Clinical Cancer Research

# Randomized Phase II Trial of Letrozole plus Anti-MUC1 Antibody AS1402 in Hormone Receptor–Positive Locally Advanced or Metastatic Breast Cancer

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## Abstract

**Purpose:** AS1402 is a humanized immunoglobulin G1 antibody that targets the aberrantly glycosylated antigen MUC1, which is overexpressed in 90% of breast tumors and contributes to estrogen-mediated growth and survival of breast cancer cells *in vitro* by modulating estrogen receptor (ER) activity. Aromatase inhibitors have been reported to enhance antibody-dependent cell-mediated cytotoxicity elicited by antibodies *in vitro*. We compared the outcomes of patients with breast cancer treated with letrozole with or without AS1402.

**Experimental Design:** The study population included 110 patients with locally advanced or metastatic hormone receptor–positive breast cancer randomized to receive 2.5 mg letrozole only once daily or with a weekly 9 mg/kg AS1402 infusion. The primary endpoint was overall response rate. Secondary endpoints included progression-free survival, time to progression, and safety. AS1402 exposure and influence of allotypes of *FcγRIIIa*, *FcγRIIa*, and *MUC1* were evaluated.

**Results:** The study was stopped early because of a trend toward worse response rates and a higher rate of early disease progression in the AS1402 + letrozole arm. Final analysis revealed no significant difference in efficacy between the study arms. Evaluated gene polymorphisms did not define patient subgroups with improved outcomes. Addition of AS1402 to letrozole was associated with manageable toxicity.

**Conclusions:** Because adding AS1402 to letrozole did not improve outcomes compared with letrozole only, blocking ER may be a better strategy for harnessing MUC1 modulation of the ER to a clinical advantage. *FcγRIIIa*, *FcγRIIa*, and *MUC1* allotype did not predict outcome for patients treated with letrozole with or without AS1402. *Clin Cancer Res; 17(21); 6822–30. ©2011 AACR.*

## Introduction

For the past 30 years, the selective estrogen receptor (ER) modulator tamoxifen has been the standard of care in much of the Western world for hormone-responsive advanced breast cancer in both premenopausal and postmenopausal women, mainly because of its favorable safety profile (1–3). Results from randomized trials (4, 5), and a meta-analysis (6), suggest that when used as first-line therapy for metastatic breast cancer, third-generation aromatase inhibitors, such as letrozole, are associated with superior response rates, time to progression (TTP), and overall survival compared with first-line tamoxifen. However, hormone receptor (HR)-positive advanced breast cancer remains an area of unmet medical need because most patients will eventually die from their disease.

MUC1 is an aberrantly glycosylated antigen overexpressed in approximately 90% of breast cancer tumors (7). It stimulates ER-α–mediated transcription and contributes to estrogen-mediated growth and survival of breast cancer cells *in vitro*. MUC1 activates and stabilizes ER-α by binding to the DNA-binding domain of the receptor (8). This MUC1-ER dynamic may be the basis for the clinical finding of prolonged survival in breast cancer patients vaccinated with Theratope and given concomitant hormonal therapy, compared with a control group receiving the

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## Translational Relevance

This article reports on a phase II study evaluating the safety and efficacy of letrozole ± AS1402 in the treatment of hormone receptor–positive ($HR^+$) metastatic breast cancer. The main conclusions of clinical relevance are as follows:

- AS1402 should not be further developed in combination with letrozole.
- The safety and efficacy of letrozole as first-line therapy for $HR^+$ metastatic breast cancer is confirmed by this study conducted without involvement of the letrozole marketing authorization holder (Novartis AG).
- Contrary to what has previously been proposed, increased antibody-dependent cell-mediated cytotoxicity (ADCC) against tumors is not likely to be a significant part of the mechanism of action of letrozole.
- There is an experimentally supported theory that MUC1 and the estrogen receptor interacts. The clinical relevance of such an interaction, if it exists at all, is called into question by this study.
- This is a useful model phase II study for the evaluation of an ADCC-dependent antibody added to a standard anticancer therapy, as it used an ambitious and useful biomarker program.

vaccination only. Median survival times in the 2 groups were 36.5 months and 30.7 months, respectively, in a retrospective subgroup analysis (9). Theratope is a vaccine based on Sialyl-Tn, a carbohydrate associated with MUC1.

