Cancer Antigen 15-3/Mucin 1 Levels in CCTG MA.32: A Breast Cancer Randomized Trial of Metformin vs Placebo

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Abstract

Background: Circulating levels of cancer antigen (CA) 15–3, a tumor marker and regulator of cellular metabolism, were reduced by metformin in a nonrandomized neoadjuvant study. We examined the effects of metformin (vs placebo) on CA 15–3 in participants of MA.32, a phase III randomized trial in early-stage breast cancer. Methods: A total of 3649 patients with T1-3, N0-3, M0 breast cancer were randomly assigned; pretreatment and 6-month on-treatment fasting plasma were centrally assayed for CA 15–3. Genomic DNA was analyzed for the rs11212617 single nucleotide polymorphism. Absolute and relative change of CA 15–3 (metformin vs placebo) were compared using Wilcoxon rank and t tests. Regression models adjusted for baseline differences and assessed key interactions. All statistical tests were 2-sided. Results: Mean (SD) age was 52.4 (10.0) years. The majority of patients had T2/3, node-positive, hormone receptor–positive, HER2-negative breast cancer treated with (neo)adjuvant chemotherapy and hormone therapy. Mean (SD) baseline CA 15–3 was 17.7 (7.6) and 18.0 (8.1 U/mL). At 6 months, CA 15–3 was statistically significantly reduced in metformin vs placebo arms (absolute geometric mean reduction in CA 15–3 = 7.7% vs 2.0%, P < .001; relative metformin: placebo level of CA 15–3 [adjusted for age, baseline body mass index, and baseline CA 15–3] = 0.94, 95% confidence interval = 0.92 to 0.96). This reduction was independent of tumor characteristics, perioperative systemic therapy, baseline body mass index, insulin, and the single nucleotide polymorphism status (all Ps > .11). Conclusions: Our observation that metformin reduces CA 15–3 by approximately 6% was corroborated in a large placebo-controlled randomized trial. The clinical implications of this reduction in CA 15–3 will be explored in upcoming efficacy analyses of breast cancer outcomes in MA.32.

Cancer antigen 15-3 (CA 15–3, the soluble moiety of the human oncoprotein, mucin 1 [MUC1]) is a transmembrane protein (composed of C and N terminal subunits that remain linked) with a heavily glycosylated extracellular domain that is present normally on many epithelial cells that has also been linked to metabolic reprogramming in cancer cells (1). Circulating CA 15–3 may be useful as a marker of prognosis and treatment response in breast cancer (2,3), but measurement of CA 15–3 is not recommended during follow-up of asymptomatic early breast cancer (4).

In a recent nonrandomized preoperative window-of-opportunity study (5) involving 39 breast cancer patients, we
identified a statistically significant reduction in CA 15–3 of 5% (95% confidence interval [CI] = −1% to −9%) (6) after metformin was administered for 2 weeks. Given that metformin has been postulated to improve breast cancer outcomes, acting indirectly through improvement of obesity-related physiology, notably insulin, or through a variety of direct antitumor effects, we sought to replicate this finding.

Here, we explore the effect of metformin (vs placebo) on levels of circulating CA 15–3 at baseline and 6 months in women enrolled in the Canadian Cancer Trials Group (CCTG) MA.32, a phase III randomized adjuvant trial of the effect of metformin vs placebo on invasive disease-free survival (IDFS) in high-risk, operable breast cancer (7), including the contribution of body mass index (BMI) and other metabolic factors to metformin effects. Based on reports that the minor allele (C) of rs11212617, a single nucleotide polymorphism (SNP) located near the ataxia telangiectasia mutated gene (ATM), may affect blood levels of metformin (8) and response to metformin in diabetic patients (9) as well as response to neoadjuvant therapy in HER2+ breast cancer (10), we also investigated whether the effect of metformin on CA 15–3 blood levels was affected by the genotype of this SNP.

