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Phase II Trial to Evaluate the Efficacy of the FASN Inhibitor, TVB-2640, in Combination with Trastuzumab plus Paclitaxel or Endocrine Therapy in Patients with HER2+ Metastatic Breast Cancer Resistant to Trastuzumab-based Therapy

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Mayo Clinic Cancer Center

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Study Chairs:	Tufia C Haddad MD* Mayo Clinic 200 First Street SW Rochester MN 55905 507/284-2511
Study Co-chairs:	Ruth Lupu PhD√ Matthew P Goetz MD Alvaro P Moreno MD (Florida) Brenda J Ernst MD (Arizona)
Statistician:	Vera J Suman PhD√
Research Monitor:	Yolanda I Garces MD√

***Investigator having DOD responsibility for this protocol**

√Study contributor(s) not responsible for patient care

Drug Availability

Commercial Agents: paclitaxel, trastuzumab, anastrozole, exemestane, fulvestrant, letrozole
Drug Company Supplied: TVB-2640 (IND# 133975)

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MCCC Amendment 7	pending

Protocol Resources

Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion and submission	Katie A Hervey Quality Assurance Specialist Phone: 507/266-6117 E-mail: hervey.katie@mayo.edu
Drug administration, infusion pumps, nursing guidelines	Lisa M La Liberte, RN Research Nurse Phone: 507/293-4968 E-mail: laliberte.lisa@mayo.edu
Forms completion and submission (MCR only)	Britni L Anderson Clinical Research Coordinator Phone: 507-266-3207 Email: anderson.britni@mayo.edu
Protocol document, consent form, regulatory issues	Lynn M Flickinger MA Senior Research Protocol Specialist Phone: 507/538-7034 Email: flickinger.lynn@mayo.edu
Paraffin-embedded tissue pathology	Amanda R Sand Pathology Coordinator Phone: 507/284-3559 E-mail: sand.amanda@mayo.edu
Non-paraffin biospecimens	Roxann M Neumann CCRP Biospecimen Resource Manager Phone: 507-538-0602 Email: neumann.roxann@mayo.edu
Serious Adverse Event Reporting	FDA Coordination Team E-mail: INDSafety@mayo.edu

*No waivers of eligibility allowed

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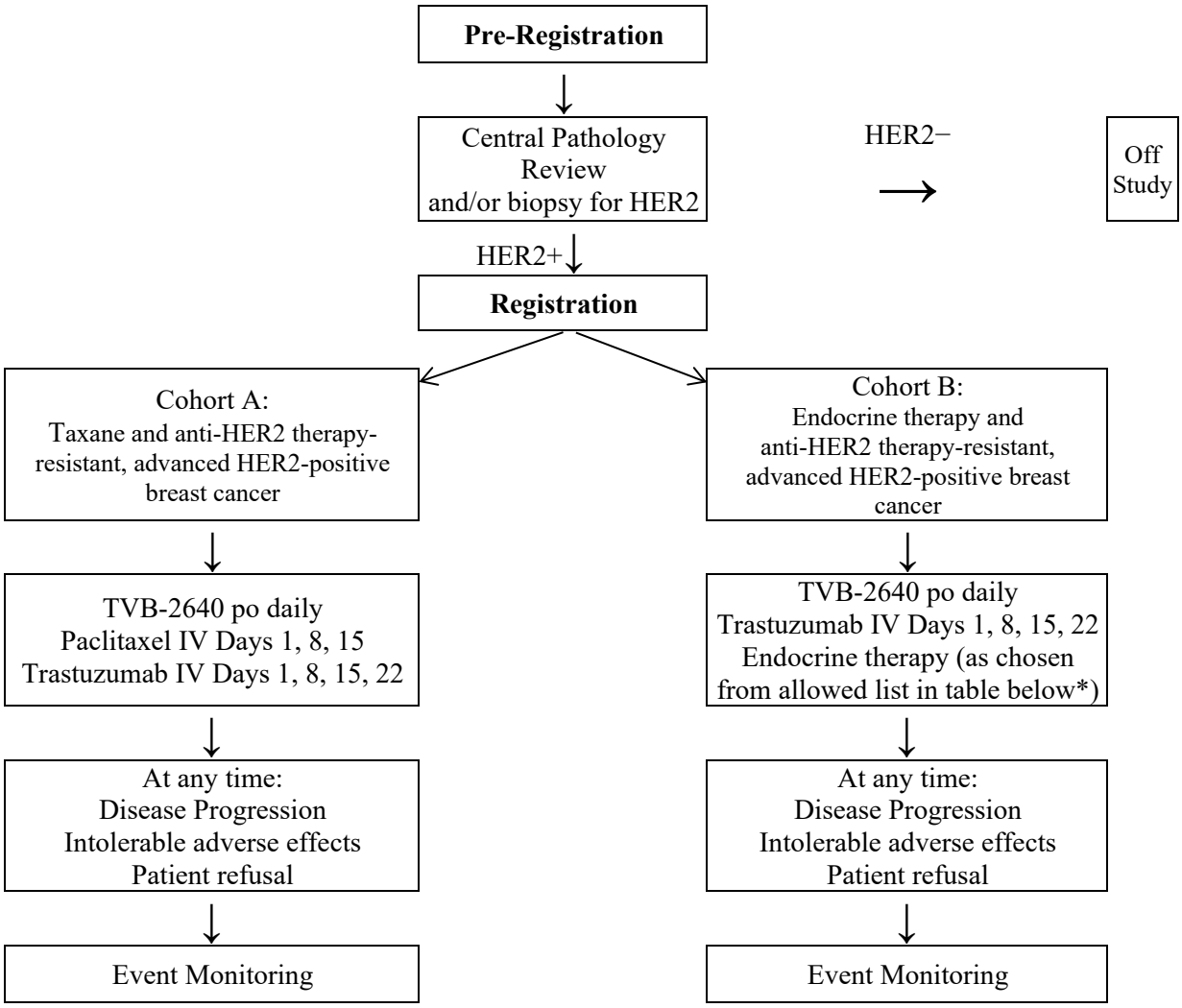
Schema

For the run-in portion only, prior to discussing protocol entry with the patient, call the MCCC Registration Office (507-284-2753) for dose level and to insure that a place on the protocol is open to the patient.



Cycle = 28 days ±3 days

Generic name: TVB-2640 Brand name(s): N/A Mayo Abbreviation: TVB-2640 Availability: Mayo Clinic Pharmacy	Generic name: Paclitaxel Brand name(s): Taxol Mayo Abbreviation: TAXOL Availability: Commercial	Generic name: Trastuzumab Brand name(s): Herceptin Mayo Abbreviation: HERCEP Availability: Commercial
*Endocrine therapy: (only allowed agents are listed) Availability: Commercial	Generic names: Anastrozole (ANA) Exemestane (EXE) Fulvestrant (FULV) Letrozole (LET)	Brand names: Arimidex Aromasin Faslodex Femara

1.0 Background

1.1 Introduction

Several agents besides trastuzumab have been developed to target HER2, including the monoclonal antibody pertuzumab, tyrosine kinase inhibitors such as lapatinib and neratinib, and the antibody-drug conjugate, T-DM1[1]. Unfortunately, none of these drugs or other targeted therapies is able to reverse trastuzumab and/or taxane resistance in patients[2,3]. To address this challenge, we seek to develop the untapped potential of interrupting the crosstalk between metabolic and oncogenic signaling pathways, as we have previously demonstrated that one of the key lipogenic enzymes, fatty acid synthase (FASN), is co-regulated with HER2. FASN is a metabolic enzyme required for *de novo* synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH in cells[4,5]. Importantly, FASN is highly expressed in all molecular subtypes of breast carcinomas, including luminal A and B, HER2+ and basal-like. In normal epithelia, however, the protein is only present at very low or negligible levels, making it an excellent therapeutic target.

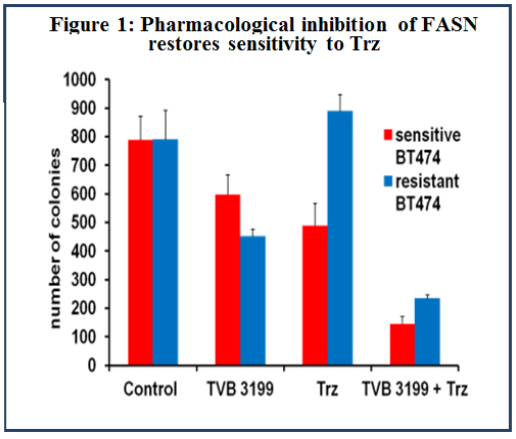
1.2 FASN Inhibition in Breast Cancer and Drug Response

Most normal cells have low levels of FASN and activity[22] in contrast to several cancers, including breast cancer[23-42]. Natural and synthetic FASN inhibitors [Cerulenin (Cer)[43-45], C75[40,46,47], and Orlistat[48-50]] induce apoptosis specifically in cancer cell lines and delay tumor growth in xenograft models; however, the mechanism by which this occurs is still unclear. Moreover, FASN inhibition induces synergistic chemosensitization of FASN+ breast cancer cells to anticancer therapies[51,52,53] including trastuzumab[54]. Most importantly, FASN blockade restores sensitivity to trastuzumab in trastuzumab-resistant, HER2+ breast cancer cells[55]. Thus, it is feasible that patients with FASN+, trastuzumab- and chemo-resistant breast cancer may benefit from FASN targeted therapies.

1.21 FASN Inhibition Restores Trastuzumab Sensitivity in Trastuzumab-resistant Cells:

Development of trastuzumab-resistance is a significant obstacle for HER2-targeted therapy, and the underlying mechanisms are still inconclusive[59]. Thus, it is critical to develop strategies to restore trastuzumab sensitivity. Since inhibition of FASN down-regulates HER2 expression and inhibits anchorage-independent growth of HER2+ breast cancer cells[54], and C75 synergizes with trastuzumab[22], we tested whether FASN inhibition restores trastuzumab sensitivity. BT474S (trastuzumab-sensitive) and BT474R (trastuzumab-resistant) cells were treated concurrently with a combination of TVB-3199 (one of the non-clinical forms of TVB-2640, 20nM) and trastuzumab (3µg/ml) based on drug-drug-interaction studies and isobologram analysis. As shown in Figure 1, FASN inhibition sensitizes BT474R to trastuzumab (Trz) and restores

Figure 1: Pharmacological inhibition of FASN restores sensitivity to Trz

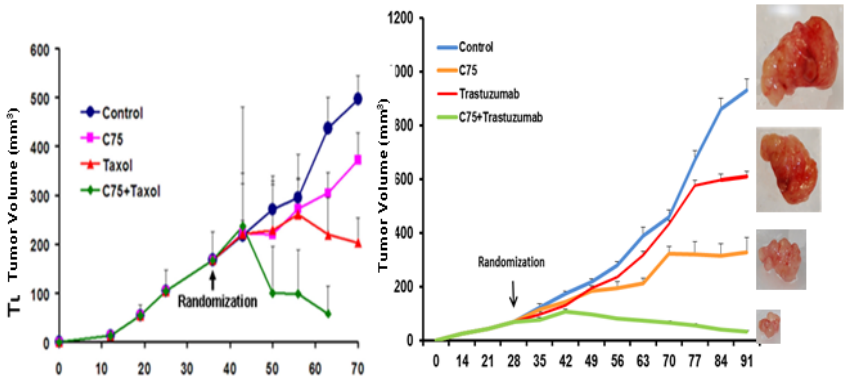


their sensitivity to levels of the BT474S cells. *(In vivo studies show similar results, pending confirmation).*

1.22 FASN inhibition sensitizes breast cancer tumors to chemo- and HER2-targeted therapy

To determine whether FASN inhibition sensitizes breast cancer tumors to paclitaxel (PXL) or trastuzumab (Trz) *in vivo,* we inoculated $4x10^6$ BC cells into the mammary fat pad of athymic nude mice, allowed tumors to reach $\leq 150mm^3$, and then randomized the mice to four arms as indicated. The mean tumor volume ± SE in each arm (n=10) is reported. The combination of C75+PXL and C75+Trz markedly reduced tumor volume compared with either treatment alone (Figure 2).

Figure 2: Inhibition of FASN significantly reduces tumor growth and synergizes with trastuzumab and paclitaxel.



Importantly, no weight loss was observed under these conditions. These data support the key proposal concept that FASN is a major player in (re)sensitizing tumors to chemo- and HER2-targeted therapy. Studies using TVB-3199 are underway yielding similar results.

1.23 Inhibition of FASN is associated with decreased synthesis of SP1, a key cancer metabolic switch

FASN-related altered lipid metabolism has been recognized to occur in tumor cell lines with acquired resistance to chemotherapeutics (e.g., doxorubicin, etoposide, cisplatin and paclitaxel) and HER2-targeted therapies (e.g., trastuzumab and lapatinib); however, lipid-metabolic traits including FASN activity are emerging as key drivers not only of ER signaling, but also of resistance to endocrine therapies including tamoxifen and aromatase inhibitors. (Benz & et al., 1992) (Pietras & et al., 1995) (Liu & et al., 1995) (Kurokawa & Arteaga, 2001)

Sphingolipid signaling mediators represent a group of extracellular and intracellular signaling molecules with pleiotropic effects on important cellular processes, including cancer. (Ogretman & Hannun, 2004) Altered sphingolipid metabolism contributes to cancer progression (Ryland & et al., 2011). The dynamic balance between intracellular sphingosine 1-phosphate, S1P, versus sphingosine and ceramide, and the consequent regulation of opposing pathways are important factors that determine whether cells survive or die (Nava & et al., 2002) (Huwiler & Zangemeister-Wittke, 2007). Estradiol (E2) was reported to mediate export of S1P from breast cancer cells leading to sphingosine 1-

phosphate receptor (S1PR) signaling, cell growth and survival (Ogretman, Sphingolipid metabolism in cancer signalling and therapy, 2018). It is hypothesized that overexpression of FASN promotes a metabolic switch that fuels bioenergetics pathways to enhance a hormone-independent breast cancer phenotype.

BT-474 and MCF-7/HER2-18 cell lines are both positive for ER expression and HER2 overexpression, and further contain high levels of FASN expression. As shown in Figure 1, in both cell lines, the FASN inhibitor, TVB3166, significantly blocks the synthesis of S1P. There was no change in S1P levels in response to TVB3166 in MCF-7/neo cells that are ER+ but do not express detectable levels of either HER2 or FASN (data not shown). Knockdown of FASN expression was established using a FASNsh in BT-474, MCF-7/neo and MCF-7/HER2-18 cells. FASNsh was associated with a profound decrease S1P in the BT-474 AND mcf-7/HER2 FASNsh cells (Figure 2), however no change was observed in MCF-7/neo cells (Gonzalez-Guerrico & et al., 2016). A decrease in S1P synthesis and signalling through S1PR can impair cancer cell growth and survival.

Figure 1

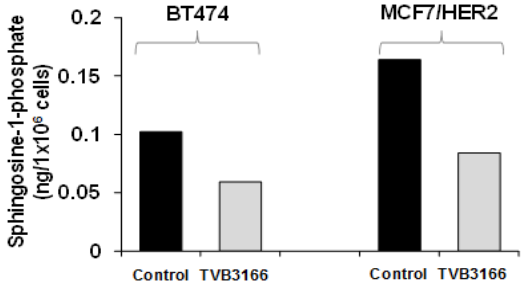
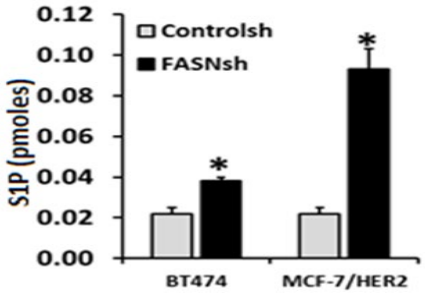


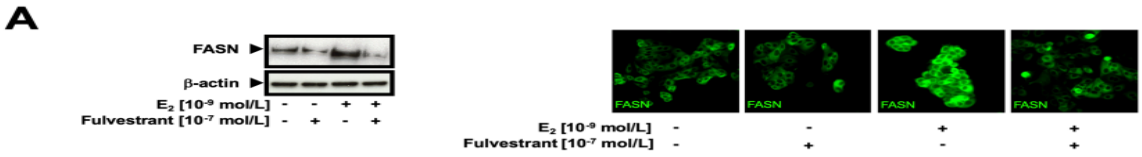
Figure 2



1.24 Estradiol modules FASN expression and promotes cross talk with PI3K/AKT pathways

Exogenous E2 supplementation at physiological concentrations modulated FASN protein levels in ER-positive, endocrine-responsive MCF-7 breast cancer cells. Immunoblotting analysis revealed that E2 significantly increased FASN protein expression (Fig. 3A, *left*). Up-regulation of FASN protein was prevented by co-exposure to the SERD, fulvestrant (Fig. 3A, *right*).

Figure 3



The stimulatory effects of estradiol on *FASN* gene promoter activity involves the PI3K/AKT/mTOR signaling cascade, a key pathway that is activated in the setting of estrogen deprivation and endocrine-resistance.