AS1402 is a humanized immunoglobulin G1 kappa (IgG1 $\kappa$) monoclonal antibody that targets MUC1. Its antitumor activity has been shown *in vitro* with an antibody-dependent cell-mediated cytotoxicity (ADCC) assay (10). AS1402 plasma concentrations similar to the concentrations necessary to elicit ADCC *in vitro* were achieved in a phase I dose escalation study (11).

Here, we report the findings of a phase II, randomized, open-label, international study designed to compare the efficacy, safety, and tolerability of AS1402 in combination with letrozole to that of letrozole only as first-line treatment in postmenopausal women with HR-positive/HER2-negative locally advanced or metastatic breast cancer. The combination of AS1402 with an aromatase inhibitor in this study was predicated upon preclinical pharmacology, showing a mechanistic interaction between the MUC1 oncoprotein and the ER (12, 13).

The *in vitro* finding that aromatase inhibitor pretreatment sensitized malignant breast tissue to monocyte-mediated ADCC further extends the body of evidence underpinning the potential clinical utility of a combined therapeutic approach (14), with AS1402 mediating ADCC at the MUC1 level and an aromatase inhibitor acting at the ER level.

Natural killer cells, the principal mediators of ADCC, constitutively express FcγRIIIa, and macrophages, also contributing to ADCC, express FcγRIIIa and FcγRIIa (15). The *FcγRIIIa* 158 V/V genotype and/or *FcγRIIa* 131 H/H genotype increases the affinity of the receptors for IgG and subsequent cell killing by immune cells (16) and correlates with higher response rates to monoclonal antibodies including rituximab and trastuzumab (17–19). A *MUC1* polymorphism at nucleotide position 568 has been shown to have functional implications in human disease (20). Participants in this study were allotyped for *FcγRIIIa* 158, *FcγRIIa* 131, and *MUC1* 568 in an attempt to identify patient subsets particularly suited for therapy with AS1402.

## Materials and Methods

### Study population

This was a randomized, international, open-label phase II study of postmenopausal women with metastatic or locally advanced breast cancer not amenable to curative therapy. One hundred ten patients were enrolled at 22 sites in the United States, Poland, Ukraine, and Russia.

Patients were eligible for the study if they had ER-positive and/or progesterone receptor (PR)-positive histologically or cytologically confirmed breast cancer, had measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), were past natural or therapy-induced menopause, had Eastern Cooperative Oncology Group performance status (ECOG PS) 0 or 1, and had a granulocyte count $\geq 1.0 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, bilirubin levels $\leq 2$ times the upper limit of normal, aspartate transaminase and alanine transaminase levels $\leq 5$ times the upper limit of normal, and creatinine clearance $\geq 30$ mL/min. Patients were excluded if they had undergone prior chemotherapy or endocrine therapy, had a relapse $\leq 1$ year after discontinuation of adjuvant therapy including an aromatase inhibitor, had HER2/neu-positive breast cancer, or had any other concurrent disease or condition precluding study compliance.

### Study conduct

The study was sponsored by Antisoma Research Ltd. Enrollment was started only after the study protocol was approved by the local Institutional Review Board, ethics committee, and/or regulatory agency for each study site. All study participants provided signed informed consent before initiating any study-related procedures.

### Treatment

All subjects received 2.5 mg letrozole orally (Novartis AG) once daily. Subjects randomized to the experimental arm received AS1402 (Antisoma Research Ltd.) as a weekly 1-hour 9 mg/kg infusion. Patients were treated until disease progression or withdrawal.

### Study assessments

The primary objective of this study was to compare the overall tumor response rates between the treatment arms.

The secondary endpoints were TTP, progression-free survival (PFS), clinical benefit rate, duration of response, and safety. Tumor assessments were to be conducted according to RECIST upon enrollment and every 12 weeks thereafter until disease progression, or when clinical examination suggested disease progression. Radiology scans were submitted for independent review.

Safety was evaluated using medical history, physical examination, electrocardiogram, concomitant medications, vital signs, weight, hematology, biochemistry, urinalysis, ECOG PS, and adverse event data. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events version 3.0. Safety and efficacy data were reviewed 3 times by an independent Data Monitoring Committee (DMC).

Pharmacokinetic (PK) samples were collected according to a previously described limited sampling scheme (21). Plasma levels of AS1402 were determined at Covance Laboratories Ltd. Samples were collected for the detection of human anti-human antibodies (HAHA) and for the allotyping of *MUC1*, *FcγIIIRa*, and *FcγIIRa*. Tissue sections were submitted for immunohistochemical staining for MUC1, if available.

