VICC BRE 1374: A Phase Ib/II Trial of Taselisib (GDC-0032), a PI3K Inhibitor, in Combination With Enzalutamide in Patients With Androgen Receptor Positive Triple Negative Metastatic Breast Cancer

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Primary Investigators

Vandana G. Abramson, MD (Protocol Chair)
Vanderbilt-Ingram Cancer Center
2220 Pierce Ave. 777 PRB
Nashville, TN 37232
[redacted]

Jennifer Pietenpol, PhD
Vanderbilt-Ingram Cancer Center
2220 Pierce Ave. 777 PRB
Nashville, TN 37232
[redacted]

Co-Investigators

Medical Oncology:

Carlos L. Arteaga, MD
Vanderbilt-Ingram Cancer Center
2220 Pierce Ave. 777 PRB
Nashville, TN 37232
[redacted]

Laboratory Correlatives:

Brian Lehmann, PhD
Vanderbilt-Ingram Cancer Center
2220 Pierce Ave. 777 PRB
Nashville, TN 37232
[redacted]

E-mail: brian.d.lehmann@vanderbilt.edu

Advanced Imaging Correlative:
Richard G. Abramson, M.D.
Vanderbilt-Ingram Cancer Center
Department of Radiology and Radiological Sciences
1161 21st Ave South, CCC-1121 MCN
Nashville, TN 37232-2675
Phone: 615-322-6759
E-mail: richard.abramson@vanderbilt.edu

H. Charles Manning, Ph.D.
Vanderbilt University Institute of Imaging Science (VUIIS)
Department of Radiology and Radiological Sciences
1161 21st. Avenue South, MCN AA-1105
Nashville, TN 37232
Phone: 615-322-3793
Email: henry.c.manning@vanderbilt.edu

Pathology:
Melinda Sanders, MD
Vanderbilt-Ingram Cancer Center
Department of Pathology 4912 TVC
1301 22nd Ave South
Nashville, TN 37232-5310
Phone: 615-343-9115
E-mail: melinda.sanders@vanderbilt.edu

Biostatistics:
Yu Shyr, PhD
Vanderbilt-Ingram Cancer Center
2220 Pierce Ave. 777 PRB
Nashville, TN 37232
Phone: (615) 322-4967
Fax: (615) 343-7602
E-mail: yu.shyr@vanderbilt.edu

Sujata Patil, PhD
Memorial Sloan Kettering Cancer Center
Department of Epidemiology & Biostatistics
1275 York Avenue
New York, NY 10065
Phone: 646-735-8182
patils@mskcc.org

Supporters Genentech

Translational Breast Cancer Research Consortium (TBCRC)

Coordinating Center Vanderbilt-Ingram Cancer Center (VICC)

Study Liaison:

VICC-CTSR Multi-Institutional Department
2141 Blakemore Avenue
Nashville, TN 37212
[redacted]

A Phase Ib/II trial of taselisib (GDC-0032), a PI3K inhibitor, in combination with enzalutamide in patients with androgen receptor positive triple negative metastatic breast cancer

- Protocol Revision Record -

Original Protocol:	January 27, 2015
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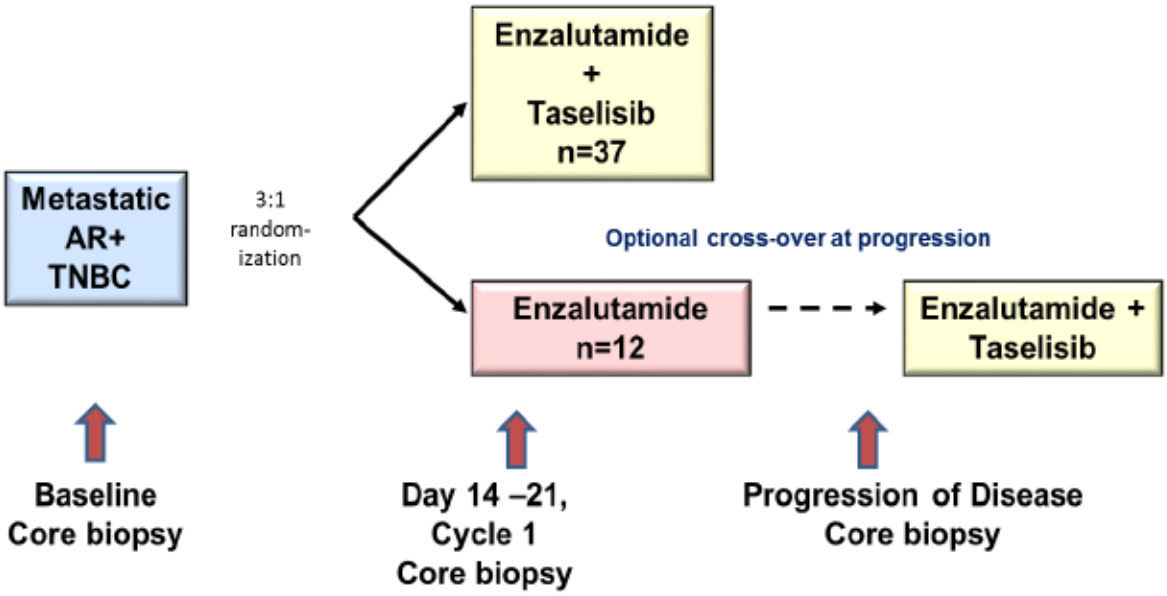
PHASE I SCHEMA:

PHASE II SCHEMA:



AR positive: ≥10% of tumor cell nuclei with immunoreactivity for AR in a CLIA certified laboratory

One cycle = 28 days, tumor assessment with scans will be performed after every 2 cycles

STUDY CALENDAR: ENZALUTAMIDE +/- TASELISIB

Evaluation[a]		Screening[b]	Treatment period								Post treatment	
				Cycle 1 (28-days)		Cycle 2 (28-days)[c]			Additional cycles[c]	Cycle 4	Every 2 cycles from C1D1		
		Day-28 – Day 1	Daily	Day 1	Day 15	Day 1	Day 8[t]	Day 15	Day 1	Day 1		End-of-treatment[d]	30 day follow-up[d]
Informed consent		X											
Inclusion/exclusion criteria		X											
Brain imaging (MRI or CT scan)[e]		X											
Demographics/ medical history		X											
Physical exam/signs & symptoms[b,f]		X		X	X	X	X	X	X			X	
Vital signs (including oxygen sat)		X		X	X	X	X	X	X			X	
12-lead ECG[g]		X											
ECOG Performance Status		X		X	X	X	X	X	X			X	
Blood collection	Clinical laboratory tests[h]	X		X	X	X	X	X	X			X	
	Fasting plasma glucose[i]	X		X	X	X	X		X			X	
	Pregnancy test[j]	X											
	HbA1C	X											
	PT (or INR)/PTT	X											
	PK assessments[k]					X		X		X			
	Circulating tumor DNA					X		X				X	
Tumor assessment CTs, bone scan[l]		X									X		
Archived tumor blocks/slides[m]		X											
Tumor biopsy (metastatic site)[n]		X						Day 14-21				X	
Imaging Correlate: CEST-MRI[o]		X						Day 14-21				X	
Enzalutamide administration (oral)[p]			X	Oral, once-daily administration									
Taselisib administration (oral): if on taselisib arm			X			Oral, once-daily administration, starting cycle 2, day 1							

Evaluation[a]	Screening[b]	Treatment period								Every 2 cycles from C1D1	Post treatment	
			Cycle 1 (28-days)		Cycle 2 (28-days)[c]			Additional cycles[c]	Cycle 4			
	Day-28 – Day 1	Daily	Day 1	Day 15	Day 1	Day 8[t]	Day 15	Day 1	Day 1		End-of-treatment[d]	30 day follow-up[d]
Pill Diary[q]		X										
Concomitant medications[r]	X		X	X	X	X	X	X				
Adverse events	X		X	X	X	X	X	X				X
Biopsy questionnaire[s]								X				

AE = adverse event; **BSA** = body surface area; **CX** = Cycle X, where X is the cycle number; **DLT** = dose-limiting toxicity; **ECG** = electrocardiogram; **ECOG** = Eastern Cooperative Oncology Group; HbA1C = glycosylated hemoglobin; **IMP** = investigational medicinal product; **INR** = international normalized ratio; **PT** = prothrombin time; **PTT** = partial prothrombin time; **SAE** = serious adverse event

a Evaluation assessments should be performed prior to administration of IMP unless otherwise indicated. Results should be reviewed by the Investigator prior to the administration of the next dose. All visits should occur within 3 days of scheduled visit unless otherwise noted.

b Screening must occur within 28 days of dosing. The following procedures must be repeated at the day 1 visit: Medical and cancer history, physical examination, weight measurement, vital signs, hematology (see h), serum chemistry panel (see h). Lab values must still meet eligibility criteria on cycle 1, day 1. Tumor assessments must be repeated at the baseline visit only if determined to be changed by clinical exam or other clinical observations. SOC procedures performed prior to consent but within the protocol defined screening window for each assessment can be used for study purposes. All research only procedures must be performed after the consent date.

c Patients may continue with daily dosing of enzalutamide with or without taselisib in the absence of unacceptable toxicities after C2 (or if the toxicity has resolved) and with no clinical evidence of disease progression.

d The 30-day follow-up assessments should be done 30 (±3 days) days after the last dose of enzalutamide and taselisib. 30 day follow-up can be done by phone or by a clinic visit. Any ongoing toxicities should be documented. Any ongoing serious adverse events will be followed every 3 months until resolution. Any AEs or SAEs considered possibly or probably related to IMP that have not stabilized, returned to baseline, or are considered irreversible will continue to be monitored as per the protocol in Section 7.

e MRI should be completed only if CT is unclear and a lesion can only be followed on MRI imaging. The CT scan should be completed with contrast. This is considered a research procedure if the patient does not have any signs or symptoms of brain metastases. If the patient must undergo brain imaging for the sole purpose of screening for this study, it is expected that a CT of the head will be done.

f The physical examination will include measurements of height and weight at screening. Weight will be measured on day 1 of each cycle and at the End-of-treatment visit. Physical examination will include examination of major body systems including neurologic, cardiac, respiratory, gastrointestinal, and skin. Clinically relevant signs and symptoms will be reported as AEs.

g ECG will be performed at screening

h Clinical laboratory tests will include hematology and serum chemistry panel including white blood cell count, differential, hemoglobin, platelet count, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, and magnesium, SGOT, SGPT, and total serum bilirubin, albumin, and total protein. Additional tests will be performed when clinically appropriate. In case of Grade 3 or higher abnormal liver function results, additional testing will be done weekly until recovery to baseline value (see section 7.2). For all other timepoints, labs can be performed up to 3 days prior to each timepoint indicated in the study calendar (other than required C1D1 timepoint). Results from labs obtained on C1D1 do have to meet eligibility criteria.

i FPG (fasting plasma glucose) will be collected at screening , on Days 1 and 15 of C1, on CXD1 of subsequent cycles, and at the End-of-treatment visit. FPG will also be monitored if patients experience Grade ≥1 hyperglycemia during the course of the study per the hyperglycemia management guidelines provided in the protocol. FPG can be obtained up to 3 days prior to each timepoint indicated in the study calendar (other than required C1D1 timepoint).Fasting plasma glucose is the same as serum glucose on days that the patient is fasting. On days the patient is getting non-fasting labs, it should be serum glucose only. (See Table 3 under section 7.2.1.5)

j Serum pregnancy test: women of child bearing potential must have a negative serum pregnancy test result within 28 days prior to the initial dose of IMP.

k PKs for patients in the phase Ib portion: plasma sample should be obtained on Cycle 1, day 8 at: 0-4 hours prior to dose of taselisib and 1, 3, and 6 hours post administration taselisib. On Cycle 2, day 15 at: 0-4 hours prior to dose of taselisib and enzalutamide and 1, 3, and 6 hours post administration taselisib and enzalutamide. On Cycle 4, day 1, plasma sample should be obtained 0-4 hours before taselisib and enzalutamide administration, and 3 hours post-administration of both drugs. PKs in phase II will occur for 10 patients at the Vanderbilt site only and should be obtained on patients receiving both enzalutamide and taselisib: C2D1, C2D15, and C4D1 at 0-4 hours prior to taselisib and enzalutamide and 2 to 4 hours post treatment.

l Tumor assessment should be performed approximately every 2 cycles (up to -5 days) while on study treatment and at End-of-treatment visit if not obtained within the past 6 weeks. Scans should consist of CT scan of the chest, abdomen, and pelvis and a bone scan at baseline. Subsequent scans should consist of CT chest, abdomen and pelvis, and bone scan if bone metastases were present on the baseline studies. At the end of treatment, all scans (baseline and through disease progression) should be sent on a CD to the Vanderbilt Cancer Imaging Support Laboratory for central review and assessment. See Section 10.5 for details and address.

m Archived tumor biopsy specimen: 20 unstained slides of archived biopsy block (unstained slides) or fresh tumor biopsy will be requested at the time of enrollment prior to initiation of treatment.

n Tumor biopsies will only be performed in the phase II portion. A tumor biopsy from a metastatic site will be obtained from patients prior to day 1, at cycle 2 day 14-21, and upon progression of disease. The third biopsy (at the end of treatment) will not be required for patients who are removed from study for reasons other than disease progression. A patient who undergoes a research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, is not obligated to undergo a repeat biopsy.

o See section 9.1. Required only for patients at Vanderbilt and only in the phase II portion. This should be performed prior to tumor biopsy, if possible. If MRI sub-study is not feasible due to scheduling, MRI weight limits, etc., patients may still continue with the remainder of the study.

p Subjects on the enzalutamide + taselisib arm should begin enzalutamide on day 1 of cycle 1, and taselisib on day 1 of cycle 2.

q Pill Diary can be found in the packet of supplemental forms.

r See Section 6.4.2.

s Phase II only. Biopsy questionnaire should be filled out by the patient on C3D1. Form can be found in the packet of supplemental forms.

t The cycle 2 day 8 visit is only needed for patients on the combination of taselisib + enzalutamide. Patients on enzalutamide only do not need a visit on this day.

STUDY CALENDAR: ENZALUTAMIDE + TASELISIB (CROSSOVER)

Crossover therapy (taselisib) must begin no later than 21 days after the clinic visit at which progression is determined.

Evaluation[a]		Treatment period							Post treatment	
		Daily	Cycle 1 (28-days)		Cycle 2 (28-days)[b]		Additional cycles[b]		End-of-treatment[c]	30 day follow-up[c]
			Day 1	Day 15	Day 1	Day 15	Day 1	Every 2 cycles from C1D1		
Physical exam/signs and symptoms[d]			X	X	X	X	X		X	
Vital signs (including oxygen sat)			X	X	X	X	X		X	
ECOG Performance Status			X	X	X	X	X		X	
Blood collection	Clinical laboratory tests[e]		X	X	X	X	X		X	
	Fasting plasma glucose[f]		X	X	X		X		X	
	Circulating tumor DNA		X			X			X	
Tumor assessment CTs, bone scan[g]								X		
Tumor biopsy (metastatic site)[h]										
Enzalutamide administration (oral)		X	Oral, once-daily administration, starting Day 1							
Taselisib administration (oral)		X	Oral, once-daily administration							
Pill Diary[i]		X								
Concomitant medications[j]			X	X	X	X	X			
Adverse events			X	X	X	X	X			X

a Evaluation assessments should be performed prior to administration of IMP unless otherwise indicated. Results should be reviewed by the Investigator prior to the administration of the next dose. All visits should occur within 3 days of scheduled visit unless otherwise noted.

b Patients may continue with daily dosing of enzalutamide with or without taselisib in the absence of unacceptable toxicities after C2 (or if the toxicity has resolved) and with no clinical evidence of disease progression.

c The 30-day follow-up assessments should be done 30 (±3 days) days after the last dose of enzalutamide and taselisib. 30 day follow-up can be done by phone or by a clinic visit. Any ongoing toxicities should be documented. Any ongoing serious adverse events will be followed every 3 months until resolution. Any AEs or SAEs considered possibly or probably related to IMP that have not stabilized, returned to baseline, or are considered irreversible will continue to be monitored as per the protocol in Section 7.

d The physical examination will include measurements of height and weight at screening. Weight will be measured on day 1 of each cycle and at the End-of-treatment visit. Physical examination will include examination of major body systems including neurologic, cardiac, respiratory, gastrointestinal, and skin. Clinically relevant signs and symptoms will be reported as AEs.

e Clinical laboratory tests will include hematology and serum chemistry panel including white blood cell count, differential, hemoglobin, platelet count, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, and magnesium, SGOT, SGPT, and total serum bilirubin, albumin, and total protein. Additional tests will be performed when clinically appropriate. In case of Grade 3 or higher abnormal liver function results, additional testing will be done weekly until recovery to baseline value. Labs do not have to be repeated on cycle 1, day 1 if they were done up to 7 days prior to day 1. For all other timepoints, labs can be performed up to 3 days prior to each timepoint indicated in the study calendar.

f FPG (fasting plasma glucose) will be collected at screening , on Days 1 and 15 of C1, on CXD1 of subsequent cycles, and at the End-of-treatment visit. FPG will also be monitored if patients experience Grade ≥>1 hyperglycemia during the course of the study per the hyperglycemia management guidelines provided in section 7.2 of the protocol. FPG does not have to be repeated on cycle 1, day 1 if it was done up to 7 days prior to day 1. FPG can be obtained up to 3 days prior to each timepoint indicated in the study calendar. The fasting plasma glucose is the same as the serum glucose on the days that the patient is fasting. On the days the patient is getting non-fasting labs, it should be serum glucose only. (see Table 3 under section 7.2.1.5)

g Tumor assessment should be performed approximately every 2 cycles (up to -5 days) while on study treatment and at End-of-treatment visit if not obtained within the past 6 weeks. Scans should consist of CT scan of the chest, abdomen, and pelvis and a bone scan at baseline. Subsequent scans should consist of CT chest, abdomen and pelvis, and bone scan if bone metastases were present on the baseline studies. At the end of treatment, all scans (baseline and through disease progression) should be sent on a CD to the Vanderbilt Cancer Imaging Support Laboratory for central review and assessment. See Section 10.5 for details and address.

h Tumor biopsies from a metastatic site will be obtained from patients prior to day 1 (this will be the biopsy obtained at disease progression on the enzalutamide only arm).

i Pill Diary can be found in the packet of supplemental forms.

j See Section 6.4.2.

