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Correlative studies investigating effects of PI3K inhibition on peripheral leukocytes in metastatic breast cancer: potential implications for immunotherapy

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Abstract

Purpose—Patients with localized breast cancer have a 5-year survival rate >99% compared to patients with metastatic breast cancer (MBC) that have a 5-year survival rate of ~27%. Unregulated PI3K/AKT signaling is a common characteristic of MBC, making it a desirable therapeutic target for tumors with activating mutations in this pathway. Interestingly, inhibition of the PI3K/AKT pathway can affect signaling in immune cells, which could potentially alter the immune phenotype of patients undergoing therapy with these drugs. The purpose of this study is to evaluate how PI3K inhibition affects the immune cells of MBC patients during treatment.

Methods—We investigated the effects of PI3K inhibition on the immune cell populations in peripheral blood of MBC patients enrolled in 4 different clinical trials utilizing PI3K inhibitors. Peripheral blood was drawn at different points in patient treatment cycles to record immune cell fluctuations in response to therapy.

Results—MBC patients who responded to treatment with a *positive fold-change in cytotoxic T-cell population*, had an average duration of treatment response of *31.4 months*. In contrast, MBC patients who responded to treatment with a *negative fold-change in cytotoxic T-cell population*, had an average duration of therapeutic response of *5 months*. These data suggest that patients with

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a more robust, initial anti-tumor T-cell response may have a longer therapeutic response compared to patients who do not have a robust, initial anti-tumor T-cell response

Conclusions—These results highlight the potential for PI3K inhibition to sensitize tumors to immune checkpoint inhibitors, thus providing additional therapeutic options for patients with MBC.

Keywords

breast; metastatic; PI3K; leukocyte; immunotherapy

Introduction

Breast cancer is a major contributor to cancer-related deaths in women worldwide ¹. In particular, metastatic spread of the primary breast tumor is the major driver of cancer-related mortality ⁴⁰. Metastatic breast cancer (MBC) can be difficult to treat due to the location and/or the characteristics of the metastatic disease. A common characteristic of MBC is the over-activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, which can result from *PIK3CA* activating mutation, *AKT* amplification or activating mutation, and/or functional loss of the tumor suppressor, *PTEN* ^{6, 9, 18, 26}. PI3Ks form an evolutionarily conserved family of lipid kinases whose members include Class IA catalytic subunits (p110 α , β , δ), Class 1B catalytic subunit (p110 γ), Class I regulatory domains (p85 α and β , p55 α and γ , p50 α , p101, p84, p87), Class II subunits (C2 α , C2 β , C2 γ), and Class III catalytic protein (Vps34) and regulatory protein (Vps15) ¹⁵. Interestingly, the Class IA PI3K known as PIK3CA (p110 α) plays a critical role in a wide range of carcinomas, and over 33% of somatic gene mutations in breast cancer patient tumors occur in this kinase family ²⁸. The PI3K/AKT signaling pathway is responsible for numerous pro-tumorigenic characteristics including, but not limited to, cell proliferation, survival, and angiogenesis ^{12, 13, 25}. Unfortunately, breast cancers harboring *PIK3CA* and/or *AKT* mutations or amplifications have shown *unfavorable prognosis and increased drug resistance* ²⁷. Moreover, breast tumors with high PIK3CA mRNA exhibit significantly lower relapse free survival (RFS; p<0.001) (Supplemental Figure 1a), and high PIK3CA protein expression significantly decreases overall survival (OS; p<0.05) (Supplemental Figure 1b).

