves ≤grade 1. Resume at next lower dose.
Grade 3 or 4 or requires hospitalization	Hold dose until toxicity resolves to ≤grade 1. Resume at next lower dose.

7.2.3 Abemaciclib Dose Modification – Hepatoxicity

Adverse Reaction Grade	**Management**
Grade 1 (>ULN-3.0 x ULN) or grade 2 (>3.0-5.0 x ULN), WITHOUT increase in total bilirubin above 2 x ULN	No dose modification
Persistent or recurrent grade 2, or grade 3 (>5-20 x ULN), WITHOUT increase in total bilirubin above 2 x ULN	Hold dose until toxicity resolves to ≤grade 1. Resume at next lower dose.
Elevation in AST and/or ALT >3 x ULN WITH total bilirubin >2 x ULN, in the absence of cholestasis	Discontinue abemaciclib
Grade 4 (>20.0 x ULN)	Discontinue abemaciclib

7.2.4 Abemaciclib Dose Modification – Other Toxicities

Adverse Reaction Grade	Management
Grade 1 or 2	No dose modification
Persistent or recurrent grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or grade 1	Hold dose until toxicity resolves to ≤grade 1. Resume at next lower dose.
Grade 3, or grade 4	Hold dose until toxicity resolves to ≤grade 1. Resume at next lower dose.

If these conditions are not met, fulvestrant and copanlisib may be continued but treatment with abemaciclib must be delayed by one week. If, after a one-week delay, all toxicities have recovered within the limits described above, treatment with abemaciclib can be resumed.

If the patient has not recovered after 3 weeks despite dose reduction to the lowest dose level, treatment with abemaciclib will be permanently discontinued.

7.3 Fulvestrant

No dose adjustments are permitted for fulvestrant, but interruptions are allowed at the discretion of the treating physician.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent

8.1.1 Material Safety Data Sheets

The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.2 Copanlisib (NSC 784727)

Chemical Name or Amino Acid Sequence: 2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamide dihydrochloride

Other Names: BAY 80-6946 (free base); BAY 84-1236 (dihydrochloride salt)

Classification: Pan class I PI3K inhibitor

Molecular Formula: C23H28N8O4 2HCl **M.W.:** 553.45 g/mol

Approximate Solubility: Freely soluble in water and 0.1 M hydrochloric acid (HCl)

Mode of Action: Copanlisib is a pan class I PI3K inhibitor with potent activity against the delta and alpha isoforms. Class I PI3K is downstream of most cancer associated tyrosine kinase growth factor receptors or mesenchymal epithelial transition factor. PI3K delta has a critical role in regulating downstream events of the B-cell receptor.

Description: The powder is white to yellow solid substance.

How Supplied: Copanlisib is supplied by Bayer HealthCare AG and distributed by the Pharmaceutical Management Branch, CTEP, DCTD, NCI. The agent is available as a lyophilized product containing 60 mg of copanlisib in a 6 mL injection vial. The excipients are mannitol, sodium hydroxide, citric acid, and water for injection.

Preparation: Using appropriate aseptic technique, reconstitute the 60 mg vial of copanlisib with 4.4 mL of 0.9% sodium chloride resulting in a concentration of 15 mg/ml. Gently shake for 30 seconds and allow the vial to stand for 1 minute to let bubbles rise to the surface. Repeat if undissolved substance is still present. The reconstituted solution may be slightly yellow and should be clear prior to being withdrawn from the vial. Withdraw the appropriate volume of the reconstituted solution and further dilute by adding to a 50-200 mL sterile 0.9% sodium chloride bag. Mix well by inverting.

Storage: Store intact vials between 2°C and 8°C.

> If a storage temperature excursion is identified, promptly return copanlisib to between 2°C and 8°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 studies of the vials are ongoing. The diluted solution should be used immediately (stored up to 4 hours at room temperature including preparation and administration). If the diluted solution for infusion is not used immediately, it is stable for up to 24 hours refrigerated between 2 °C and 8 °C. It takes approximately 60 minutes for the diluted solution to return to room temperature after refrigeration. The infusion should be completed within 24 hours of preparation.

CAUTION: The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded 4 hours after initial entry.

Route of Administration: IV infusion

Method of Administration: The diluted solution for infusion is administered IV over 1 hour. After administration, flush the line to ensure complete dose is given. No IV glucose preparations

should be administered on the days of infusion.

Potential Drug Interactions: *In vitro*, copanlisib is metabolized primarily via CYP3A4 and to a minor extent by CYP1A1. It is also a substrate of P-gp and BCRP, but not a substrate of MATEs, OCTs, OATs, or OATPs. Concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided. Use caution when administered with strong inhibitors and inducers of CYP1A1, P-gp, and BCRP.

In vitro, copanlisib is a strong inhibitor of MATE2K. Copanlisib and its metabolite M-1 have a low risk for inhibition or induction of CYP isoforms, inhibition of UGT isoforms, and inhibition of dihydropyrimidine dehydrogenase. Copanlisib does not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, bile salt export pump (BSEP), MRP2, or MATE1 at therapeutic 60 mg dose plasma concentrations. Use caution when administered with sensitive drug substrates of MATE2K.

Copanlisib is not an inducer of CYP1A2, 2B6, and 3A.

Special Handling: Copanlisib is not genotoxic *in vitro* or *in vivo*. Copanlisib is expected to adversely affect male and female reproduction.

Patient Care Implications: Females of child-bearing potential and male patients must use adequate contraception while receiving copanlisib and for 1 month after last dose of copanlisib. Do not breastfeed during treatment with copanlisib and for at least 1 month after the last dose of copanlisib.

Hypertension is frequently observed within the first 3 h after start of infusion and hyperglycemia is frequently observed persisting for approximately 1-3 days after study drug administration. Refer to protocol document for treatment and monitoring guidelines.

Availability

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

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

8.1.3 Agent Ordering and Agent Accountability

8.1.3.1 NCI supplied agents

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.

Subjects must be enrolled prior to submitting the agent request to PMB.

Submit agent requests through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems 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.

8.1.3.2 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.

Product Quality Complaint (PQC): A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.4 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems 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.

8.1.5 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 AURORA application: https://ctepcore.nci.nih.gov/aurora/login
- CTEP Identity and Access Management (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 Agents

8.2.1 Abemaciclib (NSC 783671)

Chemical Name: 2-Pyrimidinamine, N-[5-[(4-ethyl-1-piperazinyl)methyl]-2-pyridinyl]-5-fluoro-4-[4-fluoro-2-methyl-1-(1-methylethyl)-1H-benzimidazol-6-yl]

Other Names: Verzenio™.

Classification: Cyclin-dependent kinase 4 and 6 (CDK4 and CDK6) inhibitor.

Molecular Formula: $C_{27}H_{32}F_2N_8$ **M.W.:** 506.59 g/mol

Mode of Action: Abemaciclib inhibits CDK4 and CDK6 pending phosphorylation of retinoblastoma protein (Rb), cell cycle progression and cell proliferation in ER+ breast cancer.