Methods

Study Design

The CCTG MA.32 Clinical Trial (ClinicalTrials.gov identifier: NCT01101438; http://clinicaltrials.gov/show/NCT01101438) is a phase III randomized trial conducted in North America, the United Kingdom, and Switzerland that enrolled 3649 nondiabetic women receiving standard surgical, chemotherapeutic, hormonal, biologic, and radiation therapy for a T1-3, N0-3, M0 breast cancer diagnosed during the previous year (enrollment was between 2010 and 2013; those with T1a,b N0 breast cancer were not eligible). Patients with T1c N0 breast cancer were eligible if they had at least 1 of the following: histologic grade III, lymphovascular invasion, negative estrogen (ER) and progesterone (PgR) receptors, HER2 positivity, Oncotype Recurrence Score (80) of ≤18, 3%, 3%, and 4% for CA 15–3, insulin, leptin, and hsCRP, respectively. Glucose was analyzed immediately at local centers, and homeostasis model assessment (HOMA, a marker of insulin resistance) was calculated from glucose and insulin levels (glucose [mg/dL] × insulin [pmol/L]/22.5) (11). Metformin effects on blood variables other than CA 15–3 have been previously reported (12,13).

Blood for genomic analysis was drawn into ethylenediamine tetraacetic acid (EDTA) tubes that were aliquoted into 1.5-mL cryovials and stored at −80°C. One aliquot was sent on dry ice for genomic DNA extraction and genotyping for the SNP rs11212617 [Chr11(GRCh38): g.108412434C>T] at The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada, using a QIAsymphony magnetic bead DNA extraction (Qiagen, Germany) and PCR primers (S‘ACACACAGGAAAACATCCATAATACATATC3’ and 5’TATTAAGTGGTGTCTTGGATCTA3’) with TaqMan 100-mM dual-label minor groove binder (MGB) probes AGATCAGAGCTGTCCAGGAGT and AGATCAAAGATGTCAGGACG (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA).

Statistical Analyses

Statistical analyses were conducted by Drs Bingshu Chen (CCTG) and Marguerite Ennis using SAS Version 9.2. The population for this analysis included all patients who had provided blood samples at baseline (before initiation of study drug) and at 6 months (while on study drug). Patient and tumor characteristics at baseline (B) were tabulated by study arm; those included (vs excluded) from this analysis were compared using χ² tests for categorical variables and Wilcoxon rank sum tests for continuous variables. Baseline CA 15–3 levels were tabulated by baseline stage, receptor status, adjuvant treatment, and SNP status and compared using Wilcoxon rank sum tests. Spearman rank correlations with baseline weight, BMI, and CA 15–3 level was performed; when back-transformed, this gave the relative metformin:placebo levels in the 2 arms at 6 months corrected or standardized for differences in baseline BMI, BMI, and CA 15–3 level was performed; when back-transformed, this gave the relative metformin:placebo levels in the 2 arms at 6 months corrected or standardized for differences in baseline BMI, BMI, and CA 15–3, age, and BMI. By adding interaction terms to the regression model, we explored whether this outcome was differentially affected by each of baseline stage, receptor status,
adjuvant treatment, SNP status, BMI, or insulin level. A P value less than or equal to .05 was considered statistically significant, and all tests were 2-sided.

**Results**

**Study Population**

The assembly of patients in MA.32, on-treatment at 6 months and who had levels of CA 15–3 available at baseline and 6 months (CA 15–3 population) as well as genotyping information for rs11212617 (SNP population) is shown in the Consort diagram (Figure 1). Characteristics of the study population are shown in Table 1. Patients included in the CA 15–3 population (n = 2708) were more likely than excluded patients (n = 941) to have been on the placebo arm (51.7% vs 45.1%, P < .001), to be White (92.0% vs 86.4%, P < .001), to have ER- and/or PgR-positive breast cancer (70.6% vs 65.9%, P = .006), and to have received hormonal therapy (62.9% vs 57.1%, P = .002). These differences in exclusion rates may reflect our requirement that included patients be on study drug at 6 months, with available blood samples at both baseline and 6 months; greater toxicity on the metformin arm may have led to more frequent drug discontinuation at 6 months. Furthermore, differences in SNP rs11212617 allele distribution across racial groups may have led to differences in metformin levels and drug discontinuation rates at 6 months across racial groups.