1.25 Pharmacological inhibition of FASN activity fully prevents E2-stimulated breast cancer growth (Chung & al., 2002) (Shou & al., 2004) (Menendez & Lupu, 2017)

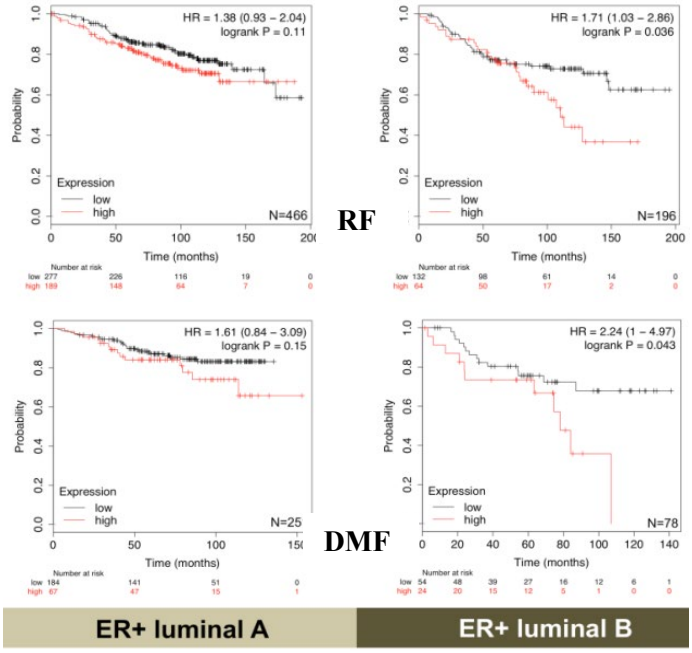
The overexpression of HER2 in ER-positive, endocrine-sensitive breast cancer has been shown to confer resistance to tamoxifen [24, 77, 78]. In the Lupu lab,

the rapid growth of MCF-7/HER2-18 cells in the presence of E2 was completely prevented in the presence of the FASN inhibitor, C75 (Fig. 4). In subsequent studies, ovariectomized nude mice were transplanted subcutaneously with tamoxifen-sensitive MCF-7/neo cells and tamoxifen-resistant MCF-7/HER2-18 counterparts, and then were randomized into four groups (vehicle, tamoxifen, C75, and tamoxifen + C75) following estrogen withdrawal (Figure 5, top panels) or with continued E2 supplementation (Figure 5, bottom panels). Growth of MCF-7/HER2-18 tumors was stimulated by E2 and inhibited by E2 withdrawal, thereby indicating E2 dependency (Figure 5, *right panels*). Tamoxifen-stimulated growth of MCF-7/HER2-18 tumors was strikingly suppressed in mice treated with the FASN inhibitor C75. Treatment with tamoxifen failed to cause any significant reduction in MCF-7/HER2-18 tumors growing in the presence of E2, thereby confirming that HER2 overexpression suffices to drive tamoxifen resistance in ER-positive MCF-7/HER2-18 BC cells *in vivo*. C75 treatment drastically antagonized E2-stimulated MCF-7/HER2-18 tumor growth, particularly when combined with tamoxifen (Figure 5, *right panels*). Similar findings were observed with C75 was combined with the SERD, fulvestrant (data not shown). The data indicate that FASN expression mediates endocrine therapy response in advanced ER+/HER2+ tumors, and inhibition of FASN can enhance the efficacy and even reverse resistance to endocrine therapy.

1.26 High FASN expression in Luminal B breast cancer is associated with poor clinical outcomes

The translational impact of FASN expression on clinical outcomes in patients with ER+ breast cancer was subsequently assessed utilizing the online Kaplan-Meier plotter tool (Gyorffy & et al., 2010). ER+ tumors were classified as luminal-A (ER+, HER2-, Ki67-low, typically endocrine-sensitive) or luminal-B (ER+, HER2-/HER2+, Ki67-high, typically endocrine-resistant), and the association of FASN low or high expression was evaluated for an association with relapse-free survival (RFS) and distant metastasis-free survival (DMFS) outcomes among patients treated with tamoxifen (Fig. 6). There was no difference in either RFS (N=466, p=0.11) or DMFS (N=251, p=0.15) between the FASN-high and FASN-low groups of patients with ER+ luminal-A breast cancer. By contrast, FASN-high, ER+ luminal B-like breast cancer patients had significantly poorer outcomes than FASN-low luminal B-like patients in terms of both RFS (N=196, p=0.036) and DMFS (N=78, p=0.043). These findings suggest that the poor outcomes associated with high FASN expression in breast cancers that are ER+ and commonly HER2+/endocrine-resistant may be associated with the SP1 metabolic switch, hormone-independent growth, and cancer cell survival.

Figure 6



1.27 FASN enables tamoxifen resistance in estrogen receptor-positive (ER+) / HER2-positive (HER2+) breast cancer cells

While both genomic and non-genomic cross-talk between the ER and HER2 has been associated with endocrine therapy resistance, combined targeting of ER and HER2 pathways has failed to improve overall survival in endocrine resistant disease (Kaufman, et al., 2009) (Johnston, et al., 2009). The role of FASN has been evaluated in the development and maintenance of estrogen-independent growth and resistance to endocrine therapy in ER+/HER2+ breast cancer (Menendez & Lupu, 2017) (Du, et al., 2018). Estradiol stimulates FASN gene promoter activity and protein expression, both of which can be blunted by anti-estrogens in endocrine-sensitive breast cancer cells. Conversely, a MAPK/AKT-related constitutive hyperactivation of FASN gene promoter activity was unaltered in response to estradiol in endocrine resistant ER+/HER2+ breast cancer cells, and could be further enhanced by tamoxifen. Pharmacological blockade of FASN activity inhibited estrogen-dependent, tamoxifen-resistant cell growth of ER+ MCF-7 cells engineered to overexpress HER2.

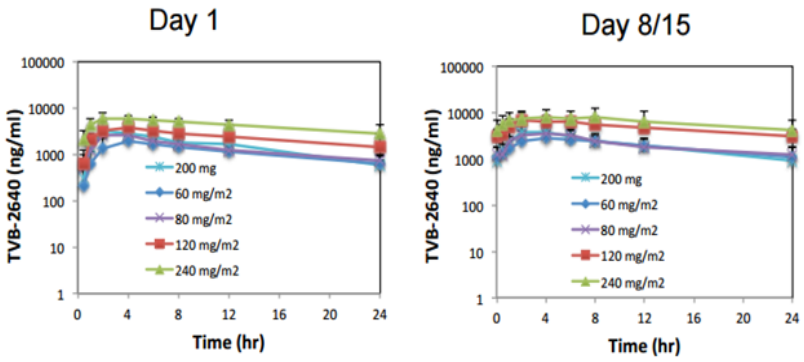
FASN overexpression has been shown to be significantly associated with poor response to tamoxifen in patients with luminal B- but not luminal A-like ER+ breast cancer (by Kaplan-Meier survival analysis). Overall, these findings reveal that FASN can be co-opted by HER2 pathway activation to circumvent the inhibition of ER signaling by endocrine therapies. As such, FASN is potentially a therapeutically targetable driver of tamoxifen resistance in luminal B-like, ER+/HER2+ breast cancer.

1.28 Clinical development of the FASN inhibitor TVB-2640

A first-in-human, first-in-class clinical trial with the only clinically available FASN inhibitor, TVB-2640, is currently treating patients with advanced solid

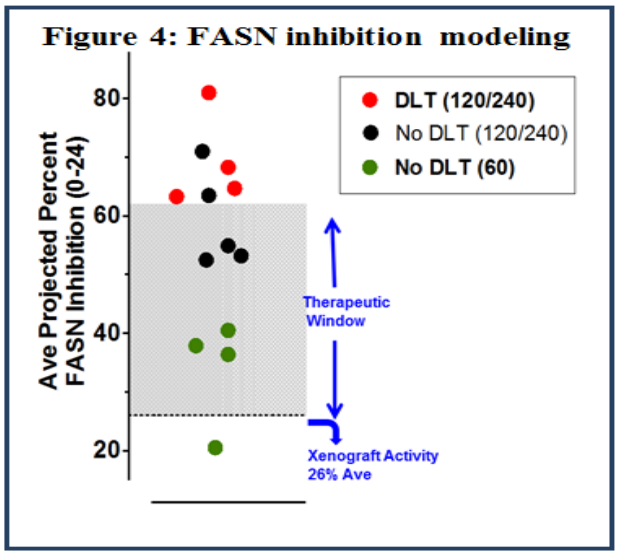
tumor malignancies (NCT 02223247). This phase I trial determined the maximum tolerated dose (MTD), recommended phase II dose (RP2D) and explored the safety profile of this drug as monotherapy and in combination with weekly paclitaxel. TVB-2640 is an oral agent administered once daily. In the most recent update of this trial (J Clin Oncol 34, 2016 (suppl; abstr 2512), 121 patients (15 with breast cancer) were treated with TVB-2640 with doses ranging from 60 to 240 mg/m^2, as well as with flat doses of 200 and 250 mg. The median patient age was 62; 68 (56%) were women and had received 3 or more prior lines of therapy. Dose limiting toxicities (DLTs) occurred at dose levels of 120 and 240 mg/m^2 and the 250mg flat dose. These included 3 Grade 3 ocular toxicities (2 corneal edema and 1 keratitis), as well as 3 skin toxicities (all hand foot syndrome) which were considered on-target effects and were reversible with drug discontinuation. Serious GI, hematologic and serum chemistry toxicities were not observed at any dose level. There was no QTc prolongation. The pharmacokinetic (PK) profile of TVB-2640 has been favorable, with predictable exposure, steady state obtained by day 8, and a half-life of approximately 15 hours (Figure 3).

Figure 3: Pharmacokinetic Plasma Levels of TVB-2640



Plasma levels of TVB-2640 measured using a validated method. On day 8/15 the predose value is plotted as the 24 hour timepoint. Mean +/- SD for n of 3 to 7 patients per monotherapy cohort.

FASN inhibition modeling at drug exposure of 60 mg/m^2 doses and above demonstrated that target modulation exceeded the minimum threshold for preclinical efficacy in all but one patient (Figure 4).



1.29a A Phase I evaluation of TVB-2640 in combination with paclitaxel

In Part B of the phase I trial, the safety and tolerability of TVB-2640 in combination with standard dose weekly paclitaxel (80 mg/m^2) was explored. Per the ASCO 2016 update, 54 heavily pre-treated patients, including 14 with breast cancer, have received combination therapy at TVB-2640 dose levels of 60 mg/m^2, 200 mg flat dose, and 100 mg/m^2. Importantly, no alterations in PK were observed when weekly paclitaxel was combined with TVB-2640.

Of all patients receiving the combination during dose-escalation, 2 DLTs were observed [1 skin (hand foot syndrome), 1 eye (uveitis)] at the 200 mg flat dose level, and both were reversible with discontinuation of TVB-2640. The MTD and RP2D of TVB-2640 as monotherapy and in combination with PXL (80 mg/m^2 weekly) are 100 mg/m^2 oral continuous once daily.

In all patients receiving combination therapy (n=54), the following adverse events were observed regardless of attribution at an incidence of ≥10% but no more than 50%: fatigue, alopecia, hand foot syndrome, nausea, peripheral neuropathy, anemia, diarrhea, anorexia, dyspnea, vomiting, cough, constipation, dry skin, UTI, back pain, peripheral edema, dry eye, increased lacrimation, mucosal inflammation, headache, abdominal pain, lower respiratory tract infection, pain in extremities and skin exfoliation. Grade 3 toxicities were uncommon and occurred in ≤5% of patients, with the exception of anemia, cough, and hand foot syndrome which occurred in 13%, 7%, and 7% of patients respectively.

Other adverse events occurring in <10% of patients receiving the combination therapy include neutropenia, rash and pneumonitis. Notably, 9% of patients receiving the combination therapy experienced pneumonitis, and this was identified as a treatment-emergent adverse event (TEAE). For one patient, pneumonitis was fatal. There were no cases of pneumonitis reported for patients receiving TVB-2640 monotherapy, whereas pneumonitis is a well-described toxicity associated with paclitaxel. Beyond this specific TEAE, there were no others observed with the combination therapy, nor was there a higher than expected incidence of other paclitaxel-associated toxicities (hypersensitivity

reaction, alopecia, cytopenias, or neuropathy). Importantly there was no observed cardiac toxicity, including no QTc prolongation, by ECG and Holter monitoring.

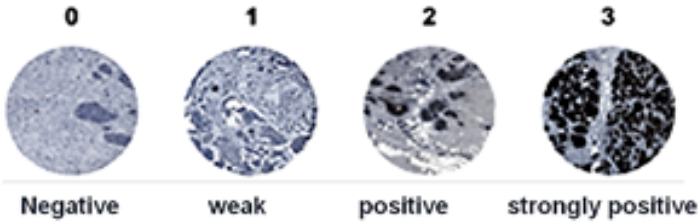
Per the ASCO 2016 update, 3 of the 14 (21%) patients with metastatic breast cancer experienced a partial response (PR) to therapy. The 16-week clinical benefit rate (PR + stable disease for minimum 16 weeks) was 62% (8/13 patients; the other patient was still on active therapy after week 10 with stable disease).

1.29b FASN Expression in Invasive Breast Cancers

We evaluated FASN expression in breast cancer in a tissue microarray (TMA) containing cores of 253 invasive breast cancers from a tumor bank created by the Breast SPORE at Northwestern University. The TMAs and corresponding patient clinico-pathological data were prepared and analyzed. FASN scoring was performed by two pathologists each blinded to the other's results. This TMA was selected for its invasive and highly metastatic tumors. All three major invasive histopathological BC types were present in the following distribution: estrogen receptor positive, ER+ (n=76, 30%), HER2+ (n=78, 31%), and triple negative, TN (n=99, 39%).

FASN was specifically stained in the breast cancer lesions by immunohistochemistry (IHC), with no observed expression in stroma or normal breast tissue. FASN expression was interpreted as negative (0), weak (1), positive (2), and strongly positive (3), using normal epithelial cells and adipose tissue as reference (Figure 5). For a more detailed value for FASN expression among invasive carcinomas, the scored specimens were separated in two groups, 0/1+ (n= 101, 40%) and 2+/3+ (n= 152, 60%). We found that high FASN levels (3+) were present in all molecular subtypes of BD: 44% of ER+, 38% of HER2+, and 20% of TN. Thus, our results demonstrate that 60% of invasive breast cancer were FASN+, and high FASN levels (3+) were found in all molecular subtypes, in particular ER+ and HER2+.

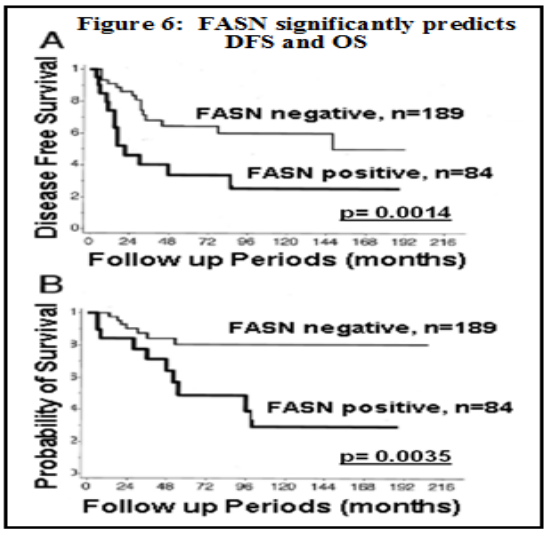
Figure 5: FASN is highly expressed in invasive BCs: Sample of cytoplasmic FASN staining in TMAs (400 x)



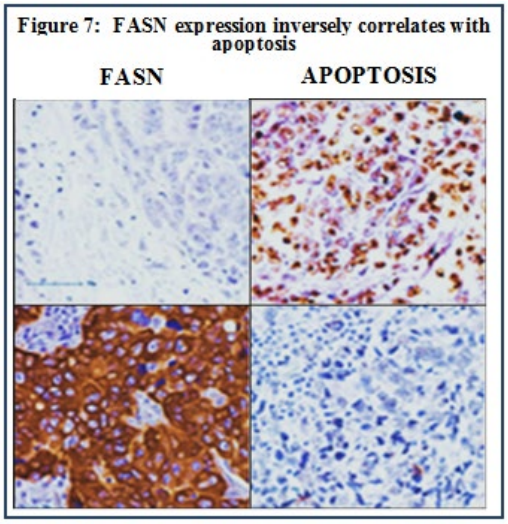
1.29c FASN Expression as a Prognostic Biomarker

In rapidly-proliferating cancer cells, *de novo* fatty acid synthesis is essential to provide lipids for membrane formation. FASN is highly expressed in breast, prostate, ovarian, endometrial and other cancers (reviewed in [23-42]) and is prominent in higher grade tumors correlating with poor prognosis[60-62]. Importantly, we demonstrated that FASN expression correlates with high HER2 levels in aggressive breast cancer [24,63,64]. As FASN was implicated as a poor prognostic indicator[65], we performed a retrospective clinical study of FASN expression in a series of 273 breast cancer tumors from patients with early stage breast cancer who all received adjuvant chemotherapy. Disease-free survival

(DFS) and overall survival (OS) were calculated using the Kaplan-Meier method, and patients were segregated by the status of FASN expression in accordance with the above described analysis. The analysis revealed that the FASN status significantly predicted DFS (log rank p=0.0014) and OS (log rank p=0.0035) as shown in Figure 6. Multivariate Cox regression analysis showed that FASN expression was associated with shorter DFS and OS, both independent of tumor grade, lymph node status, Ki67, ER and HER2 expression status. Thus, in this high risk population of patients with invasive breast cancer treated with adjuvant chemotherapy, FASN was an independent prognostic factor.



1.29d FASN Expression is Inversely Correlated with Apoptosis



To assess whether FASN expression is a marker of treatment resistance in breast cancer, we correlated FASN expression and the apoptotic status of the tissue. Apoptosis was measured using ApopTag® Peroxidase *In Situ* Apoptosis Detection Kit which detects apoptotic cells *in situ* by labeling and detecting DNA strand breaks by the TUNEL method. For this purpose we used the TMAs described in Section 1.25. Statistical analysis of the study is ongoing; however, our preliminary results indicate that tumors with FASN 2+/3+ overexpression had low number of cells staining for Apoptosis (Fig.7). Findings similar to ours have been reported in prostate cancer[66]. These data suggest that FASN overexpression exerts an anti-apoptotic effect and thus confers pro-survival properties in breast cancer.

1.3 Evolving HER2+ Landscape

Since initial protocol activation in 2017, there have been four (4) new FDA approved drugs for treatment of HER2+ metastatic breast cancer: neratinib with capecitabine (February 2020); tucatinib with capecitabine and trastuzumab (April 2020); margetuximab with chemotherapy (December 2020); trastuzumab deruxtecan (accelerated approval, December 2019 and full approval May 2022) (Exman P, 2021). Given more FDA approved options for disease management, accruals to this clinical trial

were negatively impacted and more patients were found to be ineligible due to the number of lines of prior chemotherapy despite meeting all other requirements.