Product description: Abemaciclib is available as immediate release tablets packaged in blister packs containing 14 tablets of the following strengths:
- 150 mg tablets are oval yellow tablet with “Lilly” debossed on one side and “150” on the other side.
- 100 mg tablets ate oval white to practically white tablet with “Lilly” debossed on one side and “100” on the other side.
- 50 mg tablets are oval beige tablet with “Lilly” debossed on one side and “50” on the other.

Storage requirements: Store at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F).

Route of administration: Abemaciclib is taken by mouth. Patients should be instructed to swallow tablets whole and not to chew, crush, or split them prior to swallowing. No tablets should be ingested if it is broken, cracked, or otherwise not intact. Patients should take abemaciclib with or without food and should be encouraged to take their dose at approximately the same times each day.

Agent Ordering: Abemaciclib is commercially available and will be billed to the patient or her insurance.

8.2.2 Fulvestrant (NSC 719276)

Chemical Name: 7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)-triene-3,17 beta-diol.

Other Names: Faslodex®.

Classification: Cyclin-dependent kinase 4 and 6 (CDK4 and CDK6) inhibitor.

Molecular Formula: $C_{32}H_{47}F_5O_3S$ **M.W.:** 606.77 g/mol

Mode of Action: Fulvestrant is an ER antagonist that binds to the ER in a competitive manner with affinity comparable to that of estradiol and downregulates the ER protein in human breast cancer cells.

Product description: Fulvestrant is indicated for the treatment of hormone receptor positive metastatic breast cancer in postmenopausal women with disease progression following antiestrogen therapy, and the treatment of hormone receptor positive, HER2-negative advanced or metastatic breast cancer in combination with palbociclib in women with disease progression after endocrine therapy. Fulvestrant is available as two 5 mL clear neutral glass (type 1) barrels, each containing 250 mg/5 mL of fulvestrant solution for IM injection and fitted with a tamper evident closure. The solution for injection is a clear, colorless to yellow viscous liquid.

Storage requirements: Refrigerate, 2°-8°C (36°-46°F). To protect from light, store in the original carton until time of use.

Route of administration: Fulvestrant will be given as two IM injections (one in each buttock).

Agent Ordering: Fulvestrant is commercially available and will be billed to the patient or her insurance.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

The study originally consisted of a Phase 1 portion consisting of two parts (Part A followed by Part B) and a randomized Phase 2 portion. Between June 2020 and February 2021, 10 patients were enrolled to 2 dose levels in Phase 1 (Dose Level 1 and Dose Level 2). Review of the associated data led to Amendment 6, which designated the original dosing schema for Phase 1 as Phase 1 Part A and added a second dosing schema for Phase 1 (Part B) to test an alternative schedule of abemaciclib (5 days on and 2 days off, each week) in combination with fulvestrant and copanlisib.

The phase 1 trial is designed to determine the RP2D for the randomized Phase 2 part. The CRM (O'Quigley *et al.,* 1990; Lee & Cheung, 2009; Lee & Cheung, 2011) will be used for the Phase 1 part (both Parts A and B, each having a maximum of 4 dose levels to be tested, see Section 6.1). The Phase 1 portion started at Dose Level 1 in the Dose Level Table for Part A in Section 6.1,

then escalated to Dose Level 2 prior to Amendment 6. Part A was initially conducted with 7 patients tested on Dose Level 1 and 3 patients on Dose Level 2. Based on the AE profile of the 10 enrolled patients in Part A, Part A was stopped at Dose Level 2 and Part B was added to test a maximum of 4 dose levels with abemaciclib administered at an intermittent schedule of 5-days on and 2-days off each week after the activation of Amendment 6. The MTD or RP2D for the randomized phase is defined as the dose level identified by CRM to have the highest probability of corresponding to the target DLT, *i.e.,* the estimated DLT at the selected dose level is closest to the target toxicity level (here we set at 30%, see Section 9.2). CRM will continuously monitor DLT and assign the next cohort of patients to dose level with DLT closer to the desired toxicity level using the data accumulated thus far in the trial.

During the process of the Phase 1 results being formally reviewed and approved by CTEP prior to activation of the randomized Phase 2 portion, Bayer withdrew the NDA for copanlisib and the study is closing prior to opening Phase 2 with Amendment 8d dated 07Jun2024.

9.1.1 Primary Endpoints

The primary endpoint for the Phase 1 trial is DLT. DLT will be determined based on the incidence, intensity and duration of AEs that are related to the drug combinations and occur within 28 days of drug administration. The severity of AEs will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0. All patients enrolled in the Phase 1 part who receive any dose level of the drug combination from the time of their first treatment will be evaluated for DLT.

9.1.2 Secondary Endpoints

The secondary endpoints are objective response rate (ORR) defined as proportion of response-evaluable patients who achieve complete response (CR) or partial response (PR); clinical benefit rate (CBR) defined as proportion of response-evaluable patients who achieve complete response (CR) or partial response (PR) or stable of disease (SD) for at least 6 months; Progression free survival (PFS) is defined as the time from start of therapy to the event of disease progression or death due to any cause. Overall survival defined from time of randomization to time of death due to any cause or latest follow-up, whichever earlier. All response evaluation will be based on RECIST v1.1.

9.2 Continual reassessment method (CRM) for Phase 1 dose finding

The adaptive Phase 1 dose finding method, the continual reassessment method (CRM) (O'Quigley, *et al.,* 1990; Lee & Cheung, 2009; Lee & Cheung, 2011), will be similarly used for both the Part A and Part B of the Phase 1 portion of the study. For both Part A and B, the acceptable DLT rate range is 25~35% with a target DLT rate of 30% and the DLT rate at the 4 dose levels (from lowest to highest) are assumed as 0.15, 0.2, 0.3, 0.45, respectively. The anticipated RP2D is dose level 3, with a DLT rate expected at 0.3. We allow a maximum sample size of 24, with a cohort size of 3. The number of patients who need to be tested at a dose level to stop is set at 10, and the confidence level for the safety stopping rule, at the lowest study dose level is 90%. The prior distribution on the CRM model parameter is N (0, 0.2), and the skeleton

working model is 0.2, 0.3, 0.4, 0.5. Based on 1000 simulations using a Bayesian CRM online app (https://uvatrapps.shinyapps.io/crmb/) (Wages & Petroni, 2018), the operating characteristics of the design for this set of assumed true DLT rates are provided in the table below. The MTD selection % will be 32.2% at dose level 3, and 25.6% at dose level 2.

Table 9.2.1: CRM operating characteristics at true DLT rate of as (0.15, 0.2, 0.3, 0.45) from dose level 1 to dose level 4.

Dose level	1	2	3	4
True DLT probability:	0.15	0.2	0.3	0.45
MTD selection percentage:	4.5	36.3	49.1	10.0
Average number of DLTs:	0.2	1.5	2.3	0.9
Average number of patients:	1.3	7.7	7.69	2.06
Percentage stopped for safety	0.1			

The boundaries for early stopping due to safety concern at the lowest level (Dose level -1) were computed based on Argesti-Coull binomial confidence interval (Agresti & Coull, 1998) are provided in the Table below.