1. STUDY DESIGN/SUMMARY

This is an open-label phase Ib/II multiple institution trial that evaluates the safety profile and efficacy of enzalutamide with or without taselisib in patients with androgen receptor positive triple negative metastatic breast cancer (AR+ TN MBC). The phase 1b portion will allow any ER/PR status, provided the patient is HER2 negative. Patients with AR positivity, defined as $\geq$ 10% of tumor cell nuclei with immunoreactivity for AR, will receive enzalutamide +/- taselisib. All patients in the phase Ib portion will receive both drugs. Patients in the phase II portion will undergo core biopsy of a metastatic lesion (if reasonably safe) at baseline, at day 14-21, and at the time of progression. If a fresh biopsy is deemed not safe, a paraffin block from a prior biopsy or surgery must be available for enrollment. Standard staging radiology scans will be performed at baseline and every 2 cycles (up to - 5 days) thereafter. Upon progression of disease by RECIST, patients on the enzalutamide only arm will be allowed to crossover to enzalutamide + taselisib. Those on the combination arm will be removed from study. Patients will be screened and enrolled from the oncology practice at the Vanderbilt-Ingram Cancer Center, from participating member centers of the Translational Breast Cancer Research Consortium, and from other approved sites.

2. OBJECTIVES

2.1 Primary Objectives

Phase Ib

To determine the safety and tolerability of taselisib given in combination with enzalutamide:

- Assessment of dose limiting toxicities (DLTs) during the first 4 weeks of treatment (cycle 1)
- Determination of the maximally tolerated dose (MTD) of taselisib given in combination with enzalutamide

Phase II

- To evaluate the efficacy, as measured by clinical benefit rate (CBR), of enzalutamide + taselisib in patients with AR+ TN MBC. CBR is defined as the proportion of patients with a best response of CR, PR, or stable disease (SD) $\geq$ 16 weeks.

2.2 Secondary Objectives

- To determine the progression free survival (PFS) of enzalutamide + taselisib in patients with AR+ TN MBC. PFS is defined as the time from cycle 1, day 1 until objective tumor progression.
- To assess the pharmacokinetics (PKs) of taselisib and enzalutamide in patients with AR+ TN MBC

2.3 Exploratory Objectives

- To explore predictors of biomarker response and mechanisms of resistance based on exploratory analysis of tumor tissue obtained through biopsies
  a) Levels of PTEN expression by immunohistochemistry (IHC) and qPCR
  b) Presence of mutations in the PIK3CA gene
  c) HER2 (IHC, FISH) and ER/PR levels (IHC) in tumor biopsy from a metastatic site.

d) Levels of MEK activity measured by phosphorylated p-ERK1/2 (IHC) and phosphorylated p-S6 (S235/236 and S240/244) at baseline and upon progression of disease
e) Levels of phosphorylated p-AKT (IHC) at baseline and upon progression of disease
f) Gene expression profiling to assign a triple negative subtype
g) Whole exome DNA-seq on DNA isolated at baseline and upon progression
h) Plasma for ctDNA analysis to assess PIK3CA mutation status in response and resistance

- To assess the predictive effects of PIK3CA mutations and PTEN loss on PFSand CBR
- To evaluate the ability of multi-parametric MRI performed early in therapy to predict both biological and clinical response.

3. BACKGROUND

3.1 PI3K Pathway and Breast Cancer

Molecular alterations involving this pathway are considered the most frequent in breast cancer, encompassing over 30% of invasive tumors. PI3K is aberrantly activated in many human neoplasias[1,2]. Alterations in breast cancer resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in *PIK3CA* (the gene encoding the PI3K catalytic subunit p110α)[3-10], mutations in *AKT1*[11], amplifications of *AKT2*[12], and loss of PTEN[13,14]. Mutations in *PIK3CA* cluster in two major 'hot spots' located in the helical (E542K and E545K in exon 9) and catalytic (H1047R in exon 20) domains[4,15]. Expression of these mutant p110α isoforms confers growth factor-independent growth and protection from anoikis and chemotherapy[16]. Loss of PTEN also increases PI3K activity and increased phosphorylation, activation, or membrane translocation of signal transducers downstream PI3K including Akt, mTOR, S6 kinase, and 4-EBP-1[17]. Both genetic and biochemical data suggest that activation of the PI3K/Akt survival pathway contributes to breast cancer development and tumorigenesis[18-25].

Over the past decade, the term "triple negative breast cancer" has been used to classify tumors that lack expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2. Triple negative breast cancers (TNBC) are generally more aggressive than their ER positive counterparts, with higher rates of relapse in the early stage and decreased overall survival in the metastatic setting. TNBC has been particularly difficult to treat given that the biology of the disease has not been well understood, thus molecular targets for therapeutic intervention are unknown. Recently, 104 TNBC exomes were sequenced and identified TP53 (62%) as the most frequently mutated gene, followed by alterations in PI3K pathway that included PIK3CA (10.2%) and PTEN (9.6%) mutations [26], suggesting that targeting the PI3K pathway would be clinically relevant in TNBC.

3.2 Correlative Science Background

3.2.1 PI3K pathway and triple negative breast cancer

Using gene expression (GE) analyses, we recently identified distinct TNBC subtypes displaying unique biologies [27]. We identified six TNBC subtypes including two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem–like (MSL), and a luminal androgen receptor (LAR) subtype [27]. Further, GE analysis allowed us to identify TNBC cell lines representative of these subtypes. Predicted "driver" signaling pathways were pharmacologically targeted in these cell lines as proof-of-concept and to generate pre-clinical data to inform clinical trial design. The LAR subtype was characterized by androgen receptor signaling.

LAR cell lines were uniquely sensitive to inhibition of AR signaling achieved through siRNA-mediated AR knockdown or pharmacological inhibition by bicalutamide (an AR antagonist). From our initial analysis it was evident that all LAR cell lines analyzed had an activating PIK3CA mutation and were sensitive to PI3K inhibitors. The co-evolution of PIK3CA mutations with AR-dependency is similar to observations that ER-positive breast cancers frequently contain PIK3CA mutations[28,29]. To confirm the enrichment of PIK3CA mutations in AR expressing TNBC, we isolated DNA from 26 LAR and 26 non-LAR TNBC tumors. Sanger sequencing was performed across exons 5 and 20 of amplified PIK3CA DNA from the same tumors as further validation. Seventy percent (18/26) of tumors of the LAR subtype contained activating 'hotspot' mutations in the PIK3CA gene.

AR+ tumors with a PIK3CA mutation expressed higher levels of activated p-AKT (S473). While PIK3CA mutations were detected in non-LAR TNBC (26.9%), the frequency of PIK3CA mutations was nearly double in LAR TNBC (70.0%), suggesting that these tumors may depend on PI3K and, as such, will be particularly sensitive to PI3K inhibitors. To determine the single agent effectiveness of cisplatin, bicalutamide and a PI3K inhibitor (GDC-0941), we performed viability assays evaluating the efficacy of these drugs across a panel of TNBC cell lines. Cell lines that possessed BRCA1/2 mutations and correlated to basal-like TNBC subtypes were especially sensitive to cisplatin (Figure 1A).

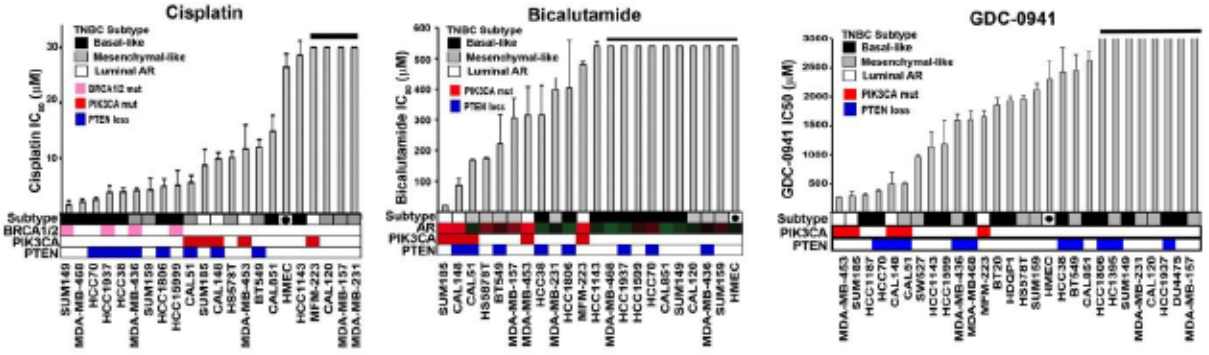


Figure 1. TNBC subtypes display differential sensitivity to cisplain, bicalutamide and GDC-0941. IC50 values for cell lines 72 h after treatment with (A) cisplatin, (B) bicalutamide or (C) GDC-0941. Black, gray, and white color bars indicate TNBC subtype; white box with black dot indicates primary HMEC; pink, red, and blue color bars indicate mutation status for BRCA1/2, PIK3CA and PTEN, respectively. Heatmap below bicalutamide graph indicates levels of AR mRNA (Log2 GE).

As anticipated, AR+ TNBC cell lines were sensitive to the AR antagonist bicalutamide and the PI3K inhibitor GDC-0941 (Figure 1B and C). Since GDC-0941 displayed activity in PIK3CA mutant cell lines and all AR-expressing cell lines, we then sought to test the hypothesis that simultaneous targeting of AR and PI3K would be more effective than either agent alone. We genetically knocked down AR using shRNA in the presence of GDC-0941. Knockdown of AR significantly increased the efficacy of GDC-0941 over a range of doses as viability was reduced in the presence of two shRNAs targeting AR (Figure 2). We advanced the combination of these inhibitors to *in vivo* xenograft studies and examined bicalutamide ± GDC-0941; representative results are shown (Figure 2D).

To determine if pharmacological inhibition of AR with bicalutamide is more effective in combination with PIK3CA inhibition, we treated AR-expressing TNBC cells with either increasing doses of GDC-0941 or in combination with fixed doses of the inhibitor (10, 25, and 50 μM) with bicalutamide. While increasing doses of bicalutamide alone decreased viability of each cell line, the combination with GDC-0941 was either additive or synergistic over a range of doses (1 -1000 nM) as measured by a viability assay (Alamar Blue detection). Bicalutamide treatment alone did not affect protein levels evaluated, however the combination with GDC-0941 decreased p-S6 more than GDC-0941 alone and decreased AR levels at 48 h.

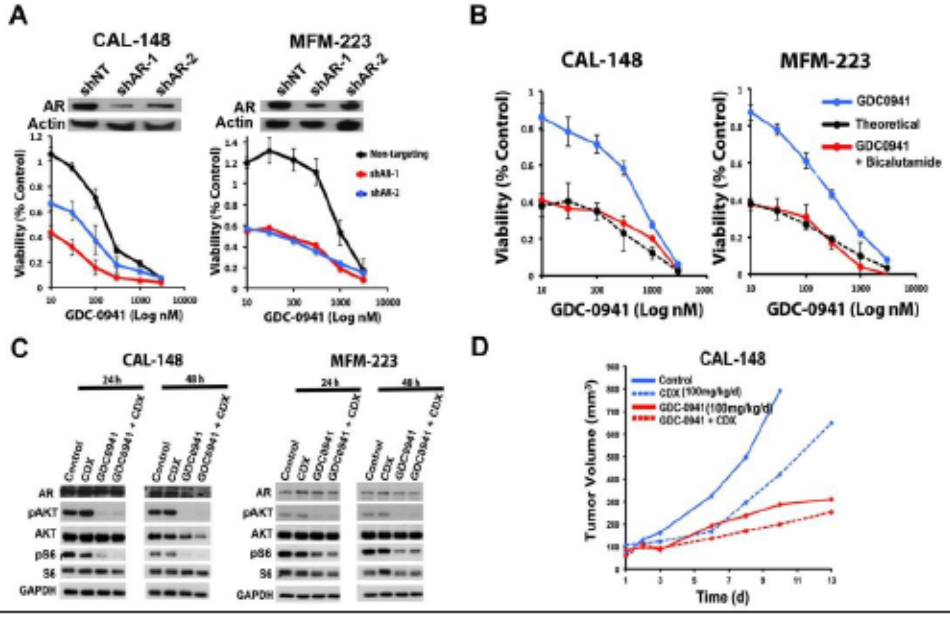


Figure 2. PI3K inhibition increases the efficacy of genetic and pharmacological targeting of AR in AR+ TNBC. (A, upper) Immunoblots display decreased AR expression 72h after lentiviral infection with shRNAs targeting AR compared to control in indicated AR+ TNBC cell lines. **(A, lower)** Relative viability of LAR cell lines infected with nontargeting or shRNAs targeting AR (shAR1 and shAR2) after 72 h treatment with GDC-0941. **(B)** Relative viability of AR+ cell lines after indicated doses of GDC-0941 alone (blue) or in combination (red) with 25 μM bicalutamide (CDX). Dashed black line depicts the theoretical line of additivity from the combined effect of bicalutamide and GDC-0941 alone. **(C)** Immunoblots show decreased pAKT and pS6 at 24 and 48 after treatment with 1 μM GDC-0941 or the combination with 25 μM CDX. **(D)** CAL-148 xenograft tumors display greater sensitivity to GDC-0941 and the combination compared to bicalutamide (CDX) alone. Mice were treated for approximately 3 weeks. Serial tumor volumes (mm^3) were measured at the indicated days. Each data point represents the mean tumor volume of 16 tumors.

We tested the effectiveness of PI3K inhibition alone or in combination with bicalutamide in mice with CAL-148 xenograft tumors. GDC-0941 alone or in combination with bicalutamide significantly decreased the growth of CAL148 xenograft tumor growth. These results provide pre-clinical rationale for a clinical trial in which AR-positive triple negative breast cancer patients will receive a combination of an anti-androgen + a PI3K inhibitor.

3.3 Background on Study Treatment

3.3.1 Taselisib

Taselisib is a potent, selective small-molecule inhibitor of Class I PI3K that is being developed by Genentech, Inc. as an anti-cancer therapeutic agent. Activating and transforming mutations in the p110α subunit of PI3K are commonly found in tumors. The beta isoform of PI3K is involved in glucose metabolism, as evidenced by increased glucose levels in conditional p110β knockout mice compared with wild-type mice following a glucose challenge test. Taselisib has approximately 30-fold biochemical selectivity of the alpha isoform relative to the beta isoform and has been shown to be a potent inhibitor of growth in nonclinical models of PI3K-mutant tumors.

Refer to the taselisib Investigator's Brochure for further information regarding the nonclinical evaluation of taselisib.

3.3.1.1 Summary of Clinical Data

As of 5 July 2013, a total of 144 patients have been treated with taselisib capsules, in the Phase I/II PMT4979g study, either as single agent (n = 90, 63%) or in combination with endocrine therapy (n = 54, 37%).

As of 5 July 2013, enrollment into the dose-escalation stage of Study PMT4979g had been completed with 34 patients enrolled at taselisib doses ranging from 3 to 16 mg daily. Taselisib was well tolerated in the first three cohorts (3, 5, and 8 mg), with no patients experiencing a dose-limiting toxicity (DLT). At the 16-mg dose level, 2 of the 11 safety-evaluable patients experienced a DLT (Grade 4 hyperglycemia and Grade 3 fatigue). At the 12-mg dose level, 1 of the 10 safety-evaluable patients experienced a DLT of Grade 3 acute renal failure. Although the single-agent taselisib maximum tolerated dose (MTD) was not exceeded at the 16-mg dose level, the recommended taselisib dose and schedule for the single-agent expansion cohorts is 9 mg daily on the basis of long-term safety data through multiple treatment cycles. As of the cutoff date, a total of 53 patients had been enrolled in the 9-mg daily dosing expansion cohorts.

As of 5 July 2013, adverse events of any grade that occurred in ≥ 10% of the 87 patients treated with daily single-agent taselisib capsules and were investigator-assessed as related to taselisib were as follows: diarrhea (47%), hyperglycemia (38%), nausea (36%), fatigue (35%), decreased appetite (31%), rash (25%), stomatitis (13%), vomiting (13%), and mucosal inflammation (11.5%). Grade 3 and 4 adverse events assessed by the investigator as taselisib related included hyperglycemia (12%), colitis (6%), rash (5%), diarrhea (3%), fatigue (3%), pneumonitis (3%), pruritus (2%), stomatitis (2%), increased alanine aminotransferase levels (1%), anemia (1%), increase in blood creatinine (1%), exfoliative rash (1%), hypokalemia (1%), hypophosphatemia

(1%), lung infection (1%), pneumonia (1%), erythematous rash (1%), generalized rash (1%), maculopapular rash (1%) and skin exfoliation (1%) and acute renal failure (1%).

As of 5 July 2013, a total of 27 patients have been enrolled in the expansion cohort of taselisib at dose levels of 6 and 9 mg daily (19 patients at 6 mg, and 8 patients at 9 mg) daily in combination with letrozole (Cohort E). No DLTs were observed at either dose level. Adverse events of any grade and assessed by the investigator as drug related that occurred in ≥ 10% of the 27 safety-evaluable patients assessed as related to taselisib were diarrhea (67%), fatigue (30%), nausea (30%), rash (30%), decreased appetite (26%), hyperglycemia (26%), stomatitis (26%), dysgeusia (22%), mucosal inflammation (19%), asthenia (15%), pruritis (15%), vomiting (15%) and dry mouth (11%). Grade 3 and 4 adverse events assessed by the investigator as taselisib related include diarrhea (11%), mucosal inflammation (7%), increased amylase (4%), hyperglycemia (4%), increased aspartate aminotransferase (AST) (4%), stomatitis (3.7%). increased blood alkaline phosphate (4%), fatigue (4%), increased gamma-glutamyltransferase in the blood (4%), hypokalemia (4%), increased lipase in the blood (4%) and papilloedema (4%).