Additionally, serial biopsies of tumor tissues have revealed downregulation of the HER2 receptor as a mechanism of resistance to HER2-directed therapy. Internalization and degradation of HER2 has been shown to be dependent on the density of HER2 at the cell surface (Ram S, 2014). Whereas high-level expression was associated with rapid recycling, increased degradation and cellular depletion of HER2 were observed in cells with lower cell surface receptor expressions. Clinical studies also support that at least a subset of HER2 breast cancers are susceptible to HER2 degradation and loss. Neoadjuvant trastuzumab treatment was associated both with loss of HER2 expression in ~47% of cases and poorer disease-free survival (Ignatov T, 2019). While it is known that tumor cell heterogeneity can result in discordant HER2 results on serial biopsies from different sites, this research team further determined that a longer interval between treatment and determination of HER2 expression in the second histology was associated with better concordance (which supports transient loss due to internalization and degradation).

Emerging research has identified HER2 downregulation as a mechanism of resistance to the newly FDA approved agent, trastuzumab deruxtecan. A small study was conducted in a HER-low cohort that received trastuzumab deruxtecan in the neoadjuvant setting. Results demonstrated that 49% of patients had a change in HER2 status, 88% of which had a decrease of HER2 by IHC from 2+ to 1+ or 1+ to 0 (Hurvitz SA, 2021). This too suggests that downregulation of the antigen target (HER2) after antibody drug conjugate treatment may be contributing to the high volume of screen failures for this clinical trial due to HER2-negative disease on the pre-registration biopsy. Despite this phenomenon, it is common practice to continue HER2-directed therapy for treatment given the potential heterogeneity of HER2 expression across all metastatic tumor sites.

1.4 Correlative Studies and Emerging Biomarkers from the Phase I Trial of TVB-2640

The preliminary biomarker and PK/Pharmacodynamic (PD) studies from the first-in-human, first-in-class, phase I clinical of TVB-2640 in patients with advanced solid tumor malignancies were presented at AACR in April 2015 (abstract #2675); an update and expanded findings were shared at the AACR Special Conference on Metabolism and Cancer in June 2015 (Abstract #4446).

Results of the PK studies are per Figure 3. A key PD biomarker that has emerged in the development of TVB-2640 is serum malonyl carnitine, reflective of the accumulated Malonyl-CoA upon FASN inhibition. As shown in Fig.8, a mean 3.4-fold increase in levels of serum malonyl carnitine was observed in 9 out of 10 patients by day 8 after initiation of TVB-2640 monotherapy ($p<0.05$ for D8 pre- versus D1 pre- for dose levels 120 and 240 mg/m^2). In contrast, changes in serum FASN levels after treatment with TVB-2640 were more variable (data not shown). Four patients consented to prospectively collected biopsies at two time points during the trial (one “pre-dose” prior to treatment and one “post-dose” after completing one cycle of TVB-2640). Per Figure 9, one patient with PIK3CA-mutant MBC treated at the 240 mg/m^2 dose level displayed a marginal change in tumor FASN expression after the first cycle of therapy; however, there was a 50% decrease in tumor pAKT expression, suggesting that FASN inhibition can modulate cell signaling pathways. A 30-45% decrease in pAKT expression after 1 cycle of therapy was replicated in all 3 of the additional patients, including one patient with triple negative BC.

Figure 8: Candidate PD biomarker, malonyl carnitine, increases in patient sera after 7 day TVB-2640 exposure

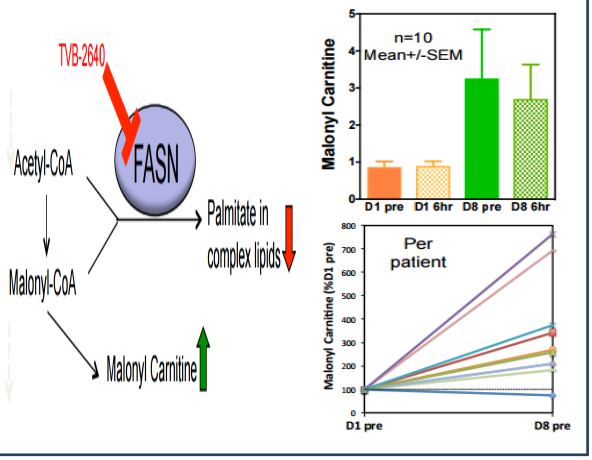
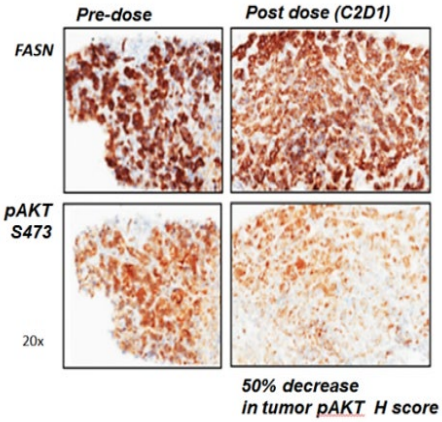


Figure 9: Tumor FASN and pAKT expression in response to TVB-2640



1.5 Palmar-Plantar Erythrodysesthesia (PPE)

Prior to April 28, 2023, 4 (30.8%) of the 13 patients enrolled onto Cohort A (Prior Taxane and anti-HER2 Therapy) and one patient enrolled onto Cohort B (Prior Endocrine and anti-HER2 Therapy) developed Grade 2 palmar-plantar erythrodysesthesia syndrome (PPE) that was considered to be possibly, probably, or definitely related to study treatment. This extent of toxicity seen in Cohort A (Prior Taxane and anti-HER2 Therapy) crossed the safety boundary and triggered a review of all the adverse event data by the study PI. Upon reviewing of the five cases with Grade 2 PPE, it became apparent that it is difficult for managing clinicians to distinguish among Grade 1 PPE (minimal skin changes or dermatitis without pain), Grade 2 PPE (skin changes with pain limiting instrumental ADL), and Grade 3 PPE (severe skin changes with pain limiting self-care ADL). Within these 'Grade 2' events, there were instances of skin changes (erythema) without pain. There were also skin changes with discomfort (ranging from mild skin peeling and scattered cracks to 'raw areas'), but it was not clear whether these skin changes limited ADLs or self-care.

The study PI spoke to each of the managing clinicians concerning the signs and symptoms observed and the importance of documenting in the patient's medical record the elements needed for the accurate grading of PPE. Moreover, the study PI developed a guide for the grading of PPE (found in Appendix III) to help in this effort. The study PI also spoke to the managing clinicians of the need to closely monitor patients with Grade 1 PPE who develop Grade 2 peripheral motor neuropathy and/or peripheral sensory that required a reduction in their paclitaxel dose as they may experience more severe PPE.

Utilizing the PPE guide for grading PPE, it appears that one (1) patient in Cohort A (Prior Taxane and anti-HER2 Therapy) and one (1) patient enrolled onto Cohort B (Prior Endocrine and anti-HER2 Therapy) developed Grade 1 PPE.

2.0 Goals

2.1 Primary Goal

2.11 To estimate the overall tumor response rate (ORR i.e. CR+PR) of the combination of TVB-2640 with paclitaxel and trastuzumab in patients with taxane and trastuzumab-resistant, advanced HER2-positive breast cancer

2.12 To estimate the ORR of the combination of TVB-2640 with paclitaxel and trastuzumab in patients with endocrine and trastuzumab-resistant, advanced HER2-positive breast cancer

2.2 Secondary Goals

2.21 For each patient cohort, to evaluate the safety profile of the combination of TVB-2640 with paclitaxel and trastuzumab

2.22 For each patient cohort, to assess the clinical benefit rate (CBR), duration of response, and progression free survival of the combination of TVB-2640 with paclitaxel and trastuzumab

2.23 To obtain a point and interval estimate of the difference in RR as well as the difference in CBR between Cohort A and Cohort B.

2.3 Correlative Research

2.31 For each patient cohort, to assess the changes in FASN, pAKT, and pS6 expression in tumor tissue after the first cycle of the combination of TVB-2640 with paclitaxel and trastuzumab from pre-treatment levels

2.32 For each patient cohort, to assess the changes in levels of cellular apoptosis in tumor tissue after the first cycle of the combination of TVB-2640 with paclitaxel and trastuzumab from pre-treatment levels

2.33 For each patient cohort, to assess the changes in serum FASN after the first cycle of the combination of TVB-2640 with paclitaxel and trastuzumab from pre-treatment levels.

3.0 Eligibility

3.1 Pre-registration – Inclusion and Exclusion Criteria

<u>Inclusion Criteria</u>

3.11 Age ≥18 years.

3.12 Measurable disease as defined by RECIST criteria (see Section 11.0) that is:

A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI and/or

A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

Note: Tumor lesions in a previously irradiated area are not considered measurable disease; Disease that is measurable by physical examination only is not eligible.

3.13 Prior treatment:

3.131 Received ≤six (6) prior chemotherapy regimens in the metastatic setting.

3.132 Cohort A (one of the following must be true):

(a) Distant disease progression during administration of combination therapy with taxane based chemotherapy and anti-HER2 therapy (trastuzumab or pertuzumab) for metastatic disease
Note: patients who began treatment with this combination and discontinued taxane-based chemotherapy due to intolerability before distant disease progression are eligible.

(b) Distant disease progression during administration or within 180 days of discontinuing combination therapy with taxane based chemotherapy and anti-HER2 therapy (trastuzumab or pertuzumab) in the adjuvant disease.
Note: Patients who began treatment with this combination and discontinued taxane-based chemotherapy due to intolerability before distant disease progression are eligible.

(c) For patients who received taxane based chemotherapy and anti-HER2 therapy (trastuzumab or pertuzumab) in the neo-adjuvant setting and underwent surgical resection of primary breast disease: Distant disease progression during or within 180 days of discontinuing anti-HER2 therapy (trastuzumab or pertuzumab) in the adjuvant setting.

3.133 Cohort B (one of the following must be true):

- Distant disease progression during administration of combination therapy with endocrine therapy and anti-HER2 therapy (trastuzumab or pertuzumab) for metastatic disease.
Permissible endocrine therapies include an aromatase inhibitor or fulvestrant.
NOTE: Tamoxifen is not permissible.
- Distant disease progression during administration of combination therapy with endocrine therapy and anti-HER2 therapy (trastuzumab or pertuzumab) in the adjuvant setting.
Permissible endocrine therapies include an aromatase inhibitor or fulvestrant.
NOTE: Tamoxifen is not permissible.

3.14 Willingness to provide mandatory tumor tissue specimens for correlative research (see Section 17.0).
NOTE: If insufficient or no tissue is obtained by the pre-registration biopsy, an archival tissue specimen (preferably from a metastatic site) from procedure performed ≤5 years prior to pre-registration must be available to submit for Central Laboratory review prior to registration.
Exception: If there is no medically safe site for biopsy, Study Chair (Dr Haddad) may waive this requirement.

Exclusion criteria

3.15 Cardiac Exclusion Criteria:
- Patients who previously discontinued trastuzumab due to unacceptable cardiac toxicity
- Patients with a history of LVEF decline to below 50% during or after prior trastuzumab or other HER2 directed therapy ≤6 months prior to pre-registration
- Patients with any class of New York Heart Association (NYHA) CHF or heart failure with preserved ejection fraction (HFPEF)
- Patients with a history of known coronary artery disease or a myocardial infarction within 12 months prior to pre-registration
- Patients with persistently uncontrolled hypertension (systolic BP >160 mm Hg or diastolic BP >100 mm Hg) despite optimal medical therapy
- Patients with known unstable angina pectoris
- Patients with a known history of serious cardiac arrhythmias requiring treatment (exception: controlled atrial fibrillation, paroxysmal supraventricular tachycardia)
- Patients with a prolonged QTc interval (≥450 ms)

3.16 Leptomeningeal disease or uncontrolled brain metastasis.
NOTE: Metastases treated by surgery and/or radiotherapy such that patient is neurologically stable and off steroids ≥4 weeks prior to preregistration are eligible.

3.17 Failure to recover from acute, reversible effects of prior therapy regardless of interval since last treatment.
EXCEPTION: Grade 1 peripheral (sensory) neuropathy that has been stable for at least 3 months since completion of prior treatment.

3.18 Tumors involving spinal cord or heart

3.19a Visceral crisis or lymphangitic spread
NOTE: Visceral crisis is not the mere presence of visceral metastases, but implies severe organ dysfunction as assessed by symptoms and signs, laboratory studies, and rapid progression of disease

3.19b Uncontrolled intercurrent non-cardiac illness including, but not limited to:
- ongoing or active infection
- psychiatric illness/social situations
- dyspnea at rest due to complications of advanced malignancy or other disease that requires continuous oxygen therapy
- or any other conditions that would limit compliance with study requirements

3.19c Immunocompromised patients and patients known to be HIV positive and currently receiving antiretroviral therapy.
NOTE: Patients known to be HIV positive, but without clinical evidence of an immunocompromised state, are eligible for this trial.

3.19d Receiving any other investigational agent which would be considered as a treatment for the primary neoplasm.

3.19e History of myocardial infarction ≤ 6 months, or congestive heart failure requiring use of ongoing maintenance therapy for life-threatening ventricular arrhythmias.

3.19f Prior history of hypersensitivity, drug or radiation-induced, or other immune-mediated pneumonitis.

3.19g Patient is unable to swallow oral medications or has impairment of GI function or GI disease that may significantly alter drug absorption (e.g., active inflammatory bowel disease, uncontrolled nausea, vomiting, diarrhea, or malabsorption syndrome). Note: Concomitant therapy with proton pump inhibitors and/or H_2-receptor antagonists is permissible.

3.19h Patient has a history of clinically significant dry eye (xerophthalmia) or other corneal abnormality, or if a contact lens wearer, does not agree to abstain from contact lens use from baseline through the last TVB-2640 dose.

3.19i Patients with a history of intolerance to trastuzumab (i.e., a Grade 3 or 4 infusion reaction) are excluded.
Note: Patients with a history of mild infusion reaction to trastuzumab who have previously been successfully re-challenged after an infusion reaction with or without prophylactic medication are allowed.

3.19j Other invasive malignancy ≤3 years prior to pre-registration.
EXCEPTIONS: Non-melanoma skin cancer, papillary thyroid cancer, or carcinoma-in-situ of the cervix which has been adequately treated.
NOTE: If there is a history of prior malignancy, patients must not be receiving other antineoplastic treatment for their cancer and the disease must be inactive/stable.

3.2 Registration - Inclusion Criteria

3.21 Registration must be completed ≤28 days of pre-registration.

3.22 ECOG Performance Status (PS) 0 or 1 (Appendix I).

3.23 Disease characteristics

3.231 Histological confirmation of HER2-expression (IHC 1-3+) on pre-registration biopsy. (HER2+ is defined by 2018 ASCO/CAP guidelines.)
NOTE: If pre-registration biopsy is HER2- by ASCO/CAP guidelines then both of the following must be true:

- The patient's most recent prior biopsy (prior to the pre-registration biopsy) must be HER2+,
  AND
- The managing provider must be planning to treat the patient with anti-HER2 therapy.

3.232 For Cohort B only: Histologic confirmation of ERα positive disease (≥1% expression).

3.24 The following laboratory values obtained ≤14 days prior to registration:

- Hemoglobin ≥9.0 g/dL
- Absolute neutrophil count (ANC) ≥1500/mm^3
- Platelet count ≥100,000/mm^3
- Direct bilirubin ≤1.5 x ULN
- Aspartate transaminase (AST) ≤3 x ULN (≤5 x ULN for patients with liver involvement)
- Calculated creatinine clearance ≥45 ml/min using the Cockcroft-Gault formula below:

Cockcroft-Gault Equation:

Creatinine clearance for males = $\frac{\textbf{(140 - age)(weight in kg)}}{\text{(72)(serum creatinine in mg/dL)}}$

Creatinine clearance for females = $\frac{\textbf{(140 - age)(weight in kg)(0.85)}}{\text{(72)(serum creatinine in mg/dL)}}$

3.25 Cardiac ejection fraction (LVEF) ≥50% by echocardiogram ≤28 days prior to registration.

3.26 Provide written informed consent.

3.27 Willing to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.28 Negative urine pregnancy test done ≤7 days prior to registration, for women of childbearing potential only.

NOTE: If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

3.29a Patient and his/her partner agree to use adequate contraception after providing written informed consent through 3 months after the last dose of TVB-2640, as follows:

- For women: Compliant with a medically-approved contraceptive regimen during and for 3 months after the treatment period or documented to be surgically sterile or postmenopausal.
- For men: Compliant with a medically-approved contraceptive regimen during and for 3 months after the treatment period or documented to be surgically sterile. Men whose sexual partners are of child-bearing potential must agree to use 2 methods of contraception prior to study entry, during the study, and for 3 months after the treatment period.

3.29b Willingness to provide mandatory tumor tissue and/or blood specimens for correlative research (see Sections 14.0 and 17.0).