Due the oncogenic nature of this signaling pathway, many studies are investigating the effects of pharmacological inhibition of targets in this pathway ^{2–5, 11, 14, 16, 21–23, 33, 34}. Recently, two PI3K inhibitors, alpelisib and tasisib (Supplemental Figures 2a–b), were utilized in clinical trials (Supplemental Table 1) for the treatment of MBC. Alpelisib (BY719) inhibits the PI3K α isoform, and tasisib (GDC-0032, RG7604) is a pan-PI3K (β -sparing) inhibitor ²⁴. *PIK3CA*-mutant human breast cancer cell lines show increased sensitivity to both alpelisib (p<0.001) (Supplemental Figure 2a) and tasisib (p<0.001) (Supplemental Figure 2b), compared to human breast cancer cells without mutation (cancerrxgene.org) ²². Accordingly, the phase III randomized clinical trial SOLAR-1 data revealed that patients with metastatic hormone receptor-positive (HR+)/human epidermal growth factor receptor-2-negative (HER2-) breast cancer harboring *PIK3CA* mutations significantly benefited from the addition of alpelisib to endocrine therapy (fulvestrant) when compared to endocrine therapy only ², which led to FDA approval of alpelisib. This trial,

which evaluated 300 mg/day alpelisib combined with fulvestrant, demonstrated a progression-free survival of 11 months for as compared to 5.7 months for fulvestrant plus placebo. The overall response for patients with *PIK3CA* mutation was 26.6% versus 12.8% in alpelisib versus placebo treated patients, respectively. The Sandpiper Phase III trial showed that *PIK3CA* mutant postmenopausal women with HR+/HER2- locally advanced, or MBC patients receiving both taselelisib plus fulvestrant demonstrated a PFS of 7.4 months in contrast to 5.4 months for patients receiving fulvestrant with placebo^{5, 30}. This minimal response combined with severe adverse events led to withdrawal of taselelisib from the market.

Human breast cancer cell lines harboring *PTEN* mutations are more sensitive to either paclitaxel (p=0.12) (Supplemental Figure 2c) or docetaxel (p<0.05) (Supplemental Figure 2d) (cancerrxgene.org), suggesting that combining either alpelisib or taselelisib with paclitaxel or docetaxel could exert synergistic anti-tumor effects for patients with *PIK3CA* mutant MBC. While initial trials with alpelisib revealed considerable toxicity at doses of 300 or 250 mg alpelisib once daily with paclitaxel (80mg/m²), and the maximum tolerated dose (MTD) was established at 150mg daily when combined with paclitaxel.³¹ However, another study found that compared to MBC patients without *PIK3CA* mutation, those with *PIK3CA* mutation had significantly better progression free survival (PFS) (7m versus 13m; p<0.05) when treated with alpelisib plus paclitaxel compared to PI3K inhibitor plus placebo, and the treatment was tolerable³⁶. Alpelisib is currently being examined for treatment of HER2+ and TNBC by Novartis in Phase III clinical trials. (Novartis. Novartis global pipeline. 2019. <https://www.novartis.com/our-science/novartis-global-pipeline>).

Orditura *et al.* generated *ex-vivo* three-dimensional cultures from 25 human breast cancer patients for selection of patients that were sensitive to PI3K/AKT inhibitors²⁷. This study found that *PIK3CA* mutant human breast cancer cells isolated from patient tumors were more sensitive to anti-PI3K agents than the non-*PIK3CA* mutant counterparts²⁷. Their findings also showed an additive synergy of anti-PI3K agent in combination with a microtubule stabilizer (e.g. paclitaxel), resulting in augmented killing of breast cancer patient isolated tumor cells paclitaxel on human breast cancer cells²³. The combination of taselelisib and paclitaxel completely suppressed PI3K/AKT signaling pathway activation²³.

Altogether, these findings formed the basis for moving agents targeting PI3K/AKT signaling pathway into clinical trials testing their efficacy in MBC. The landscape of current clinical trials investigating the effects of PI3K/AKT signaling inhibition in MBC is quite large. Currently, reports show 15 clinical trials [(1) available, (2) recruiting, (6) active, not recruiting, (5) completed, (1) unknown] investigating the effects of alpelisib on MBC and 6 clinical trials [(1) recruiting and (3) active, not recruiting, (2) completed] investigating taselelisib's effects on MBC (clinicaltrials.gov) (Supplemental Table 1).