Considering included patients, mean (SD) baseline age was 52.4 (10.0) years. Baseline and 6-month BMI were changed from 28.8 (6.6) and 28.2 (6.5) kg/m² in the metformin arm and 28.5 (6.1) to 28.7 (6.2) kg/m² in the placebo arm (Table 1) (change = −0.6 [1.4] vs 0.2 [1.6] kg/m², P < .001), respectively. Mastectomy was performed in 1357 (50.1%). In those receiving neoadjuvant therapy (n = 547), clinical tumor stage was T1 in 54 (9.9%), T2 in 312 (57.0%), and T3 in 181 (33.1%) while clinical N stage was N0 in 179 (32.7%) and N+ in 368 (67.3%). In those not receiving neoadjuvant therapy (n = 2161), pathologic tumor stage was T1 in 866 (40.1%), T2 in 1125 (52.1%), and T3 in 170 (7.9%), and pathologic N stage was N0 in 1035 (47.9%) and N+ in 1126 (52.1%). ER and/or PgR were positive in 1913 (70.6%) patients, and HER2 was positive in 464 (17.1%). Adjuvant or neoadjuvant treatment included chemotherapy in 2419 (89.3%), hormone therapy in 1706 (62.9%), and trastuzumab in 468 (17.3%) patients.

So, rs11212617 status was available for 2693 of the patients included in CA 15–3 analyses. Of these, 808 (30.0%) had the AA genotype, 1322 (49.1%) the CA genotype, and 563 (20.1%) the CC genotype (Table 1). Distributions were similar in the metformin and placebo arms.

**CA 15–3**

Plasma levels of CA 15–3 at baseline are shown in Table 2. Levels were similar in the 2 arms (mean [SD] = 17.7 [7.6] U/mL in metformin arm vs 18.0 [8.1] U/mL in placebo arm, P = .33) and most were not statistically significantly associated with T or N stage, HER2 status (which were determined at diagnosis, up to 1 year; mean [SD] = 9.2 [2.1] months) before study enrolment and before surgical excision and (neo)adjuvant chemotherapy, hormone therapy, and radiation. CA 15–3 was also not associated with treatment with adjuvant hormones or trastuzumab or with rs11212617 status. Baseline levels were statistically significantly lower in hormone receptor-negative vs –positive patients.
Table 1. Baseline patient and tumor characteristics according to the formation of the CA 15–3 population and by study arm