3.3 Registration – Exclusion Criteria

3.31 Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:

- Pregnant women
- Nursing women
- Men or women of childbearing potential who are unwilling to employ adequate contraception

3.32 Any of the following therapies prior to registration:

- Chemotherapy ≤3 weeks
- Immunotherapy ≤3 weeks
- Biologic therapy ≤3 weeks
- Monoclonal antibodies ≤3 weeks
- Radiation therapy ≤2 weeks
- CDK 4/6 inhibitors ≤4 weeks
- mTOR inhibitors ≤4 weeks

4.0 Test Schedule

4.1 Test schedule for Cohorts 1 and 2 and Cohorts A and B

Tests and procedures	After Pre-Registration	Prior to Registration			Treatment Phase			
		≤28 days prior to registration	≤14 days prior to registration	≤7 days prior to registration	Prior to Day 1 of each treatment cycle	Prior to treatment on Days 8 15, and 22 of Cycle 1	Prior to treatment on Day 15 of Cycle 2	End of all protocol treatment for any reason
Window					-3 days	±3 days	±3 days	±7 days
History and exam, weight, PS		X			X			X
Height		X						
Adverse event assessment			X		X	X	X	X
Urine Pregnancy test[1]				X				
Hematology group Hemoglobin White blood cell count Absolute neutrophil count Platelets			X		X	X	X	X
Chemistry group: aspartate aminotransaminase (AST) alanine aminotransferase (ALT) alkaline phosphatase total bilirubin direct bilirubin[2] creatinine albumin calcium sodium potassium magnesium glucose			X		X	X	X	X
Tumor measurement[3]		X			X			X
Echocardiogram[4]		X			X			
ECG		X						
Ocular assessment		X						

Tests and procedures	After Pre-Registration	Prior to Registration			Treatment Phase			
		≤28 days prior to registration	≤14 days prior to registration	≤7 days prior to registration	Prior to Day 1 of each treatment cycle	Prior to treatment on Days 8 15, and 22 of Cycle 1	Prior to treatment on Day 15 of Cycle 2	End of all protocol treatment for any reason
Window					-3 days	±3 days	±3 days	±7 days
Patient Medication Diary (Appendix II)[5]					X			X
Mandatory research blood specimens (see Section 14.0)[R,6]					X[6]	X[6]		
Mandatory research tissue specimens (see Section 17.0) [R]	X[7]				X[8]			

NOTE: Protocol minimums are specified in the table. Additional clinical testing may be done at the discretion of the treating provider.
Cycle = 28 days

R Research funded (see Section 19.0)

1. For women of childbearing potential only. Must be done ≤7 days prior to registration.
2. Direct bilirubin is only needed ≤14 days prior to registration.
3. Tumor measurements (CT or MRI) are to be done at the completion of Cycles 2, 4, 6, etc until disease progression or treatment discontinuation. Use same imaging throughout the study.
4. Echocardiogram will be performed for left ventricular ejection fraction (LVEF) assessment. The LVEF assessments are to be performed at the completion of Cycle 1 and then after each odd numbered cycle of therapy (i.e. Cycles 3, 5, 7, etc.).
5. The diary must begin the day the patient starts taking the medication and must be completed per protocol and returned to the treating institution OR compliance must be documented in the medical record by any member of the care team.
6. Blood specimens will be collected prior to treatment on D1and D8 of Cycle 1 and D1 of Cycle 2 only. Kits will be provided for MCA and MCF.
7. The pre-registration biopsy will be utilized for local HER2 evaluation and correlative studies. NOTE: If pre-registration biopsy is attempted and fails to obtain evaluable tissue, archival specimens may be allowed if there is available tissue obtained ≤5 years prior to pre-registration (See Section 17.0)
8. Tissue specimens must be collected and submitted on D1 of Cycle 2 only (prior to treatment).

4.2 Event Monitoring/Survival Follow-up

NOTE: All timelines are approximate and not meant to represent exact dates

	Event Monitoring Phase[1]				
	q. 6 months until PD	At PD	After PD q. 6 months	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

1. If a patient is still alive 3 years after registration, no further follow-up is required.

5.0 Grouping Factor

Group: Cohort A (prior taxane and anti-HER2 therapy) vs. Cohort B (endocrine and anti-HER2 therapy)

6.0 Pre-registration/Registration Procedures

6.1 Pre-Registration (Step 1)

For the run-in portion of the trial, Call the Mayo Clinic Research Site Management Office (507-284-2753) prior to discussing protocol entry with the patient to ensure that a place on the protocol is open to the patient.

6.11 Patient preregistration

To pre-register a patient, access the Mayo Clinic Research Registration Application web page at https://registration.mayo.edu/. The Research Registration Application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the Research Site Management Office at (507) 284-2753 between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the Research Registration Application are available on the Office of Clinical Trials web page (https://www.mayo.edu/research/centers-programs/center-clinical-translational-science/offices/office-of-clinical-trials/research-registration-application) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the Research Site Management Office (507) 284-2753. If the patient was fully pre-registered, the Research Site Management Office staff can access the information from the centralized database and confirm the pre-registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

6.12 IRB approval(s) is required for each treating site.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually). If the necessary documentation is not submitted in advance of attempting patient registration, the

registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals is no longer necessary.

6.13 Verification

Prior to accepting the pre-registration, the registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient pre-registration eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.14 The site has reviewed and understands the process listed in Section*s* 14 and 17 and must account for sufficient time to complete pre-registration and registration steps.

6.15 Correlative studies

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.14, and 17.0).

6.2 Registration (Step 2)

6.21 For the run-in portion

To register a patient, fax (507-284-0885) a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

6.22 Following the completion of the run-in portion

To register a patient access the Mayo Clinic Research Registration Application web page at https://registration.mayo.edu/ and enter the remote registration application. The Research Registration/ Application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the Research Site Management Office at (507) 284-2753 between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for the registration application are available on the Mayo Clinic Office of Clinical Trials web page (https://www.mayo.edu/research/centers-programs/center-clinical-translational-science/offices/office-of-clinical-trials/research-registration-application) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the Research Registration Application can be confirmed in any of the following ways:

- Contact the Mayo Clinic Research Site Management Office (507) 284-2753. If the patient was fully registered, the Research Site Management Office staff can access the information from the centralized database and confirm the registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

6.23 Verification

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval of the study
- Patient eligibility
- Grouping factor

6.24 Documentation of IRB approval

Documentation of IRB approval must be on file in the Research site Management Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Research Site Management Office (fax: 507-284-0885 or email RESEARCHSITEMANAGEMENT@mayo.edu). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Research Site Management Office is no longer necessary.

6.25 Correlative studies

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.30, 14.0 and 17.0).

6.26 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic under the supervision of a medical oncologist or endocrinologist

6.27 Treatment start

Treatment cannot begin prior to registration and must begin ≤14 days after registration.

6.28 Pretreatment

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.29a Baseline symptoms

All required baseline symptoms (see Section 10.0) must be documented and graded.

6.29b Study drug is available on site

Study drug is available on site for this patient.

6.29c Kits

Blood draw kits are available on site

7.0 Protocol Treatment

7.1 Treatment Schedule

Use actual weight or estimated dry weight if fluid retention

7.11 Pre-treatment medications allowed for paclitaxel and trastuzumab (or per institutional guidelines)

Cohort	Agent	Dose	Route	When given
A	Dexamethasone	20 mg	IV or PO	30 minutes prior to paclitaxel
A	Diphenhydramine	25-50 mg	IV	30 minutes prior to paclitaxel
A	Famotidine	20 mg	IV	30 minutes prior to paclitaxel
A and B	Acetaminophen	650 mg	PO	30 minutes prior to trastuzumab

7.12 Cohort A: Treatment medication table

Agent	Dose Level	Route	Day	ReRx
TVB-2640	100mg/m^{2*}	PO	1-28 (continuous)	Q4w
Paclitaxel	80 mg/m^2	IV	Days 1, 8, 15	Q4w
Trastuzumab	4 mg/kg load† then 2mg/kg thereafter	IV	Days 1, 8, 15, 22	Q4w

*Dose rounding down to the nearest 50 mg TVB-2640 capsule
†The loading dose of trastuzumab is required if the last dose of trastuzumab or T-DM1 was administered >4 weeks prior to Day 1 of Cycle 1.

7.13 Cohort B: Treatment medication table

Agent	Dose Level	Route	Day	ReRx
TVB-2640	100mg/m^{2*}	PO	1-28 (continuous)	Q4w
Trastuzumab	4 mg/kg load† then 2mg/kg thereafter	IV	Days 1, 8, 15, 22	Q4w
Endocrine Therapy	Continue previously prescribed treatment		1-28 (continuous)	Q4w

*Dose rounding down to the nearest 50 mg TVB-2640 capsule
†The loading dose of trastuzumab is required if the last dose of trastuzumab or T-DM1 was administered >4 weeks prior to Day 1 of Cycle 1.

7.131 Endocrine Therapy allowed

- Standard doses of letrozole, anastrozole, exemestane, or fulvestrant Permissible agents include:
  - Anastrozole 1 mg PO daily;
  - Exemestane 25 mg PO daily;
  - Fulvestrant 500 mg IM Day 1 and 14 q 28 days for first cycle, then Day 1 q 28 days for all subsequent cycles (NOTE: 250 mg may be used if clinically indicated)
  - Letrozole 2.5 mg PO daily;
  - NOTE: Tamoxifen is NOT allowed
- 28-day cycles will apply for all agents

- There are no dose modifications for these agents
- If chosen aromatase inhibitor changes during the study, the change must be documented in the medical record and in Medidata Rave

7.14 Optional treatment medication tables after completion of 3 or more cycles

The patient **may** be changed to an alternate trastuzumab dosing schedule if both of the following conditions are met:

- Patient has completed 3 or more cycles of combination treatment
- Paclitaxel has been discontinued (if applicable)

7.141 Cohort A – Alternate trastuzumab dosing (Cycle 4 and beyond)

Agent	Dose Level	Route	Day	ReRx
TVB-2640	100mg/m^{2*}	PO	1-28 (continuous)	Q4w
Trastuzumab	8 mg/kg load† then 6mg/kg thereafter	IV	Day 1	Q3w

*Dose rounding down to the nearest 50 mg TVB-2640 capsule
†The loading dose of trastuzumab should be administered as needed per local institutional guidelines

7.142 Cohort B – Alternate trastuzumab dosing (Cycle 4 and beyond)

Agent	Dose Level	Route	Day	ReRx
TVB-2640	100mg/m^{2*}	PO	1-28 (continuous)	Q4w
Trastuzumab	8 mg/kg load† then 6mg/kg thereafter	IV	Day 1	Q3w
Endocrine Therapy	Continue previously prescribed treatment	Per agent	1-28 (continuous)	Q4w

*Dose rounding down to the nearest 50 mg TVB-2640 capsule
†The loading dose of trastuzumab should be administered as needed per local institutional guidelines

7.2 Administration

7.21 For the purposes of this study, a treatment cycle is 28 days.
Patient must return to Mayo Clinic once every 28 ±3 days for testing and prescription refill.

7.22 TVB-2640

Each QD TVB-2640 dose is to be taken at the same time of the day under fasting conditions (i.e., at least 2 hours after last food consumption and at least 1 hour before next food consumption), with each dose separated by 24 hours (±4 hours).

Cohort A only: TVB-2640 is to be taken 1 hour prior to initiation of paclitaxel. It is permissible to administer paclitaxel pre-medications during this time.

7.3 Treatment by local medical doctor (LMD)

7.31 Administration of TVB-2640 by a local medical doctor (LMD) is <u>not</u> allowed.

7.32 Administration of trastuzumab (both cohorts) and paclitaxel (Cohort A only)* is allowed by LMD as follows:

- Cycle 1: none
- Cycle 2: Days 8 and 22 only
- Cycle 3 and beyond: Days 8, 15, and 22
  OR
  May change trastuzumab to 21-day cycle and give trastuzumab once every 21 ±3 days (see Section 7.14 above for conditions)

*If paclitaxel is discontinued, LMD may administer trastuzumab according to restrictions stated above.

7.33 Administration of endocrine therapy by LMD is allowed as follows:

- Standard doses of aromatase inhibitors per Section 7.131 with no dose modifications allowed
- Fulvestrant cannot be given by LMD due to the need to align cycles with TVB-2640
- If chosen aromatase inhibitor changes during the study, the change must be documented in the medical record and in Medidata Rave
- Tamoxifen use is NOT allowed

7.34 Any serious adverse events must be reported to Mayo Clinic immediately upon discovery by LMD.

7.4 Safety Run-in

For Cohort B: An initial six patients will be enrolled at the planned starting dose level in Section 7.1 and observed for one cycle for evaluation of the safety analysis. See Section 16 for specifics.

8.0 Dosage Modification Based on Adverse Events

Strictly follow the modifications in this table for the first **two** cycles, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose.

→ ***ALERT:*** ***ADR reporting may be required for some adverse events (See Section 10.0)*** ←

NOTE: If the patient experiences a significant adverse event requiring a dose reduction at the start of the next cycle, then the dose will remain lowered for that entire subsequent cycle.

8.1 Dose Levels (Based on Adverse Events in Tables 8.2 and 8.3)

Dose Level	TVB-2640**	Trastuzumab		Paclitaxel
		Weekly	Q3W (see Section 7.14)	
0*	100 mg/m^2	4mg/kg loading dose† then 2mg/kg thereafter	8mg/kg loading dose† then 6mg/kg thereafter	80mg/m^2
-1	Starting dose reduced by 50 mg	No dose reduction	No dose reduction	60 mg/m^2
-2	Starting dose reduced by 100 mg	No dose reduction	No dose reduction	Discontinue

*Dose level 0 refers to the starting dose.

** Dose rounding down to the nearest 50 mg TVB-2640 capsule.

†The loading dose of trastuzumab should be administered as needed per local institutional guidelines

NOTES:

- If TVB-2640 is discontinued, the patient will stop all study treatment and go to Event Monitoring/Survival Follow-up.
- If paclitaxel or trastuzumab or both are discontinued, the patient remains on study treatment with TVB-2640.

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) current version 4.03* unless otherwise specified*** ← ←

*Located at http:/ctep.cancer.gov/protocolDevelopment/electronic_applications.ctc.htm

8.2 Dose Modifications for TVB-2640

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Eye Disorders	≥Grade 2 Blurred vision Eye Pain Dry eye Conjunctivitis	TVB-2640	Omit TVB-2640 until AE returns to ≤Grade 1 or baseline. Once resolved resume TVB-2640 at next lower dose. If drug is withheld for 7 days and the AE does not return to at least Grade 1 or baseline, then discontinue TVB-2640 and continue on paclitaxel and trastuzumab.
	≥Grade 2 Cataract Keratitis Papilledema Retinopathy Uveitis		Discontinue TVB-2640
Skin and subcutaneous tissue disorders	≥Grade 2 Palmar-plantar erythrodysesthesia syndrome	TVB-2640	Omit TVB-2640 until AE resolves or returns to ≤Grade 1. Once resolved resume TVB-2640 at next lower dose. If drug is withheld for 14 days and the AE does not return to at least Grade 1 or baseline, then discontinue TVB-2640 and continue on paclitaxel and trastuzumab.
Respiratory, thoracic, and mediastinal disorders	Pneumonitis Grade 2	TVB-2640 paclitaxel	Omit TVB-2640, permanently discontinue paclitaxel, and consider immediate initiation of systemic steroids for management Treatment with TVB-2640 is to be omitted until symptoms and signs of possible pneumonitis resolve to ≤Grade 1. Then TVB-2640 may be resumed If symptoms do not resolve to ≤Grade 1 within 8 weeks or prednisone (or equivalent corticosteroid) is not reduced to ≤10 mg/day within 12 weeks, then treatment with TVB-2640 is to be permanently discontinued Second event: If a second pneumonitis event occurs, discontinue TVB-2640 It is at the discretion of the treating physician to maintain trastuzumab monotherapy or have the patient discontinue all study treatment

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Respiratory, thoracic, and mediastinal disorders	Pneumonitis Grade ≥3	TVB-2640 paclitaxel	Treatment with TVB-2640 and paclitaxel is to be permanently discontinued for patients who experience Grade 3 or 4 pneumonitis (see Section 9.8). It is at the discretion of the treating physician to maintain trastuzumab monotherapy or have the patient discontinue all study treatment.

8.3 Recommended Dose Modifications for Paclitaxel

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Investigations	Neutrophil count decreased ≥Grade 3 (<1,000/mm^3)	paclitaxel	Day 1 AE: • Omit paclitaxel administration • If counts recover within 4 weeks, decrease paclitaxel by 1 dose level and maintain for all subsequent doses. • If a second dose reduction is needed, discontinue paclitaxel • If counts do not recover within 4 weeks discontinue paclitaxel Day 8 or 15 AE: • Omit the Day 8 and/or 15 administration of paclitaxel (doses are not made up) • If paclitaxel is skipped, decrease paclitaxel by 1 dose level and maintain for all subsequent doses. • If a second dose reduction is needed, discontinue paclitaxel

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Investigations	Platelet count decreased Grade 2 (50,000 - <75,000/mm^3)	paclitaxel	Day 1 AE: • Omit paclitaxel administration • If counts recover to ≥75,000/mm^3 within 4 weeks, resume paclitaxel without dose reduction • If counts do not recover within 4 weeks discontinue paclitaxel Day 8 or 15 AE: • Omit Day 8 and/or 15 administration of paclitaxel (doses are not made up) • If paclitaxel is omitted no dose reduction is required for subsequent doses
	Platelet count decreased ≥Grade 3 (<50,000/mm^3)	paclitaxel	Day 1 AE: • Omit paclitaxel administration • If counts recover to ≥75,000/mm^3 within 4 weeks, decrease paclitaxel by 1 dose level and maintain for all subsequent doses • If counts do not recover within 4 weeks discontinue paclitaxel Day 8 or 15 AE: • Omit Day 8 and/or 15 administration of paclitaxel (doses are not made up) • If treatment is omitted decreased paclitaxel by 1 dose level and maintain for all subsequent doses
Investigations	Alanine aminotransferase increased	paclitaxel	See Table 8.31
Investigations	Aspartate aminotransferase increased	paclitaxel	See Table 8.31
Investigations	Total bilirubin increased	paclitaxel	See Table 8.31

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Nervous system disorders	Peripheral sensory neuropathy	paclitaxel	For Grade 2 peripheral neuropathy, decrease paclitaxel by one dose level and maintain for all subsequent doses. Omit paclitaxel administration for Grade 3 peripheral neuropathy. When the symptoms improve to ≤Grade 1, paclitaxel may resume with one dose level reduction for all subsequent doses If a second dose reduction is needed, discontinue paclitaxel Discontinue paclitaxel if Grade 4 peripheral neuropathy develops
	Peripheral motor neuropathy	paclitaxel	For Grade 2 peripheral neuropathy, decrease paclitaxel by one dose level and maintain for all subsequent doses. Omit paclitaxel administration for Grade 3 peripheral neuropathy. When the symptoms improve to ≤Grade 2, treatment may resume with one dose level reduction for all subsequent doses. If a second dose reduction is needed, discontinue paclitaxel Discontinue paclitaxel if Grade 4 peripheral neuropathy develops.
Other non-hematologic toxicity	≥Grade 3	paclitaxel	Omit until recovery to ≤Grade 1 or back to baseline then decrease by 1 dose level. If not recovered within 14 days discontinue paclitaxel. Grade ≥3 nausea, vomiting, or diarrhea with maximal supportive treatment(s) resolving ≤3 days will not necessitate a dose reduction.