In pre-clinical studies, we and others have shown that inhibition of PI3K enhances the response to immune checkpoint inhibitors (ICIs) in murine cancer models^{10, 17, 32}. These reports show that either inhibition of PI3K with a pan inhibitor or by inhibition of only PI3K γ can enhance response to ICIs. Taselelisib will inhibit PI3K α , γ , δ while alpelisib is PI3K α specific. PI3K α and β are broadly expressed in multiple cell types, while immune

cell functions rely more heavily on PI3K γ and PI3K δ -mediated signaling^{13, 39}. Thus, general inhibition of PI3K could potentially inhibit tumor growth, but also lead to the impairment of anti-tumor immune cell recruitment into the tumor.

MBC patients whose tumors are infiltrated with a high content of CD8+ T effector cells have an improved PFS and/or improved overall survival⁷. Studies have found that enhanced T-cell activation in the peripheral blood reflects T-cell activation in the spleen³⁸, and this finding could be translated to measuring intratumoral T-cell activation by analyzing T-cell activation in the peripheral blood. Moreover, there are now multiple reports showing that PI3K inhibitors enhance the activation state of CD8+ T effector cells, and this can be detected in both peripheral blood and in the tumor microenvironment^{10, 32}. Analysis of leukocytes in peripheral blood is one way to access the effects of therapy on immune cells when biopsy tissue is not available during treatment³⁸. To determine how inhibition of PI3K α, γ, δ with tasislisib compares to PI3K α inhibition with alpelisib affects the peripheral blood leukocytes in breast cancer patients treated with these inhibitors plus fulvestrant, we analyzed the peripheral blood of MBC patients enrolled in clinical trials with alpelisib or tasislisib. Here we report data obtained from multiple time points on trial from 11 patients enrolled in (4) clinical trials investigating alpelisib and tasislisib treatments for metastatic breast cancer (Table 1).

Methods

Patients

This study was conducted according to IRB Approval number 130489. Data was collected from (11) breast cancer patients who were HR+/HER2+, HR+/HER2-, or HR-/HER2- enrolled in clinical trials [NCT01862081](#), [NCT01791478](#), [NCT01872260](#), or [NCT02379247](#) at Vanderbilt University Medical Center. Efforts were made to collect blood at each cycle of therapy, though in some cases it was not possible to obtain blood at every cycle.

Patients enrolled in Phase I/II ([NCT01791478](#)) were given, 2.5mg letrozole daily for 28 days, and alpelisib 250–600mg (escalating dose) daily for 28 days. Secondary outcome measurements included clinical benefit rate, overall response (OR), progression free survival (PFS), and toxicities. Patients enrolled in [NCT01862081](#) were given tasislisib 3–10mg (escalating dose) daily and 75mg/m² docetaxel on day 1 of each 218-day cycle. Primary outcome measurements included incidence of adverse events and dose limiting toxicities. Secondary outcome measurements included area under the curve from time 0 to the last measurable concentration, time to maximum observed plasma concentration, maximum observed plasma concentration, minimum observed plasma concentration, objective response, duration of response, and PFS. Patients enrolled in [NCT01872260](#) Arm 2 received alpelisib 250–600mg (escalating dose) daily for 28 days and 2.5mg letrozole daily. Primary outcome measurements included incidence of dose limiting toxicities, safety and tolerability. Secondary outcome measurements included plasma concentration-time profiles, overall response rate (ORR), duration of response (DOR), and PFS. Patients enrolled in [NCT02379247](#) were given alpelisib 250–600mg (escalating dose) daily and 100mg/kg nab-paclitaxel on days 1, 8, 15 of each 28-day cycle. Primary outcome measurements included

incidence of dose limiting toxicities, safety and tolerability. Secondary outcome measurements included plasma concentration-time profiles, ORR, DOR, and PFS.