As of 5 July 2013, a total of 27 patients have been enrolled in the expansion cohort of taselisib at dose levels of 6 and 9 mg daily (21 patients at 6 mg and 6 patients at 9 mg) in combination with fulvestrant (Cohort F). No DLTs were observed at either dose level. One patient has been enrolled in the Phase II part of the study with 6 mg taselisib in combination with fulvestrant. Adverse events assessed by the investigator as taselisib related and of any grade that occurred in ≥ 10% of the 27 patients and were assessed as related to taselisib were diarrhea (48%), hyperglycemia (33%), nausea (33%), decreased appetite (26%), fatigue (26%), rash (26%), stomatitis (22%), asthenia (19%), muscle spasms (15%), vomiting (15%), dysgeusia (11%), gastroesophageal reflux disease (11%) and mucosal inflammation (11%). Grade 3 and 4 adverse events assessed by the investigator as related to taselisib included hyperglycemia (15%), diarrhea (7%), dyspnea (4%), flank pain (4%), hyponatremia (4%), neutropenia (4%), rash (4%) and vomiting (4%). A phase 1b study of taselisib in combination with paclitaxel is ongoing.

Refer to the taselisib Investigator's Brochure for further information regarding the nonclinical and clinical safety evaluation of taselisib.

3.3.2 Enzalutamide

Enzalutamide (Medivation) is approved by the United States Food and Drug Administration (FDA) for the treatment of men with metastatic castrate resistant prostate cancer (CRPC) who previously received docetaxel. Enzalutamide is marketed as Xtandi and was formerly known as MDV3100. Enzalutamide is an androgen receptor antagonist which acts by competitively inhibiting (1) testosterone binding to androgen receptors, (2) nuclear translocation of androgen receptors, and (3) DNA binding and activation by androgen receptors. Enzalutamide is administered orally.

Refer to the Enzalutamide Investigator's Brochure for further information regarding the nonclinical evaluation of Enzalutamide.

3.3.2.1 Summary of Clinical Data

Enzalutamide has been studied primarily in patients with prostate cancer. The pharmacokinetics (PK), tolerability, and antitumor activity of enzalutamide (then known as MDV3100) were first studied in a multicenter, open-label, first-in-human, dose-escalation study in 140 patients with CRPC[30]. Patients who were chemotherapy-naïve or who had previous docetaxel-based chemotherapy failure were treated with enzalutamide at doses of 30 to 600 mg/day until disease progression or intolerable side effects developed. The maximum tolerated dose was determined to be 240 mg daily. After review of all data available from S-3100-1-01, the optimal dose of enzalutamide for evaluation in phase 3 clinical trials was determined to be 160 mg/day. AFFIRM was a phase 3, randomized, double-blind, placebo-controlled, efficacy and safety study of enzalutamide (160 mg daily) in patients with progressive CRPC previously treated with docetaxel-based chemotherapy which was conducted in 1199 men, 800 of whom received treatment with enzalutamide[31]. The primary endpoint was overall survival. The FDA approval of enzalutamide was based on the results of this study. PREVAIL was a multinational, phase 3, randomized, double-blind, placebo-controlled, efficacy and safety study of enzalutamide in chemotherapy-naïve patients with progressive metastatic prostate cancer and prior androgen deprivation failure. Subjects included 1717 men, approximately half of whom received treatment with enzalutamide. The interim analysis at 539 deaths showed a statistically significant benefit of enzalutamide over placebo with a 30% reduction in risk of death (OS: HR 0.70; 95% CI: 0.59-0.83; P< 0.0001) and an 81% reduction in risk of radiographic progression or death (rPFS: HR 0.19; 95% CI: 0.15-0.23; P< 0.0001). At the time of the analysis, 28% of enzalutamide patients and 35% of placebo patients had died. Estimated median OS was 32.4 months (95% CI, 31.5–upper limit not yet reached) in the enzalutamide arm vs 30.2 mo (95% CI, 28–upper limit not yet reached) in the placebo arm. Median rPFS was not yest reached in the enzalutamide arm vs 3.9 mo (95% CI: 3.7-5.4) in the placebo arm. Seizure events were reported in two patients. The Independent Data Monitoring Committee considered the benefit-risk ratio to favor enzalutamide and recommended stopping the study and crossing placebo patients to enzalutamide. Protocol MDV3100-11 is evaluating single agent enzalutamide in women with triple negative breast cancer that is androgen receptor positive. Two other protocols with enzalutamide in breast cancer are ongoing: (1) MDV3100-12, investigating exemestane plus enzalutamide or placebo in hormone-receptor positive breast cancer and (2) 9785-CL-1121, investigation enzalutamide with trastuzumab in HER2+ AR+ metastatic breast cancer. More than 4200 subjects and patients have been enrolled worldwide in completed and ongoing clinical trials evaluating enzalutamide. The cumulative exposure to enzalutamide is estimated to be more than 2900 subjects and patients who received at least 1 dose in any clinical study as of January 2013.

3.4 Rationale

Therapies for breast cancer should be guided by biologic features of the tumor, such as expression of steroid hormone receptors and hormone dependence and/or HER2/neu overexpression. Notable examples are the successful use of hormonal therapy (e.g. tamoxifen or aromatase inhibitors) for patients with hormone-sensitive (estrogen receptor [ER] and/or progesterone receptor [PR] expressing) tumors[32], and the use of trastuzumab (Herceptin™), a monoclonal antibody against HER2/neu, for patients with overexpression of the aforementioned protein[33]. These are excellent examples of where appropriate patient selection is instrumental on the success of these therapies. An unfortunate problem triple in negative breast cancer is that the key drivers of this disease are not well understood, and thus molecular targets for therapeutic intervention have not been identified.

We have identified distinct subtypes of triple negative breast cancer to better understand potential drivers of disease progression and to help identify potential therapeutic targets. Considering all of our preclinical data, we propose a clinical trial in which patients with metastatic TNBC have their tumor tissue initially assessed for androgen receptor (AR) status by standard CLIA-certified AR IHC analysis. For patients who are AR positive, we propose a phase Ib/II study of enzalutamide +/- taselisib. We hypothesize that the combination of enzalutamide + taselisib will provide an improvement in outcome compared to enzalutamide alone. This study will elucidate the safety and preliminary therapeutic value of this combination.

4. PARTICIPANT SELECTION

4.1 Inclusion criteria

4.1.1 Patients must provide informed written consent.

4.1.2 Patients must be ≥18 years of age.

4.1.3 ECOG performance status 0-1.

4.1.4 Clinical stage IV invasive mammary carcinoma

4.1.5 For phase 1b: HER2 negative, as defined for phase II. Any ER/PR (negative or positive) can be enrolled in the phase 1b portion. For phase II: ER negative (defined as expression of ER in ≤ 5% cells), PR negative (defined as expression of PR in ≤ 5% cells), HER2 negative [acceptable methods of HER2 analysis include IHC (0, 1+), fluorescence in situ hybridization (FISH) with HER2/CEN-17 ratio < 2, and/or chromogenic in situ hybridization (CISH) with HER2/CEN-17 ratio <2], as previously documented by histological analysis.

4.1.6 Androgen receptor positivity, defined as ≥ 10% of tumor cell nuclei with immunoreactivity for AR on central review at Vanderbilt.

4.1.7 Measurable or evaluable disease. Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension by RECIST criteria 1.1, with radiologic scans within 28 days of day 1, cycle 1. Evaluable disease must include bone metastases (pleural effusions and ascites are not considered evaluable for this study).

4.1.8 Any number of prior therapies as long as patients have adequate performance status and meet all other eligibility criteria.

4.1.9 Prior treatment with anti-androgens other than enzalutamide is acceptable.

4.1.10 Phase 1b only: FFPB or fresh frozen tissue from the original diagnosis or the metastatic setting should be located. Tissue must be submitted with 3 weeks of study initiation.

4.1.11 Phase II only: Biopsy of a metastatic lesion in patients with reasonably accessible metastatic lesions (chest wall, skin, subcutaneous tissue, lymph nodes, skin, breast, bones, lung, and liver metastases). If a reasonably accessible metastatic lesion is not available, the patient may go on study provided that archived tissue is available. However, if a reasonably accessible site is available for biopsy, the patient must agree to biopsy. Any patients not undergoing biopsy must be approved for study enrollment by the Protocol Chair. Biopsies may be done with local anesthesia or intravenous conscious sedation, according to institutional guidelines. If a biopsy requires general anesthesia, then it is only allowed if acquisition of tissue is clinically indicated, and excess tissue may be collected for research purposes. Patients without sites available for biopsy must have available tissue [archived formalin-fixed paraffin embedded blocks (FFPB) or fresh frozen tissue from original diagnosis or metastatic setting] for correlative studies per Section 9.2.

Tissue needs to be located and available at the time of registration (tissue needs to be submitted within 3 weeks of study initiation).

4.1.12 Patients must have adequate hematologic, hepatic, and renal function. All tests must be obtained within 28 days of starting treatment. Labs are to be repeated on C1D1 and must still meet eligibility. These include:

- ANC $\geq$1500/mm^3
- Platelet count $\geq$ 75,000/mm^3
- HgB $\geq$ 9 g/dL
- Creatinine $\leq$1.5X upper limits of normal (ULN)
- INR $\leq$2
- Total serum bilirubin $\leq$ 1.5 x ULN (in patients with known Gilbert Syndrome, a total bilirubin $\leq$ 3.0 x ULN, with direct bilirubin $\leq$ 1.5 x ULN)
- AST and ALT $\leq$ 3 x ULN (or $\leq$ 5.0 x ULN if hepatic metastases are present)
- For patients without known Type II diabetes, the following is required at screening:
    - Fasting plasma glucose$\leq$160 mg/dL (7.49 mmol/L) and glycosylated hemoglobin (HbA1c)<7.5 % or International Federation of Clinical Chemistry (IFCC) < 53 mmol/mol
- For patients with Type II diabetes receiving only oral anti-hyperglycemic therapy (patients receiving insulin are not eligible), the following are required at screening:
    - HbA1c < 8.5 % or IFCC < 69.4 mmol/mol
    - Stable regimen of oral anti-hyperglycemic therapy without insulin usage for at least 3 weeks prior to first study treatment
    - Fasting plasma glucose levels $\leq$ 160 mg/dL (8.88 mmol/L) and no hypoglycemia (BS<60) during home monitoring for at least 1 week prior to study entry

4.1.13 Patients must be able to swallow and retain oral medication.

4.1.14 For patients who are not postmenopausal or surgically sterile (absence of ovaries and/or uterus), agreement to remain abstinent or to use two adequate methods of contraception(e.g., condoms, diaphragm, vasectomy/vasectomized partner, tubal ligation), during the treatment period and for at least 30 days after the last dose of study treatment or 3 months after discontinuation of taselisib and/or enzalutamide, whichever is longer. Hormone based oral contraceptives are not allowed on study. Postmenopausal is defined as:

- Age $\geq$ 60 years
- Age $\leq$ 60 years and amenorrheic for 12 months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression; or follicle stimulating hormone and estradiol in the postmenopausal range

4.1.15 Patients may have received radiation therapy to painful bone metastases or areas of impending bone fracture as long as radiation therapy is completed ≥ 2 weeks prior to day 1 of cycle 1 of treatment. Patients who have received prior radiotherapy must have recovered from toxicity (≤ grade 2) induced by this treatment. Baseline radiologic scans must be obtained after completion of radiation.

4.1.16 Patients must complete all screening assessments as outlined in the protocol.

4.2 Exclusion Criteria

4.2.1 Any kind of malabsorption syndrome significantly affecting gastrointestinal function, including history of Crohn's disease or inflammatory bowel disease.

4.2.2 Concurrent anti-cancer therapy (chemotherapy, radiation therapy, surgery, immunotherapy, hormonal therapy, biologic therapy) other than the ones specified in the protocol. Patients must have discontinued the above cancer therapies for 1 week prior to the first dose of study medication. Any investigational drugs should be discontinued 2 weeks prior to the first dose of study medication and radiotherapy must have been completed ≥ 2 weeks prior to initiation of study drug (Cycle 1, Day 1).

4.2.3 Prior use of PI3K or Akt inhibitors in the metastatic setting for the treatment of cancer. These include, but are not limited to: taselisib, GDC-0941, GDC-0980, BEZ235, BKM120, LY294002, PIK-75, TGX-221, XL147, XL765, SF1126, PX-866, D-87503, D-106669, GSK615, CAL101. Patients who have received PI3K/Akt inhibitors previously for <4 weeks will be eligible.

4.2.4 Prior treatment with enzalutamide.

4.2.5 Current or previously treated brain metastasis or active leptomeningeal disease; head imaging is required during screening in all patients to exclude the presence of central nervous system (CNS) metastatic disease.

4.2.6 History of seizure or any condition that may predispose to seizure; history of loss of consciousness or transient ischemic attack within 12 months before day 1.

4.2.7 Pregnant or lactating women.

4.2.8 Insulin-dependent diabetes. Patients with Type II diabetes must meet the inclusion criteria outlined above.

4.2.9 Uncontrolled intercurrent illness including, but not limited to:

- Ongoing or active infection requiring parenteral antibiotics

- Impairment of lung function (COPD > grade 2, lung conditions requiring oxygen therapy) or current dyspnea at rest
- Symptomatic congestive heart failure (class III or IV of the New York Heart Association classification for heart disease)
- Known Left Ventricular Ejection Fraction (LVEF) < 50%
- Unstable angina pectoris, angioplasty, stenting, or myocardial infarction within 6 months
- Uncontrolled hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure > 100 mm Hg, found on two consecutive measurements separated by a 1 or 2-week period despite adequate medical support)
- Clinically significant cardiac arrhythmia (multifocal premature ventricular contractions, bigeminy, trigeminy, ventricular tachycardia that is symptomatic or requires treatment [National Cancer Institute -Common Terminology Criteria for Adverse Events, Version 4.0, grade 3]
- QTcF ≥ 480 msec on screening EKG
- Known history of QT/QTc prolongation or Torsades de Pointes (TdP)
- ST depression or elevation of ≥ 1.5 mm in 2 or more leads
- Diarrhea of any cause ≥ CTCAE grade 2
- Active autoimmune disease that is not controlled by nonsteroidal or steroidal (< 10 mg of prednisone per day) anti-inflammatory drugs or active inflammatory disease, including small or large intestine inflammation such as active Crohn's disease or ulcerative colitis, which requires immunosuppressive therapy
- Psychiatric illness/social situations that would compromise patient safety or limit compliance with study requirements including maintenance of a compliance/pill diary
- Known history of chronic liver disease including cirrhosis, current alcohol abuse, or infection with hepatitis B virus or hepatitis C virus (active or carrier) or renal failure
- Known history of chronic pancreatitis

- Conditions that affect lymphocyte counts, such as HIV infection or immunosuppressive therapy

4.2.10 Use of prohibited drugs, as described in section 6.4.

4.3 Inclusion of Underrepresented Populations

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of men and women.

5. REGISTRATION PROCEDURES

Patients must be AR positive to register for the study. Sections 5.1, 5.2, and 5.3 describe how AR testing can be done. Once AR positivity is confirmed, all patients MUST be registered with the Vanderbilt-Ingram Cancer Center (VICC) prior to the start of protocol treatment (see Section 5.4). Each participating site must also be registered with their own institution according to their institutional guidelines prior to start of protocol treatment.

5.1 Pre-Screening

Patients with TNBC may submit archival tissue to test for AR expression. A separate prescreening consent will be available to enable testing of archival tissue for AR expression before initiating other screening activities. Patients must sign the separate prescreening consent form if AR status is not already known (see section 5.2). Study site personnel must document the informed consent process for tissue analysis in the patient's clinical record.

If archival tissue is not available for AR analysis and the patient is otherwise eligible for the study and willing to go on study within 4 weeks, the patient may undergo a biopsy of metastatic site to assess AR status after signing the prescreening consent (see Section 5.3). The prescreening consent form allows extra study-related research biopsy specimens to be obtained so that the patient would not have to undergo a repeat biopsy at baseline for study purposes. Patients must be informed that analysis of tissue will not guarantee eligibility for this study, as patients found to have AR+ disease must meet all other eligibility criteria at the time of enrollment.

5.2 AR testing for registration

AR testing can be performed on a prior breast cancer biopsy (primary or local recurrence), surgical specimen, or biopsy of a metastatic site.

- For participants who are known to have androgen receptor-positive triple negative breast cancer by local testing in a CLIA certified lab on any one of the above samples (primary, local recurrence or metastasis), after discussion with the Protocol Chair, the prescreening consent does not need to be signed and he/she can sign the main consent form to proceed with eligibility screening. Patients who are found to be AR negative upon central review at Vanderbilt will be considered screen failures.

- For participants in whom androgen receptor status is not known upfront and who have archival tissue for testing: participants can sign the prescreening AR testing consent form to allow for AR testing. Please send:
    - A tissue block to the address below and indicate on the requisition that AR testing needs to be performed for registration.

      OR
    - If a participating institution does not allow release of formalin-fixed paraffin-embedded tissue blocks, send 3 unstained slides cut, 5 □ n each, on charged (plus) slides for AR testing by immunohistochemistry.
- For participants who are undergoing biopsy of a metastatic site as standard of care or specifically for AR testing: participants can sign the prescreening AR testing consent form to allow for AR testing from the biopsy, and to obtain the extra biopsies required for the study so that the participant would not have to undergo another biopsy if he/she is found to AR+ and qualifies for the study. Tissue must be sent to Vanderbilt-Ingram Cancer Center, along with the tissue block registration form for central testing of androgen receptor. See section 9 of the protocol for details on what should be obtained.

  Please note that a patient can only undergo a biopsy specifically for AR testing for this study if he/she otherwise meets all eligibility criteria and is able to go on study within 4 weeks of obtaining the AR results.
- The coordinating center must be notified if a sample is being sent for AR testing (see lab manual for details). Once the tissue to be tested is received at Vanderbilt, AR testing results will be provided to the ordering institution within 7-10 days. If the patient is AR+, the patient will be eligible to sign the treatment consent form and continue screening for protocol participation; the steps in section 5.4 should be followed.