Table 9.2.2: The boundary for early stopping due to safety concern at the lowest level (Dose level -1) based on Argesti-Coull binomial confidence interval.

# of patents treated	# of DLTs
2	2
3	3
4	3
5	4
6	4
7	5
8	5
9	5
10	6
11	6
12	7
13	7
14	8
15	8
16	8
17	9
18	9
19	9
20	10
21	10
22	11
23	11
24	11

Under other scenarios of DLT rates at the four dose levels, the operating characteristics based on 1000 simulations indicate that the Bayesian CRM selects the target DLT with the greatest probability.

Table 9.2.3: CRM operating characteristics at other scenarios of true DLT rate of dose levels 1-4.

Dose level	1	2	3	4
Scenario (1)				
True DLT probability:	0.3	0.4	0.5	0.6
MTD selection percentage:	45.7	37.8	6.2	0.1
Average number of DLTs:	2.1	3.3	1	0.1
Average number of patients:	6.98	8.25	2.06	0.18
Percentage stopped for safety	10.2			
Scenario (2)				
True DLT probability:	0.15	0.3	0.4	0.5
MTD selection percentage:	15.7	60.2	20.7	3.2
Average number of DLTs:	0.5	2.9	1.7	0.3
Average number of patients:	3.48	9.62	4.35	0.71
Percentage stopped for safety	0.2			
Scenario (3)				
True DLT probability:	0.04	0.1	0.2	0.3
MTD selection percentage:	0.1	8.6	42.9	48.4
Average number of DLTs:	0	0.5	1.6	1.9
Average number of patients:	0.19	5.15	8.18	6.37
Percentage stopped for safety	0			

9.3 Criteria for stopping of the phase 1 trial

CRM will continuously monitor DLT and assign the next cohort of patients to the dose level with DLT closer to the desired toxicity level using the data accumulated thus far in the trial. After each cohort of patients complete the DLT evaluation, the CRM analysis will be performed to determine the dose level to the next cohort of patients. The RP2D will be the dose with the highest probability of lying in the acceptable DLT range of 25~35% and being close to the target DLT rate of 30%.

The criteria for stopping the phase 1 trial are:

(1) Before 24 patients are enrolled to the phase 1 trial, if 10 consecutive patients are assigned to receive the same dose level, then it is at least 90% certain that the current dose is the MTD and the trial will stop early with the dose level identified as MTD.
(2) After 24 DLT evaluable patients have been enrolled to the phase 1 and have completed

the first cycle of treatment and the DLT observation window, an MTD will be estimated;
(3) The trial will stop and the protocol will be amended if the estimated probability of all the four dose levels having a DLT rate above (or below) the target DLT level is at least 90%.

9.4 Sample Size/Accrual Rate

9.4.1 Sample Size for the Phase 1 Trial

The primary objective of the Phase 1 trial is to determine the RP2D. A maximum of 34 DLT evaluable patients (as defined in Section 9.7) will be treated in the Phase 1 trial, including 10 patients enrolled in Phase 1 Part A prior to Amendment 6, and a maximum of 24 patients expected to enroll in Phase 1 Part B. At time of Amendment 8, 24 patients have enrolled to Phase 1.

The RPTD for FAC will be defined by the Bayesian CRM method. The maximum sample size for part 1 of the study will depend on the number of patients treated at each dose level and the observed DLTs at each level.

ORIGINAL PLANNED ENROLLMENT REPORT*

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	1	0	1	0	**2**
Asian	2	0	0	0	**2**
Native Hawaiian or Other Pacific Islander	0	0	1	0	**1**
Black or African American	38	0	0	0	**38**
White	141	2	18	0	**161**
More Than One Race	0	0	0	0	**0**
Total	**182**	**2**	**20**	**0**	**204**

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*Includes Phase 2 portion, which did not open to accrual.

9.5 Analysis of Primary Endpoints

For Phase 1, AEs will be summarized by counts and percentages, overall as well as by dose levels and by patient characteristics.

9.6 Analysis of Secondary Endpoints

The secondary endpoints ORR and CBR will be estimated with a 95% exact CI and difference between patient groups will be compared by Fisher's exact test. Raw and adjusted odds ratio (OR) will be derived with 95% CI from logistic regression without and with adjustment for patient characteristics. OS will be analyzed similarly as PFS.

9.6.1 Demographics and Baseline Characteristics

Frequency distributions of gender, race, ethnicity, target gene mutation and other categorical baseline characteristics will be tabulated. Baseline body mass index (BMI) will be derived from measurements of baseline body weight and height. Summary statistics for age, body weight, height, and BMI will be provided using mean, median, standard deviation, inter-quartile range and range. Baseline disease characteristics will be summarized overall and by patient groups of interest, as appropriate.

9.6.2 Correlative Biomarker Analysis

Descriptive statistics will be provided for selected demographic, safety, PK, candidate gene mutations, PTEN loss, and pharmacodynamic data (RPPA) by dose and time as appropriate. Descriptive statistics on continuous data (*e.g.,* gene expression, proteomics, metabolomics, TILs) will include means, medians, standard deviations, and ranges, while categorical data (*e.g.,* mutations) will be summarized using frequency counts and percentages. Graphical summaries of the data will be presented as appropriate. Prognostic effect of biomarker for PFS will be examined by the KM method or/and Cox proportional hazard model and for ORR using logistic regression model. For markers measured along multiple time points, generalized linear mixed effects model will be used to model longitudinal trajectories along time, without and with adjustment for other covariates. Two sample t-test or Wilcoxon rank sum test will be applied to compare time-matched biomarkers between two groups. Paired sample t-test or Wilcoxon signed rank test will be applied to compare subject-specific biomarkers between two time points. Multiple comparisons will be adjusted to control false discovery rate. Proposed subset analyses will be applicable to biomarkers

All data will be evaluated as they are, and no missing value imputations will be conducted.

9.7 Populations for Analysis

- All Enrolled Subjects Analysis Set: this analysis set contains all subjects who signed an informed consent for the study
- DLT-evaluable set: This set will be used for the DLT assessment in the Phase 1 part.

This analysis set refers to all subjects who receive at least 80% of the drug combinations during the first 28 days of therapy from the time of their first treatment, or those who experience DLT within the first 28 days. The patients who have no observed DLTs but drop out of the study before the completion of the first 28 days due to reasons other than treatment-emergent toxicities, will be replaced.

- Safety/toxicity evaluable analysis set: this analysis set contains all subjects who receive any dose level of the drug combinations from the time of their first treatment.
- Response-Evaluable Subjects: this analysis set contains all subjects who receive any amount of the study drugs, and have a baseline tumor assessment and at least one post-baseline tumor assessment, or who were discontinued due to toxicity (counted toward PD). This analysis set will be used for RECIST response analysis.
- Copanlisib PK Analysis Set: This analysis set includes all subjects who receive Copanlisib and have at least one valid PK parameter to be included in statistical analysis of the PK data.