Patient Peripheral Blood Leukocyte (PBL) Isolation

Peripheral blood was collected from patients at several points in each cycle of patient treatment. Leukocytes were isolated from peripheral blood samples. Because the number of cycles on therapy varied considerably among patients, we present here the data from cycle 1 where 8/11 patients had peripheral blood collected at the beginning and end of cycle (21–28 day cycles). Patients RS006 and RS007 did not have complete cycle 1 data and patient RS014 did not have metastatic disease.

Multicolor FACS Analysis

Isolated leukocytes from 8/11 MBC patients, with complete cycle 1 data, were stained with Biolegend antibodies including (A Group) CD66b (Pacific Blue), CD163 (FITC), CD336 (PE), CD123 (Alexa Fluor 647), CD20 (PE/Cy7), and CD45 (Alexa Fluor 700), (B Group) CD25 (Alexa Fluor 647), CD69 (APC/Cy7), CD154 (Alexa Fluor 488), CD4 (PE within CD45 Alexa Fluor 700 population), and (C Group) CD107a (Alexa Fluor 488), PD-1 (Alexa Fluor 700), CD137 (PE/Cy7), and CD45RO (Pacific Blue within CD8 APC population [CD8 APC within CD45 PE population]). Cell populations were identified as CD45+CD66b+ (Neutrophil), CD45+CD163+ (Macrophage), CD45+CD336+ (Natural Killer cell), CD45+CD123+ (Dendritic cell), CD45+CD20+ (B-cell), CD45+CD25+ (Treg), CD45+CD69+ (T-cell), CD45+CD8+ (T-cell), and CD45+CD107a (T-cell).

Killing Assay

Supplemental peripheral blood was collected from patients RS013 and RS010, and CD8+ T-cells were isolated from each sample. The Dynabeads CD8+ Isolation Kit (Invitrogen) was used to capture CD8+ T-cells (cultured in HBSS with $\text{Ca}^{2+}/\text{Mg}^{2+}$ with 2% HAS media), and they were then co-cultured at a 30:1 ratio with MDA-MB-231-Fluc-GFP2 (cultured in DMEM with 10% FBS and 1% pen/strep media), human triple-negative breast cancer cell line, for 18 hours followed by measurement of MDA-MB-231-Fluc-GFP2 cell death.

Statistics

Fold change was calculated for the change in CD45+/CD8+/CD107a+ cell population in the peripheral blood from the 8 MBC patients with complete cycle 1 data. In addition, duration of response was calculated for each of the 8 patients, and a correlation analysis was performed on the fold change of CD45+/CD8+/CD107a+ cell population in relation to the duration of patient response.

Results

MBC patient population characteristics

All patients enrolled in the four clinical trials harbored metastatic disease at the time of trial enrollment except patient RS014, who did not have metastatic disease at the start of the trial. Somatic mutations of the tumors were obtained for the 8 MBC patient tumors and 6/8

harbored mutations in PI3K/AKT signaling pathway, 1/8 harbored *CHEK2* mutation, and for 1/8 the PI3K mutational status was unknown (Table 1). Interestingly, we observed that patients whose tumors exhibited increased somatic mutations exhibited longer therapeutic responses (Table 1). However, we did not observe a significant correlation between the number of somatic mutations and duration of therapeutic response across all clinical trials.

The duration of therapeutic response (months) for patients varied. The average duration of therapeutic response for each respective clinical trial was 7.5m for letrozole and alpelisib, 22m for nab-paclitaxel and alpelisib, 30.7m for ribociclib, letrozole, and alpelisib, and 5.2m for docetaxel and taselesib (Figure 1). The two clinical trials with the longest therapeutic responses were (1) ribociclib, letrozole, and alpelisib and (2) nab-paclitaxel and alpelisib. The clinical trial with the shortest therapeutic response administered taselesib with docetaxel. Two patients exhibited duration of response >30 months, including one patient with *PIK3CA* mutation who continues to respond (60 months at time of publication).