**Statistical analysis**

Patients were randomized 1:1 to receive letrozole and AS1402 (experimental arm) or letrozole only (control arm). The randomization was stratified for the presence of visceral disease and for the administration of prior adjuvant endocrine therapy.

Simon's minimax 2-stage design was used with the stopping criteria applied to the AS1402 + letrozole arm only (22). If three or fewer confirmed responses were seen within the first 6 months of treatment in the first 31 patients randomized to the experimental arm, the trial was to be stopped. Otherwise, additional patients were to be randomized until 55 patients were accrued for each treatment arm. The final sample size was selected to have a 90% power to detect a true response rate $\geq$ 25% in the experimental arm. The efficacy hurdle was designed to ensure the combination therapy of AS1402 + letrozole was not inferior to the standard letrozole therapy in terms of historical response rates.

The response rates for each arm and 95% confidence intervals (CI) for the estimates were calculated. PFS and TTP were calculated from the date of randomization. Median PFS and TTP were calculated with Kaplan–Meier analyses.

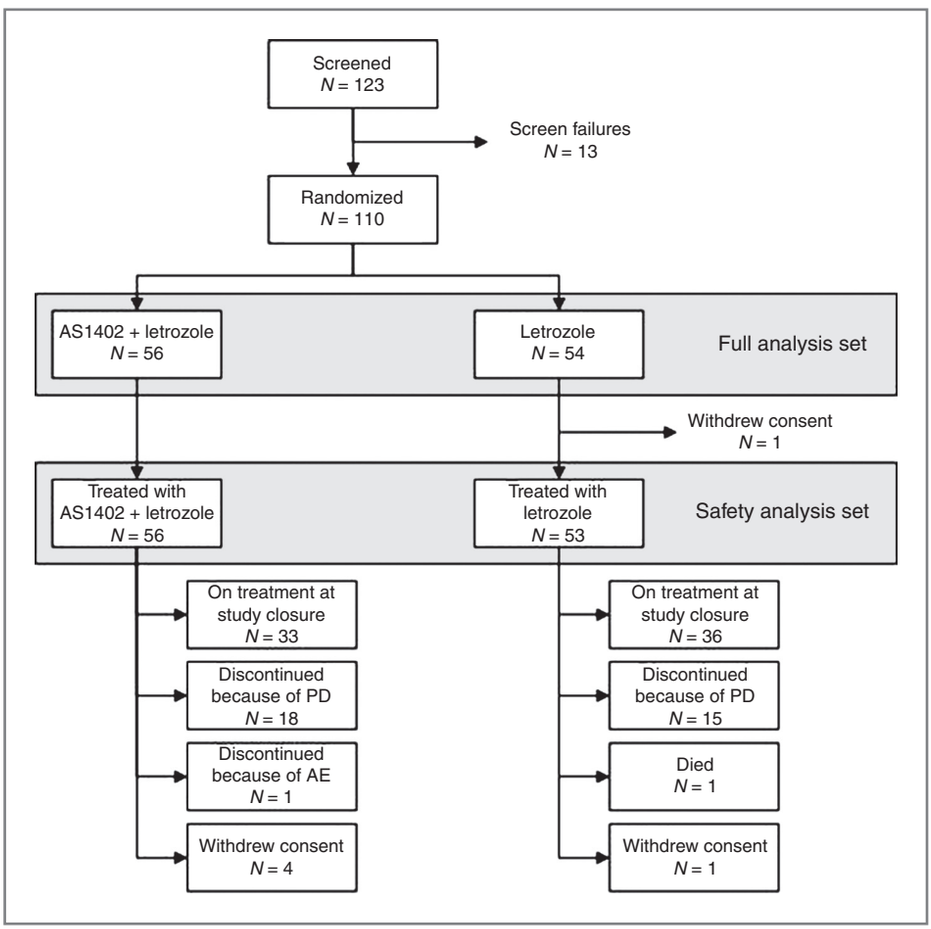


**Figure 1.** CONSORT diagram of the trial. PD, progressive disease; AE, adverse event.

The CIs for median estimates were based on the Brookmeyer and Crowley method. Treatment effect estimates and 95% CIs were based on a Cox proportional hazards regression model, both unadjusted and adjusted for randomization stratification factors. All outcomes related to disease progression and responses were based on investigator-reported data collected on case report forms.