8.31 Liver Function dose modifications for paclitaxel

Transaminase level		Bilirubin level	Dose modification
AST or ALT ≥2.5	**and**	T. bilirubin ≥1.5 x ULN, but ≤3 x ULN	Reduce one dose level♦
AST or ALT ≥2.5, but ≤10 x ULN	**and**	T. bilirubin ≤1.5 x ULN	Reduce one dose level♦
AST or ALT ≥2.5 but ≤10 x ULN	**and**	T. bilirubin ≥1.5 x ULN, but ≤3 x ULN	Reduce two dose levels*♦
AST or ALT >10 x ULN	**or**	T. bilirubin >3 x ULN	Discontinue paclitaxel

* No reductions below dose level -2.
♦ Dose reductions stay at the same level unless hepatic function worsens (*i.e.*, for abnormal LFTs that remain at the same level, another dose reduction should not be made).

8.4 Dose Modifications for Trastuzumab

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
Cardiac disorders	Left ventricular systolic dysfunction	trastuzumab	See Figure 8.4.1
General disorders and administration site conditions	Infusion related reaction, Grade ≥3	trastuzumab	Infusion related reactions should be managed per institutional protocol For Grade 3 infusion reaction, trastuzumab will be discontinued if: • the infusion reaction lasts more than 24 hours, or • the patient cannot receive the entire dose due to the infusion reaction, or • a Grade 3 infusion reaction occurs in spite of pre-medication For Grade 4 infusion reaction, trastuzumab will be discontinued.

If the patient misses a dose (or doses) of trastuzumab, a re-loading dose of trastuzumab should be given as outlined in Section 8.1.

Trastuzumab can be held for a maximum of 42 days from last administered dose. Patients who require longer dose delays may not restart trastuzumab. Patients who discontinue trastuzumab may continue treatment with paclitaxel and/or TVB-2640 or discontinue all study treatment.

Any patient who develops clinical signs and symptoms suggesting congestive heart failure or adverse events that may be related to cardiac dysfunction should have LVEF measurement preformed. If CHF is confirmed, the patient should discontinue all protocol treatment and go to event monitoring. CHF should be treated and monitored according to institutional guidelines

8.41 Management of cardiac toxicity related to trastuzumab

Asymptomatic decrease in left ventricular ejection fraction (LVEF): The decision to continue or stop trastuzumab is based on: measured ejection fraction as it relates to the radiology facility's lower limit of normal (LLN) **and** change in ejection fraction from baseline. Guidelines for performing MUGA scan/echocardiogram and management of HERCEP patients who have an ***asymptomatic*** decrease in LVEF from baseline are in the table below:

Asymptomatic decrease in LVEF percentage points from baseline			
Relationship of LVEF to the radiology facility's LLN	**Decrease of <10 percentage points**	**Decrease of 10 to 15 percentage points**	**Decrease of ≥16 percentage points**
Within normal limits	Continue	Continue	Hold and repeat echocardiogram after 4 weeks
1 to 5 percentage points below the LLN	Continue and repeat echocardiogram after 4 weeks	Hold and repeat echocardiogram after 4 weeks	Hold and repeat echocardiogram after 4 weeks
≥6 percentage points below the LLN	Continue and repeat echocardiogram after 4 weeks	Hold and repeat echocardiogram after 4 weeks	Hold and repeat echocardiogram after 4 weeks

- Trastuzumab must be permanently discontinued when two consecutive "hold" categories occur.
- Trastuzumab must be permanently discontinued when three intermittent "hold" categories occur. (At the discretion of the investigator, trastuzumab may also be permanently discontinued prior to the occurrence of three intermittent "hold" categories.)
- If LVEF is maintained at a "continue and repeat echocardiogram" or improves from a "hold" to a "continue and repeat echocardiogram" category, additional echocardiogram prior to the next scheduled echocardiogram will be at the discretion of the investigator.

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Blood products and growth factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the Journal of Clinical Oncology, Volume 33, No 28 (October 1), 2015: pp. 3199-3212 (WBC growth factors) AND Journal of Clinical Oncology, Volume 28, No 33 (November 20), 2010: pp. 4955-5010 (darbepoetin/epoetin).

9.3 Antiemetics

Antiemetics may be used at the discretion of the attending physician.

9.4 Diarrhea

Diarrhea could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of Grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

9.5 Hand-foot syndrome (palmar-plantar dysesthesia)

Prophylaxis: patient can use a daily moisturizing cream on hands and feet with a high emollient factor such as Eucerin, Vanicream, O'Keefe's Working Hands (or similar product), 4-6 times a day. Patient can also use a non-drying daily wash on hands and feet such as Cetaphil or Vanicream Soap (or similar product).

If hand-foot symptoms/signs are detected despite the above prophylaxis recommendations:

Treatment: to be symptomatic. At the first sign (deemed a grade 1; per CTCAE 4.03; minimal skin changes or dermatitis (e.g. erythema, edema, or hyperkeratosis without pain):

- **Moisturizing:** daily cream on hands and feet to continue;
- **Urea cream:** can be considered if scaling, peeling present;
- **Antihistamines:** oral antihistamine medication to be considered for reduction of itchiness.

If hand-foot symptoms/signs present at or escalate to Grade 2 or above:

- **Steroid Ointment (N.B. not cream):** patient can apply ointment based barrier layer of topical steroid cream Diprolene (or similar product) once daily.

- **Dermatologist consult:** should be considered for further evaluation and treatment recommendations.

9.6 Dry Eye

Treatment: to be symptomatic. At the first sign (deemed a grade 1 per Common Terminology Criteria for Adverse Events CTCAE 4.03; asymptomatic, clinical or diagnostic observations only, mild symptoms relieved by lubricants) of dryness, itchiness, redness or otherwise "gritty" eye.

- **Eye drops:** soothe XP-Xtra Protection Emollient (Lubricant) Eye Drops (or similar product) can be used 4-6 times daily. If the patient finds this to be too cumbersome and/or altering to his/her vision, then a less oily eye drop such as Soothe Hydration Lubricant Eye drop or Visine (or similar product) can be used during the day with the mineral oil based [Soothe XP-Xtra Protection Emollient (Lubricant) Eye Drops or similar products] drops to be used nightly before bed.
- **Warm compresses:** patient can apply warm compresses to eyes 4-6 times a day or as needed to unblock tear ducts and reduce inflammation. Patient should be instructed to place a warm, wet washcloth over the eyelids (a fresh/sterile cloth to be used with each compress application) for up to 10 minutes.
- **Azasite:** patient can apply to eyelid with a cotton swab such as Q-Tip once daily to reduce inflammation (as needed).
- **Ophthalmologist consult:** should be considered for further evaluation and treatment recommendations.

9.7 Bone Modifying Agents

Zolendronic acid, pamidronate, or denosumab may be administered per institutional guidelines as appropriate for patients with bone metastases.

9.8 Pneumonitis

9.81 Grade 1-2

It is the treating provider's choice whether empiric antibiotic therapy should be instituted, even in the absence of a causative organism being isolated.

9.82 Grade 3-4

For patients who experience Grade 3 or 4 pneumonitis, intravenous steroid treatment as well as additional anti-inflammatory measures should be considered and administered, as needed. It is the treating provider's choice whether empiric antibiotic therapy should be instituted, even in the absence of a causative organism being isolated. Prophylactic antibiotics for opportunistic infections should be considered in the case of prolonged steroid administration.

10.0 Adverse Event (AE) Monitoring and Reporting

The site principal investigator is responsible for reporting any/all serious adverse events and adverse events of special interest to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDA, 3-V Biosciences, Inc., and all participating investigators in a written safety report of any of the following:

a. Any suspected adverse reaction that is both serious and unexpected.

b. Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.

c. Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug

d. Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf	Mayo Sites – attach to MCCC Electronic SAE Reporting Form
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56	Will automatically be sent to CANCERCROSAFETYIN@mayo.edu

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

- Identify the grade and severity of the event using the CTCAE version 4.0.
- Determine whether the event is expected or unexpected (see Section 10.2).
- Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
- Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
- Determine if other reporting is required (see Section 10.5).
- Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.
An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.
NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME (Combination) Arm

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the **entire combination (arm) is then considered an investigational intervention for reporting.**

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for **investigational** agents/interventions.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report, unless hospitalization is required. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

- An increased incidence of an expected adverse event (AE) is based on the patients treated for this study at their site. A list of known/expected AEs is reported in the package insert or the literature, including AEs resulting from a drug overdose.
- Commercial agent expedited reports must be submitted to the FDA via MedWatch 3500A for Health Professionals (complete all three pages of the form).

  http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf

  or

  http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/ListFormsAlphabetically/default.htm

  Instructions for completing the MedWatch 3500A:
  http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/UCM387002.pdf

10.32 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be reported in an expedited manner[1]
General disorders and administrations site conditions	Fatigue	≤Grade 3
	Malaise	≤Grade 3
Skin and subcutaneous tissue disorders	Alopecia	Any Grade

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.
Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for IND/IDE Agents

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

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

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

NOTE: Refer to Section 10.32 for exceptions to Expedited Reporting

10.42 **General reporting instructions**

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB plus Sagimet Biosciences Inc. (formerly 3-V Biosciences, Inc.).

Use Mayo Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56 for investigational agents or commercial/investigational agents on the same arm.

For commercial agents (Trastuzumab and Paclitaxel):

Submit form MedWatch 3500A to the FDA, 5600 Fishers Lane, Rockville, MD 20852-9787, by fax at 1-800-332-0178 or online at http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm.

Submit SAEs to Industry Partner Contacts:

dsafety@sagimet.com

Katharine Grimmer (Katharine.Grommer@sagimet.com)

Dr. Bill McCulloch (Bill.McCulloch@sagimet.com)

George Kemble (George.Kemble@sagimet.com)

10.43 **Reporting of re-occurring SAEs**

ALL SERIOUS adverse events that meet the criteria outlined in table 10.41 MUST be immediately reported to 3-V Biosciences within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 **Other Required Reporting**

The following study events must be promptly reported to the HRPO in one of the following manners:

- Phone: 301-619-2165
- Email: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil
- Fax: 301-619-7803
- Mail: US Army Medical Research and Materiel Command
  ATTN: MCMR-RP
  810 Schreider St
  Fort Detrick, MD 21702-5000

a) All unanticipated problems involving risk to subjects or others.
b) Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies.
c) Any instances of serious or continuing noncompliance with the federal regulations or IRB requirements.
d) The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research.
e) The issuance of inspection reports, FDA Form 483, warning letters, or actions taken by any government regulatory agencies.

f) Change in subject status when a previously enrolled human subject becomes a prisoner must be promptly reported to the USAMRMC ORP HRPO. The report must include actions taken by the institution and the IRB.

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets all of the following criteria:

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

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56, to submit to CANCERCROSAFETYIN@mayo.edu. The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 **Death**

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 **Secondary Malignancy**

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 **Second Malignancy**

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 **Pregnancy, Fetal Death, and Death Neonatal**

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent

form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf

10.551 Pregnancy

Pregnancies occurring in the patient or patient's partner while the patient is receiving study drug or within 3 months after the patient's last dose of study drug will not be considered serious, but are to be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Study drug must be discontinued immediately in the event of a pregnancy in the patient. The patient should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. Pregnancy should be followed until the outcome is known and must be reported to the Medical Monitor within 5 days.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the study drug should also be reported.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 **Baseline and Adverse Events Evaluations**
Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v4.03 **unless** alternate grading is indicated in the table below:

CTCAE System Organ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Blood and Lymphatic System Disorders	Anemia	X	X
Eye Disorders	Dry Eye	X	X
	Eye Pain	X	X
Gastrointestinal Disorders	# of stools	X	
	Diarrhea		X
	Nausea	X	X
	Vomiting	X	X
	Mucositis Oral	X	X
General disorders and administration site conditions	Fatigue	X	X
Investigations	Platelet Count Decreased	X	X
	Neutrophil Count Decreased	X	X
	White Blood Cell Decreased	X	X
Nervous system disorders	Peripheral motor neuropathy	X	X
	Peripheral sensory neuropathy	X	X
Respiratory, thoracic and mediastinal disorders	Pneumonitis	X	X
Skin and subcutaneous tissue disorders	Rash maculo-papular	X	X
	Palmar-plantar erythrodysesthesia syndrome*	X	X

*See Appendix III for PPE Grading Worksheet

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study

treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

10.8 3V Biosciences Inc. Additional Event Reporting Instructions

Adverse Events of Special Interest

Any pneumonitis AE and/or treatment-emergent respiratory symptoms or signs experienced by patients receiving TVB-2640 are to be reported as adverse events of special interest (AESI). All AESIs that occur after any patient has been enrolled, before treatment, during treatment, or within 28 days following the cessation of treatment, whether or not they are related to the study drug, must be reported within 24 hours of discovery using the procedures as described for SAEs

11.0 Treatment Evaluation/Measurement of Effect

11.1 Schedule of Evaluations

Evaluations using RECIST will then be performed at completion of Cycles 2, 4, 6, etc until disease progression or discontinuation of all protocol treatment.

11.2 Definitions of Measurable and Non-Measurable Disease

11.21 Measurable Disease

11.211 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

11.212 A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: Tumor lesions in a previously irradiated area are not considered measurable disease.

11.22 Non-Measurable Disease

- All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/ pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: '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. In addition, lymph nodes that have a short axis <1.0 cm are considered non- pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.31 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up.

11.32 Acceptable Modalities for Measurable Disease:

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

- As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

Body scans should be performed with breath-hold scanning techniques, if possible.

- PET-CT: CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

11.33 Measurement at Follow-up Evaluation:

- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.
- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in ATC after chemoradiation can represent fibrotic tissue)

11.4 Measurement of Effect

11.41 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. <u>These lesions can be non-nodal or nodal (as defined in 11.21)</u>, where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.
- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.
- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.

- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.42 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with 11.433.

11.43 Response Criteria

11.431 All target lesions and target lymph nodes followed by CT/MRI/PET-CT must be measured on re-evaluation Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment,

11.432 Evaluation of Target Lesions

Complete Response (CR):All of the following must be true:

- Disappearance of all target lesions.
- Each target lymph node must have reduction in short axis to <1.0 cm.

Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (*see* Section 11.41).

Progression (PD): At least one of the following must be true:

- At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.
- At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.433 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

Complete Response (CR):	All of the following must be true: a. Disappearance of all non-target lesions. b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.
Non-CR/Non-PD:	Persistence of one or more non-target lesions or non-target lymph nodes.

Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.

b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)

11.44 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

11.441 For Patients with Measurable Disease

Target Lesions & Target Lymph Nodes	**Non-Target Lesions & Non-Target Lymph Nodes**	**New Sites of Disease**	**Overall Objective Status**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported

as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration.

12.0 Descriptive Factors: None

13.0 Treatment/Follow-up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who have not had disease progression and have experienced acceptable toxicity are to continue treatment per protocol until documentation of progressive disease, unacceptable toxicity or refusal.

13.2 Criteria for discontinuation of all protocol treatment

- Disease progression
- Request by patient to discontinue all protocol treatment
- Unacceptable toxicity
- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or require discontinuation of all protocol treatment
- Administration of radiotherapy, non-protocol chemotherapy, immunotherapy, biological agents, or an experimental drug
- Development of new primary cancer
- Ineligibility

Patients who discontinue protocol treatment due to any of the above reasons will proceed to event monitoring phase of the trial where patient and disease status until death or a maximum of 3 years post-registration. Further treatment is at the discretion of the patient's medical team.

13.3 Ineligible

A patient is deemed ineligible if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry.

- If the patient received protocol treatment and is deriving benefit, the patient may continue on study per protocol as if the patient was eligible. If the patient chooses to end protocol treatment, then all data (except biospecimens) up until the point of treatment discontinuation must be submitted. No further data submission is necessary.
- If the patient never received any protocol treatment, on-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.4 Cancel

A patient who withdraws consent before any study treatment is given. On-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	C1D1	C1D8	C2D1	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping
FASN	Mandatory	Serum	Gold top SST tube	5mL	X	X	X	yes	Freeze

14.2 Collection and Processing

14.21 Serum FASN

- *NOTE: all pre-dose samples should be collected BEFORE daily TVB-2640 administration.*
- *The steps listed should be performed as quickly as possible after each blood draw:*
    1. Label one serum separator vacutainer tube (Gold top (SST tube)), and two storage tubes (Sample A and Sample B)
    2. Collect 3 mL of blood into the SST vacutainer.
    3. Immediately and gently, invert the tube 5 times to mix.
    4. Stand the tube upright in a vertical rack for 30 to 60 minutes at room temperature to allow clotting to occur.
    5. Record the actual sampling date and time (hours and minutes using 24 hour clock time) for each sample collected onto the appropriate CRF.
    6. Centrifuge at 1300 g for 15 min. While directing away from your face, remove the rubber top from the tube. Two separate layers should be readily visible.
    7. Transfer ~half of serum (approx. 0.6 ml) into a clean storage tube (labeled as "Sample A", and transfer the remaining serum into another clean storage tube labeled as "Sample B". ("Sample B" is the back-up sample.)
    8. Store serum samples in an upright position in a frost-free freezer, at approximately 70°C or lower.
    9. The time between blood collection and freezing the serum should not exceed 4 hours. Following collection if processing will take longer than 2 hours the sample will need to be refrigerated until processing can occur.
    10. Ship specimens on dry ice according to instructions below. Ship "Sample A" serum samples every month by the 15th on Monday, Tuesday or Wednesday for US sites.
    11. Store "Sample B" specimens at -70°C or lower, and ship to the storage facility (EPL) every month by the 15th on Monday, Tuesday or Wednesday for US sites.

14.3 Shipping and Handling

14.31 Kits *will* be used for this study.

14.311 Kits will be supplied to MCA and MCF by the Biospecimen Accessioning and Processing Shared Resource (BAP). Cards will be generated for MCR.

14.312 The kit contains supplies and instructions for collecting, processing and shipping specimens.

14.313 Participating institutions may obtain kits by faxing the Supply Order Form to the number listed on the form. Because we are charged for all outgoing kits, a small, but sufficient, supply of the specimen collection kits should be ordered prior to patient entry. **Supply Order Forms must be filled in completely and legibly for quick processing.**

14.314 Kits will be sent via Fed Ex® Ground at no additional cost to the participating institutions. **Allow at least two weeks to receive the kits.**

14.315 Kits will not be sent via rush delivery service unless the participating institution provides their own Fed Ex® account number or alternate billing number for express mail. **Cost for rush delivery of kits will not be covered by the study.**

14.316 All specimens must be collected and shipped Monday – Thursday ONLY.

14.32 Shipping

14.321 Staff from MCA and MCF should ship all their serum FASN samples to:

BAP Freezer
Mayo Clinic
St SL-16
150 Third Street SW
Rochester, MN 55902
507-538-0602

Fed-Ex is the selected courier for Serum (for free FASN) samples.