| Characteristics | Included vs excluded from CA 15–3 population | CA 15–3 population by study arm |
|-----------------|---------------------------------------------|---------------------------------|
| Treatment arm, No. (%) | Included (n = 2708) | Excluded (n = 941) | P<sup>a</sup> | Metformin (n = 1307) | Placebo (n = 1401) |
| Metformin | 1307 (48.3) | 517 (54.9) | — | — |
| Placebo | 1401 (51.7) | 424 (45.1) | — | — |
| Total | 2708 (100) | 941 (100) | — | — |
| Age, mean (SD), y | 52.41 (10.01) | 52.25 (10.3) | .73 | 52.11 (10.0) | 52.7 (10.1) |
| BMI, mean (SD), kg/m² | 28.7 (6.3) | 28.6 (6.6) | .42 | 28.8 (6.6) | 28.5 (6.1) |
| Race, No. (%) | | | | | |
| Asian | 65 (2.4) | 34 (3.6) | 27 (2.1) | 38 (2.7) |
| Black or African American | 99 (3.7) | 68 (7.2) | 48 (3.7) | 51 (3.6) |
| Native Hawaiian, or Pacific Islander | 24 (0.9) | 6 (0.6) | 12 (0.9) | 12 (0.9) |
| White | 2491 (92.0) | 813 (86.4) | 1206 (92.3) | 1285 (91.7) |
| Not reported (or refused) or unknown | 29 (1.1) | 20 (2.1) | 14 (1.1) | 15 (1.1) |
| T stage (any neoadjuvant), No. (%) | .36 | | | | |
| cT1a+cT1b+cT1c | 54 (9.9) | 30 (13.4) | 16 (6.3) | 38 (12.9) |
| cT2 | 312 (57.0) | 122 (54.5) | 153 (60.5) | 159 (54.1) |
| cT3 | 181 (33.1) | 72 (32.1) | 84 (33.0) | 97 (33.0) |
| Total | 547 (100) | 224 (100) | 253 (100) | 294 (100) |
| N stage (any neoadjuvant), No. (%) | .58 | | | | |
| cN0 | 179 (32.7) | 78 (34.8) | 79 (31.2) | 100 (34) |
| cN1+cN2+cN3 | 368 (67.3) | 146 (65.2) | 174 (68.8) | 194 (66.0) |
| Total | 547 (100) | 224 (100) | 253 (100) | 294 (100) |
| T stage (no neoadjuvant), No. (%) | .12 | | | | |
| T1a+T1b+T1c+T1mic | 866 (40.1) | 289 (40.3) | 409 (43.8) | 457 (41.3) |
| T2 | 1125 (52.1) | 384 (53.6) | 555 (53.7) | 570 (51.5) |
| T3 | 170 (7.9) | 43 (6.0) | 90 (8.5) | 80 (7.2) |
| T4 | 0 (0) | 1 (0.1) | 0 (0) | 0 (0) |
| Total | 2161 (100) | 717 (100) | 1054 (100) | 1107 (100) |
| N stage (no neoadjuvant), No. (%) | .13 | | | | |
| pN0+pN0(i+) | 1035 (47.9) | 320 (44.6) | 490 (46.5) | 545 (49.2) |
| pN1+pN1mi+pN2+pN3 | 1126 (52.1) | 397 (55.4) | 564 (53.5) | 562 (50.8) |
| Total | 2161 (100) | 717 (100) | 1054 (100) | 1107 (100) |
| Hormone receptor status, No. (%) | .006 | | | | |
| ER-negative and PgR-negative | 795 (29.4) | 321 (34.1) | 372 (28.5) | 423 (30.2) |
| ER-positive and/or PgR-positive | 1913 (70.6) | 602 (65.9) | 935 (71.5) | 978 (69.8) |
| Total | 2708 (100) | 941 (100) | 1307 (100) | 1401 (100) |
| HER2 status, No. (%) | .70 | | | | |
| Negative | 2244 (82.9) | 785 (83.4) | 1078 (82.5) | 1166 (83.2) |
| Positive | 464 (17.1) | 156 (16.6) | 229 (17.5) | 235 (16.8) |
| Total | 2708 (100) | 941 (100) | 1307 (100) | 1401 (100) |
| Most extensive primary surgery, No. (%) | .68 | | | | |
| Mastectomy | 1357 (50.1) | 479 (50.9) | 690 (52.8) | 667 (47.6) |
| Partial mastectomy, lumpectomy, or excisional biopsy | 1351 (49.9) | 462 (49.1) | 617 (47.2) | 734 (52.4) |
| Total | 2708 (100) | 941 (100) | 1307 (100) | 1401 (100) |
| Adjuvant chemotherapy, No. (%) | .23 | | | | |
| Missing | 0 (0) | 1 (0.1%) | 0 (0) | 0 (0) |
| No | 289 (10.7) | 102 (10.8) | 135 (10.3) | 154 (11.0) |
| Yes neoadjuvant and/or yes postoperative | 2419 (89.3) | 838 (89.1) | 1172 (89.7) | 1247 (89.0) |
| Total | 2708 (100) | 941 (100) | 1307 (100) | 1401 (100) |
| Adjuvant hormone therapy, No. (%) | .002 | | | | |
| Missing | 1005 (37.1) | 404 (42.9) | 475 (36.3) | 530 (37.8) |
| No | 1706 (62.9) | 538 (57.1) | 832 (63.7) | 871 (62.2) |
| Yes neoadjuvant and/or yes postoperative | 2711 (100) | 942 (100) | 1307 (100) | 1401 (100) |
| Total | 2708 (100) | 941 (100) | 1307 (100) | 1401 (100) |