### Allotyping

Venous blood was collected from each patient and frozen. DNA was extracted at the central laboratory from 200 μL blood using the QIAamp Blood Mini Kit from Qiagen. The DNA yield was measured using an ND-1000 Nanodrop spectrophotometer from Thermo Fischer Scientific.

For each polymorphism, Applied Biosystems' TaqMan SNP real-time PCR assays were used (part numbers: C-25815666-10 for *FcγRIIIa* 158 V/F; C-9077561-20 for *FcγRIIa* 131 H/R; and C-27532642-10 for *MUC1* 568 A/G). The reactions were set up with TaqMan Universal PCR Master Mix, No AmpErase UNG (part number: 4324018) and run in an ABI Prism 7000 real-time PCR detection system. The reactions were 25 μL reactions as follows: 12.5 μL of Universal PCR Master Mix 2×, 1.25 μL of SNP detection assay 20×, and 11.25 μL of $ddH_2O$ + DNA mixture (providing 10 ng of DNA). The thermal cycling conditions consisted of an initial denaturation step at 95°C for 10 minutes, followed by 50 cycles of denaturation at 92°C for 15 seconds, and annealing/extension step at 60°C for 1 minute. Every DNA sample was run in duplicate. Every reaction plate had a triplicate nontemplate control (NTC). Genotypes were detected by allele-specific fluorescence using the SDS 1.2.3 Software (Applied Biosystems).

Selected samples were sequenced and the PCR was carried out in 2 separate laboratories to ensure reliable results (data not shown).

## Results

### Demographics and treatment

A total of 110 patients were enrolled and 109 treated in the study between August 29, 2008, and April 15, 2009 (Fig. 1). There were no notable differences between the

**Table 1.** Demographic parameters, full analysis set

| | AS1402 + letrozole (*N* = 56) | Letrozole only (*N* = 54) | Total (*N* = 110) |
|---|---|---|---|
| Age, mean (SD) | 60.9 (7.76) | 59.9 (9.49) | 60.4 (8.63) |
| Race., white/Caucasian | 56 (100) | 54 (100) | 110 (100) |
| Weight, mean (SD) | 72.4 (13.43) | 73.9 (12.46) | 73.1 (12.93) |
| Height, mean (SD) | 160.7 (5.68) | 159.9 (6.15) | 160.3 (5.90) |
| Clinical stage | | | |
| IIIA | 2 (3.6) | 4 (7.4) | 6 (5.5) |
| IIIB | 5 (8.9) | 7 (13.0) | 12 (10.9) |
| IIIC | 2 (3.6) | 0 | 2 (1.8) |
| IV | 47 (83.9) | 43 (79.6) | 90 (81.8) |
| Mean time from diagnosis, mo (SD) | 28.0 (47.58) | 27.7 (51.13) | 27.9 (49.12) |
| Histologic type | | | |
| Mucinous | 0 | 2 (3.7) | 2 (1.9) |
| Lobular | 5 (8.9) | 7 (13.0) | 12 (10.9) |
| Tubular | 1 (1.8) | 1 (1.9) | 2 (1.8) |
| Ductal | 32 (57.1) | 30 (55.6) | 62 (56.4) |
| Other | 18 (32.1) | 14 (25.9) | 32 (29.1) |
| Hormone receptor status | | | |
| $ER^+$ | 54 (96.4) | 51 (94.4) | 105 (95.5) |
| $PR^+$ | 46 (82.1) | 48 (88.9) | 94 (85.5) |
| $ER^+$ and $PR^+$ | 44 (78.6) | 45 (83.3) | 89 (80.9) |
| ECOG PS | | | |
| 0 | 27 (48.2) | 29 (53.7) | 56 (50.9) |
| 1 | 29 (51.8) | 25 (46.3) | 54 (49.1) |
| Prior therapies | | | |
| Radiotherapy | 18 (32.1) | 15 (27.8) | 33 (30.0) |
| Surgery | 27 (48.2) | 25 (46.3) | 52 (47.3) |
| Endocrine therapy | 9 (16.1) | 11 (20.4) | 20 (18.2) |
| Visceral disease at baseline | 42 (75) | 40 (74.1) | 82 (74.5) |

NOTE: Values in table are number of patients (percentage) unless otherwise indicated.