All "Sample A" serum samples should be shipped frozen on dry ice using appropriate packaging to avoid damage and maintain temperature.

1. Unless other arrangements are made, samples should be shipped on Monday, Tuesday or Wednesday for US sites for next day delivery, excluding holidays.
2. Complete one requisition form per sample type and per patient. Note any missing samples.
3. Place the frozen samples for each patient in Cryoboxes, and label box with patient numbers. Include the requisition form with the shipment.
4. Pack the frozen samples in a sufficient quantity of dry ice in shipping containers to maintain a frozen state for approximately 2-3 days.
5. Label the package with 3-V Biosciences, Inc., and study number (MC1633).
6. Include a return address (which includes the investigator's name) on the outside of each shipping container.
7. Comply with all courier regulations for the shipment of biological specimens (include all paperwork).
8. Retain all documents indicating date, time, and signature(s) of person(s) making the shipment, in the study files.
9. As soon as shipment day and air bill number(s) are available, fax, or e-mail the completed requisition form to the recipient, including the shipment tracking or reference number.

Shipping address:

Leslie Couch
BioRepository Archivist
EPL BioRepository
615 Davis Drive
Suite 300
Durham, NC 27713 USA
Tel: 1-919-998-9009 Ext 653
Fax: 1-919-998-9008
e-mail: LCouch@epl-inc.com

14.33 Reminder: Sample A's should always be shipped independently of Sample *B's.*

14.34 Handling Specimens

RSTP tickets should be completed for each time samples are shipped

14.4 Background and Methodology

Evaluation of Serum FASN Levels: We hypothesize that levels of serum FASN are strongly correlated with tumor tissue levels of FASN. This would be an important finding should tumor FASN emerge as a relevant biomarker of response, as a serum study (as opposed to a tumor biopsy) for FASN determination would eliminate the need for an invasive procedure. These studies will be performed at the 3-V Biosciences laboratories (or by a contracting site TBD). For the FASN ELISA, a commercially available human FASN kits from Immtech will be used. FAS-detect ELISA is an enzyme-linked immunosorbent assay that can be used to detect soluble FASN in human serum. We run serum of neat, 1/5 and 1/20 dilutions in duplicate. Developed by FASgen Diagnostics LLC, FAS-detect ELISA is a highly sensitive, specific, and quantitative enzyme-linked immunosorbent assay that can be used to detect soluble FASN in human serum, secondary to production and release by FASN-positive tumors.

15.0 Drug Information

15.1 TVB-2640

15.11 Background

TVB-2640 is a small-molecule, orally-bioavailable, reversible inhibitor of the human FASN enzyme. Many solid and hematopoietic tumors overexpress FASN, including breast, ovarian, prostate, colon, and pancreatic cancers as well as non-Hodgkin lymphoma. FASN tumor expression has been found to be increased in a stage-dependent manner that is associated with diminished patient survival. This expression-prognosis relationship suggests that FASN plays an important role in affecting tumor cell biology and therapeutic response.

15.12 Formulation

TVB-2640 is currently supplied for oral administration as immediate release 50 mg strength capsules.

15.13 Preparation and storage

TVB-2640 should be stored in a secure, limited access storage area at room temperature, 20-25°C (77°F). Excursions to 15°C - 30°C (59°F - 86°F) are permitted. TVB-2640 should not be refrigerated or frozen.

15.14 Administration

Administer on an empty stomach, one hour before or two hours after a meal. Swallow the capsules whole, do not chew, crush, or open. Dose should be taken at the same time each day, ±4 hours.

15.15 Pharmacokinetic information

There is limited PK data in humans. The half –life has been determined to be approximately 15 hours at steady state.

15.16 Potential Drug Interactions

TVB-2640 showed no significant inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C19, or CYP2D6. It showed weak inhibition of CYP2C9 and CYP3A4 with testosterone as a substrate. In a screening assay in human hepatocytes from 3 donors, TVB-2640 at a concentration of 1 and 10 µM caused little or no significant induction of catalytic activity of CYP1A2, CYP2B6, and CYP3A4; the extent of induction was less than 40% of the positive controls. These data suggest that TVB-2640 is not an inducer of drug-metabolizing enzymes. TVB-2640 is a time-dependent inhibitor of CYP3A4 and may reduce the clearance of substrates of this enzyme.

15.17 Known potential toxicities

Of 73 patients treated with TVB-2640 as monotherapy, the most common (incidence ≥ 10%) adverse events reported were alopecia (56%), palmar-plantar erythrodysesthesia syndrome (40%), fatigue (33%), decreased appetite (25%), dry skin (22%), constipation (18%), nausea (18%), diarrhea (17%), vomiting (17%), cough (15%), dry eyes (15%), abdominal pain (14%), conjunctivitis (14%), anemia (13%), asthenia (13%), skin exfoliation (13%), urinary tract infection (13%), dehydration (11%), dyspnea (11%), increased lacrimation (11%), dysgeusia (10%), peripheral edema (10%), pyrexia (10%), rash (10%).

Six dose-limiting toxicities (DLTs) were observed, including corneal edema (Grade 3, n = 2), keratitis (Grade 2, n = 1) and palmar-plantar erythrodysesthesia (PPE) syndrome (Grade 3, n = 2; Grade 2, n = 1), across a range of doses.

With TVB-2640 in combination with paclitaxel, the most common TEAEs were fatigue (49%), alopecia (45%), PPE syndrome (44%); nausea (38%), and peripheral neuropathy (36%). 2 DLTs were observed, uveitis (Grade 2, n=1) and PPE syndrome (Grade 3, n=1), both at a flat dose of 200 mg. Serious cases of pneumonitis in 9% of patients (5/55) have been reported with TVB-2640 in combination with paclitaxel, which were all considered by the Investigator to be study drug-related, including 1 fatal case that occurred ~6 months after initiation of TVB-2640 and paclitaxel. Pneumonitis is known to be associated with paclitaxel, but any contribution of TVB-2640 is not clear. No cases of pneumonitis have occurred among patients treated with TVB-2640 monotherapy. Nonetheless, respiratory symptoms, and specifically pneumonitis, constitute Adverse Events of Special Interest (AESI) which should be reported to the FDA as such, should they occur.

No Carcinogenicity, reproductive, developmental, local tolerance or other special toxicity studies have been conducted with TVB-2640.

15.18 Drug procurement

TVB-2640 is supplied by 3-V Biosciences, Inc. An initial supply is provided once the study is finalized and contracts have been signed. TVB-2640 will then be proactively supplied by 3-V Biosciences based on enrollment, dose and patient duration on study. If an intermediate drug resupply is needed, an order request must be made through an appropriate 3-V Biosciences contact, either Clinical Operations or CMC representatives. A list of 3-V Biosciences authorized drug resupply contacts will be maintained with the site study coordinator.

15.19 Nursing guidelines

15.19a Agent must be administered on an empty stomach. Instruct patients to take one hour before or two hours after a meal. Do no crunch, chew, or open capsules, they must be swallowed whole.

15.19b Instruct patients to take agent at the same time each day.

15.19c Due to the early investigational nature of this agent, not all side effects can be known at this time. Instruct patients to report all side effects to the study team.

15.19d Warn patients of possible hair loss.

15.19e PPE (hand-foot syndrome) is common. Instruct patients to report any redness, swelling, pain, numbness, tingling, blistering and/or peeling of the hands/feet to study team immediately. Patients should be encouraged to keep areas well moisturized with alcohol free lotion, and to avoid harsh soaps. Additional skin toxicities may occur, including dermatitis, dry skin, and nail disorders. Assess patient's skin and instruct them to report any changes to the team.

15.19f Fatigue can be seen. Encourage patient in energy-conserving lifestyle.

15.19g GI side effects can be seen – diarrhea, constipation, vomiting, nausea, etc. Treat symptomatically and assess for effectiveness.

15.19h Monitor CBC w/diff as cytopenias have been seen.

15.19i Patients may experience ocular toxicity. Instruct them to report any changes in vision, or pain to the study team immediately.

15.19j Mild LFT elevation may occur. Monitor liver function tests.

15.19k Arthralgias and musculoskeletal pain can be seen. Treat symptomatically and monitor for effectiveness.

15.19l Pneumonitis has been reported when TVB-2640 has been given in conjunction with paclitaxel. The exact relationship is not clear. Instruct patients to report any shortness of breath, DOE or chest pain to study team immediately.

15.2 Trastuzumab (Herceptin®)

15.21 **Background**: Trastuzumab is a monoclonal antibody which binds to the extracellular domain of the human epidermal growth factor receptor 2 protein (HER-2); it mediates antibody-dependent cellular cytotoxicity by inhibiting proliferation of cells which over express HER-2 protein.

15.22 **Formulation**: Commercially available injection, powder for reconstitution: 440 mg [packaged with bacteriostatic water for injection; diluent contains benzyl alcohol]

15.23 **Preparation, storage, and stability**: Refer to package insert for complete preparation and dispensing instructions. Prior to reconstitution, store intact vials under refrigeration at 2ºC to 8ºC (36ºF to 46ºF). Reconstitute each vial with 20 mL of bacteriostatic sterile water for injection (SWFI) to a concentration of 21 mg/mL. Swirl gently; do not shake. Allow vial to rest for ~5 minutes. The solution in the vial (reconstituted with bacteriostatic SWFI) is stable refrigerated for 28 days from the date of reconstitution; do not freeze. If the patient has a known hypersensitivity to benzyl alcohol, trastuzumab may be reconstituted with sterile water for injection without preservatives, which must be used immediately. Determine the appropriate volume for the trastuzumab dose and further dilute in 250 mL 0.9% Sodium Chloride for injection prior to administration. Refer to the treatment section of the protocol for any changes in dilution or dispensing instructions. Avoid rapid expulsion from the syringe; gently invert bag to mix. The solution for infusion is stable for 24 hours refrigerated; do not freeze.

15.24 **Administration:** Administered by I.V. infusion; loading doses are infused over 90 minutes; maintenance doses may be infused over 30 minutes if tolerated. Do not administer with D_5W. Do not administer I.V. push or by rapid bolus. Treatment with acetaminophen, diphenhydramine, and/or meperidine is usually effective for managing infusion-related events. Do not mix with any other medications.

15.25 **Pharmacokinetic information**:
Distribution: V_d: 44 mL/kg; not likely to cross the (intact) blood brain barrier (due to the large molecule size)
Half-life elimination: Weekly dosing: Mean: 6 days (range: 1-32 days); every 3 week regimen: Mean: 16 days (range: 11-23 days)

15.26 **Potential Drug Interactions**:
Increased Effect/Toxicity: Paclitaxel may result in a decrease in clearance of trastuzumab, increasing serum concentrations. Combined use with anthracyclines may increase the incidence/severity of cardiac dysfunction. Monoclonal antibodies/abciximab may enhance the potential for allergic or hypersensitivity reactions to trastuzumab due to the presence of human antichimeric antibodies (HACA); may also cause thrombocytopenia or diminished effects. Trastuzumab may increase the incidence of neutropenia and/or febrile neutropenia when used in combination with Myelosuppressive chemotherapy.

15.27 **Known potential adverse events:** Consult the package insert for the most current and complete information. Refer to the package insert pertaining to the following boxed warnings: Trastuzumab is associated with symptomatic and asymptomatic reductions in left ventricular ejection fraction (LVEF) and severe heart failure (HF) and may result in mural thrombus formation and stroke, and even cardiac death. Serious adverse events, including hypersensitivity reaction, infusion reactions, have been associated with trastuzumab.

Common known potential toxicities, >10%:
Cardiovascular: LVEF decreased
Central nervous system: Pain, fever, chills, headache, insomnia, dizziness
Dermatologic: Rash
Gastrointestinal: Nausea, diarrhea, vomiting, abdominal pain, anorexia
Neuromuscular & skeletal: Weakness, back pain
Respiratory: Cough, dyspnea, rhinitis, pharyngitis
Miscellaneous: Infusion reactions (chills, fever), infection.

Less common known potential toxicities, 1% - 10%:
Cardiovascular: Peripheral edema, CHF, tachycardia, hypertension, arrhythmia, palpitation
Central nervous system: Depression, paresthesia, peripheral neuritis, neuropathy
Dermatologic: Acne, nail disorder, pruritus
Gastrointestinal: Constipation, dyspepsia
Genitourinary: Urinary tract infection
Hematologic: Anemia, leukopenia
Neuromuscular & skeletal: Paresthesia, bone pain, arthralgia, myalgia, muscle spasm, peripheral neuritis, neuropathy
Respiratory: Sinusitis, nasopharyngitis, upper respiratory infection, epistaxis, pharyngolaryngeal pain
Miscellaneous: Flu-like syndrome, accidental injury, influenza, allergic reaction, herpes simplex

Rare known potential toxicities, <1%: Acute respiratory distress syndrome (ARDS), amblyopia, anaphylaxis, anaphylactoid reaction, angioedema, apnea, ascites, asthma, ataxia, bone necrosis, bronchospasm, cardiac arrest, cardiomyopathy, cellulitis, coagulopathy, colitis, confusion, deafness, esophageal ulcer, gastroenteritis, glomerulo-nephritis, glomerulopathy, glomerulosclerosis, hematemesis, hemorrhage, hemorrhagic cystitis, hepatic failure, hepatitis, herpes zoster, hydrocephalus, hydronephrosis, Hypercalcemia, hypersensitivity, hypotension, hypothyroidism, hypoxia, ileus, intestinal obstruction, interstitial pneumonitis, laryngitis, leukemia, lymphangitis, mania, mural thrombosis, sympathy, nephrotic syndrome, neutropenia, pancreatitis, pancytopenia, paroxysmal nocturnal dyspnea, pathological fracture, pericardial effusion, pleural

effusion, pneumonitis, pneumothorax, pulmonary edema, pulmonary fibrosis, pulmonary hypertension, pulmona5ry infiltrate, pyelonephritis, radiation injury, renal failure, respiratory distress, respiratory failure, seizure, sepsis, shock, skin ulcers, stroke, syncope, stomatitis, thyroiditis, vascular thrombosis, ventricular dysfunction, volume overload.

15.28 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.29 **Nursing Guidelines:**

15.291 The most common adverse events related to trastuzumab are fever (>38°C), chills, and occasional rigors, most often infusion-related after the initial dose. Treat with acetaminophen as necessary. Meperidine may be needed for rigors. Instruct patient and family to report any fever >101°F. Patients with underlying pulmonary pathology should be closely monitored.

15.292 Transient, localized tumor-site pain may be experienced within 8 hours of infusion. Advise patient that acetaminophen is helpful.

15.293 Provide symptomatic management of the possible mild-to-moderate nausea/vomiting/diarrhea.

15.294 Assess heart and lung sounds. Monitor vital signs (resting pulse, BP). Be alert to early signs to cardiotoxicity, i.e., dyspnea, steady weight gain, nonproductive cough, arrhythmias, tachycardia, and pulmonary rales. Cardiotoxicity is increased when co-administered with doxorubicin/cyclophosphamide (AC).

15.295 Advise patient of possible fatigue, loss of strength, and weakness. Have patient pace activities with frequent rest periods.

15.296 Allergic reactions may occur, therefore emergency equipment should be easily accessible especially during initial infusion. Monitor VS at baseline and frequently throughout infusion. Patient should be observed for one hour after the initial dose.

15.3 Paclitaxel (Taxol)

15.31 **Background**: Antineoplastic Agent, Antimicrotubular, Taxane derivative. Paclitaxel promotes microtubule assembly by enhancing the action of tubulin dimers, stabilizing existing microtubules, and inhibiting their disassembly, interfering with the late G_2 mitotic phase, and inhibiting cell replication. In addition, the drug can distort mitotic spindles, resulting in the breakage of chromosomes. Paclitaxel may also suppress cell proliferation and modulate immune response.

15.32 **Formulation**: Commercially available for injection 6 mg/mL (5 mL, 16.7 mL, 25 mL, and 50 mL) [contains alcohol and purified Cremophor EL (polyoxyethylated castor oil)].

15.33 **Preparation, storage, and stability**: Refer to package insert for complete preparation and dispensing instructions. Store intact vials at room temperature and protect from light. Dilute in 250-1000 mL D_5W or 0.9% NaCL to a concentration of 0.3 – 1.2 mg/mL. Solutions in D_5W and 0.9% NaCl are stable for up to 3 days at room temperature. Chemotherapy dispensing devices (e.g., Chemo Dispensing Pin) should not be used to withdraw paclitaxel from the vial.

Paclitaxel should be dispensed in either glass or non-PVC containers (e.g., Excel/PAB). Use nonpolyvinyl (non-PVC) tubing (e.g., polyethylene) to minimize leaching.

15.34 **Administration:** Infuse IV over 1-96 hours. When administered as sequential infusions, taxane derivatives should be administered before platinum derivatives (cisplatin, carboplatin) to limit myelosuppression and to enhance efficacy. Infuse through a 0.22 micron in-line filter and nonsorbing administration set.

15.35 **Pharmacokinetic information**:
Distribution: $V_{d:}$ Widely distributed into body fluids and tissues; affected by dose and duration of infusion
V_{dss}: 1- to 6-hour infusion: 67.1 L/m^2
V_{dss}: 24-hour infusion: 227-688 L/m^2
Metabolism: Hepatic via CYP2C8 and 3A4; forms metabolites (primarily 6α-hydroxypaclitaxel).
Half-life elimination: 1- to 6-hour infusion: Mean (beta): 6.4 hours,
3-hour infusion: Mean (terminal): 13.1-20.2 hours
24-hour infusion: Mean (terminal): 15.7-52.7 hours
Excretion: Feces (~70%, 5% as unchanged drug); Urine (14%)
Clearance: Mean: Total body: After 1- and 6-hour infusions: 5.8-16.3 L/hour/m^2; after 24-hour infusions: 14.2-17.2 L/hour/m^2

15.36 **Potential Drug Interactions**:
Cytochrome P450 Effect: Substrate (major) of CYP2C8, CYP3A4; **Induces** CYP3A4 (weak).
Increased Effect/Toxicity: CYP2C8 inhibitors may increase the levels/effects of paclitaxel. Refer to the package insert or LexiComp[1] for example inhibitors.
Decreased Effect: CYP2C8 inducers may decrease the levels/effects of paclitaxel. Refer to the package insert or LexiComp[1] for example inducers.
Herb/Nutraceutical Interactions: Avoid black cohosh, dong quai in estrogen-dependent tumors. Avoid valerian, St John's wort (may decrease paclitaxel levels), kava kava, gotu kola (may increase CNS depression).