Outside sites should ship specimens along with Tissue Registration Form directly to:

For all tissue and blood/plasma:

[redacted]

5.3 Biopsy of metastatic lesion

After signing informed consent for the research biopsy, potential participants should have 2-3 paraffin-embedded diagnostic core biopsy specimens and 2-3 core biopsy specimens suspended in RNAlater solution from a metastatic site if reasonably safe submitted prior to randomization.

Please see section 9 for further details. If AR status has already been confirmed centrally on a prior biopsy, tissue from the metastatic biopsy needs to be available, but not evaluated centrally, prior to beginning treatment on study.

Outside sites should ship specimens along with Tissue Registration Form directly to:

For all tissue and blood/plasma:

[redacted]

5.4 Ship ALL paraffin material, RNAlater tubes or slides as detailed in the lab manual. **Specimens should be mailed to arrive at VUMC from Monday 8AM through Friday 1PM**Registration

Once patients are confirmed to AR positive and if they meet all other eligibility criteria, patients will be centrally registered with the Vanderbilt-Ingram Cancer Center (VICC) at study entry by emailing the **Multi-Institutional Team at** coordinating.center@vanderbilt.edu . At the time of registration, the following documents must be emailed to the **VICC CTSR office** (coordinating.center@vanderbilt.edu):

- Copy of the patient's signed and dated Informed Consent including documentation of the consent process
- Patient Enrollment From
- Eligibility Checklist including source documents to verify eligibility

Prior to registration a copy of IRB approval at the respective sites will be requested and on file at VICC.

CTSR requests 24-48 hours to review all documents and confirm eligibility. [redacted]

In the phase II portion, the VICC Coordinating Center will assign sequence numbers to all patients in screening. Only patients deemed eligible will be randomized to a treatment arm. Sequence numbers will not be re-used if a patient screen fails. The Center will randomize eligible patients to enzalutamide + taselisib or enzalutamide alone. Following registration and randomization (after AR results are released to the treating institution, see below), eligible participants should begin protocol treatment within 2 weeks. Issues that would cause treatment delays should be discussed with the Protocol Chair. If a participant does not receive protocol therapy following randomization within allowed time period, the participant will become ineligible and will be cancelled from the study. Such patients will have to undergo screening

again to participate in the study in the future. Any requests for eligibility exceptions and/or deviations must be approved in writing by the Protocol Chair and the local IRB prior to execution. As is generally accepted, standard of care procedures performed prior to consent, but within the protocol defined screening window for each assessment can be used for study purposes. All research only procedures must be performed after the consent date.

6. TREATMENT PLAN

6.1 Overview

This is an open-label phase Ib/II multi-institution trial that evaluates the safety profile and anti-tumor activity of enzalutamide +/- taselisib in patients with androgen receptor positive metastatic triple negative (ER negative, PR negative, HER2Neu negative by IHC or FISH) breast cancer (AR+ TN MBC).

As taselisib has not been evaluated with enzalutamide, we will proceed with a phase Ib portion to ensure safety of the combination prior to open enrollment on the study.

6.2 Phase Ib component of the trial

The phase Ib component is complete. The primary objective of this portion of the trial was to determine the safety profile [dose-limiting toxicities (DLTs) during the first cycle of treatment, and maximum tolerated dose (MTD)] of the combination of taselisib and enzalutamide. The phase Ib portion allowed patients who are estrogen and/or progesterone receptor positive or negative, provided they are HER2 negative (see eligibility criterion 4.1.5).

- Enzalutamide + taselisib: the taselisib dose will start at 2 mg (dose level 1). Dose escalation proceeded among cohorts of 3 patients according to a standard 3+3 algorithm beginning at the lowest dose level.

In addition, pharmacokinetic (PK) sampling was collected from patients enrolled into the Phase Ib part of the study to allow for comparison within the study and to previous observations when taselisib was administered as a single agent in the Phase Ia trials. All open sites were able to participate in the phase Ib component.

6.2.1 Phase I Agent Dose Escalation

Dose level	Enzalutamide	Taselisib
1	160 mg PO daily	2 mg PO daily

2	160 mg PO daily	4 mg PO daily
3	160 mg PO daily	6 mg PO daily
4	160 mg PO daily	8 mg PO daily

1 cycle = 28 days

All pills were self-administered (by the patients themselves). A pill diary was given to all patients enrolled in the study.. The investigator will instruct the patient to take the study drugs exactly as specified in the protocol. Taselisib and enzalutamide were administered on a continuous once daily dosing schedule. To assist in PK assessment, for patients enrolled in the combination arm (phase Ib or II) at Vanderbilt only, taselisib was started on day 1, and enzalutamide was added on day 9.

6.2.2 Definition of Dose-Limiting Toxicity (DLT)

Any of the following events that occur during Cycle 1 (first 4 weeks) was considered a DLT when possibly, probably or definitively classified as drug-related (according to NCI CTCAE v 4.0 Requirements). Whenever a patient experienced toxicity that fulfilled the criteria for a DLT, treatment with taselisib was held and the toxicity was followed up, Dose reductions as listed in Section 7.1 were followed. No DLTs were noted in the phase Ib.

Table 1: DLT criteria and Follow-up Evaluation (CTCAE v 4.0)

Toxicity	DLT criteria	Follow-Up Evaluation
Hematologic	• Anemia CTCAE Grade ≥ 3 will not be considered a DLT unless judged to be a hemolytic process secondary to study drug. • Febrile neutropenia CTCAE Grade ≥ 3 • ANC CTCAE Grade 3 for > 7 consecutive days. G-CSF use is permitted during the DLT window. • ANC CTCAE Grade 4 • Platelet count CTCAE Grade 3 for > 7 consecutive days and/or with signs of excessive bleeding • Platelet count CTCAE Grade 4	If ≥ CTCAE grade 3 neutropenia or ≥ CTCAE grade 3 thrombocytopenia have been demonstrated, these parameters must be repeated at least once a week until resolution to ≤ CTCAE grade 1 neutropenia or ≤ CTCAE grade 1 thrombocytopenia to allow for initiation of re-treatment, and then at least weekly until either resolution or until stabilization.

Table 1: DLT criteria and Follow-up Evaluation (CTCAE v 4.0)		
Toxicity	DLT criteria	Follow-Up Evaluation
Cardiac disorders	• Cardiac toxicity CTCAE Grade ≥ 3 or cardiac event that is symptomatic or requires medical intervention • Clinical signs of cardiac disease, such as unstable angina or myocardial infarction, or Troponin CTCAE Grade 3 (confirmed with a repeat Troponin within 24 hrs) • ECG QTc interval prolonged CTCAE Grade ≥ 3 (after repeat confirmation on at least 2 more ECGs at the same time point)	Patients who experience ECG abnormalities indicative of an ischemic cardiac event should be evaluated by cardiology and have EGCs repeated at least once a week until normalization or stabilization of ECG findings. If troponin ≥ CTCAE grade 3 has been demonstrated, the patient should be evaluated by cardiology and this parameter must be repeated once a week or as clinically indicated until resolution to ≤ CTCAE grade 1, and then at least weekly until either resolution or until stabilization. Patients who experience QTc prolongation should be followed as per Dose Modification section guidelines and should be evaluated by cardiology.
Vascular disorders/ Hypertension	Persistent hypertension CTCAE Grade ≥ 3 requiring more than one drug or more intensive therapy than previously	
Fatigue	Fatigue CTCAE Grade 3 for > 7 consecutive days	
Rash and/or photosensitivity	Rash or photosensitivity CTCAE Grade 3 for > 7 consecutive days despite skin toxicity treatment, including oral steroid and antihistamines Photosensitivity CTCAE Grade ≥ 4	Rash should be evaluated weekly until resolution.

Table 1: DLT criteria and Follow-up Evaluation (CTCAE v 4.0)		
Toxicity	DLT criteria	Follow-Up Evaluation
Endocrine: Hyperglycemia	• Hyperglycemia Grade 3 (FPG 250 – 399 mg/dL; 13.9 - 22.2 mmol/L) (confirmed with a repeat FPG within 24 hrs) for > 7 consecutive days despite oral anti-diabetic treatment • Hyperglycemia Grade 4 (FPG ≥ 400 mg/dL; ≥ 22.3 mmol/L) • Hyperglycemia leading to diabetic keto-acidosis, hospitalization for IV insulin infusion, or non-ketotic coma • Hyperglycemia occurring during corticosteroid administration will be only considered DLT if not resolved within 2 days after the end of corticosteroid treatment.	For details of the follow-up and treatment of ≥ grade 3 hyperglycemia refer to the Guidelines for the treatment of study drug-induced hyperglycemia provided in the Dose Modification Section
GI disorders	• Diarrhea CTCAE Grade ≥ 3 for ≥ 48 hrs, despite the use of optimal anti-diarrhea therapy • Nausea/vomiting CTCAE Grade ≥ 3 for ≥ 48 hrs, despite the use of optimal anti-emetic therapy • Pancreatitis CTCAE Grade ≥ 3	If amylase and/or lipase ≥ CTCAE grade 3 (> 2 x ULN) has been demonstrated, these parameters must be assessed once at 2 to 4 days and once again at 7 days (± 1 day) and be repeated twice a week until resolution to ≤ CTCAE grade 2 to allow for initiation of re-treatment, and then at least weekly until either resolution to ≤ CTCAE grade 1 or until stabilization. A CT scan or other imaging study to assess the pancreas, liver, and gallbladder must be performed within 1 week of the first occurrence of any ≥ CTCAE grade 3 of amylase or lipase. Amylase and lipase should be followed weekly until resolution to ≤ CTCAE grade 1.

Table 1: DLT criteria and Follow-up Evaluation (CTCAE v 4.0)		
Toxicity	DLT criteria	Follow-Up Evaluation
Hepatic	• Total bilirubin CTCAE Grade 2 for > 7 consecutive days, if less normal-grade 1 at baseline • Total bilirubin CTCAE Grade ≥ 3, if grade 2 at baseline • AST or ALT CTCAE Grade ≥ 2 in conjunction with blood bilirubin d CTCAE Grade ≥ 2 of any duration • AST or ALT CTCAE Grade 3 for > 7 consecutive days • AST or ALT CTCAE Grade 4 • Serum alkaline phosphatase CTCAE Grade 4 • Serum lipase and/or serum amylase (asymptomatic) CTCAE Grade 3 for > 7 consecutive days • Serum lipase and/or serum amylase (asymptomatic) CTCAE Grade 4 • ALT or AST of 3X ULN and concomitant bilirubin of >2 X ULN (Hy's Law) with no other explanation other than study drug should be permanently discontinued from treatment with study drug.	Both taselisib and enzalutamide should be held. If total bilirubin ≥ 2 x ULN or ≥ CTCAE grade 3 AST/ALT has been demonstrated, these parameters must be repeated at least twice a week until resolution to ≤ CTCAE grade 1 (or ≤ grade 2 for AST or ALT, if liver metastasis are present) to allow for initiation of re-treatment, and then at least weekly until either resolution or until stabilization. Patients with total bilirubin > ULN (any duration) should have fractionation of bilirubin into total/direct or indirect/direct components and any additional work-up as clinically indicated by these results. Follow-up of hyperbilirubinemia should proceed as per the guidelines above, irrespective of the results of fractionation. For any hepatic toxicity CTCAE Grade 4, or CTCAE Grade 3 that does not resolve within 7 days to CTCAE Grade ≤ 1 (or CTCAE Grade ≤ 2 if liver infiltration with tumor present), an abdominal CT scan should be performed to assess if it is related to disease progression.
Renal	Serum creatinine CTCAE Grade ≥ 3	If serum creatinine ≥ 3 x ULN has been demonstrated, this parameter must be repeated at least twice a week until resolution to ≤ CTCAE grade 1 to allow for initiation of re-treatment, and then at least weekly until either resolution or until stabilization. Serum creatinine ≥ 2.0 x ULN and [+3] proteinuria or hematuria ≥ CTCAE grade 2 has been demonstrated, a 24-hour urine collection for total protein and total creatinine must be done at least weekly until either resolution to baseline value or until stabilization. Whenever a measured CrCl is obtained, a serum creatinine should

Table 1: DLT criteria and Follow-up Evaluation (CTCAE v 4.0)		
Toxicity	DLT criteria	Follow-Up Evaluation
		be obtained within ≤ 72 hours of the urine collection.
Other hematologic and non-hematologic toxicities	Any other CTCAE ≥ Grade 3 toxicity except: • Lymphocyte count decreased (lymphopenia) CTCAE grade ≥ 3 unless clinical significance • Alopecia of any grade	Patients who experience neurotoxicity should be followed as per Dose Modification Section Patients who experience non-laboratory DLTs must be evaluated at least once a week following demonstration of the toxicity until resolution of the toxicity to allow for re-treatment, stabilization of the toxicity, or study treatment completion.
Apart from the criteria listed above, if a lower grade AE leads to a dose interruption of taselisib of more than 7 consecutive days, this AE may be considered as a DLT. Such cases will be considered individually. Patients who withdraw or are withdrawn from the study prior to completing the DLT assessment window for any reason other than a DLT or miss > 7 days for non-drug related AEs will be replaced.		

The following were instructions for DLTs in the phase Ib. Patients who meet DLT criteria must stop taselisib. Patients who meet DLT criteria for hepatoxicity should hold both taselisib and enzalutamide. Patients whose treatment is interrupted or permanently discontinued due to an adverse event or clinically significant laboratory value must be followed as outlined in the table above, at least once a week for 4 weeks, and subsequently at 4-week intervals, until resolution or stabilization of the event, whichever comes first. Patients that require a dose delay of > 28 days due to a cause unrelated to study participation will have the opportunity to continue study if deemed appropriate to do so by the Protocol Chair of the study. If patients experience a DLT considered related to taselisib, the patient may remain on enzalutamide on-study after resolution of the DLT at the discretion of the treating physician after receiving counseling about alternate treatment options. If patients experience a DLT considered related to enzalutamide, they have the option of remaining on single agent GDC0032 on-study at the discretion of the treating physician after receiving counseling about alternative treatment options. After resolution of the DLT, patients may be able to restart taselisib or enzalutamide at a lower dose at the discretion of the treating physician. However, such cases will be considered a DLT for the purposes of defining the MTD. All patients who continue taking taselisib and/or enzalutamide as a single agent will not be evaluable.

Patients who withdraw or are withdrawn from the study prior to completing the DLT assessment window for any reason other than a DLT or miss ≥ 7 days for non-drug related AEs will be replaced. This includes patients whose disease progresses within 30 days of starting the study. Patients requiring dose reduction of enzalutamide or taselisib for reasons other than a DLT in cycle 1 of the phase Ib portion will not be included in the DLT evaluation. After the MTD is reached,

patients in the phase Ib on lower dose levels can dose escalate to the MTD at the discretion of the treating physician and after discussion with the Protocol Chair.

6.2.3 Definition of Maximum Tolerated Dose (MTD)

The MTD was defined as the highest dose of taselisib tested in which a DLT is experienced by 0-1 out of 6 patients among the dose levels. The dose of enzalutamide remained constant. The first cohort of patients (3 patients) in each arm started at dose level 1, and each patient was observed for 4 weeks on the specified dose and instructions were followed as below:

- If no patient in the first cohort of 3 experiences a DLT, dose escalation will proceed and patients will be accrued at the next dose level. If 1 of these patients experiences a DLT, the cohort will be expanded to 6 patients. If <33% of these patients experiences a DLT (e.g., 1 of 6 patients), dose escalation may proceed.
- If a DLT is observed in ≥ 33% of the patients in a given cohort (e.g. ≥ 2 of 6 patients), the MTD will have been exceeded and the lower dose level will be evaluated.

  An exception to the above rule would be in the case in which the two DLTs observed out of 6 patients are both hematologic in nature and potentially reflect poor bone marrow reserve in this heterogeneous patient population and not the true MTD for the combination. In this instance, up to 3 additional patients may be enrolled to expand the total cohort size to 9 patients to assess for further DLT. In this situation, if a third DLT is observed, the MTD will have been exceeded and the lower dose level will be evaluated.
- If the 2 mg dose does not exceed the MTD, then dose escalation will proceed to the 4 mg dose. If the 4 mg dose does not exceed the MTD, then dose escalation will proceed to the 6 mg dose, and if the 6 mg dose does not exceed the MTD, then dose escalation will proceed to the 8 mg dose. If <33% of patients experience a DLT in the 8 mg cohort, then this dose level will be the maximum administered dose (MAD).

No new cohort of patients will be treated until the previous cohort has been fully evaluated for toxicity. Patients requiring dose reduction for reasons other than a DLT in cycle 1 of the phase Ib portion will not be included in the DLT evaluation. If the frequency of Grade 3 or 4 toxicities or other unacceptable lower grade chronic toxicities in the phase 1b portion suggests that the MTD has been exceeded at that dose level, any remaining accrual at that dose level will be halted and a lower dose of taselisib may be examined.

If taselisib dosing is not well tolerated, a less frequent dosing schedule may be evaluated at the same or lower dose of taselisib in that arm. Consistent with the dose escalation rules above, the lowest dose level will be 2 mg, and the highest dose level will be 8 mg.

6.3 Phase II component of the trial

Based on the phase Ib and data from other studies of taselisib, the recommended dose of taselisib for phase II is 4 mg. Note that dose reduction within patients (individually) is allowed. Dose reduction will be required for a given patient in case of grade 3 or 4 toxicities.

The primary efficacy endpoint is clinical benefit rate (CBR) of enzalutamide + taselisib in patients with androgen receptor positive triple negative metastatic breast cancer. Secondary objectives are to determine the ORR and progression free survival (PFS). CBR is defined as the proportion of patients achieving a complete response (CR) plus partial response (PR) plus stable disease (SD)

for at least 16 weeks. To assess objective responses, we will estimate the overall tumor burden at baseline to which subsequent measurements [performed every 2 cycles using the Solid Tumor Response Criteria (RECIST) v1.1] will be compared.