(continued)
Sample for rs11212617 SNP, No. (%)

| Characteristics | Included (n = 2708) | Excluded (n = 941) | P* |
|-----------------|---------------------|-------------------|----|
| Available       | 2693 (99.4)         | 633 (67.3)        | <.001|
| Unavailable     | 15 (0.6)            | 308 (32.7)        | .32 |
| Total           | 2708 (100)          | 941 (100)         |    |
| rs11212617 SNP, No. (%) |
| AA              | 808 (30.0)          | 194 (30.6)        | 392 (30.1) |
| CA              | 1322 (49.1)         | 292 (46.1)        | 645 (49.6) |
| CC              | 563 (20.9)          | 147 (23.2)        | 264 (20.3) |
| Total, No. (%)  | 2693 (100)          | 633 (100)         | 1301 (100) |

*Statistical tests: χ² tests for categorical variables and Wilcoxon rank sum tests for continuous variables. All P values are 2-sided. A = A allele of the rs11212617 SNP; BMI = body mass index; C = C allele of the rs11212617 SNP; CA = cancer antigen; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; N = nodal stage; PgR = progesterone receptor; SNP = single nucleotide polymorphism; T = tumor stage.

**Notes**
- (mean [SD] = 17.6 [7.6] vs 18.5 [8.5] U/mL, P = .03) and in those who had not received (vs had received) (neo)adjuvant chemotherapy (mean [SD] = 16.6 [6.5] vs 18.0 [8.0] U/mL, P = .03). The Spearman correlations of baseline CA 15–3 with baseline weight, BMI, insulin, glucose, HOMA, leptin, and hsCRP were low (0.03, 0.04, 0.05, 0.03, 0.06, 0.04, and 0.07, respectively); all Ps less than .11 (data not shown).
- Considering change in CA 15–3, geometric means (used because of skewness in the distribution of CA 15–3 at follow-up) showed a 7.7% reduction in CA 15–3 levels in the metformin arm vs a 2.0% reduction in the placebo arm (P < .001; Table 3). After correcting for differences in baseline CA 15–3, age, and BMI, the relative metformin:placebo level of CA 15–3 at 6 months was estimated to be 0.94 (95% CI = 0.92 to 0.96).

**Discussion**
Using data from a large prospective randomized trial, we have confirmed our earlier observation that metformin is associated with a reduction in circulating levels of CA 15–3, independent of T and N stage, ER/PgR, HER2, and perioperative systemic treatment received as well as baseline BMI, fasting insulin, and
rs11212617 status. The observed reduction was modest (just less than 6% during 6 months); the modest reduction may reflect, in part, the low mean baseline levels of CA 15–3 in both metformin and placebo arms (17.7 and 18.0 U/mL, respectively). The clinical relevance of the observed reduction is unclear; it will be explored in future efficacy analyses of MA.32.

To our knowledge, our work is the first demonstration of reduction in CA 15–3 by metformin in the clinical breast cancer setting. An effect of metformin on MUC1 expression has been previously reported in preclinical studies. Metformin (in combination with salomargine) has been reported to lead to AMP-activated protein kinase–mediated suppression of MUC1 expression in castration-resistant prostate cancer cells (14). Metformin has also been found to reduce insulin-mediated increases in MUC1 expression in diabetic rat models (15). These observations are consistent with current understanding of metformin effects in cancer, notably 1) direct effects, including liver kinase B1–mediated activation of AMP-activated protein kinase, a negative regulator of phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling and protein synthesis; and 2) indirect (insulin-mediated) effects leading to reduced signaling through PI3K and ras pathways (16). Observations that transcriptome reprogramming that included increased expression of MUC1 (among other genes) is associated with in vitro acquired resistance to metformin in breast cancer suggest MUC1 may potentially modulate metformin effects in breast cancer (17).