9.8 Reporting and Exclusions

9.8.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with fulvestrant, abemaciclib and copanlisib.

9.8.2 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 of the 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. Subanalyses 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.

9.9 Data Safety Monitoring Board

The Siteman Cancer Center independent standing Data and Safety Monitoring Board (DSMB) will review toxicity data for phase I of this trial. The SCC standing DSMB includes clinical investigators and biostatisticians who are subject to the Washington University School of Medicine policies regarding standards of conduct and who have disclosed any potential conflicts of interest in accordance with institution policies.

The DSM report for the SCC standing DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to institutional safety monitoring committees as required. The DSMB must review phase I data at least every six months beginning six months after study activation. This report will include:

- Study demographics (protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician)
- Date of initial IRB approval, date of most recent consent, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site, and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites and separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported as described in the reporting requirements Section 9.11.

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 AEs 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 Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride, NSC 784727)

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 684 patients.* Below is the CAEPR for Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride).

> **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 2.3, April 2, 2023[1]

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 684]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
		Febrile neutropenia	
GASTROINTESTINAL DISORDERS			
Diarrhea			***Diarrhea (Gr 2)***
	Mucositis oral		
	Nausea		***Nausea (Gr 2)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
INFECTIONS AND INFESTATIONS			
Infection[2]			***Infection[2] (Gr 2)***
INVESTIGATIONS			
		Electrocardiogram QT corrected interval prolonged	
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
Hyperglycemia			***Hyperglycemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Muscle cramp		***Muscle cramp (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis[3]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythroderma	
		Pruritus	
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 2)***

[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.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Pneumonitis is a group term that includes interstitial lung disease, dyspnea, dyspnea at rest, and dyspnea exertional.

Adverse events reported on Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Eosinophilia
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Sinus tachycardia
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Constipation; Dry mouth; Dyspepsia; Dysphagia; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Oral dysesthesia; Oral pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; General disorders and administration site conditions - Other (failure to thrive); Multi-organ failure; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure
IMMUNE SYSTEM DISORDERS - Allergic reaction; Autoimmune disorder
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture; Infusion related reaction; Injury, poisoning and procedural complications - Other (drug eruption)
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; Ejection fraction decreased; Electrocardiogram T wave abnormal; INR increased; Investigations - Other (electrocardiogram U wave abnormal); Investigations - Other (Hepatitis B DNA increased); Lipase increased; Serum amylase increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hypertriglyceridemia; Hyperuricemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (diabetes mellitus)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (psoriatic arthropathy); Myalgia; Pain in extremity; Soft tissue necrosis upper limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor hemorrhage
NERVOUS SYSTEM DISORDERS - Amnesia; Dizziness; Dysesthesia; Dysgeusia; Headache; Paresthesia; Peripheral sensory neuropathy; Presyncope; Reversible posterior leukoencephalopathy syndrome; Syncope
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Aspiration; Cough; Dyspnea[3]; Hypoxia; Pleural effusion; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (pulmonary congestion); Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Eczema; Purpura; Rash acneiform; Stevens-Johnson syndrome
VASCULAR DISORDERS - Hypotension; Thromboembolic event; Vascular disorders - Other (circulatory collapse)

Note: Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) 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 CAEPR for Abemaciclib

Below is the CAEPR for Abemaciclib (LY2835219). Frequency is provided based on 950 patients.

Version 2.0, October 3, 2019[1]

Adverse Events with Possible Relationship to Abemaciclib (LY2835219) (CTCAE 5.0 Term) [n= 950]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		
	Mucositis oral		
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
INVESTIGATIONS			
		Alanine aminotransferase increased	
		Aspartate aminotransferase increased	
	Creatinine increased		
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		
	Headache		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
VASCULAR DISORDERS			
		Thromboembolic event	

[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 abemaciclib (LY2835219) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that abemaciclib (LY2835219) caused the adverse event:

EYE DISORDERS - Watering eyes
GASTROINTESTINAL DISORDERS - Constipation; Dyspepsia

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs; Fever; Pain
INFECTIONS AND INFESTATIONS - Lung infection; Sepsis; Upper respiratory infection; Urinary tract infection
INVESTIGATIONS - Blood bilirubin increased; GGT increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypertriglyceridemia; Hypoalbuminemia; Hypokalemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Bone pain; Generalized muscle weakness; Myalgia
NERVOUS SYSTEM DISORDERS - Somnolence
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Pruritus
VASCULAR DISORDERS - Lymph leakage

Note: Abemaciclib (LY2835219) 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.

10.1.3 Adverse Event List for Fulvestrant

The most common AEs reported in the fulvestrant study groups, regardless of causality, were GI symptoms including nausea and vomiting (26%/13%), constipation (12.5%), diarrhea (12.3%) and abdominal pain (11.8%); as well as headache (18.9%); back pain (15.4%); hot flashes (17.7%); and pharyngitis (16.1%). Vaginal bleeding was reported in <1% of patients and occurred most commonly during the first 6 weeks after changing from existing hormonal treatment to fulvestrant. If vaginal bleeding persists, further evaluation is required. Injection site reaction with mild transient pain (9.1-11.6%) and inflammation were seen with fulvestrant. Seven percent of patients (1% of treatments) given a single 5 ml IM injection and 27% of patients (4.6% of treatments) given 2 x 2.5 ml IM injections experienced reactions. Additional AEs occurring in >5% of patients treated with fulvestrant during clinical trials include asthenia (weakness) (68.3%), bone pain (15.8%), dyspnea (14.9%), increased cough (10.4%), pelvic pain (10%), anorexia (9%), peripheral edema (9%), rash (unspecified) (7.3%), chest pain (unspecified) (7.1%), dizziness (6.9%), insomnia (6.9%), fever (6.4%), paresthesias (6.4%), urinary tract infection (6.1%), depression (5.7%), anxiety (5%), diaphoresis (5%), and anemia (4.5%).

Other AEs reported as fulvestrant-related and occurring infrequently (<1%) include thromboembolism, myalgia, vertigo, and leukopenia. In addition, hypersensitivity reactions such as angioedema and urticaria have been infrequently reported

10.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP 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

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.

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 (i.e., checking the box *Send All AEs for Evaluation* and save the 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: Principal Investigator and Adverse Event 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 as long as the death occurred within 30 days after the last administration of the investigational agent. 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 h
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 h	10 Calendar Days	24-H 5 Calendar Days
Not resulting in Hospitalization ≥ 24 h	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-H; 5 Calendar Days" - The AE must initially be submitted electronically within 24 h of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-h 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-h 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

10.3.4 Copanlisib Adverse Events of Special Safety Interest (AESI)

AESI are events of scientific and medical interest specific to the further understanding of copanlisib's safety profile and require close monitoring and rapid communication by the investigators to CTEP. An AESI may be serious or non-serious. The AESI for copanlisib are the following:

- Non-infectious pneumonitis

Non-infectious pneumonitis has been observed in studies with copanlisib. As soon as there is a reasonable suspicion of a patient experiencing non-infectious pneumonitis, the investigator should report it within 24 h *via* CTEP-AERS regardless of whether the event is assessed as causally related/not related to the study therapy, or as serious/non-serious by an investigator.