Section 6.3.1 and 6.3.2 discuss administration of taselisib and enzalutamide. Patients on the combination arm can take both drugs at the same time. Patients on the enzalutamide only arm should take enzalutamide only as directed below.

6.3.1 Taselisib

Taselisib tablets are administered once daily until disease progression or intolerable toxicity. Taselisib administration will begin on C2D1 for patients enrolled on the combination arm (with enzalutamide alone beginning on C1D1). Each dose should be taken with a minimum of 3–4 ounces (90–120 mL) of water with or without food in the morning (before noon). If a dose is missed (not taken by noon), the patient will resume dosing with the next scheduled dose. Missed or vomited doses will not be made up. The occurrence and frequency of any vomiting during a treatment cycle must be noted as an adverse event. Patients should swallow the capsules as a whole and not chew or crush them.

A sufficient number of taselisib tablets should be provided to the patient to last until the next cycle. Patients will be instructed to bring their bottles of study drug and their medication diary with them to each study visit.

6.3.2 Enzalutamide

The daily dose of enzalutamide is 160 mg/day given in 4 capsules (40 mg each) by mouth. Enzalutamide capsules are administered once daily until disease progression or intolerable toxicity. The first ten subjects on the enzalutamide + taselisib arm at Vanderbilt will have blood draws for PKs as outlined in the study calendar.

Patients should be instructed to take the dose of enzalutamide at the same time in the morning (before noon) every day, with or without food. Enzalutamide should be taken with a glass of water. Patients should swallow the capsules as a whole and not chew, crush, or open them.

If the patient forgets to take her/his dose and it is past noon, then the dose should be withheld that day and enzalutamide should be restarted the following day. Missed or vomited doses will not be made up. The occurrence and frequency of any vomiting during a treatment cycle must be noted as an adverse event.

A sufficient number of enzalutamide capsules should be provided to the patient to last until the next visit or, at the investigator's discretion, to last until the next cycle. Patients will be instructed to bring their bottles of study drug and their medication diary with them to each study visit.

6.4 Concomitant Treatment and Supportive Care Guidelines

6.4.1 Concomitant Therapy and Drug-drug Interactions

Concomitant therapy includes any prescription medications or over-the-counter preparations used by a patient between the 7 days preceding day 1 of treatment and the treatment completion visit.

All concomitant medications should be documented. Patients who experience toxicities may be treated symptomatically as clinically indicated. Patients treated with anti-convulsant medications (see below for restrictions) should have levels monitored regularly.

Enzalutamide is a strong inducer of CYP3A4 and a moderate inducer of CYP2C9 and CYP2C19. Taselisib is a substrate of CYP3A4, and results of a clinical study with a potent CYP3A4 inhibitor (itraconazole) suggest that there is a moderate potential for a drug-drug interaction between taselisib and any medication that strongly inhibits CYP3A4. Therefore, the concomitant use of strong CYP3A4 inhibitors should be avoided. The effects of strong/moderate CYP3A4 inducers on the pharmacokinetics of enzalutamide have not been evaluated in vivo. Co-administration of enzalutamide may decrease the plasma exposure of enzalutamide and should be avoided. If use of one of these drugs is necessary, the risks and benefits should be discussed with the Protocol Chair and documented prior to its concomitant use with taselisib and enzalutamide:

• Strong/moderate CYP3A4 inhibitors or inducers (which should be avoided): atazanavir, bosentan, carbamazepine, ciprofloxacin, clarithromycin, cyclosporine, efavirenz, erythromycin, etravirine, fluconazole, hyperforin (St. Johns Wort), indinavir, itraconazole, ketoconazole, modafinil, nafcillin, nefazodone, nelfinavir, oxcarbazepine, phenobarbital, phenytoin, posaconazole, quinidine, rifabutin, rifampin, rifapentine, ritonavir, saquinavir, sirolimus, tacrolimus, telithromycin, troleandomycin, verapamil, and voriconazole.
• CYP2C8 inhibitors or inducers: gemfibrozol should be avoided.
• Strong P-glycoprotein inhibitors, including cyclosporine, amiodarone and quinidine should be avoided
• *In-vitro* protein binding studies have shown that enzalutamide can displace coumarin anticoagulants from binding sites. Patients already on coumarin anticoagulants should have closely monitored Prothrombin times while taking enzalutamide.
• Grapefruit juice or grapefruit supplements should be avoided.

See the following websites for updated lists of CYP inhibitors, inducers, and substrates:

- http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#potency
- [redacted]

6.4.2 Excluded Therapy

Use of the following therapies is prohibited for≤ 14 days prior to Cycle 1 Day 1 dosing and during the study.

• Any experimental concomitant therapy intended for the treatment of cancer , including chemotherapy, radiotherapy, immunotherapy, biologic therapy, herbal therapy, or hormonal therapy. Bisphosphonate and denosumab therapy for bone metastases is allowed.
• Quinidine or other anti-arrhythmic agents are prohibited

6.5 Assessments during Treatment

All visits must occur within ± 3 days from the scheduled date, unless otherwise noted. Please see the Study Calendar at the beginning of this protocol for the schedule of treatment period assessments.

6.6 Cross-over post-progression

Patients who are randomized to enzalutamide alone can crossover to receive enzalutamide + taselisib upon disease progression. Patients who are randomized to enzalutamide alone and who discontinue enzalutamide for toxicity cannot crossover to receive single agent taselisib. However, patients on the enzalutamide + taselisib arm can continue to receive single agent taselisib if they discontinue enzalutamide due to toxicity. Patients who cross over to receive taselisib in combination with enzalutamide will follow the assessments and procedures as outlined in the study calendar. The duration of each cycle will continue to be 28 days and the cycle numbers will "reset" to 1 and will be followed by the "X" suffix (e.g., Cycles 1X, 2X, etc.). Crossover therapy (taselisib) must begin no later than 21 days after the clinic visit at which progression is determined, but can begin as early as C1XD1.

Patients must have labs and procedures performed as outlined in the cross-over study calendar and must meet the values as described in the Inclusion Criteria, prior to receiving taselisib based on assessments and procedures performed within 28 days prior to Day 1 of Cycle 1X. Labs and procedures not included in the cross-over study calendar do not have to be repeated. Patients must also agree to a tumor biopsy, as outlined in the eligibility criteria, prior to initiating taselisib. Patients will receive crossover treatment until progression, intolerable toxicity, or elective withdrawal from the study. Patients who crossover will be evaluated with radiologic assessments every 2 cycles until disease progression.

6.7 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of criteria in Section 6.9 applies.

6.8 Duration of Follow-Up

Participants will be followed for 30 days after the final dose of study drug. The 30 day follow-up can be done by phone or through a clinic visit. Patients who at the treatment completion visit have an ongoing serious adverse event or an adverse event leading to treatment discontinuation will be followed every 3 months until the event resolves, the investigator assesses the event as stable, or the patient is lost to follow-up. Participants will also be followed annually for disease progression by phone, and if necessary, by chart review. If the patient is no longer coming to the institution for care, then the patient may be called as needed for follow-up.

6.9 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed below apply. All patients who initiate protocol treatment will be included in the overall evaluation of response (intent-to-treat analysis). All reasons for discontinuation of therapy should be documented clearly in the medical record.

If a subject discontinues or withdraws from the study, every attempt will be made to get a tissue/tumor biopsy at the end of treatment, along with blood if the subject is able and willing to do so.

Patients may be withdrawn from the study if they experience any of the following:

- Disease progression, per investigator assessment
- Intolerable toxicity to taselisib or enzalutamide. Patients experiencing toxicity to taselisib or enzalutamide can continue on study with single agent enzalutamide or taselisib, respectively, at the discretion of the treating physician. Patients will be followed for disease progression and should continue radiologic assessments every 2 cycles as per the study.

Other reasons for patient discontinuation may include, but are not limited to, the following:

- Non-compliance with the study protocol, including, but not limited to not attending the majority of scheduled visits. The Protocol Chair will determine when non-compliance should lead to removal from study. Note: The patients will still be included in the overall evaluation of response (intent-to-treat analysis).
- Patient personal decision. The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of response (intent-to-treat analysis) if any protocol therapy was administered prior to withdrawal.
- If the patient becomes pregnant
- Patient is lost to follow-up
- Study is terminated for any reason

The investigator has the right to discontinue a patient from the study for any medical condition that the investigator determines may jeopardize the patient's safety if he or she continues in the study; for reasons of noncompliance (e.g., missed doses, visits); or if the investigator determines it is in the best interest of the patient. The Protocol Chair should be notified in such cases.

7. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

7.1 Dose Modifications/ Delays

Table 2 below lists dose level reductions for taselisib and enzalutamide. Please see section 7.2 to 7.4 for thresholds to reduce doses.

Table 2: Dose Modifications			
Taselisib dose level reductions			
Starting dose	First reduction	Second reduction	
4 mg daily	2 mg daily	Discontinue	
Enzalutamide dose level reductions			
Starting dose	First reduction	Second reduction	Third reduction
160 mg daily	120 mg daily	80 mg daily	Discontinue

7.2 Taselisib

See Table 3 at the end of this section for taselisib dose modification criteria

Patients who experience an SAE or whose treatment is interrupted or permanently discontinued due to an adverse event or clinically significant laboratory value must be followed as outlined in Table 3 below, at least once a week for 4 weeks, and subsequently at 4-week intervals, until resolution or stabilization of the event, whichever comes first. If a patient requires a dose delay of > 28 days from the intended day of the next scheduled dose, then the patient should be discontinued from the study. All patients must be followed for adverse events and serious adverse events for 30 days following the last dose of taselisib. This can be done as either an in-office visit or by phone. If the taselisib dose is reduced due to an adverse event which is later determined to be secondary to enzalutamide and enzalutamide is discontinued, taselisib can be re-escalated at the discretion of the treating physician after consultation with the Protocol Chair. If both taselisib and enzalutamide are held for an adverse event, enzalutamide should be restarted at the original dose. Taselisib should be restarted at one dose lower than the last dose. After 28 days, if the patient is tolerating both medications well, taselisib may be increased back to the original dose.

7.2.1 Anticipated Toxicities and Management

7.2.1.1 *Hyperglycemia and Metabolic Effect*

Hyperglycemia has been observed in patients who received single-agent taselisib in the single-agent Phase I study. Hyperglycemia has been reversible upon holding taselisib and/or initiation of anti-hyperglycemic medication (e.g., metformin). HbA1c and fasting glucose levels will be monitored at baseline, and as per the study calendar. Patients should be advised to report symptoms associated with hyperglycemia, such as thirst, frequent urination, and blurred vision. Guidelines for dosage modification and treatment interruption or discontinuation for patients who experience hyperglycemia are outlined in Table 3.

7.2.1.2 Potential Pulmonary Toxicity

Noninfectious pneumonitis has been observed in patients treated with taselisib. For example, in the single-agent Phase I study in a lung adenocarcinoma patient treated at the 16-mg daily dose level 1 week after discontinuing taselisib. The patient was treated with corticosteroids, and associated symptoms resolved. Patients who require any daily supplemental oxygen are not eligible for the study. Patients will be assessed for pulmonary signs and symptoms throughout the study. Patients will be monitored carefully (including physical examinations, oxygen saturation measurement, and periodic CT scans) for changes in pulmonary status during treatment. Diffusion capacity of the lung for carbon monoxide (DLCO) and computed tomography (CT) scans may be used to confirm and evaluate changes in pulmonary status. Patients experiencing symptomatic or asymptomatic pneumonitis should be treated per standard-of-care and guidelines in Table 3 that were adapted from recommendations by White et al. (2010) for the management of pneumonitis in cancer patients receiving everolimus. Use of corticosteroids should be considered for symptomatic cases of noninfectious pneumonitis.

7.2.1.3 Dermatological Toxicity

Treatment-related rash, including cases of Grade 3 rash, has occurred in patients who received taselisib. This rash is commonly manifested as maculo-papular with or without pruritus. Incidence rate and severity of rash appear to be dose dependent. The rash has resolved upon holding of taselisib and/or supportive therapy (e.g., topical or systemic steroids). Rash and other dermatological events should be closely monitored, and patients with severe rash should be monitored for associated signs and symptoms such as fever and hypotension that may be suggestive of a systemic hypersensitivity reaction. For severe rash, dosing of taselisib should be interrupted, and patients should be treated with supportive therapy per standard of care. Use of topical antihistamine, as well as topical or systemic corticosteroids, may be considered. Dose delay and modification guidelines for patients who experience rash are outlined in Table 3. Of note, no grade 3 or 4 rashes were noted in the phase Ib portion of this study with dose escalation to 8 mg taselisib. However, in the phase II portion with taselisib at 4 mg, 7 of 8 patients on the combination arm experienced a grade 3 or 4 rash with fever within the first 14 days of treatment. No patients who crossed over from enzalutamide to taselisib + enzalutamide experienced the rash, thus the study is amended so that all patients have a 28 day lead-in with enzalutamide prior to starting taselisib (similar to the cross-over arm). The rash resolved in all patients with IV steroids, anti-histamines and supportive care.

7.2.1.4 *Gastrointestinal Toxicity*

Nausea, vomiting, stomatitis, and diarrhea have been observed in patients receiving taselisib. Colitis was diagnosed by several methods, including endoscopy and abdominal imaging (computed tomography [CT] scans). Pathology from biopsies obtained from endoscopy has confirmed colitis. Patients usually present with Grade 2 or Grade 3 diarrhea that has been refractory to antidiarrheals. The time (from the first dose of study treatment) to onset (formal diagnosis of colitis) ranged from approximately 99–250 days. Patients had resolution or improvement of gastrointestinal toxicities upon holding study drug and/or initiating corticosteroid therapy. Perforated duodenal ulcer has been observed in 2 patients (1 patient at 6 mg in combination with letrozole; another patient at 6 mg in combination with fulvestrant. Appropriate caution should be taken with the administration of medications such as aspirin, NSAIDs, and corticosteroids, which can increase the risk of gastritis, peptic ulcers, or GI perforation.

Patients should be closely monitored for gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, abdominal pain, stomatitis, and changes in stool, including checking for blood in stool if clinically indicated). Patients should be advised to inform the investigator if any diarrhea occurs, even if it is mild. Prompt management of diarrhea with antidiarrheal medications should be implemented. Because of the approximately 40 hour half-life of taselisib, investigators should hold taselisib for Grade ≥ 2 diarrhea. Patients who resume treatment should be monitored closely for sign of renewed diarrhea.

Patients with inflammatory bowel disease, such as Crohn's disease or ulcerative colitis, are excluded from this study. Steroid-responsive diarrhea and colitis have been difficult to distinguish in patients treated with taselisib. All cases of colitis have been reversible with corticosteroid treatment. Prompt initiation of corticosteroids for persistent diarrhea despite anti-diarrheal treatment can decrease the severity of the diarrhea and prevent the need for hospitalization. Patients who develop severe steroid-responsive diarrhea usually have been on taselisib treatment for at

least 2 months, with an average onset 4 to 6 months of treatment. A stool culture is helpful in identifying concurrent infections, and patients have been successfully treated with concurrent steroids and appropriate antibiotics, if needed.

If a patient is being treated with corticosteroids, total parenteral nutrition is discouraged, as this increases the risk for severe hyperglycemia. Discontinuation of non-steroidal inflammatory medications or other medications that exacerbate colitis are also recommended during colitis episodes.

Management recommendations for GI toxicities are outlined in Table 3.

7.2.1.5 *Liver Toxicity*

If clinical symptoms or signs suggestive of liver dysfunction occur (e.g., nausea, vomiting, abdominal pain, fatigue, anorexia, "flu-like" symptoms, dark urine, jaundice, or right upper quadrant tenderness), the serum transaminases should be measured immediately. Elevations above baseline should prompt an evaluation should occur to determine the cause of the elevation, including progressive disease and hepatotoxicity from concurrent medications.

If transaminases (ALT/AST) are elevated > 3X ULN and are thought to be secondary to disease progression, a CT scan should be obtained to confirm disease progression and the patient should be removed from study. If transaminases (ALT/AST) are elevated > 3X ULN and not thought to be secondary to disease progression, treatment should be withheld until levels fall to ≤ 3X normal levels. If a patient has known liver metastases and baseline transaminases were 3-5X ULN, treatment should be held until levels fall to less than or equal to baseline levels. If total bilirubin is elevated > 1.5 x normal level, treatment should be withheld until level falls to ≤ 1.5 x normal levels. If alkaline phosphatase is elevated > 2.5 x normal value, treatment should be withheld until levels fall to ≤ 2.5 x normal level (unless bone metastases are present in the absence of liver metastases). A dose reduction should then be administered following resolution of any or all of these liver enzyme abnormalities.

If transaminases are subsequently elevated > 3X ULN or, if total bilirubin is elevated > 1.5X normal value, or if alkaline phosphatase is elevated > 2.5 x normal value (unless bone metastases are present in the absence of liver metastases), treatment will again be withheld until AST/ALT decrease to ≤ 3X normal, total bilirubin decreases to ≤ 1.5 x normal value and alkaline phosphatase decreases to ≤ 2.5 x normal value. A second dose reduction should then be administered. If a patient has known liver metastases and baseline transaminases were 3-5X ULN, treatment should be held until levels fall to less than or equal to baseline levels. For any hepatic toxicity CTCAE Grade 4, or CTCAE Grade 3 that does not resolve within 7 days to CTCAE Grade ≤ 1 (or CTCAE Grade ≤ 2 if liver infiltration with tumor present), an abdominal CT scan should be performed to assess if it is related to disease progression.

If, after 2 dose reductions, AST, ALT, total bilirubin and/or alkaline phosphatase remain elevated, patient should be taken off study, and laboratory abnormalities should be reported as an unacceptable toxicity.