AS1402 + letrozole arm and the letrozole-only arm in any baseline demographic variable (Table 1).

The mean actual dose intensity of administered AS1402 was 8.8 mg/kg/wk or 98% of the target dose intensity. The mean cumulative actual dose of administered letrozole was 406.4 mg for patients in the AS1402 + letrozole arm and 431.2 mg for patients in the letrozole-only arm. The 6% lower mean cumulative actual letrozole dose in the AS1402 + letrozole arm is unlikely to represent a clinically relevant difference.

### Early termination of the study

The study recruited faster than expected; hence, the study was fully recruited before the stage I analysis could be conducted. A scheduled DMC review of the data on August 3, 2009, revealed a trend toward worse response rates in the AS1402 + letrozole arm. In addition, the number of patients who had withdrawn from the study because of disease progression was considerably higher in the AS1402 + letrozole arm, resulting in a shorter estimated PFS in the AS1402 + letrozole arm than in the letrozole-only arm. After discussion with the DMC, a detailed analysis of the reasons of discontinuation (see Fig. 1), available biomarker and PK data, and a thorough statistical analysis, the study sponsor concluded that the study was highly unlikely to yield a positive final result, and administration of AS1402 was stopped on August 7, 2009, to prevent unnecessarily exposing subjects to AS1402, when it was highly unlikely that the final results of the study would support further development of AS1402.

### Safety results

There was no notable difference between the AS1402 + letrozole arm and the letrozole-only arm in the incidence or severity of adverse events, as displayed in Table 2. Adverse events were reported in 60.7% and 66.0% of patients, respectively. No grade 5 adverse events were reported during safety assessments in either arm. However, 5 patients on the letrozole arm died from disease progression before the study was terminated. The only grade 4 adverse event reported was one case of anemia in the letrozole-only arm. Grade 3 adverse events were reported in one patient (1.8%) in the AS1402 + letrozole arm compared with 9 patients (17.0%) in the letrozole-only arm. The most frequently

**Table 2.** Adverse events reported in more than 5% in either arm irrespective of relationship to the study drugs, safety analysis set[a]

| System organ class | Preferred term | AS1402 + letrozole ($N = 56$) | Letrozole only ($N = 53$) |
|---|---|---|---|
| Gastrointestinal disorders | | 8 (14.3) | 7 (13.2) |
| | Nausea | 3 (5.4) | 2 (3.8) |
| | Vomiting | 3 (5.4) | 2 (3.8) |
| General disorders and administration site disorders | | 14 (25.0) | 10 (18.9) |
| | Asthenia | 7 (12.5) | 3 (5.7) |
| | Fatigue | 5 (8.9) | 5 (9.4) |
| | Pyrexia | 3 (5.4) | 3 (5.7) |
| Infections and infestations | | 10 (17.9) | 2 (3.8) |
| | Rhinitis | 6 (10.7) | 0 |
| Investigations | | 7 (12.5) | 8 (15.1) |
| | Alanine transaminase increased | 2 (3.6) | 3 (5.7) |
| | Aspartate transaminase increased | 2 (3.6) | 4 (7.5) |
| | γ-Glutamyltransferase increased | 1 (1.8) | 5 (9.4) |
| | Weight decreased | 3 (5.4) | 0 |
| Musculoskeletal and connective tissue disorders | | 10 (17.9) | 8 (15.1) |
| | Arthralgia | 4 (7.1) | 5 (9.4) |
| | Back pain | 3 (5.4) | 1 (1.9) |
| Respiratory, thoracic, and mediastinal disorders | | 9 (16.1) | 4 (7.5) |
| | Cough | 6 (10.7) | 3 (5.7) |
| Vascular disorders | | 17 (30.4) | 13 (24.5) |
| | Hot flush | 9 (16.1) | 10 (18.9) |
| | Hypertension | 6 (10.7) | 2 (3.8) |
| | Hypotension | 5 (8.9) | 0 |

[a]Values in table are number of patients (percentage).