15.37 **Known potential adverse events:** Consult the package insert for the most current and complete information. Percentages reported with single-agent therapy. **U.S. Boxed Warning**: Bone marrow suppression is the dose-limiting toxicity; do not administer if baseline absolute neutrophil count (ANC) is <1500 cells/mm^3 (1000 cells/mm^3 for patients with AIDS-related KS); reduce future doses by 20% for severe neutropenia. **U.S. Boxed Warning**: Severe hypersensitivity reactions have been reported.

Common known potential toxicities, >10%:
Cardiovascular: Flushing, ECG abnormal, edema, hypotension.
Dermatologic: Alopecia, rash.
Gastrointestinal: Nausea/vomiting, diarrhea, mucositis, stomatitis, abdominal pain (with intraperitoneal paclitaxel)
Hematologic: Neutropenia, leukopenia, anemia, thrombocytopenia, bleeding.
Hepatic: Alkaline phosphatase increased, AST increased.
Local: Injection site reaction (Erythema, tenderness, skin discoloration, swelling).
Neuromuscular & skeletal: Peripheral neuropathy, arthralgia, myalgia, weakness.
Renal: Creatinine increased.
Miscellaneous: Hypersensitivity reaction, infection.

Less common known potential toxicities, 1% - 10%:
Cardiovascular: Bradycardia, tachycardia, hypertension, rhythm abnormalities, syncope, venous thrombosis.
Dermatologic: Nail changes.
Hematologic: Febrile neutropenia.
Hepatic: Bilirubin increased.
Respiratory: Dyspnea.

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Anaphylaxis, ataxia, atrial fibrillation, AV block, back pain, cardiac conduction abnormalities, cellulitis, CHF, chills, conjunctivitis, dehydration, enterocolitis, extravasation recall, hepatic encephalopathy, hepatic necrosis, induration, intestinal obstruction, intestinal perforation, interstitial pneumonia, ischemic colitis, lacrimation increased, maculopapular rash, malaise, MI, necrotic changes and ulceration following extravasation, neuroencephalopathy, neutropenic enterocolitis, ototoxicity, pancreatitis, paralytic ileus, phlebitis, pruritus, pulmonary embolism, pulmonary fibrosis, radiation recall, radiation pneumonitis, pruritus, renal insufficiency, seizure, skin exfoliation, skin fibrosis, skin necrosis, Stevens-Johnson syndrome, supraventricular tachycardia, toxic epidermal necrolysis, ventricular tachycardia (asymptomatic), visual disturbances.

15.38 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.39 **Nursing Guidelines**

15.391 Premedicate with steroids, antihistamines, and H2 blockers as per institutional guidelines.

15.392 Mix the infusion bag well. Thorough admixture of this drug often prevents a hypersensitivity reaction. An inline filter of <0.22 micron must be used distal to the infusion pump. Filter may need to be

changed if infusion is to be prolonged >12 hours. Inspect solution for excessive particulate matter, if present do not use.

15.393 Caution patients that the alcohol contained in the infusion may cause impairment in operating heavy equipment or in driving a vehicle and to assess their ability before trying either. Advise avoidance of any alcohol or depressants such as sedatives and opiates if not necessary.

15.394 Assess the patient frequently for the first 30 minutes. Taxol® hypersensitivity reactions, which may include chest pain, back pain, flushing, diaphoresis, dyspnea, pruritus, hypotension, hypertension, bronchospasm and/or urticaria, usually occur early in the infusion. Have the anaphylaxis tray available.

15.395 If a reaction occurs, stop the infusion immediately. Epinephrine, IV fluids, diphenhydramine, and methylprednisolone may be used as per MD's order.

15.396 Approximately 60% of patients experience peripheral sensory neuropathy (numbness, tingling, burning pain, fine motor skills impairment, paresthesias, distal sensory loss). Patients receiving higher doses at shorter infusion times are at greater risk. Most cases have been reported at doses >170 mg/m^2/day and with cumulative doses over multiple courses of therapy. The nerve damage may take months to resolve. Nonsteroidal anti-inflammatory agents and opiates have not been effective in treating neuropathic pain. Consult MD about trying tricyclic antidepressants or possibly Neurontin.

15.397 Increased risk of cardiotoxicity when given in combination with doxorubicin, with a sharp increase in risk of CHF once cumulative dose of doxorubicin is > 380 mg/m^2. At this point taxol should be continued as a single agent only. Monitor for sign/symptoms of CHF. Instruct patient to report any swelling in the hands, arms, feet, or legs, and any chest pain.

15.398 Mucositis can usually be managed with a salt and soda mouthwash (1 tsp. Salt, 1 tsp. Soda and 1 quart boiled water) or try OTC oral Lysine or Vitamin E.

15.399a Narcotics and nonsteroidal anti-inflammatory drugs may be used to manage the myalgias.

15.399b Monitor CBC closely. Neutropenia is most severe in patients who have had previous chemotherapy. Instruct patient to report signs or symptoms of infection, unusual bruising or bleeding to the health care team.

15.399c There is an increased risk of neutropenia and stomatitis when given prior to doxorubicin. Therefore Taxol should always be given after doxorubicin administration.

15.399d Monitor IV site closely and establish patency before administration. Paclitaxel is an irritant, however rarely rash, radiation recall, and ulceration have occurred with infiltration of drug.

15.399e Monitor liver function tests

15.399f Inform patient about total alopecia.

15.399g If given on the same day as a platinum agent, paclitaxel should be administered first to limit myelosuppression and enhance efficacy of agent.

15.4 Endocrine Therapy

NOTE: Cohort B patients will be on **one** of these agents.

15.41 **Anastrozole** commercial supply (Arimidex®)

15.411 Formulation and storage

Anastrozole is commercially available from AstraZeneca as 1 mg tablets. Store at 15 C to 30 C (59 F to 86 F).

15.412 Category and mechanism of action

Anastrozole is a potent and selective nonsteroidal aromatase inhibitor. It significantly lowers serum estradiol concentrations and has no detectable effect o formation of adrenal corticosteroids or aldosterone. In postmenopausal women, the principal source of circulating estrogen is conversion of adrenally generated androstenedione to estrone b aromatase in peripheral tissues.

15.413 Administration

Oral. Anastrozole is well absorbed and not affected by food.

15.414 Dose:

1 mg PO once daily without regard to meals.

15.415 Known potential adverse events:

Likely (occurring greater than 20% of the time):
Cardiovascular: Flushing

Less Likely (occurring less than or equal to 20% of the time):
Cardiovascular: peripheral edema, chest pain, hypertension
Central nervous system: Headache, dizziness, anxiety, depression, insomnia,
Dermatologic: Rash, pruritus
Endocrine & metabolic: Increased cholesterol levels
Gastrointestinal: Nausea, vomiting, diarrhea, abdominal pain, anorexia, constipation, gas and cramping
General effects: Asthenia, weakness, flu-like symptoms, back pain, arthralgia, carpal tunnel syndrome
Neuromuscular & skeletal: Osteoporosis, pathological fracture, breast pain
Respiratory: Dyspnea, cough, pharyngitis, sore throat

Rare but serious (occurring less than 2-3% of the time):
Cardiovascular: Thrombophlebitis, heart attack, stroke, low supply of blood to the heart and brain, blood clots
Central nervous system: Confusion
Dermatologic: Hair thinning
Endocrine & metabolic: Increase in liver enzymes
Gastrointestinal: Weight gain or loss

General effects: Allergic reaction (Stevens Johnson Syndrome)
Genitourinary: Vaginal dryness or bleeding
Hematologic: Anemia, leukopenia
Neuromuscular & skeletal: Paresthesia

15.416 Drug procurement

Commercially available.

15.417 Nursing guidelines

15.4171 May take with food if needed for nausea. Instruct patient to report unrelieved nausea or vomiting.

15.4172 Instruct patient that hot flashes may occur. Manage hot flashes with non-hormonal interventions (ie: venlafaxine XR 75 mg daily).

15.4173 Assess for changes in bowel patterns. Manage diarrhea with non-prescription drugs. Instruct patient to report unrelieved diarrhea.

15.4174 Headache may occur. Can be managed with non-prescription analgesics. Instruct patient to report headaches that are not relieved.

15.4175 Vaginal dryness may occur. Instruct patient in the use of lubricating agents.

15.4176 Mild swelling may occur in the arms and legs. Instruct patient to elevate extremities when at rest to relieve the swelling.

15.4177 While thrombophlebitis is rare, instruct patient to report any pain, redness, marked swelling in the arms and/or legs, dizziness, or shortness of breath to their health care provider immediately or seek medical attention in an emergency room.

15.42 **Exemestane** (Aromasin)

15.421 Background:

Exemestane is an irreversible, steroidal aromatase inactivator. It prevents conversion of androgens to estrogens by tying up the enzyme aromatase. In breast cancers where growth is estrogen-dependent, this medicine will lower circulating estrogens.

15.422 Formulation

Commercially available in 25 mg tablets for oral administration.

15.423 Preparation, storage, and stability

Store at room temperature of 25ºC.

15.424 Administration

Administer after a meal. Patients on aromatase inhibitor therapy should receive vitamin D and calcium supplements.

15.425 Pharmacokinetic information:

Absorption: Rapid and moderate (~42&) following oral administration; absorption increases ~40% following high-fat meal
Protein Binding: 90%, primarily to albumin and α_1-acid glycoprotein
Metabolism: Extensively hepatic; oxidation (CYP3A4) of methylene group, reduction of 17-keto group with formation of many secondary metabolites; metabolites are inactive
Distribution: Extensive
Half-life elimination: 24 hours
Time to peak: Women with breast cancer: 1.2 hours
Excretion: Urine (<1% as unchanged drug, 39% to 45% as metabolites); feces (36% to 48%)

15.426 Potential Drug Interactions:

Cytochrome P450 Effect: Substrate of CYP3A4 (major)
Decreased Effect: CYP3A4 inducers may decrease the levels/effects of Exemestane. See package insert for example CYP3A4 inducers. Adjustment of dosage required with potent inducers.
Ethanol/Nutrition/Herb Interactions:
Food: Plasma levels increased by 40% when Exemestane was taken with a fatty meal.
Herb/Nutraceutical: St John's wort may decrease Exemestane levels. Avoid black cohosh, dong quai in estrogen-dependent tumors.

15.427 Known potential adverse events

Consult the package insert for the most current and complete information.

Common known potential adverse events, > 10%:
Cardiovascular: Hypertension
Central nervous system: Fatigue, pain, headache, depression
Dermatologic: Hyperhidrosis, alopecia
Endocrine & metabolic: Hot flashes
Gastrointestinal: Nausea, abdominal pain
Hepatic: Alkaline phosphatase increased
Neuromuscular & skeletal: Arthralgia

Less common known potential adverse events, 1% - 10%:
Cardiovascular: Edema, cardiac ischemic events, chest pain
Central Nervous System: Dizziness, anxiety, fever, confusion, hypoesthesia
Dermatologic: Dermatitis, itching, rash
Endocrine& metabolic: Weight gain
Gastrointestinal: Diarrhea, vomiting, anorexia, constipation, appetite increased, dyspepsia
Genitourinary: Urinary tract infection
Hepatic: Bilirubin increased
Neuromuscular & skeletal: Back pain, limb pain, osteoarthritis, weakness, osteoporosis, pathological fracture, paresthesia, carpal tunnel syndrome, cramps
Ocular: Visual disturbances
Renal: Creatinine increased

Respiratory: Dyspnea, cough, bronchitis, pharyngitis, rhinitis, sinusitis, upper respiratory infection
Miscellaneous: Influenza-like symptoms, diaphoresis, lymphedema, infection

Rare known potential adverse events, <1% (Limited to important or life-threatening):
Cardiac failure, endometrial hyperplasia, GGT increased, neuropathy, osteochondrosis, thromboembolism, transaminases increased, uterine polyps

15.428 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.429 Nursing Guidelines

15.4291 Advise patients to take after a meal.

15.4292 Manage hot flashes with non-hormonal interventions (ie. venlafaxine XR 75 mg daily).

15.4293 Instruct patient to report uncontrolled nausea, insomnia.

15.4294 Assess for changes in bowel patterns and manage diarrhea or constipation. Instruct patient to report unrelieved diarrhea or constipation.

15.4295 Monitor for drug interactions. Aromasin is metabolized by the P450 3A4 system. While it is unlikely that inhibitors of this system will significantly increase aromasin level, the manufacturer cautions about concommitent use with inducers of the CYP3A4 system as this may decrease serum levels, and thereby it's effectivness. Assess all of patients prescription and non-prescription medications. Instruct patient to check with physician before starting any new medications, including OTC.

15.43 **Fulvestrant** (Faslodex®)

15.431 Background

Fulvestrant is an estrogen receptor antagonist. Fulvestrant competitively binds to estrogen receptors on tumors and other tissue targets, producing a nuclear complex that causes a dose-related down-regulation of estrogen receptors and inhibits tumor growth.

15.432 Formulation:

Commercially available for injection as:
Injection, solution: 50 mg/mL (5 mL) [contains benzyl alcohol, benzyl benzoate, castor oil, ethanol 10% w/v]

15.433 Preparation, storage, and stability

Refer to package insert for complete preparation and dispensing instructions. Store in original carton under refrigeration at 2°C to 8°C (36°F to 46°F). Protect from light.

15.434 Administration

Refer to the treatment section for specific administration instructions. Administer IM only; do not administer IV, SubQ, or intra-arterially. Administer 500 mg dose as two 5 mL injections (one in each buttocks) slowly over 1-2 minutes per injection.

15.435 Pharmacokinetic information:

Distribution: V_d: ~3-5 L/kg
Protein binding: 99%; to plasma proteins (FLDL, LDL and HDL lipoprotein fractions)
Metabolism: Hepatic via multiple biotransformation pathways (CYP3A4 substrate involved in oxidation pathway, although relative contribution to metabolism unknown); metabolites formed are either less active or have similar activity to parent compound.
Half-life elimination: 250 mg: ~40 days
Excretion: Feces (~90%); urine (<1%)

15.436 Potential Drug Interactions:

Cytochrome P450 Effect: Substrate of CYP3A4 (minor). There are no known interactions where it is recommended to avoid concomitant use.

15.437 Known potential adverse events

Consult the package insert for the most current and complete information.

Common known potential toxicities, > 10%:
Endocrine & metabolic: Fertility: Hot flushes
Hepatic: Alkaline phosphatase increased, transaminases increased
Local: Injection site pain
Neuromuscular & skeletal: Joint disorders

Less common known potential adverse events, 1% - 10%:
Cardiovascular: Ischemic disorder
Central nervous system: Fatigue, headache
Gastrointestinal: Nausea, anorexia, vomiting, constipation, weight gain
Genitourinary: Urinary tract infection
Neuromuscular & skeletal: Bone pain, arthralgia, back pain, extremity pain, musculoskeletal pain, weakness
Respiratory: Cough, dyspnea
Rare known potential adverse events, <1% (Limited to important or life-threatening):
Angioedema, hypersensitivity reactions, leukopenia, myalgia, osteoporosis, thrombosis, urticaria, vaginal bleeding

15.438 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.439 Nursing guidelines:

15.4391 Rarely a blood-tinged vaginal discharge has been reported infrequently during therapy. Advise patients that this is a

possibility and that studies have shown no effects on vagina or endometrium.

15.4392 Monitor LFTs based on protocol requirements. Report increased LFTs to treating physician.

15.4393 Hot packing the injection site for a short while after injection may prevent the possible bruising, tenderness, and/or erythema at the IM injection site.

15.4393 It is recommended that pregnancy be avoided during treatment. Instruct patient and partner on adequate methods of birth control.

15.4393 Instruct patient to report any signs or symptoms of blood clots. Patients with calf tenderness or burning, and/or chest pain (pulmonary embolus) should be evaluated by a physician immediately.

15.4393 Warn patient of possible hot flashes. Assess severity and discuss non-hormonal treatments with treating physician. Assess treatment for efficacy.

15.4393 May cause mild GI symptoms (nausea, anorexia, vomiting, constipation). Treat symptomatically and monitor for effectiveness of intervention.

15.44 **Letrozole** for Oral Administration (Femara®)

15.441 Background

Letrozole is an aromatase inhibitor. Letrozole is a nonsteroidal, competitive inhibitor of the aromatase enzyme system which binds to the heme group of aromatase, a cytochrome P450 enzyme which catalyzes conversion of androgens to estrogens. This process leads to inhibition of the enzyme and a significant reduction in plasma estrogen levels. Letrozole does not affect synthesis of adrenal or thyroid hormones, aldosterone, or androgens. Patients treated with letrozole do not require glucocorticoid or mineralocorticoid replacement therapy.

15.442 Formulation

Commercially available for oral administration as: Tablets: 2.5 mg

15.443 Preparation, storage, and stability

Refer to package insert for complete preparation and dispensing instructions. Store letrozole at room temperature of 77°F (25°C); excursions permitted to 59°F to 86°F (15°C to 30°C).

15.444 Administration

Refer to the treatment section for specific administration instructions. Administer orally with or without food.

15.445 Pharmacokinetic information:

Distribution: V_d: ~1.9 L/kg
Protein binding: weak
Metabolism: Hepatic via CYP3A4 and CYP2A6 into an inactive carbinol metabolite

Half-life elimination: Terminal: ~ 2 days
Excretion: Urine (90%; 6% as unchanged drug, 75% as glucuronide carbinol metabolite, 9% as unidentified metabolites)

15.446 Potential Drug Interactions:

Cytochrome P450 Effect: Substrate of CY2A6 (minor) CYP3A4 (minor). **Inhibitor** of CYP2A6 (strong), CYP2C19 (weak)
Increased Effect/Toxicity: CYP2A6 substrates, methadone, vitamin K antagonists (eg, warfarin)
Decreased Effect: digoxin, tegafur, vitamin K antagonists (eg, warfarin)

15.447 Known potential adverse events

Consult the package insert for the most current and complete information.