Table 3: Dosage Modification Criteria and Guidelines for Management of TASELISIB Related Toxicities	
Toxicity NCI-CTCAE (v 4.0)	**Actions**
Diarrhea	
Grade 1	• Manage with anti-diarrheals[a]. • No dose reduction necessary. • For persistent Grade 1 diarrhea occurring after Cycle 2, recommend evaluation for infectious causes via stool culture[b]. For non-infectious diarrhea, consider colonoscopy to evaluate for colitis.
Grade 2	• Hold taselisib and initially manage with per institutional standard of care until Grade ≤ 1. These include antidiarrheals[a]. • Obtain stool culture for infectious workup[b] Infections (e.g. Clostridium difficile, enteric bacteria, CMV) should be treated with the appropriate antibiotic. • For persistent Grade 2 non-infectious diarrhea lasting longer than 48 hours despite treatment with antidiarrheals, treat with oral corticosteroids (20-40 mg prednisone QD starting dose with taper) or budesonide 9mg PO QD. • If Grade 2 diarrhea occurred after Cycle 2, was a recurrent episode, or improved with corticosteroid treatment, resume taselisib treatment at one dose level lower upon improvement to Grade ≤ 1 and after completion of corticosteroid treatment. • If Grade 2 diarrhea occurred before Cycle 2, did not require corticosteroid treatment, and was an initial episode, resume taselisib treatment at the same dose upon improvement to Grade ≤ 1. • For Grade 2 colitis, resume taselisib treatment at one dose level lower upon improvement to Grade ≤ 1 and after completion of corticosteroid treatment. • If Grade 2 diarrhea does not improve after 48 hours of corticosteroid treatment, a colonoscopy is recommended to evaluate for other causes of diarrhea (e.g., CMV colitis)
Grade 3	**First Occurrence:** • Hold taselisib and initially manage with antidiarrheals. • Obtain stool culture for infectious workup. • For Grade 3 diarrhea or colitis, treat with systemic corticosteroids (prednisone 60-80 mg QD equivalent or solumedrol 16-20 mg IV q 8 h to start). Can increase steroid dosage if diarrhea does not improve. • Concurrent infections (e.g., Clostridium difficile, enteric bacteria, CMV) should be treated with the appropriate antibiotic. • For patients that do not improve upon 48 hours of corticosteroid treatment, a colonoscopy is recommended to evaluate for other causes of diarrhea (e.g. CMV colitis) If diarrhea or colitis improves to Grade ≤1 and upon completion of any steroid taper or antibiotic treatment, resume taselisib treatment at one dose level lower. **Second Occurrence:** • Discontinue taselisib
Grade 4	• Discontinue taselisib.
Rash	

Special considerations	• Permanently discontinue for any rash with concurrent signs/symptoms strongly suggestive of a severe Type 1 hypersensitivity or anaphylactic/anaphylactoid reaction, fever, or with painful desquamation or mucosal involvement suggestive of Stevens-Johnson Syndrome/ toxic epidermal necrolysis, or with other life-threatening complications
Grade 1	• Continue dosing at current dose and monitor for change in severity (consider contacting patient between study visits). Consider prescribing topical steroids[c].
Grade 2	• Monitor closely for change in severity and hypersensitivity-related systemic symptoms (contact patient weekly between study visits) • Consider prescribing topical steroids[c] • Consider dermatological consultation • Consider one or more of the following: - Hold dose until resolution to ≤ Grade 1, then resume at next lower dose level - Dose reduction - Initiation of oral steroid treatment unless contraindicated[d]; monitor blood sugar carefully if initiating steroids while continuing taselisib
Grade 3: first event	• Hold dose and monitor closely for changes in severity and for hypersensitivity-related systemic symptoms • Recommend topical steroids[c] • Recommend initiating oral steroids unless contraindicated[d] • Upon resolution to ≤ Grade 1, reduce to next lower dose level • Consider dermatological consultation • Consider obtaining photographs of rash if permitted by local regulations
Grade 3: first recurrence after dose reduction	• Hold dose and monitor closely for changes in severity and for hypersensitivity-related systemic symptoms • Recommend initiating oral steroids unless contraindicated[d] • Discuss with Protocol Chair if would like to consider restarting at next lower level upon resolution to ≤ Grade 1, or permanently discontinue • Recommend dermatological consultation, if not already performed • Consider obtaining photographs of rash if permitted by local regulations
Recurrent Grade 3 or Grade 4	• Manage as described above. • Discontinue taselisib permanently.
Hyperglycemia	
For all grades	• Instruct patient to follow dietary guidelines provided by the American Diabetes Association • For ≥ grade 2, initiate, continue or intensify medication with appropriate anti-diabetic treatment (Note: some oral anti-diabetic drugs are CYP3A inducers or inhibitors and should be used with caution) • Metformin is the first antihyperglycemic medication of choice because of the lower risk of hypoglycemia with this agent. Because metformin in some patients may also cause diarrhea and not be well tolerated, other antihyperglycemic medications such as sulfonylureas (e.g., glimepiride, glipizide) can be used. Extra caution should be used with other drugs such as sulfonylureas because of the increased risk for hypoglycemia with these agents. Consultation with an endocrinologist can be helpful in managing hyperglycemia. • If Grade worsens start following recommendations for the worsened grade
Grade 1	• Maintain dose level, check FPG every week for 2 weeks, then continue checking every 2 weeks until resolved

Grade 2	• Maintain dose, re-check FPG within 24 hours, if no worse than Grade 2, initiate or increase the dose of an anti-hyperglycemic (e.g. metformin) • If FPG does not resolve to ≤ Grade 1 within 14 days after initiation/ intensifying anti-diabetic treatment: consider endocrine evaluation, consider increasing anti-hyperglycemic therapy further. • Dosing with taselisib may either be held or continued per investigator evaluation.
Asymptomatic Grade 3	• Initiate/increase anti-diabetic treatment, re-check FPG within 24 hours, and if no worse than Grade 3: • Hold taselisib until hyperglycemia resolves to Grade ≤ 2. Anti-hyperglycemics may be initiated or intensified. Consider endocrine evaluation. After this, taselisib may be restarted at the same dose or a lower dose at the discretion of the treating physician. If another grade 3 hyperglycemia event occurs, the above steps should be followed, but the dose of taselisib must be decreased by one dose level.
Symptomatic Grade 3 or Grade 4	If a fasting patient experiences a symptomatic Grade 3 hyperglycemia event (e.g., blurred vision, frequent urination, excessive thirst) or any Grade 4 hyperglycemia event: • Taselisib dosing should be suspended until the hyperglycemia resolves to Grade ≤2. • The patient should be managed as per standard care, including implementation of additional glucose monitoring and initiation and/or increase of anti-hyperglycemic therapy. The patient will be discontinued from the study if such therapy fails to control their hyperglycemia. Dosing with taselisib may otherwise resume at a lower dose level and after discussion with the Protocol Chair.
Hematologic	
Grade 3	• Hold taselisib until resolution to ≤ grade 1. • If resolved in ≤ 7 days, resume taselisib at the current dose level. • If resolved in > 7 days, resume taselisib at one lower dose level.
Grade 4	• Discontinue taselisib permanently.
Pneumonitis	
Grade 1	• CT scans with lung windows and pulmonary function testing including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 8 weeks until return to within normal limits. • Administer 100% of taselisib dose. No specific therapy required.
Grade 2	• CT scan with lung windows. Pulmonary function testing including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 8 weeks until return to within normal limits. Consider a bronchoscopy with biopsy and / or BAL • Consider corticosteroids if symptoms are troublesome and infectious etiology ruled out. • Reduce taselisib dose by 1 dose level until recovery to ≤ Grade 1. Study treatment may also be interrupted if symptoms are troublesome. Patients will discontinue study treatment if they fail to recover to ≤ Grade 1 within 28 days. • Hold taselisib as long as corticosteroids are being given.

Grade 3	• CT scan with lung windows and pulmonary function testing including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 6 weeks until return to within normal limits. Bronchoscopy with biopsy and / or BAL is recommended. • Oral corticosteroids if infection is ruled out. Taper as medically indicated. • Hold taselisib as long as corticosteroids are being given. • Hold treatment with taselisib until recovery to ≤ Grade 1. May restart study treatment within 28 days at a reduced dose (by one level) if evidence of clinical benefit. Discontinue taselisib if recovery to Grade ≤ 1 is not evident within 28 days.
Grade 4	• CT scan with lung windows and required pulmonary function testing, including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 6 weeks until return to within normal limits. Bronchoscopy with biopsy and / or BAL is recommended if possible. • Consider corticosteroids if infective origin is ruled out. Taper as medically indicated. • Discontinue treatment with taselisib.
Other clinically significant toxicities	
Grade 3	• Hold taselisib until resolution to ≤ grade 1 and then restart at one dose level lower.
Grade 4	• Discontinue taselisib permanently.

a: Suggested antidiarrheals include the following: 1) loperamide (initial: 4 mg, followed by 2 mg after each lose stool, up to 16 mg/day); 2) diphenoxylate and atropine (diphenoxylate 5 mg, four times daily [QID], until control achieved (maximum: 20 mg/day), then reduce dose as needed; some patients may be controlled on doses of 5 mg/day; 3) tincture of opium (6 mg of undiluted opium tincture (10 mg/mL) QID.

b: Non-infectious diarrhea can be diagnosed by stool culture with work-up for various enteric bacteria and C. difficile. Fecal calprotectin is a possible marker for bowel inflammation. Blood-based CMV PCR test can also be used to detect CMV infection.

c: Suggested topical steroids include hydrocortisone 2.5% to face bid, triamcinolone 0.1% or fluocinonide 0.1% cream to body bid.

d: Suggested oral steroids include methylprednisolone dose pack or prednisone 60 mg daily followed by a taper (e.g., 60 mg X 2 days, 40 mg X 2 days, 20 mg X 2 days, etc.

7.3 Enzalutamide

The most common adverse reactions (≥ 10%) in patients treated with enzalutamide are asthenia/fatigue, back pain, decreased appetite, constipation, arthralgia, diarrhea, hot flush, upper respiratory infection, peripheral edema, dyspnea, musculoskeletal pain, weight decreased, headache, hypertension, and dizziness/vertigo.

7.3.1 Hot flashes

Hot flashes are common with treatment with enzalutamide. If ≥ grade 3 hot flashes occur, enzalutamide may be reduced to the next lower dose level, at the discretion of the treating physician. Pharmacologic intervention with low-dose SSRI (or an alternative oral therapy that has been demonstrated to have beneficial effect for treatment of hot flashes greater than that reported for placebo e.g. clonidine, buproprion) may be administered. If ≥ grade 3 hot flashes persist at the lower dose level and pharmacologic intervention with the above treatments has been unsuccessful, a second dose reduction may be instituted. If ≥ Grade 3 hot flashes persist at lowest treatment

dose (50mg), patient will be taken off study, and adverse effect will be reported as intolerable toxicity. If ≤ grade 2 hot flashes persist at lowest treatment dose (50mg), patient may be taken off study at discretion of treating physician. This toxicity will be reported, but it will not constitute unacceptable toxicity for purposes of adverse event reporting.

7.3.2 Other toxicities

Asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension have all occurred on studies of enzalutamide.

Though not expected, if ≥ grade 3 diarrhea occurs that is not controlled with anti-diarrheal agents (e.g. loperamide), enzalutamide should be withheld until resolution to ≤ grade 1, then reinstituted at the next lower dose level.

If toxicities are ≤ grade 2, manage symptomatically if possible and retreat without dose reduction.

If toxicities are ≥ grade 3, except for anemia, treatment should be withheld until resolution to ≤ grade 1 or baseline if baseline was greater than grade 1, then reinstituted, if medically appropriate, at the next lower dose level.

For any grade 3 or 4 toxicity not mentioned above, treatment should be withheld until the patient recovers completely or to grade 1 toxicity. The treatment should then be resumed at the next dose level. For grade 1 or 2 toxicities, no dose reduction should be made.

A maximum of two weeks will be permitted for resolution of grade 3 or 4 toxicities. If toxicity has not resolved by the end of two weeks, patient will be taken off study.

7.4 Special Considerations

- For toxicities which are considered by the treating investigator unlikely to develop into serious or life–threatening events (e.g. alopecia, altered taste etc.), treatment may be continued at the same dose without reduction or interruption.
- The treating investigator may reduce a subject's dose for a toxicity of any grade/duration where s/he believes it to be in the best interests of the subject.
- Any consideration to modification of the above dose modification guidelines should be discussed with the Protocol Chair for approval or disapproval in advance.
- Study participants should be given instructions to minimize sun exposure (i.e. use SPF ≥ 30 sunblock, wear hats and long-sleeve clothing) due to the possibility of photosensitivity.
- As referenced above, pneumonitis has rarely been reported in clinical trials with taselisib. All patients should be routinely asked about the occurrence of adverse events which could include new or changed pulmonary symptoms (consistent with lung abnormalities). CT scans and pulmonary function tests should be done, as clinically indicated, or if there are symptoms that indicate that the patient has developed pneumonitis. In case of a documented pneumonitis, the guidelines (including dose

modifications) in the table above should be followed. Consultation with a pulmonologist is highly recommended for any pneumonitis case during the study.

8. DRUG FORMULATION/STORAGE/SUPPLY

8.1 Taselisib

Taselisib is an investigational agent and will be supplied free-of-charge from Genentech.

8.1.1 Taselisib Formulation and Storage

Drug Product taselisib (GDC-0032) is intended for oral administration. The taselisib tablet is a white, film-coated, immediate-release formulation of 2 mg strength, consisting of taselisib API, lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, and Opadry 2 white film coating.

Taselisib tablets should be stored at 59°F–86°F (15°C–30°C) and should be protected from light.

For additional details, see the taselisib Investigator's Brochure.

8.2 Enzalutamide

Enzalutamide will be provided free of charge by the study.

8.2.1 Enzalutamide Formulation and Storage

The drug is supplied as 40 mg capsules by Medivation and Astellas. The drug substance is formulated in the surfactant caprylocaproyl polyoxylglycerides, or Labrasol. The product will be supplied as white to off-white gelatin capsules containing 40 mg of enzalutamide.

Enzalutamide is to be handled and stored safely and properly in accordance with the study drug label.

8.2 Drug Accountability

Accountability for the drug at all study sites is the responsibility of the site Principal Investigator and designated Pharmacy representative. The investigator will ensure that the investigational drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Genentech or Medivation will be maintained by the clinical site. Of note, only unopened medication packages (i.e. not dispensed to patients) will be returned to Genentech or Medivation if not used. Drugs returned by patients will be destroyed according to each institution's policy. Accountability records will include dates, quantities, lot numbers, expiration dates, and patient numbers.

9. CORRELATIVE/SPEIAL STUDIES

9.1 Advanced imaging correlative (Vanderbilt only)

Patients enrolling at Vanderbilt will be screened for participation in an advanced imaging correlative study, to be conducted through collaboration with investigators at the Vanderbilt University Institute for Imaging Science (VUIIS). The advanced imaging correlative will consist of multiparametric magnetic resonance imaging (MRI) to be performed at the VUIIS facility at three timepoints, chosen to coincide with the timing of required core biopsies: baseline, at day 14-21 following treatment initiation, and at the end of treatment.

Multiparametric MRI examinations will be designed and made available for four body sites – breast/axilla, lower neck/supraclavicular region, liver, and pelvic bone marrow – and patients will be eligible to participate in the advanced imaging correlative if their required core biopsies are obtained from one of these four body sites. Participation will be mandatory for patients accruing at Vanderbilt who meet the criteria outlined below, including presence of measurable disease (defined as at least one lesion measuring at least 10 mm in long axis diameter) at the body site of interest. If participation in the MRI sub-study is not possible due to scheduling issues, MRI weight limits, eligibility, or safety concerns over implanted medical devices, subjects may still continue with the rest of the study.

9.1.1 Background and rationale

Oncologic imaging is in the midst of a paradigm shift in which a number of advanced quantitative techniques reporting on tissue function and composition at the molecular level are now being advanced as candidate biomarkers for cancer detection, characterization, and treatment response assessment. This advanced imaging correlative deploys several promising MRI techniques within a single multiparametric MRI examination. The overall hypothesis for the advanced imaging correlative is that these techniques, either individually or in combination, can provide clinically useful information beyond that provided by standard-of-care imaging in the context of metastatic TNBC. The components of the multiparametric MRI examination, and hypotheses related to these components, are outlined briefly below:

- **Dynamic contrast enhancement MRI (DCE-MRI)** is a method for characterizing tissue vascularity. The technique involves rapid acquisition of serial MR images before, during, and after injection of an intravenous contrast agent; these images are then analyzed with mathematical models to estimate quantitative values for pharmacokinetic and tissue properties. We hypothesize that early changes in these derived DCE-MRI parameters (i.e., from baseline to day 14-21) will predict treatment response. We will also test whether baseline DCE-MRI parameters by themselves predict patient response to treatment, whether baseline DCE-MRI parameters can discriminate between AR+ and AR- subtypes of TNBC, and whether DCE-MRI parameters at the time of progression are significantly different from DCE-MRI parameters at baseline and early during therapy. Finally, we will evaluate the correlation between DCE-MRI parameters and phosphorylated p-ERK1/2, p-S6, and p-AKT S-473 levels at serial core biopsies.

- **Diffusion-weighted MRI (DW-MRI)** is a method for quantifying tissue cellularity. The technique reports on the ability of water molecules to move randomly, or diffuse, within tissue; more cellular tissues tend to have more barriers to water movement and, therefore,

restricted water diffusion when measured by MRI. We hypothesize that early changes in quantitative DW-MRI parameters will predict treatment response. We will also test whether baseline DW-MRI parameters by themselves predict patient response to treatment, whether baseline DW-MRI parameters can discriminate between AR+ and AR- subtypes of TNBC, and whether DW-MRI parameters at the time of progression are significantly different from DW-MRI parameters at baseline and early during therapy. Finally, we will evaluate the correlation between DW-MRI parameters and phosphorylated p-ERK1/2, p-S6, and p-AKT S-473 levels at serial core biopsies.

- **Chemical exchange saturation transfer (CEST) MRI.** CEST is a method for the detection and characterization of specific low-concentration metabolites in tissue. Recent attention has focused on amide proton transfer (APT), a form of CEST reporting on amide groups in proteins and peptides, as a novel cancer imaging biomarker. The technique exploits the base-catalyzed chemical exchange between amide protons and water molecules and is sensitive to both protein concentration and pH. We hypothesize that early changes in quantitative APT will predict treatment response. We will also test whether baseline APT measurements can by themselves predict patient response to treatment, whether baseline APT measurements can discriminate between AR+ and AR- subtypes of TNBC, and whether APT measurements at the time of progression are significantly different from APT measurements at baseline and early during therapy. Finally, we will evaluate the correlation between APT measurements and phosphorylated p-ERK1/2, p-S6, and p-AKT S-473 levels at serial core biopsies.