**Table 3.** Adverse events possibly, probably or definitely related to AS1402 and reported in more than 3%, safety analysis set[a]

| System organ class | Preferred term | AS1402 + letrozole ($N = 56$) |
|---|---|---|
| Gastrointestinal disorders | | 5 (8.9) |
| | Nausea | 2 (3.6) |
| | Vomiting | 2 (3.6) |
| General disorders and administration site disorders | | 10 (17.9) |
| | Asthenia | 6 (10.7) |
| | Fatigue | 3 (5.4) |
| Investigations | | 3 (5.4) |
| | Alanine transaminase increased | 2 (3.6) |
| | Aspartate transaminase increased | 2 (3.6) |
| Respiratory, thoracic, and mediastinal disorders | | 3 (5.4) |
| | Cough | 2 (3.6) |
| Vascular disorders | | 4 (7.1) |
| | Hypotension | 3 (5.4) |

[a]Values in table are number of patients (percentage).

reported adverse events were hot flush, asthenia, fatigue, arthralgia, cough, and hypertension.

Adverse events considered to be related to AS1402 were reported in 18 patients (32.1%; Table 3). All were classified as ≤ grade 2. There was no notable difference between the experimental and control arms in the incidence of adverse events considered to be related to letrozole (28.6% and 32.1%, respectively; data not shown).

## Efficacy results

Partial response to treatment was reported in a lower proportion of patients in the AS1402 + letrozole arm (7 patients, 12.5%) than in the letrozole-only arm (14 patients, 25.9%). Stable disease was reported in 32 patients (57.1%) in the AS1402 + letrozole arm and in 27 patients (50.0%) in the letrozole-only arm, and progressive disease was reported in 13 patients (23.2%) in the AS1402 + letrozole arm and in 10 patients (18.5%) in the letrozole-only arm. The difference in response rates (AS1402 + letrozole vs. letrozole only) was 13.43% with a CI of −27.975% to 1.12%, implying a lack of treatment difference between the arms.

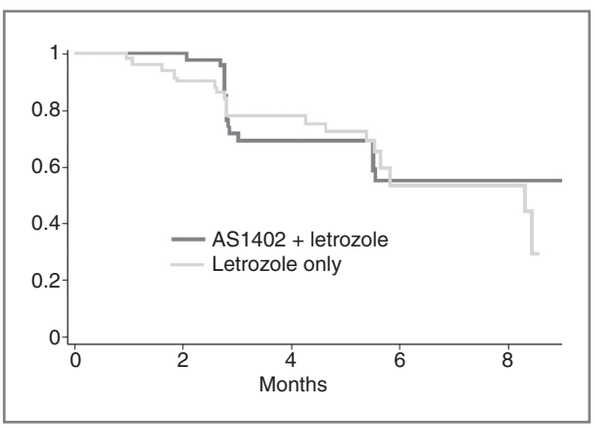


Figure 2. Kaplan–Meier estimates of time to disease progression or death in patients treated with letrozole only and with AS1402 plus letrozole.

Results of the PFS and TTP analyses were the same, as everyone who died had disease progression (Fig. 2). There was no notable difference between the arms in the number of patients for whom death or disease progression was recorded [18 patients (32.1%) in the AS1402 + letrozole arm and 19 patients (35.2%) in the letrozole-only arm]. The estimated HR in the unadjusted analysis was less than 1 (0.947), but the CI contained 1 (0.496–1.805), indicating a lack of treatment difference between the arms. The estimated HR (CI) in the adjusted analysis was 0.925 (0.483–1.771).

## *FcγRIIIa*, *FcγRIIa*, and *MUC1* allotypes

Samples from 105 patients (95%) were successfully allotyped. For *FcγRIIIa* 158, the allotypes were F/F for 46 patients (44%), F/V for 51 patients (49%), and V/V for 8 patients (8%). For *FcγRIIa*, the allotypes were H/H for 30 patients (29%), H/R for 58 patients (55%), and R/R for 17 patients (16%). For *MUC1*, the allotypes were G/G for 32 patients (30%), A/G for 51 patients (49%), and A/A for 22 patients (21%). The distribution of the evaluated allotypes was thus in line with previous reports in this white/Caucasian population (20, 23).

The waterfall diagrams presented in Fig. 3 are color coded for each allotype. Analysis of the allotypes did not reveal any specific subgroup associated with a favorable or adverse outcome when change in tumor burden measured according to RECIST was used as measure of outcome. This result provided additional support for stopping the study early.