Immune response to alpelisib and taselesib therapy in MBC patients

In order to examine the effects of PI3K inhibition on leukocyte composition in the 8 MBC patients with complete cycle 1 data, peripheral blood of patients was collected at the beginning and end of cycle 1 of treatment, and multicolor-fluorescence-activated cell sorting (FACS) was used to identify leukocyte subpopulations. We did not find any significant trends in early neutrophil, macrophage, dendritic, natural killer, B-, or T-regulatory cells over cycle 1 of treatment. However, we found a strong correlation (*correlation coefficient* = 0.84; *p* < 0.05) between the cytotoxic T-cell (CD45/CD8/CD107a) population relative to duration of therapeutic response (months) (Figure 1). Of the MBC patients who responded to treatment with a *positive fold-change in cytotoxic T-cell population* (5/8 – black bars: RS012, RS013, RS011, RS009, and RS001), there was an average duration of treatment response of 31.4 months (Figure 1). In contrast, the patients who responded to treatment with a *negative fold-change in cytotoxic T-cell population* (3/8 – grey bars: RS010, RS004, and RS002) had an average duration of therapeutic response of 5 months (Figure 1). The data suggest that increased cytotoxic T-cell response increases patient duration of therapeutic response.

In addition, we found a positive correlation (*correlation coefficient* = 0.48; *p* = 0.10) between the cytotoxic T-cell (CD45/CD8/CD107a) population relative to progression-free survival (PFS) (months) (Figure 2). Of the MBC patients who responded to treatment with a positive fold-change in cytotoxic T-cell population (4/8 – black bars: RS012, RS011, RS009, and RS001), there was an average PFS of 32.1 months (Figure 2). In contrast, the patients who responded to treatment with a negative fold-change in cytotoxic T-cell population (3/8 – grey bars: RS010, RS004, and RS002) had an average PFS of 21.8 months (Figure 2). The data suggest that increased cytotoxic T-cell response increases patient PFS.

While all the patients except patient 7 had some evidence of toxicity, it is clear that the group of patients who responded to treatment with a positive fold-change in cytotoxic T cells did not exhibit more severity in adverse events than those patients who responded to treatment with a negative fold-change in cytotoxic T cells (Table 1). For example, patient 12 (stronger T cell response) did have G1 diarrhea, G1 fatigue, G1 hyperglycemia, G1 brittle

nails (but no rash), G2 cramping of hands/feet, G1 alopecia, and G1 QTc prolongation. In comparison, patient 4 and patient 10, who both had a negative fold- change in cytotoxic T cell population, experienced toxicity as follows: Patient 4: G1 peripheral neuropathy, G2 paronychia, G2 pneumonia, G2 fatigue, G1 onycholysis; Patient 10: G1 nausea, G2 fatigue, G1 diarrhea, G1 rash, G2 mucositis.

Furthermore, CD8+ T-cells isolated from patient RS013 exhibited higher killing capacity of human triple-negative breast cancer cells compared to CD8+ T-cells isolated from patient RS010 (Figure 3). These data suggest that patients with a more robust, initial anti-tumor T-cell response may have a longer therapeutic response compared to patients who do not have a robust, initial anti-tumor T-cell response. However, this will need to be confirmed in a larger data set. Overall, alpelisib-treated MBC patients had a more robust initial anti-tumor T-cell response, which was associated with a longer therapeutic response.

Discussion

Elucidating tumor and microenvironment characteristics that are associated with therapeutic response and/or resistance is a major goal in the current era of personalized cancer medicine. Conventional cancer treatments such as cytotoxic and targeted chemotherapy can have immunomodulatory effects, and many studies have investigated their clinical utility with ICIs⁴¹. Common ICIs include anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4), anti-programmed cell death protein 1 (anti-PD-1), and anti-programmed death-ligand 1 (anti-PD-L1)¹⁰. Typically, breast cancers are considered immunologically cold, and recent studies show that this phenotype can be switched through the use of certain targeted therapies³². In late 2018, PD-L1 inhibitor atezolizumab became the first ICI FDA-approved for use in combination with nab-paclitaxel in un-resectable or metastatic triple-negative breast cancer patients³⁵.