- **Multiparametric MRI** is itself an imaging technique under intensive investigation. We hypothesize that tumor characterization may be even further improved by combining information derived from a number of quantitative imaging techniques to assemble a multifaceted, multidimensional construct of tumor phenotype and behavior. We will test different permutations of quantitative DCE-MRI, DW-MRI, and CEST parameters for detecting lesions, characterizing lesions, and monitoring response to treatment in the study population.

9.1.2 Participant selection: MRI Advanced Imaging Correlative

9.1.2.1 Inclusion criteria

- Patients must be enrolled at Vanderbilt University Medical Center.
- Patients must have planned core biopsies from one of the following sites: breast/axilla, lower neck/supraclavicular region, liver, or pelvic bone marrow.
- Patients must have measureable disease at the site selected for core biopsies. Measureable disease is defined as presence of at least one lesion measuring greater than 10 mm in long axis diameter.

9.1.2.2 Exclusion criteria

- Patients with inadequate renal function (creatinine ≥1.5 times upper limit of normal) or acute or chronic renal insufficiency (glomerular filtration rate <30 mL/min).

- Patients who have any type of bioimplant activated by mechanical, electronic, or magnetic means (e.g., cochlear implants, pacemakers, neurostimulators, biostimulators, electronic infusion pumps, etc), because such devices may be displaced or malfunction.
- Patients who have any type of ferromagnetic bioimplant that could potentially be displaced.
- Patients who have cerebral aneurysm clips.
- Patients who may have shrapnel imbedded in their bodies (such as from war wounds), metal workers and machinists (potential for metallic fragments in or near the eyes).
- Patients who have exhibited past allergic or other adverse reactions in response to intravenous injection of Magnevist® (gadopentetate dimeglumine) or other gadolinium-containing contrast agents.
- Patients who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the magnet bore.
- Patients incapable of giving informed written consent, for the following reasons:
  - Inability to adhere to the experimental protocols for any reason
  - Inability to communicate with the research team
  - Limited ability to give informed consent due to mental disability, altered mental status, confusion, or psychiatric disorders
  - Prisoners or other individuals deemed to be susceptible to coercion

9.1.3 MRI Imaging procedures

Patients will undergo multiparametric MRI at baseline, at day 14-21, and at the end of treatment. The multiparametric MRI examinations should be scheduled to coincide with the planned core biopsies as much as is reasonably practical. At any given timepoint, however, ***every effort should be made to complete the MRI examination before the core biopsy***, because hemorrhage from the core biopsy may interfere with the MRI measurements.

The general scan order and anticipated scan durations are summarized below:

1. Scout images, transmitter tuning, shimming, slice prescription – 5 min
2. T_1 map – 2.5 min
3. Diffusion-weighted MRI (DW-MRI) – 5 min
4. Chemical exchange saturation transfer (CEST-MRI) – 10 min
5. Pre-contrast T1-weighted anatomic MRI – 2 min
6. Dynamic contrast-enhanced MRI (DCE-MRI) – 8 min
   *Intravenous injection of the FDA-approved contrast agent (CA) Magnevist® (gadopentetate dimeglumine, 0.1 mmol/kg) 60 sec after the start of the scan. Infusion time is 5-10 sec, depending on patient weight. CA will be followed by saline flush.
7. Post-contrast T_1- and T_2-weighted anatomic MRI – 10 min

9.2 Tissue Samples (All participating sites)

9.2.1 AR testing

Please see Section 5 for AR testing and requirements for tissue for AR testing.

9.2.2 Archival tissue

Once a patient is confirmed to be AR positive, meets all eligibility requirements, and is registering for the study, formalin-fixed paraffin embedded tissue blocks from the patient's primary tumor or metastatic lesion collected for diagnostic and therapeutic purposes is requested. Blocks will be returned within 8 weeks (or earlier if requested); tissue taken from these blocks will not be returned.

In case the participating institutions are not allowed to release clinical paraffin embedded blocks, ideally we will need the following cut in this order:

1. H&E slide
2. 5 peels cut at 10 µm each in a single **DNAse-free/RNAse-free** tube
3. H&E slide
4. 3 peels cut at 10 µm each in a single **DNAse-free/RNAse-free** tube
5. H&E slide
6. 10 unstained slides cut at 5 µm each on charged slides
7. H&E slide

If remaining tissue is insufficient for the complete request, please send:
1. H&E slide
2. 3 peels cut at 10 µm each in a single **DNAse-free/RNAse-free** tube
3. H&E slide
4. 2 peels cut at 10 µm each in a single **DNAse-free/RNAse-free** tube
5. H&E slide

If remaining tissue is limited, please send:
1. H&E slide
2. 2 peels cut at 10 µm placed in a single DNAse-free/RNAse-free tube
3. H&E slide

After removing all stray tissue from the area, the microtome blade and surrounding area should be wiped with 70% ETOH before starting to section the block and between patients to prevent contamination with extraneous tissue.

All formalin-fixed paraffin embedded tissue and/or slides should be sent with a cold pack to avoid melting that could damage tissue analysis.

9.2.3 Fresh biopsy of metastatic site

Once a patient is confirmed to be AR positive, meets all other eligibility, and registers for the study, **collection of tissue from a metastatic site, if reasonably safe, is also a requirement for entry to this study. A biopsy can also be used for AR testing for the study (see Section 5). Pre-trial and day 14-21 biopsies should be collected.** Research tissue blocks prepared from

these metastatic biopsies will not be returned. **All patients must have a biopsy of a metastatic site at the end of treatment if the patient is removed for disease progression or is on the enzalutamide only arm and crossing over to enzalutamide + taselisib. See section 9.2.4 below for details on collecting tissue.**

9.2.4 Guidelines for Tissue Acquisition on Biopsies of Metastatic Lesions

Tissue specimens, when feasible, will be collected from recurrent or metastatic lesions using standard institutional procedures. The amount of tissue collected will follow the guidelines listed below. If a patient has more than one site of disease, only one site needs to be biopsied, and the site is left to the discretion of the patient and the treating physician. If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue (roughly equivalent to the goal amount of tissue listed in the guidelines above, i.e. the equivalent of two 5-mm punch biopsies of the skin, or 3-6 18-gauge core biopsies) stored for research at the time of the procedure.

Listed below are the goal amounts of tissue for patients who undergo core biopsy or punch biopsy, or who have either ascites fluid or pleural fluid accessible for collection. Please note that the below are guidelines for the amount of tissue to be obtained at the baseline biopsy, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

- Breast: A goal of 3-6 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass
- Skin/chest wall: A goal of one to two 5-mm punch biops(ies)
- Lymph node: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle
- Liver: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle
- Lung: A goal of 3 core biopsy specimens will be obtained using an 18-gauge needle. Due to the risk of pneumothorax associated with core needle biopsies of lung nodules, peripheral lesions should be carefully selected.
- Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.
- Pleural Fluid (known to be due to malignancy): A goal of 500 cc of pleural fluid will be obtained with a standard thoracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored at Vanderbilt.

- Ascites fluid: A goal of 500 cc of ascites fluid will be obtained with a standard paracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored at Vanderbilt.

9.2.5 Instructions on fresh tissue specimen handling

1) Core biopsy specimens

Tissue obtained at Vanderbilt will be handled differently as it does not need to be shipped. Please see the Vanderbilt lab manual for handing if obtaining a biopsy at Vanderbilt

- First core biopsy should be suspended in 10% buffered formalin.
- Second core biopsy should be suspended in "RNAlater" solution*
- Third core biopsy should be suspended in "RNAlater" solution*
- Fourth core biopsy should be suspended in 10% buffered formalin.
- Fifth core biopsy should be suspended in "RNAlater" solution* (outside sites)
- Fifth core biopsy should be placed in cell culture media (Vanderbilt only)
- Sixth core biopsy should be suspended in 10% buffered formalin

If only 2 core biopsies are obtained, one should be placed in "RNAlater solution"* and the other should be suspended in 10% buffered formalin.

*RNAlater kits will be provided. 1 core should be placed in one 1.5ml specimen tube. All samples should be shipped immediately, according to the lab manual.

2) Resected lesion (chest wall, lymph node, etc).

- For specimens greater than 5mm, the specimen should be bisected and one half placed in RNA later and the other in 10% buffered formalin

3) Fine needle aspiration (FNA)

For patients in whom a core biopsy is not possible and who thus undergo fine needle aspiration (FNA), 3 passes should be collected.

- First pass should be evacuated and rinsed directly into 10% buffered formalin
- Second pass should be evacuated and rinsed directly into 5-10 volumes "RNAlater" solution
- Last pass should be evacuated and rinsed directly into 10% buffered formalin

9.3 Blood/plasma collection (All Participating Sites)

Streck Cell-Free DNA BCT kits will be provided for circulating tumor DNA. One vial of whole blood should be collected in a Cell-Free DNA BCT tube (Streck tube). Fill the tube completely. Remove the tube from the adapter and immediately mix by gentle inversion 8 to 10 times. Collection times are: cycle 1/day 8, cycle 2/day 15, and end-of-treatment (also cycle 1/day 1 for those crossing over to enzalutamide + taselisib - see study calendar).

Tube should be packed according to the lab manual and sent immediately (within 24 hours) to VICC at the address below with a tissue/blood registration form. **Do not freeze specimens collected in Streck tube.**

9.4 Tissue specimen and blood/plasma labeling and documentation

Label each collection containers with Patient ID sequence number/code letter, site and location of biopsy, date and time.

If sample comes in contact with contaminate, make note in information section of paperwork.

Enter time core biopsy was collected on paperwork.

If not retrieved on site by VICC CTSR personnel, outside sites should ship specimens along with Tissue Registration Form directly to:

[redacted]

The specimens will be logged in as a consented specimen and available for molecular pathology studies.

Ship ALL samples with a cold pack. If specimens are obtained on a Friday or holiday weekend, they should be refrigerated (not frozen) and sent out the following week.

****Specimens should be mailed to arrive at VUMC from Monday 8AM through Friday 1PM****

9.5 Pharmacokinetic Sampling

Pharmocokinetic sampling will occur in the phase Ib portion and in 10 patients in the phase II portion (phase II at Vanderbilt only). PKs for patients in the phase Ib portion: will be obtained on cycle 1, day 8 at: 0-4 hours prior to dose of taselisib and 1, 3, and 6 hours post administration taselisib; on cycle 2, day 15 at: 0-4 hours prior to dose of taselisib and enzalutamide and 1, 3, and 6 hours post administration taselisib and enzalutamide; on cycle 4, day 1, plasma sample should be obtained 0-4 hours before taselisib and enzalutamide administration, and 3 hours post-administration of both drugs.

The first ten subjects on the enzalutamide + taselisib arm at Vanderbilt should initiate taselisib on day 1 and enzalutamide on day 9 to facilitate PKs, which will be done on the first 10 Vanderbilt patients on the combination arm only. PKs in phase II (Vanderbilt only) should be obtained on patients receiving both enzalutamide and taselisib on C1D8, C2D15, and C4D1 at 0-4 hours prior to taselisib and enzalutamide and 2 to 4 hours post treatment. Please see the lab manual for details of collection and for information about shipping the samples.

9.6 Specimen Banking

Any leftover study tissue or blood samples may be stored for future research studies. The subjects will consent to the future use of samples in the consent form for the study. The study PI and collaborators have approval by the TBCRC, which has custodial oversight of all biospecimens collected as part of a TBCRC trial, to address the research questions described in the protocol document. All future use as part of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the TBCRC according to its established policies, whether the specimens are stored in a central site or at a local institution or in a virtual repository.

9.7 Biopsy Questionnaire

A questionnaire will be given to each participant to complete at cycle 2, day 1. This questionnaire is designed to assess the impact of the multiple biopsies on the patient's quality of life and overall experience with the study.

10. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response after every 2 cycles. Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) Committee (version 1.1).[34] Changes in only the largest diameter (unidimensional measurement) of the tumor lesions (and short axis for lymph nodes) are used in the RECIST criteria.

10.1 Definitions

10.1.1 Evaluable for toxicity:

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

10.1.2 Evaluable for objective response:

Only those patients who have evaluable 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.)

10.2 Disease Parameters

10.2.1 Measurable disease

10.2.1.1 Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20mm by chest X-ray.

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

10.2.2 Non-measurable disease

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

10.2.3 Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

10.2.3.1 Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

10.2.3.2 Cystic lesions:

- 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.

10.2.3.3 Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

10.2.4 Specifications by methods of measurements

10.2.4.1 Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 3 weeks before the beginning of the treatment.

10.2.4.2 Method of assessment

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 should always be done rather than 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 and ≥10mm diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. Guidelines have defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Intravenous contrast should be used if appropriate.

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, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.

PET scans: PET scans are not able to be used for RECIST measurements. Similarly, the CT portion of PET/CT scans cannot be used for RECIST measurements.

Bone scan: Bone scan can be used to evaluate for disease progression by the presence of new lesions.

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

10.3 Response Criteria

10.3.1 Target lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as **target lesions** and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).

10.3.1.1 Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

10.3.2 Non-target lesions:

All other lesions (or sites of disease) including pathological lymph nodes should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

10.3.3 Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease (PD): Unequivocal progression of existing non-target

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

10.3.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

Time point response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non CR/Non PD	No	PR
CR	Not evaluated	No	PR
PR	Non PD or not all evaluated	No	PR
SD	Non PD or not all evaluated	No	SD
Not all evaluated	Non PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR= complete response, PR= partial response, PD= progressive disease, SD= stable disease, NE= not evaluable.
*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.

10.4 Duration of Response

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

time measurement criteria are first met for CR until the first date that recurrent 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.

10.5 Central tumor measurements

At the end of treatment, all scans (baseline and through disease progression, along with crossover scans) should be sent on a CD to the Vanderbilt Cancer Imaging Support Laboratory for central review and assessment. These do not need to be overnighted. Please include patient's study ID number the CD/disc.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 General

Adverse event collection and reporting is a routine part of every clinical trial. This study will use the descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) that is available at http://ctep.cancer.gov/reporting//ctc.html.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication. Participants who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study as per local requirements.

11.2 Definitions

11.2.1 Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered an investigational product. AEs do not necessarily have a causal relationship with the treatment. An adverse event (AE) can, therefore, be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.2.2 Serious adverse event (SAE)

A serious adverse event is any untoward medical occurrence resulting in one or more of the following:

- results in death
- is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity

- is a congenital anomaly or birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.2.3 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk

11.2.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

11.3 Reporting Procedures

11.3.1 General

All adverse events will be captured on a centralized electronic case report form called ON-line Clinical Oncology Research Environment = Oncore (http://www.vicc.org/ct/research/oncore.php). Oncore is a highly secure, web based, cancer specific, and customizable system that provides fully integrative clinical data management and study administration capabilities developed in an ongoing collaborative effort with NCI designated Comprehensive Cancer Centers. It fully integrates study administration functionality including protocol tracking, patient registration, NCI reporting, review committee tracking, and SAE tracking, with clinical data management functionality including electronic case report forms (eCRF) design, clinical data capture, protocol and regulatory compliance monitoring. Specified members at each participating site will submit all regulatory documents to the Coordinating Center Data Manager, who will upload it in Oncore.

11.3.2 Serious Adverse Events

All serious adverse events, regardless of causality to study drug, occurring after the patient's first dose of study treatment will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center. Serious adverse events that occur during screening do not require reporting unless they are due to a screening procedure (eg biopsy).

All serious adverse events must be reported to the Coordinating Center within 1 business day after the treating institution becomes aware of the event. Events should be reported using the Vanderbilt Serious Adverse Event Form as well as Form FDA 3500A (Mandatory Reporting Form for investigational agents). The FDA form can be found online at http://www.fda.gov/safety/medwatch/howtoreport/default.htm and the Vanderbilt SAE form is part of the packet of supplemental forms.

Both forms must be fully completed and emailed, faxed, or scanned to:

[redacted]

Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding unexpected serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

11.3.2.1 Death and Immediately Life-Threatening Events

Any death and immediately life-threatening event from any cause while a patient is receiving trial treatment on this protocol or up to 30 days after the last dose of trial treatment, or any death and immediately life-threatening event occurring more than 30 days after trial treatment has ended but which is felt to be treatment related must be **reported within 24 hours of discovery of the event**. All deaths must be reported primarily for the purposes of SAE reporting; however, deaths due unequivocally to progression are not SAEs.

Your local IRB should be notified as per institutional policies and their reporting procedure followed. The completed SAE Reporting Form and Form FDA 3500A should be emailed or faxed to the VICC Coordinating Center **within one working day of discovery of the event.**

VICC Coordinating Center will report all SAEs to the regulatory authorities (FDA) per federal guidelines.

11.3.2.2 Pregnancy

The effect of enzalutamide and/or taselisib in pregnant and lactating women is not known, and the exposure of a fetus or nursing infant is considered a potential risk. Enzalutamide and taselisib can cause fetal harm when administered to a pregnant woman based on their mechanism of action. Subjects receiving the study drugs are advised to use 2 acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of screening and continuing throughout the course of treatment and for at least three months after the study drugs are discontinued.

If a female patient or the female partner of a male patient becomes pregnant while receiving investigational therapy or within 30 days after the last dose of investigational product, a pregnancy should be reported to the VICC coordinating center within 24 hours of learning of the pregnancy. As applicable, the investigator should discontinue study drug and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Abortion, whether therapeutic or spontaneous, should always be classified as serious, recorded as an SAE, and expeditiously reported to the VICC Coordinating Center. Any congenital anomaly/birth defect in a child born to a female patient or female partner of a male patient exposed to the investigational product should be recorded and reported as an SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

The Investigator should report the outcome of the pregnancy (independent of outcome, e.g. full term delivery, pre-term delivery, spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a miscarried fetus, etc] in accordance with the same reporting procedure as for SAEs. The date of outcome of the pregnancy, gestational age, date of birth and neonatal data etc., should be included in this information.