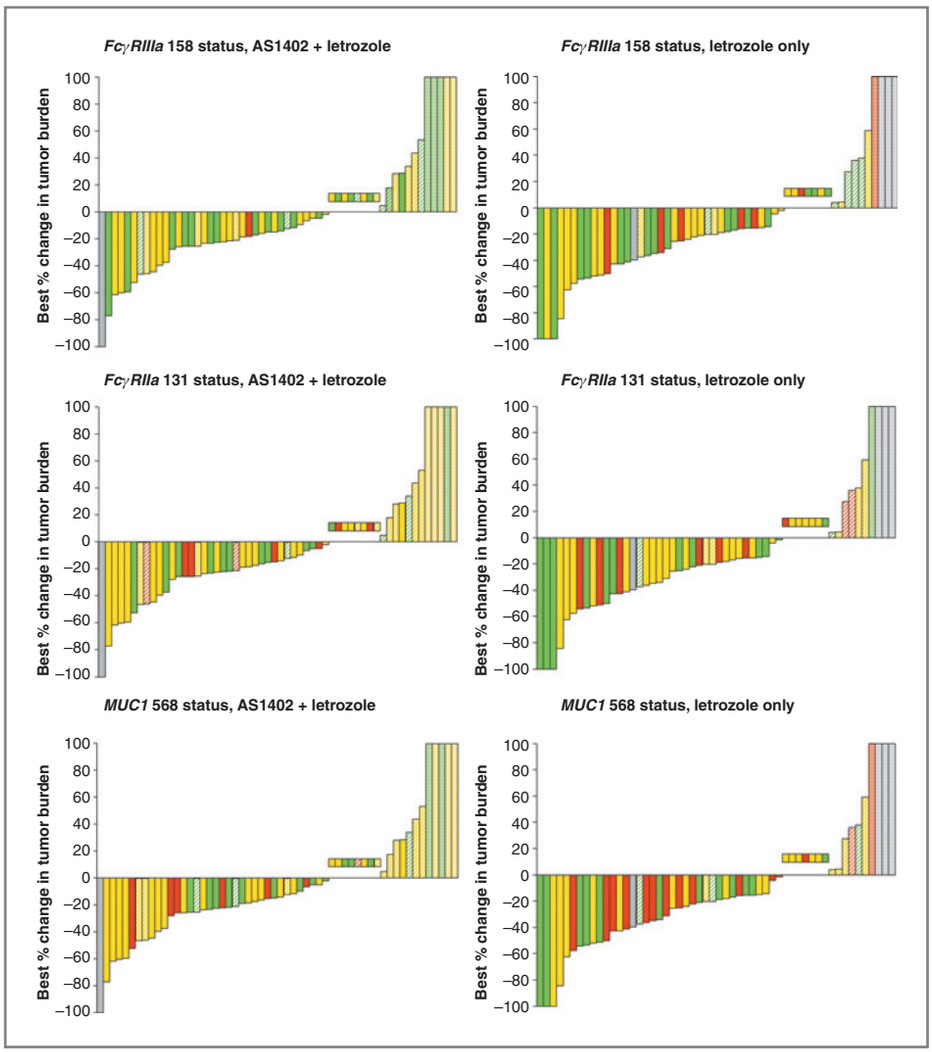


**Figure 3.** Waterfall diagrams describing the change in tumor burden and color coded for allotype. The shape of the waterfall diagram was similar between both arms, and the distribution of the allotypes for all 3 evaluated single-nucleotide polymorphisms appeared random. The tumor burden was calculated as the sum of unidimensional measurements of the target lesions selected and measured according to RECIST. Bars to the far right extending up to 100% marked with a horizontal pattern represent unevaluable patients who progressed or came off the study for other reasons before having an evaluable computed tomographic scan. Patients with exactly 0% change are displayed as squares elevated from the *x*-axis for clarity. Bars marked with a diagonal pattern represent patients with concurrent disease progression in nontarget lesions or new lesions, for which the displayed tumor burden may be an underestimate. Gray bars indicate the allotype is not available. For *FcγRIIIa*, green is F/F, yellow is V/F, red is V/V; for *FcγRIIa*, green is H/H, yellow is H/R, red is R/R; and for *MUC1*, green is G/G, yellow is A/G, red is A/A.

### Evaluation of PK, HAHA, and MUC1 expression

Because the study was terminated early, the planned full evaluation of population PK parameters, HAHA levels, and MUC1 expression was not conducted. PK samples evaluated prior to the termination of the study exhibited AS1402 concentrations in line with expectations based on the prior phase I study (11). MUC1 expression has previously been reported in more than 90% of patients with breast cancer (7), and in the phase I study of AS1402, none of the 29 treated patients developed HAHA (11). HAHA levels and MUC1 expression were therefore not evaluated, as they were unlikely to affect the overall interpretation of the trial results.