De Henau *et al.* reported that ICI resistance was directly related to PI3K-associated immune suppressive function of infiltrating cells, and that targeting PI3K increased ICI sensitivity in murine melanoma, breast, and colon cancer models¹⁰. Additionally, Kaneda *et al.* found that PI3K inhibition synergizes with ICI therapy to promote tumor regression and increase survival in various murine cancer models¹⁷. This synergy is made possible via the reprogramming of pro-tumor infiltrating cells via PI3K inhibition that ablates their immune suppressive function, thus increasing the ability of ICI therapy to work efficiently¹⁷.

Importantly, Sai *et al.* showed that PI3K inhibition reduces/abrogates breast cancer tumor growth and increases activated T-cells in the tumor microenvironment (TME) compared to vehicle treatment³². Altogether, this study found that ICI therapy in combination with PI3K inhibition, synergistically inhibited breast cancer tumor growth and enhanced anti-tumor immunity³². In addition, Schmid *et al.* found that paclitaxel plus ICI therapy significantly increased PFS compared to paclitaxel plus placebo (7.2m versus 5.5m; $p < 0.01$)³⁵. In addition, this study found that of the patients who were PD-L1 positive, PFS was 7.5m (paclitaxel plus ICI therapy) compared to 5m (paclitaxel plus placebo; $p < 0.01$)³⁵.

Evaluation of the patients enrolled in the four clinical trials in our above study suggests that real-time assaying of a patient's cytotoxic T cells from peripheral blood could serve as a predictive biomarker of response to therapeutic regimens that treat with PI3K pathway antagonists. The results obtained here suggest that an increase in patient's initial anti-tumor T-cell population in peripheral blood in response to alpelisib treatment may correlate with increased progression free survival or duration of therapeutic response. However, since we were able to study only a limited number of patients, our results will need to be validated in a larger study examining the therapeutic response to alpelisib in combination with other indicated therapeutics for treatment of MBC patients.

Data from our analysis of peripheral immune cells in breast cancer patients undergoing treatment with PI3K inhibitors combined with other therapies suggest that if *PI3K inhibition promotes an early anti-tumor T-cell response there is longer duration of therapeutic response and PFS*. Previously, studies have shown significant increases in PFS of MBC patients when treated with either (1) paclitaxel plus ICI, (2) PI3K inhibitor plus ICI, or (3) alpelisib plus paclitaxel^{32, 35, 36}. Moreover, it has been previously demonstrated that therapy induced changes in T cell populations in peripheral blood of patients has a predictive value for prognosis. Increased levels of CD8+CD28+ T cells in peripheral blood was an independent predictor for increased overall survival (OS) of non-small cell lung cancer patients who were treated with chemo-radiotherapy, while elevated CD8+CD28- T cells was associated with poorer OS²⁰. Likewise, in metastatic breast cancer in the course of chemotherapy elevated CD8+CD28- T cells predict shorter PFS³⁷. In addition, increased peripheral regulatory T cells in NSCL cancer patients correlated with poor PFS in response to radiotherapy¹⁹. In contrast in a melanoma study, Treg frequency in PBMCs did not correlate with prognosis, but increased suppressive activity of Tregs after ipilimumab therapy did correlate with poorer patient outcomes²⁹. Patients with increased CD4+ and CD8+ T cell ratios had better PFS and OS after chemoradiotherapy in patients with locally advanced esophageal squamous cell carcinoma⁸. Altogether these data indicate that when response to therapy is associated with an increase in circulating T effector cells, there is an indication for heightened immune surveillance of tumor cells, which may contribute to the enhanced progression free survival in patients. In contrast, reduction in CD8+T cells and increased populations of suppressor cells in response to therapy is associated with poorer prognosis, as we observed in this study of breast cancer patients treated with therapies that target the PI3K pathway combined with chemotherapy. Moving forward, a larger cohort of patients need to undergo similar studies to prove concept is valid.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Correlation between CD45/CD8/CD107a T-cells fold-change during cycle 1 treatment, and duration of therapeutic response in MBC patients enrolled in various clinical trials