10.4 Routine Adverse Event Reporting

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

Adverse event 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 Adverse Event 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 90 days 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: Adverse Event 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.

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

1. Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
2. Myelodysplastic syndrome (MDS)
3. 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 each protocol.

10.7 Second Malignancy

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.

11. STUDY CALENDAR

Screening evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Hepatitis panel, scans, ECHO/MUGA and x-rays must be done ≤ 4 weeks prior to the start of therapy (C1D1). In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 h prior to initiation of the next cycle of therapy.

After registration to the study, redacted clinical records will be collected. Records will include clinical tumor/blood sequencing result reports and prior breast cancer diagnosis and treatment history (a physician progress note is acceptable).

	Screening	C1D1*	C1D8*	C1D15*	C1D22*	Subsequent cycles*			EOT[19]	Follow-Up[19]
						D1	D8	D15		
Informed consent	X									
Physical exam w/ECOG PS	X	X	X[16]	X[16]		X				
Disease Assessment Imaging**	X					End of C3, then every 3 cycles thereafter			X	
CBC	X	X	X[16]	X		X		X[16]		
Serum chemistry[1]	X	X	X[16]	X	X[21]	X	X[16]	X[16]		
Lipase	X									
INR and PTT	X									
HbA1c	X									
Glucose[2,4]		X[12]	X[13]	X[13]		X[13]	X[13]	X[13]		
Echo/MUGA	X									
ECG	X	As clinically indicated								
Serum β-hCG[3]	X					X[3]				
Hepatitis panel	X									
Urinalysis[14]	X									
Blood pressure[2,4]		X	X[16]	X[16]		X	X[16]	X[16]		
Fulvestrant[15]		X		X		X				
Abemaciclib[15]		Dosing per Section 6.1								
Copanlisib[5]		X	X[5]	X		X	X	X		
Archival tumor tissue[6]	X									
Most Recent Tumor and/or blood genomic testing report if available[22]	X									
Fresh tumor biopsy	X[7]			X[17]					X[8, 17]	
Whole blood (cfDNA Streck tube), unprocessed[6]	X[20]		X	X		X[9]			X[8]	
Blood (EDTA purple top tube) for plasma[6]	X		X	X		X[9]			X[8]	
Blood (red top) for serum[6]	X		X	X		X[9]			X[8]	
Blood (LiHep) for PKs[10]		X[11]		X[11]	X[18]					
Concomitant medications[5]	X ---------- X									
AE assessment	X ---------- X									
Survival										X

1. Serum chemistry is albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

2. Only if receiving copanlisib
3. Women of childbearing potential only which is performed at screening and then every 3 cycles on study.
4. Refer to Section 7.1
5. All patients in the Phase 1 portion will receive copanlisib. Note copanlisib is not administered on day 8 in some dose levels (administered within 1 h (+/-10 minutes).
6. Mandatory
7. Mandatory if there is accessible tumor
8. At time of progression
9. C2D1, C4D1, and every 3 cycles after
10. Patients enrolled in the Phase 1 portion.
11. a) Prior to copanlisib infusion and (b) After copanlisib infusion (10 min, 1 hr, 2 hr, 3 hr, 5 hr, 24 hr [before C1D2's abemaciclib and fulvestrant])
12. Before infusion, 60 minutes post-start of infusion (end of infusion), 1 hr post-end of infusion, and 2 hr post-end of infusion
13. Before infusion and at end of infusion. Post-dose glucose monitoring after C1D1 is performed as clinically indicated at the investigator's discretion.
14. If proteinuria is present, a 24h urine protein quantification or a random urine protein and creatinine test is needed to determine eligibility.
15. Note that patients enrolled in the Phase 1 portion who have Copanlisib PK studies, the first two doses of abemaciclib on C1D1 are skipped and fulvestrant will be delayed to C1D2 following the 24 hr PK blood draw. The C1D15 dose of fulvestrant will be administered on C1D16 following the 24 hr PK draw.
16. If copanlisib is to be administered.
17. Optional. For patients on the FAC regimen, collect biopsy within 2-8 hrs post the administration of copanlisib, for scheduling issues, up to 24 hrs following the copanlisib is acceptable. Note that for patients on copanlisib, the tumor biopsy schedule should follow that of copanlisib in C1D15, in case copanlisib is delayed. For patients on FA regimen, collect biopsy on C1D15 at least 1 hr after the first dose of abemaciclib that day.
18. Prior to, 1 hr, 2 hr, 4 hr, 23 hr post the morning dose of abemaciclib on C1D22
19. Follow up for survival status occurs every 3 months for 5 years after removal from the study or until death, whichever occurs first. In addition, patients removed from study due to unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. There is a +/- 7 day window for EOT and follow up for survival status visits.
20. Whole blood (cfDNA streck tube) collected at baseline will be processed to buffy coat for germline DNA in addition to the cfDNA plasma at the EET Biobank.
21. Phase 1 patients only
22. Upload in RAVE. Tumor or blood genomic testing per SOC does not fall under 2 week window for baseline evaluations.

*Visits can occur in ± 2 days window to allow for holidays, inclement weather, etc.
** Tumor imaging for response assessment occurs at screening (within 28 days of C1D1), the completion of cycle 3 (+/- 7 days), then every 3 cycles.

12. MEASUREMENT OF EFFECT

Although the clinical benefit of this drug combination has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor regression and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated for response every 12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

12.1 Antitumor Effect – Solid Tumors

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 regimen.

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 CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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 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.

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

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

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

complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers 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 PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

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

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

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

12.1.4 Response Criteria

12.1.4.1 Evaluation of Target Lesions

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).

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

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).

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

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.

PD: Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.1.4.3 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	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Confirmation of PR, CR is not required in this trial.
*** 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

* '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

It is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event 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 Principal Investigator 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 adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly, or more frequently, conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and adverse events and unanticipated problems.

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 the 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:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems, 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 or Rave SLA role 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.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in the Regulatory application, 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 who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application 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 or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

13.2.1 Method

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, 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. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available on the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website.

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

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.

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.

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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.

APPENDIX B LIST OF PROHIBITED MEDICATIONS WHILE ON COPANLISIB TREATMENT

This list is not comprehensive. 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 minimize use of.

Category	Drug name
Strong CYP3A Inhibitors	Voriconazole, Boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycin,
Strong CYP3A Inducers	Avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum)
Herbal Preparations/ Medications	Herbal preparations/medications are prohibited throughout the study. These herbal medications include, but are not limited to: St. John’s Wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug

APPENDIX C PATIENT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINCIAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10287

Study Drug(S)/regimen:

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX D THE AVERAGE GLYCEMIC INDEX OF COMMON FOODS DERIVED FROM MULTIPLE STUDIES BY DIFFERENT LABORATORIES

Foods are categorized as having a low-glycemic index if the glucose reference index is ≤55. High-Glycemic Index foods have a glucose reference index >55. The summary table below contains glucose reference for common foods.