## Discussion

The final data from this study failed to provide evidence of increased efficacy when AS1402 is added to letrozole. The study was a randomized phase II study designed to compare AS1402 in combination with letrozole to letrozole only as a first-line treatment in postmenopausal women with locally advanced or metastatic breast cancer. The study was terminated when a review indicated a trend toward a higher proportion of patients with disease progression and a smaller proportion of patients responding to treatment in the AS1402 + letrozole arm than in the letrozole-only arm.

AS1402 has previously been shown to localize to MUC1-positive tumor sites (24), MUC1 has been shown to be present in approximately 90% of breast cancers (7), and the expected AS1402 plasma concentrations were achieved in this study. There was no evidence of an increase in the size of lymph node lesions compared with other target lesions, and thus, the lower response rate and increased rate of disease progression in the AS1402 + letrozole arm were not due to AS1402 causing reactively enlarged lymph nodes (data not shown). Furthermore, the sites and types of disease progression were similar in the AS1402 + letrozole arm and the letrozole-only arm. The lack of efficacy noted in this study is therefore unlikely to be due to methodologic issues.

AS1402 can conceptually increase the efficacy of letrozole in 2 ways, by triggering ADCC directed at cancer cells or by decreasing tumor cell growth by abrogating the MUC1-dependent modulating effects on ER-mediated transcription.

The failure of this study to meet the primary objective shows a lack of clinical synergy between an anti-MUC1 agent and an aromatase inhibitor. The finding conflicts with preclinical data describing the modulation by MUC1 of the ER (8, 25). The mere inhibition of systemic estrogen production may not influence the regulation pathway affected by the modulation of ER by MUC1. It is conceivable that blockage of the ER, which may be achieved with the use of tamoxifen or fulvestrant, would synergize with AS1402. Therefore, combining AS1402 with tamoxifen or fulvestrant may yield different clinical results. Alternatively, one may conclude that the preclinical data could not be substantiated by this study and might call for redefining the significance of ER modulation by MUC1.

Other antibodies than AS1402 relying primarily on ADCC for tumor cell kill are particularly efficacious in certain patient subsets with specific *FcγIIIRa* and *FcγIIRa* allotypes (17–19). This study did not show enhanced efficacy, measured as change in tumor burden from baseline, in any patient subgroup defined by the tested allotypes. This was true for both study arms. It is therefore likely that ADCC does not contribute significantly to the efficacy of estrogen depletion in HR-positive breast cancer, irrespective of AS1402 treatment.

The lower response rate noted in the AS1402 + letrozole arm than in the letrozole-only arm was likely due to random chance in a small study, as the waterfall diagrams were similar for both arms (Fig. 3) and as the 95% CI for the difference in response rate contained 0.

The higher number of patients who withdrew consent during therapy in the AS1402 + letrozole arm may be explained by the requirement for weekly visits during therapy with AS1402.

Overall, the incidence of adverse events reported in the study was low compared with the results of previous reports on the safety of letrozole in the advanced breast cancer setting (4). This finding may be explained by the early termination of the study, which limited the duration of safety follow-up. Adverse events considered to be related to AS1402 were reported in only 32% of patients receiving AS1402 and all were of low grades. Liver enzyme elevation was noted in both arms, but primarily in patients with liver metastases and disease progression.

In conclusion, the combination of AS1402 and letrozole exhibited a favorable safety profile but insufficient efficacy to warrant further development at the tested dose and schedule in the setting of first-line therapy for HR-positive locally advanced or metastatic breast cancer treated with letrozole. Future clinical development of AS1402 for the treatment of MUC1-positive cancers will likely depend on using novel technologies that can enhance the tumor cell kill elicited by the antibody or on combining AS1402 with drugs other than letrozole.

## Disclosure of Potential Conflicts of Interest

F. Erlandsson, G. Acton, D. Jones, S. Senderovich, and A. Chau were all employees of Antisoma and owned Antisoma stock at the time that the article was written. In addition, M. Pegram and N.K. Ibrahim served at an Antisoma advisory board as Antisoma consultants. The other authors disclosed no potential conflicts of interest.

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