Common known potential adverse events, > 10%:
Cardiovascular: Edema
Central nervous system: Headache, dizziness, fatigue
Endocrine & metabolic: Hypercholesterolemia, hot flashes
Gastrointestinal: Nausea, weight gain, constipation
Neuromuscular & skeletal: Weakness, arthralgia, arthritis, bone pain, back pain, bone mineral density decreased/osteoporosis, bone fracture
Respiratory: Dyspnea, cough
Miscellaneous: Diaphoresis, night sweats

Less common known potential adverse events, 1% - 10%:
Cardiovascular: Chest pain, hypertension, chest wall pain, peripheral edema, cerebrovascular accident including hemorrhagic stroke, thrombotic stroke, thromboembolic event including venous thrombosis, thrombophlebitis, MI, angina, transient ischemic attack
Central nervous system: Insomnia, pain, anxiety, depression, vertigo, somnolence
Dermatologic: Rash, alopecia, pruritis
Endocrine & metabolic: Breast pain, hypercalcemia
Gastrointestinal: Diarrhea, vomiting, weight loss, abdominal pain, anorexia, dyspepsia
Genitourinary: Urinary tract infection, vaginal bleeding, vaginal dryness, vaginal hemorrhage, vaginal irritation
Neuromuscular & skeletal: Limb pain, myalgia
Ocular: Cataract
Renal: Renal disorder
Respiratory: Pleural effusion
Miscellaneous: Infection, influenza, viral infection, secondary malignancy

Rare known potential adverse events, <1% (Limited to important or life-threatening):
Anaphylactic reaction, angioedema, arterial thrombosis, cardiac failure, carpal tunnel syndrome, endometrial cancer, endometrial hyperplasia, endometrial proliferation, erythema multiforme, hepatitis, leukopenia, memory impairment, stomatitis, tachycardia, thrombocytopenia, toxic epidermal necrolysis, trigger finger

15.448 Drug procurement

Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.449 Nursing Guidelines

15.4491 Manage hot flashes with non-hormonal interventions (e.g., venlafaxine XR 75 mg daily).

15.4492 Manage pain (arthralgias). Instruct patient to report unrelieved pain.

15.4493 May take with food if needed for nausea. Instruct patient to report unrelieved nausea or vomiting.

15.4494 Assess for changes in bowel patterns. Manage diarrhea or constipation with non-prescription drugs. Tell patients to report unrelieved diarrhea or constipation.

15.4495 If patient experiences difficulty breathing or sudden onset chest pain, instruct them to seek emergency medical attention immediately.

15.4496 Monitor for signs of edema, instruct patient to report any swelling in legs, feet, or hands.

16.0 Statistical Considerations and Methodology

16.1 Study Design

A two-stage phase II trial will be conducted to assess the tumor response rate and safety profile of TVB-2640 in combination with paclitaxel and trastuzumab in two patient cohorts, namely, patients with HER2+ MBC resistant to taxane and HER2-directed therapy and patients with ER+/HER2+ MBC resistant to endocrine and HER2-directed therapy

For each cohort, *the primary endpoint is overall response rate (ORR)* defined as the number of patients whose disease meets the RECIST criteria for a partial (PR) or complete (CR) on two consecutive evaluations at least 8 weeks apart divided by the total number of patients in that cohort who started protocol treatment.

For each cohort, the following two-stage Simon minimax design with a safety run-in period will be used to test the null hypothesis that the true tumor response rate is at most 5% against the alternative it is at least 20%.

For each cohort, a maximum of 6 patients may be enrolled in the run-in period before accrual is temporarily suspended. If 2 or more run-in patients develops one of the following toxicities during the first two cycles of treatment: febrile neutropenia Grade ≥3, neutropenia Grade 4, thrombocytopenia with any Grade ≥3 bleeding, thrombocytopenia Grade ≥4, hand/foot syndrome Grade 3, ocular toxicity Grade ≥3, and Grade 3 CHF, accrual will be suspended to that cohort. All toxicity data will be reviewed by the study team and consideration will be given to changing the dosing schedule for that cohort. Study recommendations will be forwarded to the IRB for approval. If the dosing schedule is changed and enrollment reopened to the cohort, the initial 6 patients will be not included in the assessment of tumor response (but will be included in all safety reports) and another run-in period of 6 patients will be undertaken.

After the 6 patient run-in demonstrates patient tolerability, another 12 patients will be enrolled to that cohort. If none of the 18 patients has a partial response (PR) or complete response (CR) on two consecutive evaluations at least 8 weeks, further enrollment to that cohort will be closed and the treatment regimen will not be considered promising in that patient cohort. If at least one tumor response is documented among the 18 patients from a given cohort, an additional 14 patients will be enrolled onto that cohort. If at least 4 tumor responses are seen among the total 32 patients enrolled onto that cohort, we will conclude that the tumor response rate is at least 20% in that patient cohort. The proposed design has a 90% chance of rejecting the true response rate is less than 5% when the true response rate is 20% or more, at a 0.10 level of significance.

NOTE: If the second run-in period does not demonstrate tolerability, enrollment to that cohort will be closed.

16.11 Secondary Endpoints:

Secondary endpoints will be evaluated for each cohort independently.

16.111 Duration of response is defined as the time from the first radiologic finding of a PR or CR to disease progression among those patients whose disease meets the RECIST criteria for CR or PR on 2 consecutive evaluations approximately 8 weeks apart.

16.112 Clinical Benefit Rate (CBR) is defined as the proportion of patients who have completed 6 cycles of treatment without disease progression

(that is, their objective disease status is a CR, PR, or stable for 6 cycles or more)

16.113 Progression-free survival (PFS) - time from randomization to the first of these disease events: local/regional or distant breast recurrence, DCIS or invasive breast disease in contralateral breast, non-breast second primary, or death due to any cause.

16.12 Analysis Plans for each cohort independently

A point and interval estimate of the response rate as well as the clinical benefit rate will be constructed using the Duffy-Santner approach to take into account the sequential nature of the study design.

The distribution of response times and progression-free survival times will be estimated using the Kaplan-Meier approach.

Toxicities will be tabulated by severity.

16.13 Adverse Event Stopping Rules

The adverse event stopping rule will be applied to each cohort independently. Enrollment to a given cohort will be temporarily suspended for close examination of treatment safety and tolerability:

- at each instance of a treatment related death;
- if 2 or more of the first 6 patients develops one of the following toxicities that is considered to be possibly, probably, or definitely related to protocol treatment with TVB-2640: a Grade 4 neutropenia, or any clinically significant Grade 4 event (other than expected cytopenias or transient laboratory abnormalities); or Grade 3 or worse fatigue, malaise, mucositis, pneumonitis; or any Grade 2 or worse eye disorder or Grade 3 or worse palmar-plantar erythrodysesthesia syndrome (hand-foot syndrome);
- or at any time after the first 6 patients have been enrolled, if 25% or more patients develops one of the following toxicities that is considered to be possibly, probably or definitely related to protocol treatment with TVB-2640: a Grade 4 neutropenia, or any clinically significant Grade 4 event (other than expected cytopenias or transient laboratory abnormalities); or Grade 3 or worse fatigue, malaise, mucositis, pneumonitis; or any Grade 2 or worse eye disorder, or Grade 3 or worse palmar-plantar erythrodysesthesia syndrome (hand-foot syndrome).

NOTE: Adverse events normally associated with treatment with paclitaxel (e.g., peripheral neuropathy) and/or trastuzumab (e.g., LVEF decreased) will not count toward the stopping rules, as these events are expected.

All toxicity data will be examined to assess whether changes should be made in the treatment or eligibility criteria to increase the tolerability of the treatment. All modifications to the protocol will be forwarded to the IRB for review.

16.14 Correlative Endpoints

The changes in H score FASN, pAKT, and pS6 expression and levels of cellular apoptosis in tumor tissue after the first cycle of the combination of TVB-2640 with paclitaxel and trastuzumab will be examined graphically by plotting the difference in pre and post levels against pre-levels of the biomarker, with patients who derived clinical benefit represented by dashed line and those who did not by solid line.

For each patient cohort, Wilcoxon signed rank tests will be used to assess the changes in serum FASN after the first cycle of the combination of TVB-2640 with paclitaxel and trastuzumab from pre-treatment levels.

16.2 Research Monitoring

Specific to meeting Department of Defense requirements

An independent research monitor will be designated for complying with Department of Defense funding regulations. This designee will monitor protocol deviations, adverse events and data analysis.

16.21 Research Monitor Tasks

1. Receive and review expedited adverse event reports as defined in section 10.0 of the protocol.
2. Receive and review unanticipated problems and major protocol violations meeting the criteria for real-time reporting
3. Receive and review Mayo Clinic Cancer Center data and safety monitoring board reports
4. Receive and approve the content of continuing review reports prior to IRB submission
5. Receive and review protocol data analyses
6. Is required to review all unanticipated problems involving risks to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor must comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and report of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO.

16.22 Research Monitor Responsibilities

1. The research monitor will agree to promptly report problems or discrepancies found to the IRB.
2. The research monitor will agree to have the authority to stop the study's research in progress, remove individual subjects from the research study, and take whatever steps deemed necessary to ensure the safety and well-being of the study subjects until the IRB can make a determination based upon the monitor's report.
3. May discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research.
4. Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's report.
5. Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official.

Assuming one cohort will require an additional run-in period and as many as 5 patients may be needed to account for patients found to be ineligible or cancel participation:

16.3 Gender and Minority Accrual

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	4		
Not Hispanic or Latino	76		
Ethnic Category: Total of all subjects	80		
Racial Category			
American Indian or Alaskan Native	1		
Asian	3		
Black or African American	2		
Native Hawaiian or other Pacific Islander			
White	74		
Racial Category: Total of all subjects	80		

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Summary Table of Research Tissue Specimens to be Collected for this Protocol

Correlative Study (Section for more information)	**Mandatory or Optional**	**Type of Tissue to Collect**	**Block, Slides, Core, etc. (# of each to submit)**	Following Pre-registration*	C2D1	**Process at site? (Yes or No)**	**Temperature Conditions for Storage /Shipping**
Tumor FASN Expression	Mandatory	FFPE	3 unstained slides	X	X		
Tumor pAKT and pS6	Mandatory	FFPE	2 unstained slides	X	X		
Tumor HER2 Expression	Mandatory	FFPE	2 unstained slides	X	X		
Tumor ER and PR expression	Mandatory	FFPE	2 unstained slides	X			
Tumor Apoptosis Evaluation	Mandatory	FFPE	3 unstained slides	X	X		
H&E	Mandatory		1 slide	X	X		

A: All tumor specimens will be collected prior to administration of therapy on Cycle 2 Day 1.

Note: Please submit a pathology report with the pre-registration biopsy in which HER2 status will be tested. HER2 status can be tested and confirmed at local site.

*If pre-registration biopsy is attempted and either aborted or insufficient tissue is obtained, the patient may still be eligible. In this case, the site may submit slides from archival specimens (preferably from a metastatic site) from biopsy or surgery completed ≤5 years prior to pre-registration for central lab review.
NOTE: If biopsy does not obtain evaluable tissue AND no recent archived tissue is available for Central Laboratory review, patient is not eligible. A repeat biopsy may be attempted at the discretion of the treating physician and patient.

17.2 Diagnostic Slides from Original and /or Recurrent Tissue

Along with original diagnostic slides, include pathology reporting form, surgical pathology report and operative report.

17.3 Correlative Tissue Collection

17.31 Tissue Kits will not be provided for this protocol.

17.32 Paraffin Embedded Tissue

17.321 For both the pre-registration and C2D1 biopsies, collection should occur per standard site procedures and at the radiologist discretion. One core will be formalin-fixed, paraffin-embedded (FFPE) and sections (5 micron) will be cut from the tumor tissue block to obtain 10 unstained slides that contain at least 75% tumor tissue. These slides will be used for FASN expression, pAKT and pS6 expression, HER2 expression, and apoptosis evaluation. One slide from each biopsy will also be cut for H&E.

17.322 All slides should be shipped to the pathology coordinator at the following address:
Mayo Clinic Cancer Center Operations Office
Attn: PC Office (MC1633)
RO_FF_03_24-CC/NW Clinic
200 First Street SW
Rochester, MN 55905

17.4 Background and Methodology

17.41 Evaluation of Tumor FASN Expression

We hypothesize that tumor expression of FASN is associated with clinical benefit from targeted treatment with TVB-2640. Paraffin-embedded tissue biospecimens will be processed and assessed by immunohistochemistry (IHC) for total expression of FASN. Staining will be performed by the Lupu laboratory with scoring and interpretation by Dr. Visscher. Results will be utilized to evaluate an association between the changes in FASN levels after 1 cycle of treatment and subject response to treatment.

17.42 Evaluation of Tumor pAKT and pS6

We hypothesize that a reduction in tumor expression of pAKT and pS6 are associated with clinical benefit from targeted treatment with TVB-2640. Paraffin-embedded tissue biospecimens will be processed and assessed by IHC for phosphorylated expression of AKT and ribosomal protein S6. Staining will be performed by 3V-Biosciences, Inc. with $pAKT^{S473}$ (Dako, rabbit clone 14-5) (CST rabbit Ab C20G5) using a validated IHC method. H-score in tumor will be quantitated by standard pathology review (staining intensity graded 0-3+ and % cells positive). Results will be utilized to evaluate an association between the change in these biomarker levels after 1 cycle of treatment and subject response to treatment. Scoring and interpretation of the IHC analysis will be performed by Dr. Visscher.

17.43 Evaluation of Tumor HER2 Expression:

As HER2 levels are positively correlated with FASN levels, and we have previously shown that inhibition of FASN results in decrease in HER2 expression. *We hypothesize that tumor expression of HER2 will decrease after*

treatment with TVB-2640. Paraffin-embedded tissue biospecimens will be processed and assessed by IHC for HER2 expression at the MCCC PRC. Testing is performed using FDA-approved Ventana Pathway HER2 (4B5) rabbit monoclonal primary antibody and a proprietary detection system. The percentage of invasive cancer cells exhibiting complete membrane staining and the uniformity of staining will be reported. Final scoring (0 – 3+) and interpretation per ASCO/CAP guidelines will be performed by Dr. Visscher . For equivocal results by IHC (2+), standard reflex testing by FISH will be performed using probes for HER2 (17q12) and a D17Z1 control probe. Two technologists will score signals in 60 total nuclei from tumor and concurrent controls. Dr. Visscher will oversee interpretation of results.

17.44 valuation of Tumor Apoptosis:

We hypothesize that Apoptosis (the percentage of apoptotic cells) will be inversely correlated with the expression of FASN. Apoptosis will be detected using the ApopTag Peroxidase In Situ Apoptosis Detection Kit from Millipore. This evaluation will be conducted in the Lupu laboratory. The assay detects apoptotic cells *in situ* by labeling & detecting DNA strand breaks by the TUNEL method. It is important to note that ApopTag Kits detect single-stranded and double-stranded breaks associated with apoptosis. Drug-induced DNA damage is not identified by the TUNEL assay unless it is coupled to the apoptotic response. In addition, this technique can detect early-stage apoptosis in systems where chromatin condensation has begun and strand breaks are fewer, even before the nucleus undergoes major morphological changes. The association between FASN and apoptosis will serve for future correlative and clinical studies.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Event monitoring

See Section 4.0 and data submission table for the event monitoring schedule.

18.3 CRF completion

This study will use Medidata Rave for remote data capture (rdc) of all study data.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for diagnosis prior to study entry. Supporting documentation for diagnosis will include either a pathology report or a laboratory report. These reports should be submitted within 14 days of registration.

A copy of the report stating the size of the lesions being followed for tumor response should be provided prior to start of treatment and at time disease progression is first documented.

18.6 Labelling of materials

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Incomplete materials

Any materials deemed incomplete by the MCCC Operations Office will be considered "not received" and will not be edited or otherwise processed until the missing information is received. A list of the missing documents will be made available to the appropriate co-sponsor/participant.

18.8 Overdue lists

A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to obtain the overdue material.

18.9 Corrections forms

If a correction is necessary the QAS will query the site. The query will be sent to the appropriate site to make the correction and return the query and documentation of correction back to the QAS.

19.0 Budget

19.1 Costs charged to patient:

Routine clinical care; every other echocardiogram; tumor assessment scans; blood work (labs) related to toxicity assessment

19.2 Tests to be research funded:

Baseline ocular assessment and ECG; tumor biopsies; all research-related serum (FASN) and tumor tissue studies; every other echocardiogram

19.3 Other budget concerns: None.

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

Appendix II Patient Medication Diary

The Patient Medication Diary is provided as a standalone document as required by the IRB.

Appendix III Palmar-Plantar Erythrodysesthesia (PPE) Grading Worksheet

For each PPE event, please use this worksheet for AE grading, per CTCAE v4.03.

Grade 1: Minimal skin changes or dermatitis (e.g., erythema, edema, or hyperkeratosis) without pain

Interpretation – Skin changes such as erythema, edema or peeling that does not cause pain or interfere with self-care and/or ADLs. If neuropathic pain from taxane-chemotherapy is present *without* pain associated with the skin changes, then PPE would be grade 1.

Grade 2: Skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL

Interpretation – skin peeling, bleeding, blistering, fissures, or edema, *with* pain that is associated with PPE. If neuropathic pain from taxane-chemotherapy is present *without* pain associated with the skin changes, then PPE would be grade 1. The skin changes with PPE-associated pain may or may not limit ADLs. If self-care is constrained, then PPE would be grade 3.

Grade 3: Severe skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL

Interpretation – skin bleeding, blistering, fissures, or edema constraining the patient's ability to perform self-care (dressing, bathing, grooming, eating, toileting, and mobility).

Are the following present (Y/N)?

Signs & Symptoms Present	**YES**	**NO**
Erythema		
Edema (not due to lymphedema)		
Peeling		
Blisters		
Fissures		
Bleeding		
Pain in hands and/or feet that is neuropathic and/or due to taxane-induced neuropathy		
Pain in hands and/or feet that is due to PPE (skin changes)*		
PPE constrains ability to perform self-care (dressing, bathing, grooming, eating, toileting, mobility)**		

**If yes, then grade is 2*
***If yes, then grade is 3*