There is an evolving understanding of the biologic effects of CA 15–3/MUC1. Aberrantly glycosylated and sialylated MUC1 is overexpressed in cancer cells. MUC1 causes transcriptional changes that lead to metabolic reprogramming, interacting with both p53 and HIF-1 alpha and leading to changes in metabolic flux during glycolysis and the pentose phosphate and tricarboxylic pathways (1,18,19). Tumor-associated MUC1 expression directly promotes cancer growth and invasion and reduces apoptosis. Thus, it is possible reductions in CA 15–3/MUC1 may enhance other beneficial effects of metformin in cancer.

It is not clear whether the reductions in CA 15–3 we have identified reflect direct biologic effects of metformin on MUC1 expression (with potential subsequent MUC1-mediated beneficial effects on breast cancer outcomes) or whether they reflect metformin-induced reductions in the burden of microscopic cancer in our breast cancer patients. In a recent study, circulating levels of CA 15–3/MUC1 in patients with newly diagnosed but unresected breast cancer were statistically significantly correlated with metabolic tumor volume and tumor lesion glucose on FDG-PET, providing evidence that CA 15–3 levels can reflect tumor burden (20). Additionally, in a case-control study that was nested in a cohort of patients who had undergone treatment for operable breast cancer, increases of more than

### Table 3. Change in CA 15–3 (U/mL) after 6 months of treatment with the study drug (metformin or placebo)*

| CA 15–3       | Metformin (n = 1307) | Placebo (n = 1401) | P     |
|---------------|----------------------|--------------------|-------|
| Baseline geometric mean (SD) | 16.12 (1.55) | 16.35 (1.57) | —     |
| Follow-up geometric mean (SD) | 14.94 (1.54) | 16.02 (1.60) | —     |
| Change (follow-up—baseline)/baseline, % | —7.7 | -2.0 | <.001b |
| Metformin/placebo standardized ratio (95% confidence interval) | 0.94 (0.92 to 0.96) | <.001c |

*aGeometric means, percent change, and the metformin/placebo standardized ratio, which gives the relative levels in the 2 arms at 6 months, were corrected for differences in baseline CA 15–3, age, and body mass index. CA = cancer antigen. 

*bPercent change: study arms compared using a t test applied to log-change, adjusted for baseline differences in the variable, body mass index, and age. 

*cStandardized ratio: study arms compared using a regression model for log-change, adjusted for baseline differences in the variable, body mass index, and age.
analyses of the MA.32 trial. This velocity is similar to the change of 1.18 U/mL during 6 months we observed (extrapolating to 2.36 U/mL during 12 months), and it suggests that even small changes in CA 15–3 during a short period of time have the potential to be clinically important. Our failure to find an association between baseline CA 15–3 levels and tumor size or uninvolved (vs involved) axillary nodes does not preclude an association of CA 15–3 with tumor and nodal stage at diagnosis. It may simply reflect the fact that ascertainment of tumor stage occurred before surgical excision and adjuvant systemic and radiation therapy, whereas CA 15–3 was measured up to 1 year later (mean = 9.1 months), after these treatments (which were administered to reduce both macroscopic and microscopic cancer) had been administered. Thus, it is possible (although not proven) that the modest reduction in CA 15–3 we observed may reflect reduced tumor burden. If this is correct, or if the reductions in CA 15–3 led to direct antitumor effects that were independent of burden of microscopic disease, fewer recurrences should be seen in those experiencing reductions in CA 15–3. This will be explored in upcoming efficacy analyses in MA.32.

The clinical utility of our findings will be explored in future planned analyses investigating the effects of metformin-induced CA 15–3 reduction on breast cancer outcomes in MA.32. In the meantime, we believe our findings are novel and of relevance to ongoing clinical research. We anticipate they will lead to attempts to replicate our observations in other settings and stimulate research into the mechanisms by which metformin lowers CA 15–3. Importantly, our findings may be of relevance in both the metastatic breast cancer setting (where CA 15–3 levels may guide therapy) and in situations when metformin is administered to manage treatment-induced hyperglycemia (eg, with the PI3K-alpha inhibitor apelisib), where CA 15–3 levels may reflect both metformin effect and tumor response.