High-carbohydrate foods		Breakfast cereals		Fruit and fruit products		Vegetables	
White wheat bread*	75 ± 2	Cornflakes	81 ± 6	Apple, raw†	36 ± 2	Potato, boiled	78 ± 4
Whole wheat/whole meal bread	74 ± 2	Wheat flake biscuits	69 ± 2	Orange, raw†	43 ± 3	Potato, instant mash	87 ± 3
Specialty grain bread	53 ± 2	Porridge, rolled oats	55 ± 2	Banana, raw†	51 ± 3	Potato, french fries	63 ± 5
Unleavened wheat bread	70 ± 5	Instant oat porridge	79 ± 3	Pineapple, raw	59 ± 8	Carrots, boiled	39 ± 4
Wheat roti	62 ± 3	Rice porridge/congee	78 ± 9	Mango, raw†	51 ± 5	Sweet potato, boiled	63 ± 6
Chapatti	52 ± 4	Millet porridge	67 ± 5	Watermelon, raw	76 ± 4	Pumpkin, boiled	64 ± 7
Corn tortilla	46 ± 4	Muesli	57 ± 2	Dates, raw	42 ± 4	Plantain/green banana	55 ± 6
White rice, boiled*	73 ± 4			Peaches, canned†	43 ± 5	Taro, boiled	53 ± 2
Brown rice, boiled	68 ± 4			Strawberry jam/jelly	49 ± 3	Vegetable soup	48 ± 5
Barley	28 ± 2			Apple juice	41 ± 2		
Sweet corn	52 ± 5			Orange juice	50 ± 2		
Spaghetti, white	49 ± 2						
Spaghetti, whole meal	48 ± 5						
Rice noodles†	53 ± 7						
Udon noodles	55 ± 7						
Couscous†	65 ± 4						

Dairy products and alternatives		Legumes		Snack products		Sugars	
Milk, full fat	39 ± 3	Chickpeas	28 ± 9	Chocolate	40 ± 3	Fructose	15 ± 4
Milk, skim	37 ± 4	Kidney beans	24 ± 4	Popcorn	65 ± 5	Sucrose	65 ± 4
Ice cream	51 ± 3	Lentils	32 ± 5	Potato crisps	56 ± 3	Glucose	103 ± 3
Yogurt, fruit	41 ± 2	Soya beans	16 ± 1	Soft drink/soda	59 ± 3	Honey	61 ± 3
Soy milk	34 ± 4			Rice crackers/crisps	87 ± 2		
Rice milk	86 ± 7						

Data are means ± SEM. *Low-GI varieties were also identified. †Average of all available data.

APPENDIX E MANAGEMENT OF TRANSIENT GLUCOSE INCREASE ON THE DAY OF COPANLISIB INFUSION

Criteria	Recommendation	Suggested Treatment
Asymptomatic glucose increases ≤250mg/dL	Does not generally require treatment with glucose lowering medication.	**None**
Asymptomatic glucose increase > 250 mg/dl	• Should have repeated laboratory glucose determination. • If the repeated glucose value is decreasing, the glucose may be followed without glucose lowering medication treatment if hydration status is normal as clinically assessed. • Consultation with endocrinologist is recommended	• Hydration if appropriate • **When planning next infusion consider prophylaxis with oral glucose lowering medication**
Symptomatic or persisting glucose increases >250mg/dL	• Hydration status should be clinically assessed. • If clinical assessment is consistent with dehydration, fluids should be given as clinically appropriate (orally or IV). • Laboratory test confirming increase should be repeated. If the repeated glucose value is persistent and/or patient is symptomatic and/or the hydration status indicates the need for hydration, glucose lowering medication should be administered. • Prompt input from a diabetes specialist should be obtained.	• Hydration if appropriate • Rapid/ short acting insulin may be given for glucose persisting at >250 mg/dL, or if the patient is symptomatic during the infusion day. • According to the institution sliding scale coverage of glucose persisting at >250 mg/dL is recommended, with oral or IV hydration as clinically appropriate **When planning next infusion consider prophylaxis with oral glucose lowering medication**

APPENDIX F MANAGEMENT OF TRANSIENT GLUCOSE INCREASE ON SUBSEQUENT DAYS FOLLOWING COPANLISIB INFUSION

Criteria	Recommendation	Suggested Treatment
Max post infusion glucose >200 mg/dL noted on subsequent days	• Oral Glucose Lowering Medication Recommended on subsequent days. • **Consultation with endocrinologist is recommended.**	• The use of sulphonylurea/metaglinides, insulin secretagogues medications to manage increased glucose levels post drug infusions is not recommended. • Treatment with glucose lowering medication suggested according the local standards of practice. • Based on mechanisms of action and decreased risk of hypoglycemia, metformin, SGLT-2-inhibitor or DPP4-inhibitor might be useful treatment options

APPENDIX G DOSE MODIFICATION OF COPANLISIB FOR ARTERIAL HYPERTENSION

Toxicity (CTCAE)	Study drug action	Recommendation
Pre-dose measurements BP ≥150/90 mmHg	No dose should be given until recovery to <150/90 mmHg.	Consider BP lowering medication. Dosing can proceed on the scheduled day if after at least 2 consecutive measurements BP returns to <150/90 mmHg. If BP doesn't return to <150/90 mmHg, delay dosing until next visit.
During infusion: CTCAE hypertension of grade 3 or ≥160/100 mmHg	Infusion can be interrupted or slowed down and administration of BP lowering therapy should be initiated.	Infusion may be resumed when BP has returned to <150/90 mmHg at the investigator's discretion or skipped. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]
Post-dose: Drug-related CTCAE hypertension of grade 3 or ≥160/100 mmHg [a]	–	Administration of BP lowering therapy should be initiated according to local standard of care. Additional measurements to be performed as clinically indicated until recovery to <150/90 mmHg. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]
CTCAE hypertension of grade 4	Permanent discontinuation	–

CTCAE = Common Terminology Criteria for Adverse Events; BP = Blood pressure
[a]: Not manageable despite optimal antihypertensive treatment.
[b]: The lowest dose level is 30mg.

APPENDIX H ABEMACICLIB MEDICATION DIARIES

Today's Date: _____ Agent: Abemaciclib – Daily Dosing Cycle: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take _____mg (___tablets) of abemaciclib at approximately the same time twice a day each day. Swallow the tablets whole and do not chew them.
2. Take abemaciclib with or without food.
3. Record the date, the number of tablets taken, and when you took them.
4. If you forgot to take your dose, then skip the dose and start taking at the next dosing time.
5. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit. If you vomit after taking a dose, DO NOT retake the dose. Take the next dose at the usual time. If you mistakenly take an extra dose, DO NOT take the next day's dose.
6. At the first sign of loose stools, start antidiarrheal medication (such as loperamide), increase your fluid intake, and tell your doctor.
7. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
8. Avoid St. John's Wort, Seville oranges, grapefruit, grapefruit juice, grapefruit hybrids, pummelos, and exotic citrus fruits from 7 days before you start taking abemaciclib and throughout the entire study.