Fig. 2.
Fold change in the killing ability of CD45/CD8/CD107a T-cells on metastatic breast cancer (MBC) cells in relation to duration of therapeutic response

Fig. 3.
Fold change in the killing ability of CD45/CD8/CD107a T-cells on metastatic breast cancer (MBC) cells in relation to duration of therapeutic response

Table 1.

Clinical characteristics of patients enrolled in clinical trials at VUMC.

Patient ID	Gender	Age	Initial Diagnosis	Stage	Grade	Mutation status	Hormone receptor status	Time on study (months)	Disease progression on study	Reason for withdrawal	Deceased	PFS (months)	OS (months)	Toxicity
RS001	Female	68	2012	III	High	PIK3CA, CCND1, TP53, CHD2, CYLD, FGF19, FGF3, FGF4	AR+	11	Yes (lungs)	Drug-related adverse event	Yes	11	11.1	G2 endocrine
RS002	Female	35	2011		High	PIK3CA, CDK, HER2	ER+/PR+/HER2-	2	No	Quality of life	res	4	7.8	G3 vascular
RS004	Female	64	2004	II/III	High	Unknown	HER2+/E R+	6.5	No	Drug-related adverse event	No	55+	NR	G3 respiratory, G1 neurologic, G2 dermatologic, G2 fatigue
RS006	Female	58	2009	I	Intermediate	None	HER2+/E R+(h)/PR-	8.5	Yes (adrenal, bone)	Disease progression	Yes	8.5	38.5	G1 hepatic, G2 mucositis
RS007	Female	53	2014	IV	Intermediate	No info	TNBC	2.5	Yes (bone, brain)	Disease progression	Yes	2.5	3.3	none
RS009	Female	57	2001		Low	PIK3CA H1047R, APC exon 16/110307K	HER2-/E R+/PR+/AR+	22	Yes (bone, liver)	Disease progression	Yes	22	24.3	G1 hepatic, G1 gastrointestinal, G1 mucositis, G1 endocrine
RS010	Female	59	1999	IV	Low	PIK3CA	HER2-/E R+/PR+	6.5	Yes (liver)	Disease progression	Yes	6.5	NR	G1 gastrointestinal, G2 fatigue, G2 mucositis, G1 dermatologic
RS011	Female	69	1989	III		CHEK2	HER2-/E R+/PR+	32.5	Yes (bone)	Disease progression	No	32.5	NR	G1 gastrointestinal, G1 vascular, G2 endocrine, G1 hepatic
RS012	Female	59	2009		Intermediate	PIK3CA	HER2-/E R+/PR+	60+	No	*Still on trial	No	60+	NR	G1 gastrointestinal, G1 fatigue, G1 endocrine, G1 dermatologic, G1 cardiac

Patient ID	Gender	Age	Initial Diagnosis	Stage	Grade	Mutation status	Hormone receptor status	Time on study (months)	Disease progression on study	Reason for withdrawal	Deceased	PFS (months)	OS (months)	Toxicity
RS013	Female	56	2013	III	High	AKT, MYC, MCCL1, NRAS amplification	HER2-/E R+ (lo)/PR-	1.5	No	Deceased	Yes	Unknown	1.9	G3 hepatic, G1 endocrine, G1 gastrointestinal, G2 fatigue, G2 hematologic
RS014	Female	70	2016	II	Intermediate	PIK3CA	HER2-/E R+/PR-	5.5	No	Completed adjuvant therapy	No	44+	NR	G1 endocrine, G1 fatigue, G1 mucositis

* Patients excluded from correlation analysis (RS006 and RS007 - cycle 1 data incomplete; RS014 - non-metastatic BC).