Strengths of our study include its conduct in a large prospective randomized clinical trial, with detailed information on tumor and treatment characteristics, body size, and key metabolic variables. Limitations include our inability to examine effects of metformin (vs placebo) on MUC1 expression in tumor tissue or to examine the correlation of CA 15–3 change with other potential markers of microscopic tumor burden, including disseminated tumor cells in bone marrow, circulating tumor cells, or cell-free tumor DNA.

In conclusion, we have confirmed our earlier observation that metformin modestly reduces CA 15–3 independent of tumor and treatment characteristics. We will examine the potential impact of metformin-induced reductions in CA 15–3 on breast cancer outcomes, including IDFS, in upcoming efficacy analyses of the MA.32 trial.

Funding

This work was supported by the Canadian Cancer Society Research Institute (#021039); National Cancer Institute (US) (#CA077202, CA180868, CA180822); The Breast Cancer Research Foundation (New York); Canadian Breast Cancer Foundation—Ontario Region, Ontario Institute for Cancer Research (#10NOV-467); Apotex Canada (in kind donation of placebo and metformin); and Hold’em for Life Charity.

Notes

Role of the funders: The study sponsors have no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

Disclosures: Dr Whelan reports non-financial support from Genomic Health, outside the submitted work. Dr Ryan Dowling is currently an employee at Hoffmann-La Roche Limited, Mississauga, ON. Dr Julie Lemieux reports honoraria from Novartis, Pfizer, Eli Lilly. Dr Ingrid Mayer reports honoraria from Novartis, grants and personal fees from Pfizer, grants and personal fees from Genentech, personal fees from Lilly, personal fees from Puma, personal fees from Abbvie, personal fees from Immunomedics, personal fees from Macrogenics, personal fees from Seattle Genetics, personal fees from Astra-Zeneca, personal fees from GSK. All of these are outside the submitted work. The other authors report no conflicts of interest.

Author contributions: Pamela J. Goodwin—Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing—original draft preparation, Writing—review and editing, Visualization, Supervision, Project administration, Funding acquisition. Ryan J.O. Dowling—Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing—original draft preparation, Writing—review and editing, Visualization, Supervision, Project administration. Marguerite Ennis—Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Writing—original draft preparation, Writing—review and editing, Visualization. Bingshu E. Chen—Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Writing—original draft preparation, Writing—review and editing, Visualization. Wendy R. Parulekar—Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing—original draft preparation, Writing—review and editing, Funding acquisition. Lois E. Shepherd—Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing—original draft preparation, Writing—review and editing. Karen A. Gelmon—Investigation, Writing—review and editing. Timothy J. Whelan—Investigation, Writing—review and editing. Jennifer A. Ligibel—Investigation, Writing—review and editing. Dawn L. Hershman—Investigation, Writing—review and editing. Ingrid A. Mayer—Investigation, Writing—review and editing. Timothy J. Hobday—Investigation, Writing—review and editing. Priya Rastogi—Investigation, Writing—review and editing. Manuela Rabaglio-Poretti—Investigation, Writing—review and editing. Julie Lemieux—Investigation, Writing—review and editing. Alastair M. Thompson—Investigation, Writing—review and editing. Daniel W. Rea—Investigation, Writing—review and editing. Vuk Stambolic—Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing—original draft preparation, Writing—review and editing, Visualization, Supervision, Project administration, Funding acquisition.

Data Availability

The primary efficacy analysis will be available from the Canadian Cancer trials Group (Kingston, Ontario) after the results of the analysis have been published. The associated data will be uploaded to the NCI data archive website: http://nctn-data-archive.nci.nih.gov/view-trials and will be searchable via NCT Trial Number NCT1101438. Further information regarding
that analysis and the data analyzed in this sub-study can be obtained from the corresponding author.

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