Day	Date	What time was dose taken?		# of capsules taken	Comments
		AM dose	PM dose		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
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13					
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APPENDIX H ABEMACICLIB MEDICATION DIARIES

Today's Date: _____ Agent: Abemaciclib – 5 Days On/2 Days Off Cycle: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take _____mg (___tablets) of abemaciclib at approximately the same time twice a day each day for 5 days, then do not take any pills for 2 days. Repeat the 5 days of dosing followed by 2 days off throughout the cycle. Swallow the tablets whole and do not chew them.
2. Take abemaciclib with or without food.
3. Record the date, the number of tablets taken, and when you took them.
4. If you forgot to take your dose, then skip the dose and start taking at the next dosing time.
5. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit. If you vomit after taking a dose, DO NOT retake the dose. Take the next dose at the usual time. If you mistakenly take an extra dose, DO NOT take the next day's dose.
6. At the first sign of loose stools, start antidiarrheal medication (such as loperamide), increase your fluid intake, and tell your doctor.
7. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
8. Avoid St. John's Wort, Seville oranges, grapefruit, grapefruit juice, grapefruit hybrids, pummelos, and exotic citrus fruits from 7 days before you start taking abemaciclib and throughout the entire study.

Day	Date	What time was dose taken?		# of tablets taken	Comments
		AM dose	PM dose		
1					
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APPENDIX I PK SAMPLING MANUAL

NorthEast BioAnalytical Laboratories

Pharmacokinetic (PK) Sampling Manual

Study Title

A randomized phase II trial of fulvestrant and abemaciclib in combination with copanlisib (FAC) versus fulvestrant and abemaciclib alone (FA) for endocrine resistant, hormone receptor positive, HER2 negative metastatic breast cancer (FAC vs FA)

Study Number: NCI Protocol #: 10287

Version 1.0
Dated: Nov 17, 2018

Table of Contents

ATTACHMENTS

NCI Protocol #: 10287
Version Date: June 13, 2024

1. Introduction

The purpose of this laboratory manual is to specify all the instructions for the collection, processing, storing and shipping of plasma samples for drug concentration / pharmacokinetic measurements.

2. Laboratory Equipment and Supplies

1.1 Required Site Equipment and Supplies:

- –70°C freezers to store all samples until shipment and a temperature control log to ensure stable storage conditions.
- Refrigerated centrifuge (4° C) for centrifuging blood samples to obtain plasma.
- Dry ice.

Notify vipin.agarwal@nebiolab.com or jonathan.quick@nebiolab.com *if you do not have any of the above supplies.*

2.2 Pharmacokinetic Sample Collection Kits

Kits will be provided by NorthEast Bioanalytical Laboratories and will contain the following supplies**:**

- Pharmacokinetic blood sample draw tubes (4 ml Li-Heparin BD vacutainers, green cap, Cat. No.367884) – (19 count)
- 2 mL cryovials – (19 count)
- Labels for blood draw tubes and cryovials – 1 sheet
- A cardboard box to place tubes – 1 count
- Requisitions (Sample Shipment Form) – 1 count
- Styrofoam boxes and shipping containers – 1 count
- Disposable plastic transfer pipettes – (Phase Ib-12 count/Phase II 6 count)
- KPA/biohazard bags, Saf-T Pak bag – 1 count
- *FedEx shipping labels*

For Re-ordering of Supplies please contact vipin.agarwal@nebiolab.com or jonathan.quick@nebiolab.com at least one week in advance to ensure that supplies are shipped in time.

3. Plasma Sample Collection, Storage and Shipment

3.1 Plasma Sample Collection for Pharmacokinetic Analysis

- Collect one 4 mL blood sample for analysis by venipuncture at the following time points:

Visit	**Scheduled time point**
Cycle 1 Day 1 (C1D1)	Pre-infusion (up to 30 minutes prior to the start of the infusion)
Cycle 1 Day 1	10 minutes (± 2 minutes after the start of the infusion)

Cycle 1 Day 1	1 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 1	2 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 1	3 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 1	5 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 2 (C1D2)	24 hour (± 1 hr after the start of the infusion; prior to the first dose of palbo and fulvestrant)
Cycle 1 Day 15 (C1D15)	Pre-infusion (up to 30 minutes prior to the start of the infusion)
Cycle 1 Day 15	10 minutes (± 2 minutes after the start of the infusion)
Cycle 1 Day 15	1 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 15	2 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 15	3 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 15	5 hour (± 5 minutes after the start of the infusion)
Cycle 1 Day 16 (C1D16)	24 hour (± 1 hr after the start of C1D15 infusion; prior to the C1D16 palbo and fulvestrant)
Cycle 1 Day 22 (C1D22)	Prior to the morning dose of abemaciclib
Cycle 1 Day 22	1 hour after taking the morning dose of abemaciclib
Cycle 1 Day 22	2 hour after taking the morning dose of abemaciclib
Cycle 1 Day 22	4 hour after taking the morning dose of abemaciclib
Cycle 1 Day 22	23 hour (1 hour before C1D23's abemaciclib)

- Pharmacokinetic Plasma Sample Processing

Note: Read instructions entirely before processing samples. Keep vacutainer tubes on ice when not being used.

1. Collect 4 mL blood in a vacutainer tube (as specified above) at each time point using adequately labeled vacutainer tubes containing Li-Heparin (green top tube).
2. Gently invert vacutainer tubes several times to mix with anticoagulant.
3. Immediately place vacutainer tubes on ice (baggie, cup or basin with ice).
4. Within 10-15 minutes after collecting blood, centrifuge each vacutainer tube at 4ºC for 10 minutes at 1000 g to separate the plasma. **If a refrigerated centrifuge is not available, it is imperative that the vacutainer tubes are placed on ice for a minimum of 10 minutes prior to centrifugation**.
5. Using disposable plastic transfer pipettes, aspirate off the plasma layer from one vacutainer tube. (If you are processing samples collected from more than one time point, use a new pipette for each vacutainer tube.)
6. Pipette the plasma into a 2-ml screw cap polypropylene cryovial.
7. Complete sample label for "PK-Plasma" and affix to the cryovial.

8. Freeze samples in cryovial upright at -60° C to -80° C in the cardboard storage box provided no later than 60 minutes after blood draw.
9. If you have successfully processed the samples according to the above instructions, appropriately dispose of vacutainers and prepare to ship samples within 24 hours of completing Cycle 1 Day 22.
10. ***Note*:** Document any significant deviations from the collection or processing instructions in the space provided on the Sample Shipment Form.