VICC Coordinating Center will report such circumstances to Genentech as defined in the Safety and Data Exchange Agreement (SDEA), and to Medivation/Astellas.

11.3.3 IND Safety Reports Unrelated to This Trial

IND safety reports not occurring on this trial but involving the study intervention (outside SAEs) received from outside sources will be reviewed by the Protocol Chair and will be forwarded to participating sites for submission to their Institutional Review Boards per their guidelines.

11.3.4 Institutional Review Board

All adverse events and serious adverse events will be reported to the participating institution's IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

11.3.5 Food and Drug Administration (FDA)

Vanderbilt will report to the FDA any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

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

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-301-796-9845) using Form FDA 3500A (Mandatory Reporting Form for investigational agents).

11.3.6 Genentech

All SAEs must be reported to the Coordinating Center (Vanderbilt) within 24 hours. Vanderbilt will report all SAEs regardless of causal relationship to study treatment using the MedWatch form (using the online form available at http://www.fda.gov/safety/medwatch/howtoreport/default.htm) to Genentech as defined in the Safety and Data Exchange Agreement (SDEA). If the SAE is linked to taselesib only, the form will only be submitted to Genentech.

11.3.7 Medivation/Astellas

All SAEs must be reported to the Coordinating Center (Vanderbilt) within 24 hours. Vanderbilt will report all SAEs related to enzalutamide to Astellas using the Medwatch form (using the online form available athttp://www.fda.gov/safety/medwatch/howtoreport/default.htm). The Investigator will submit a copy of this MedWatch form to Astellas by either e-mail or fax, within the same timeframe. If submission of this SAE to FDA or Astellas or is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone. If the SAE is linked to enzalutamide only, the form will only be submitted to Astellas.

The SAE documentation, including the Medwatch 3500A Form and available source records should be emailed or faxed to:

[redacted]

The following minimum information is required:

- Study number/IIT regulatory identifier
- Subject number, sex and age
- The date of report
- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug

Follow-up information for the event should be sent within promptly (within 7 days) as necessary.

12. DATA SAFETY AND MONITORING

12.1 Data Management and Reporting

Participating institutions will be collaborating with Vanderbilt in patient accrual. Data will be collected using a centralized electronic case report form called ON-line Clinical Oncology Research Environment = Oncore[redacted]. Oncore is a highly secure, web based, cancer specific, and customizable system that provides fully integrative clinical data management and study administration capabilities developed in an ongoing collaborative effort with NCI designated Comprehensive Cancer Centers. It fully integrates study administration functionality including protocol tracking, patient registration, NCI reporting, review committee tracking, and SAE tracking, with clinical data management functionality including electronic case report forms (eCRF) design, clinical data capture, protocol and regulatory compliance monitoring. Also the system is capable in storing basic protocol information (e.g., IRB approval dates, dates for annual renewals, etc) and clinical trials research data. Oncore allows the investigator to define specific protocol requirements and generate data collection forms. Creation of the data collection form is done with a single button click after the parameters of an individual protocol have been specified. Oncore permits specification of study protocols, management of patient enrollment, clinical data entry and viewing, and the generation of patient or study-specific reports based on time stamping. OnCore is embedded with a comprehensive domain repository of standard reference codes and forms to promote standardization. The sources for the repository include CDUS, CTC, CDEs from NCI, ICD, MedDRA and various best practices from contributing NCI-designated Comprehensive Cancer Centers. OnCore provides several reporting features specifically addressing NCI Summary 3 and Summary 4 and other reporting requirements. Data may also be exported in a format suitable for import into other database, spreadsheets or analysis systems (such as SPSS). This system will be used to manage all VICCC clinical trials data. OnCore is maintained and supported in the VICC Clinical and Research Informatics Resource.

Specified members at each participating site will submit all pertinent regulatory documents to the Coordinating Center Data Manager, who will store it in a secure location.

The Principal Investigator or designee will inform Genentech as defined in the Safety and Data Exchange Agreement (SDEA) of any serious adverse event, and will inform the IRB in accordance with each institution's IRB policy. The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE, as provided in this protocol. During the study when there is a safety evaluation, the treating investigator or site staff will be responsible for detecting, documenting, and report AEs and SAEs, as detailed in the protocol. If any problem is identified related to the conduct of this research, the VICC Data Safety and Monitoring Committee (DSMC) will be formally asked to review the study and the situation that required DSMC intervention.

12.2 Meetings

This trial will be monitored by the VICC Breast Cancer Research Team. The Breast Cancer Research Team is composed of the Clinical Core Director of the Breast Cancer Program and Team Leader, Surgical Oncologists, Radiation Oncologists, Medical Oncologists, Research Nurses, the Data Manager, and our Regulatory Specialist. The Breast Cancer Research Team meets on a monthly basis to discuss all AEs/SAEs, accrual, compliance, safety issues, adherence to protocol, reviews, etc. pertaining to all breast cancer studies. This particular study will be thoroughly reviewed during these meetings. These monthly meetings have minutes recorded each time and those are also reviewed on a monthly basis by the Breast Cancer Research Team Physician Leader.

Subsequent to the Breast Cancer Research Team Monthly Meeting, monthly teleconferences between participating site and the Breast Cancer research team at Vanderbilt will be held to discuss all issues related to the trial (AEs/SAEs, accrual, compliance, safety issues, adherence to protocol, reviews, etc.).

12.3 Monitoring

This trial will be monitored continuously by the study's Protocol Chair and by the Breast Cancer Research Team at VUMC. Quarterly safety and monitoring reports are available to the Data and Safety Monitoring Committee (DSMC) as determined by the VICC Scientific Review Committee (SRC). The DSMC reviews all adverse events reported during the previous month for all clinical trials active at the VICC and makes recommendations to address concerns of patient safety. The DSMC of the VICC SRC will submit an annual report to the VICC Director on activities of the preceding year and will make recommendations to improve data and safety monitoring activities as needed.

A Quality Assurance auditor under the direction of the DSMC will audit this clinical trial quarterly for compliance with adverse event reporting, regulatory and studies requirements, and data accuracy and completion. Audit reports detailing the findings are provided to the DSMC. Site visits to ensure compliance will be performed on an *ad hoc* basis, notably in high accruing centers.

12.4 Data Handling and Record Keeping

12.4.1 Case Report Forms

An electronic case report form (eCRF) is required and must be completed for each included participant. The completed dataset should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from Vanderbilt.

12.4.2 Record Retention

To enable evaluations and/or audits from Health Authorities and Vanderbilt, each site investigator agrees to keep records including: The identity of all participants (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. To comply with international regulations, the records should be retained by the investigator in compliance with regulations.

During data entry, range and missing data checks will be performed on-line. The checks to be performed will be documented in the Data Monitoring Plan for the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the eCRF's will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel. This will be done on an ongoing basis.

13. REGULATORY CONSIDERATIONS

13.1 Informed Consent and Confidentiality

The investigator (or his/her designee) will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it, and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the Investigator Sponsor, Genentech and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

The investigator agrees to keep all information provided by this study in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents

provided (protocols, investigators' brochures, CRFs and other material) will be stored appropriately to ensure their confidentiality.

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. This medical information must be made available to the IRB and DSMC, upon request, for source verification of study documentation. Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, local health authorities, Dr. Vandana Abramson and her authorized representative(s), collaborators and licensees, and the IRB for each study site, if appropriate. We will make all reasonable efforts to keep patient's protected health information (PHI) private and confidential. We will only utilize or relinquish this kind of information according to federal privacy guidelines. There are many safeguards in place to prevent the unintentional disclosure of information obtained for or produced by this study. Research data, including the data collected from the medical charts will be entered into a password-protected database. Any publications or public disclosure of data relating to the patient's tumor will be done without any identifying information.

PHI will be collected and stored in the OnCore system. The coordinating center will have access to all research data, which will be kept for at least 2 years after the study is completed. Any research data entered in a patient medical record will be stored for an indefinite amount of time. There are no plans to destroy data at this time.

Confidentiality and security will be maintained for the tissue collection within this study. All tissue samples obtained for this study will be assigned a code and this code used to identify the sample. The samples will not be labeled with the patient's name, address or other information that would identify them. All information will be coded to maintain privacy. Research data, including the data collected from the medical charts will be entered into a password-protected database. The database (Breast Cancer Program Database) in which this study data is going to be stored has a firewall (in addition to the institutional firewall) with the highest level of protection, i.e. the same level of protection as the on-line hospital information system at Vanderbilt. This means that users must log on to a web server that sits between the institutional firewall and the firewall to the database, and only this application server is allowed to query the database. Information, including the identifier and password for the authorized users, is transmitted via a secure shell protocol using 128k encryption. Only Dr. Vandana Abramson, the PI, and the Breast Team Data Manager, approved through our institutional review board will be allowed access to patient identifiers. Other levels of authorization may exist for approved users, e.g. access to de-identified data. This database will store a de-identified link to the patient data and will not otherwise store patient data, even de-identified. The safety monitoring will be performed by the groups deemed appropriate by the Vanderbilt University Medical Center Institutional Review Board for reviewing the clinical trials procedure. Safety monitoring for the database is also performed by the Networking and Security Services of the Vanderbilt University Medical Center. Audit trails for access to the web server and the databases behind the dual firewall system are maintained in accordance with the practices of the Networking and Security Services of the Vanderbilt University Medical Center.

13.2 Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

14. MULTI-CENTER GUIDELINES

14.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB of each institution prior to implementation.

The Protocol Chair (or her designee) is responsible for the coordination and development of all protocol amendments. Once approved by the Protocol Chair, Vanderbilt will disseminate this information to the participating centers.

14.2 Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), each participant's informed consent, enrollment form, eligibility checklist and tissue block registration, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

The requirements for data management, submissions, and monitoring are outlined below. The participating sites will submit all the research related information (source documents and research records – IRB approval documents, patient registration list, CRF info, toxicity assessments, tumor measurements/ responses, etc.) to the Coordinating Center Data Manager, who will upload it on Oncore at the appropriate time points: prior to study initiation, when patients are enrolled, and monthly during study duration. Personnel from the VICC Clinical Trial Shared Resource will monitor the trial and may periodically visit the investigative site to assure

proper conduct of the trial and proper collection of the data. The investigators at other sites will allow the monitor to review all source documents used in the preparation of the case reports.

14.3 Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by each Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

14.4 Publication

It is understood that any manuscript or releases resulting from the collaborative research will be circulated to the TBCRC Central Office and all participating sites and Genentech prior to submission for publication or presentation.

15. STATISTICAL CONSIDERATIONS

15.1 Study Design/ Endpoints

This is an open-label phase Ib/II multi-institution trial that evaluates the safety profile/ tolerability and efficacy of enzalutamide + taselisib in patients with metastatic triple negative (ER/PR/HER2 negative) breast cancer who are AR positive. As taselisib has not been evaluated with enzalutamide, we would begin with a phase Ib portion to ensure safety of the combination and to determine a recommended dose for phase II of taselisib when given in combination with enzalutamide at 160 mg/day. In the Phase II portion, we will randomize patients in a 3:1 ratio to enzalutamide + taselisib or enzalutamide only. AR positivity is defined as $\geq$ 10% of tumor cell nuclei with immunoreactivity for AR. Upon progression of disease by RECIST, patients on the combination arm will be discontinued from the study. Those on the enzalutamide alone arm can cross-over to receive enzalutamide + taselisib.

15.2 Phase Ib Study

The taselisib dose will start at 2 mg/day (dose level 1) and enzalutamide at 160 mg/day. Dose escalation will proceed among cohorts of 3 patients according to a standard 3+3 algorithm beginning at the lowest dose level as described in Section 6.2.

In the phase Ib component of the trial, the MTD will be defined as the highest dose tested in which a DLT is experienced by 0 out of 3 or 1 out of 6 patients among the dose levels. The first cohort of patients (3 patients) in each arm will be started at dose level 1, and each patient will be observed for 4 weeks on the specified dose.

No new cohort of patients will be treated until the previous cohort has completed one cycle of therapy without any DLTs.

Once the phase Ib portion is completed, the safety data will be analyzed and the phase II component of the study will be completed to assess tolerability and efficacy of the study combination. Patients in the phase II component of the study will initiate study treatment at the MTD. Note that dose reduction within patients (individually) is allowed in the phase II component of the study (see dose modification section). Dose reduction will be required for a given patient in case of grade 3 or 4 toxicities. The phase Ib study will require approximately 15-24 patients.

15.3 Phase II Study

The primary endpoint of the phase II portion is to assess the CBR of enzalutamide + taselisib at 16 weeks. This will be studied using a Simon two-stage design. Because there is sparse data on single agent enzalutamide, we also plan to accrue patients to a single agent enzalutimide arm. Patients will be randomized to the dual therapy vs monotherapy arms using a 3:1 randomization. The coordinating center (VICC) will randomly assign patients to an arm. No direct comparison of the two arms will be conducted. Rather, the monotherapy arm will serve as an informal anchor when interpreting results, and will be especially informative when interpreting many of the secondary analyses.

We have designed the enzalutamide + taselisib arm as a Simon optimal two stage. This design allows for early termination for inefficacy. The antiandrogen bicalutamide, in a prior study with a similar patient population, resulted a CBR of approximately 20%[35]. Thus, for this study, we aim to show an improvement in CBR from 20% to 40%. With a Type I error of 10% and a Type II error of 10%, we will accrue 17 patients in the first stage. After the first 17 patients in the phase II portion have completed 2 cycles and obtained scans, the data will be analyzed. If 4 out of the 17 have a response (CR, PR, or SD), we will expand the study to a total of 37. If, at the end of the trial, there are 11 out of 37 patients with response, we will deem the regimen worthy of further study. If there are 3 or few responses in the first 17 patients, the study will terminate early. If 10 or few responses are seen in the total of 37, the regimen will be deemed unworthy of further study. This study has a 55% probability of stopping early under the null hypothesis. Patients whose disease progresses within 30 days of starting the study without a DLT will be replaced during the phase I portion.

Concurrently, an arm of single agent enzalutimide will be accrued. For every 3 patients randomized to the enzalutamide + taselisib arm, 1 patient will be randomized to the enzalutamide only arm. If the dual therapy arm terminates early due to inefficacy, we will also terminate the monotherapy arm. Thus, this arm will have a minimum of 6 and a maximum of 12 patients. We do not plan to do any formal analysis on this group of patients.

15.4 Sample Size

Based on preliminary data on these therapies, we expect to require 15 patients for the Phase Ib portion of the trial. However, with 4 dose levels it is possible, though not likely that 24 patients will be required. For the phase II portion of the trial, we will require 17+6=23 to 37+12=49 (23-59) patients depending on whether the number of responses reach the threshhold at the interim analysis. Patients in the phase I portion who are the recommended dose for phase II will be included in the evaluable patients for phase II. The total sample size for the study will be approximately 73 patients.

15.5 Reporting

Demographic information such as age and race will be tabulated. Descriptive statistics, including means, medians, standard deviations, and ranges for continuous parameters, as well as percents and frequencies for categorical parameters, will be presented. Investigation for outliers and assumptions for statistical analysis, e.g., normality and homoscedasticity will be made. Adverse medical events will be tabulated. NCI toxicity Grade 3 and Grade 4 laboratory abnormalities will be listed.

All patients who receive any study drugs will be included in the toxicity evaluation. All patients who receive study drugs for at least 4 weeks will be included in the efficacy evaluation (modified intention to treat). Deaths, early progression, and other reasons for drop-out of the study prior to 4 weeks will be reported.

15.6 Analysis of Secondary Endpoints

We will calculate the ORR and corresponding 95% confidence intervals at the dose recommended for phase II. The overall PFS data for the patients at the dose recommended for phase II will be estimated using the Kaplan-Meier method with 95% confidence intervals.

Biopsy analysis will be performed in conjunction with the Pathology and Tissue Informatics Core to assess baseline, therapy-induced changes and status upon progression of the following: proliferation (Ki67), mitosis (mitotic index), apoptosis (cleaved caspase 3 levels by IHC), genomic instability (H2AX by IHC), levels of PTEN by IHC, PI3KCA mutational analysis, HER2 (IHC, FISH) and ER/PR levels (IHC). Levels of MEK [total and phosphorylated ERK1/2 (pERK1/2)] and AKT [phosphorylated AKT, S473 (pAKT)] will be measured by IHC.

RNA-seq will be performed on all biopsies to assign a triple negative subtype[36], to determine baseline and treatment-induced changes in GE after 5-10 days of therapy and define mutations present in the tumors both at baseline and upon progression of the disease. We expect an enrichment of drug resistance-associated mutations/gene amplifications particularly in tumors that regress and later recur while on therapy. Somatic SNPs and structural variants will be validated by whole exome sequencing (WES) of genomic DNA from the tumor and matched blood. We will validate mutations by targeted capture approaches that allow high-level coverage (greater than 200X) and analysis of clonality. The status of p53, BRCA1/2, PIK3CA, PTEN, INPP4B and other mutations associated with TNBC in the tumor tissue is critical for testing our hypothesis. This genetic information is key for determining the prognostic significance of mutations relative to response of agents being tested and for understanding mutation selection relative to the underlying genetic milieu of each tumor at time of entry into the trial. We anticipate significant opportunity for discovery of novel mutations as well as those arising from selection during resistance to the therapies under investigation. Statistics on the correlative endpoints (tissue assays and MRI scan) will be primarily descriptive. Therefore, these studies are considered to be exploratory and potentially hypothesis-generating.

Pharmacokinetic samples are being collected in all patients to assess concentrations of GDC-0032 and enzalutamide and estimate PK parameters, as appropriate. Plasma samples may be used for exploratory analysis to evaluate potential taselisib-related metabolites and/or exploratory biomarker development.

15.7 Reporting and Exclusions

All patients included in the study must be assessed for safety, tolerability, and 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).

All of the patients who met the eligibility criteria and received study drugs for 4 weeks should be included in the main analysis of the clinical benefit 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 and clinical benefit rate.

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