3.2 Sample Shipments:

The site will complete the FedEx shipping airway bill and e-mail or fax a copy of the completed sample shipment cover (pg. 8) to NEBA as a pre-shipment notification. Then, the site will arrange sample pick up by courier. The site is responsible for obtaining dry ice for shipment.

The following instructions need to be followed for sample pick up and shipment.

1. Verify that each sample is labeled properly, the label is completed and affixed to the cryovial, and the corresponding Sample Shipment Form has been completed. **Please make sure that samples are organized in boxes by Patient and collection time points.**
2. Remove cardboard storage box containing cryovials from freezer.
3. Place box into large biohazard bag. Seal bag completely.
4. Insert sealed biohazard bag into white "Saf-T-Pak" envelope.
5. Place original Sample Shipment Forms into the "Saf-T-Pak" envelope, between the plastic biohazard bag and envelope. Retain a copy in your site file.
6. Place envelope containing storage box inside cooler.
7. Fill cooler completely with dry ice.
8. Place cover on the cooler.
9. Place cooler in the fiberboard container, close the cardboard flaps and secure with tape.
10. Complete Shipper's Information and Dry Ice Information on the outside of the container (name, address, phone, amount of Dry Ice).
11. Affix completed Airbill on top of container in designated area.

The package is now ready for shipping.

- Batch ship frozen samples on Monday, Tuesday or Wednesday for overnight delivery after all samples for a subject have been obtained. **Do not ship samples on Thursday or Friday.**
- Notify recipient via e-mail or Fax at least one day in advance of shipping the samples by faxing **Sample Shipment Cover.**
- Ship samples to:

NCI Protocol #: 10287
Version Date: June 13, 2024

Vipin Agarwal, PhD
NorthEast Bioanalytical Laboratories
925 Sherman Avenue
Hamden, CT 06514
USA
Tel: +1-203-361-3768
Fax: +1-203-407-0703
E-mail: vipin.agarwal@nebiolab.com or jonathan.quick@nebiolab.com

Prior to shipment, please ensure:

- *all cryovials are stored in a cardboard storage box. Each box can accommodate 81 2 mL cryovials; up to two cardboard storage boxes may be included in each styrofoam shipping container filled with dry ice.*
- *all tubes are packaged according to the instructions in section 3.2.*
- *all tubes are labeled appropriately and match shipping forms*
- *all corresponding Sample Shipment Forms and Sample Shipment Cover are included*
- *Provide notification of shipment along with FedEx tracking number to :*
    - vipin.agarwal@nebiolab.com *and* jonathan.quick@nebiolab.com

NCI Protocol #: 10287
Version Date: June 13, 2024

Sample Shipment Cover

Key Study Data

Study Identifier : NCI Protocol 10287 (FAC vs FA)
FedEx Tracking Number:_____

Responsibilities/Contacts

Function:	Investigator	Study Manager	Other (e.g.CRO)	Receiving Lab
Name:				Vipin Agarwal, PhD
Address:				NorthEast Bioanalytical Laboratories 925 Sherman Ave Hamden, CT 06514
Site ID:			N/A	
Phone:				203-361-3768
Fax:				203-407-0703
Email:				Vipin.agarwal@nebiolab.com

Key Shipment Data Analyte*: N/A

Shipment from this site: First: ☐ Interim: ☐ Last shipment: ☐		No. of Boxes ____
No. of shipped samples: Plasma ____ ____ Aliqout 1/Aliqout 2 (circle one)_____		
Samples with known infectious agents (Yes / No):		
If Yes, which infectious agents: HIV___ Hepatitis ___ Other _____		
Additional Info:		
Name of Shipper:	Date of Shipment:	Signature:

Confirmation of Receipt (to be faxed to Investigator/Shipper and XXXX-US)

Comments: Shipment OK ? (Yes ☐ / No ☐) If No, specify: Some Samples thawed ☐ . Some Samples broken/open ☐ . Other:_____ Total no. of samples received (N= __) deviates from schedule (N= ___). Sample lists missing ☐ . Further Comments:		
Name of Recipient:	Date:	Signature:

Distribution:
Original: Please attach at top inside of the shipment container
Fax: Send in parallel to 203-407-0703
Copy: Retain in Investigator Site Fil

Sample Shipment Form

For Pharmacokinetic Samples

Key Study Data

Study Identifier: **NCI Protocol 10287 (FAC vs FA)**		
Investigator Name:		
Date of Shipment: ____/____/200__ (mm/dd/yyyy)	Matrix: **Plasma**	Analyte:
Additional Info for all shipped samples:		

Sample Details

Site Number: ___ ___ Subject Number___ ___ ___ ___

Sample	Visit	Planned Collection Time	Collection date (mm/dd/yyyy)	Actual Collection time (Per Clock)	Rec'd at NEBL
1	Cycle 1 Day 1	Pre-infusion (up to 30 minutes prior to the start of infusion)			☐
2	Cycle 1 Day 1	10 minutes (± 2 minutes after the start of the infusion)			☐
3	Cycle 1 Day 1	1 hour (± 5 minutes after the start of the infusion)			☐
4	Cycle 1 Day 1	2 hour (± 5 minutes after the start of the infusion)			☐
5	Cycle 1 Day 1	3 hour (± 5 minutes after the start of the infusion)			☐
6	Cycle 1 Day 1	5 hour (± 5 minutes after the start of the infusion)			☐
7	Cycle 1 Day 2	24 hour (± 1 hr after the start of the infusion; prior to the first dose of palbo and fulvestrant)			☐
8	Cycle 1 Day 15	Pre-infusion (up to 30 minutes prior to the start of infusion)			☐
9	Cycle 1 Day 15	10 minutes (± 2 minutes after the start of the infusion)			☐
10	Cycle 1 Day 15	1 hour (± 5 minutes after the start of the infusion)			☐
11	Cycle 1 Day 15	2 hour (± 5 minutes after the start of the infusion)			☐
12	Cycle 1 Day 15	3 hour (± 5 minutes after the start of the infusion)			☐
13	Cycle 1 Day 15	5 hour (± 5 minutes after the start of the infusion)			☐
14	Cycle 1 Day 16	24 hour (± 1 hr after the start of C1D15 infusion; prior to the C1D16 palbo and fulvestrant)			☐
15	Cycle 1 Day 22 (C1D22)	Prior to the morning dose of abemaciclib			☐
16	Cycle 1 Day 22	1 hour (± 5 minutes) after taking the morning dose of abemaciclib			☐
17	Cycle 1 Day 22	2 hour (± 5 minutes) after taking the morning dose of abemaciclib			☐
18	Cycle 1 Day 22	4 hour (± 5 minutes) after taking the morning dose of abemaciclib			☐
19	Cycle 1 Day 22	23 hour (± 1 hr) after the morning dose of abemaciclib			☐

Distribution:
Original: Please attach at top inside of the shipment container
Fax: Send to (203)- 407-0703
Copy: Retain in Investigator Site File

Signature: _____

APPENDIX J 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): Archival Pre-treatment C1D15 Progression

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.