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Article

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

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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 (Clinical Trials.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 of at least 25, or Ki-67 greater than 14%. In May 2012, after 2382 patients were enrolled, eligibility criteria were amended to mandate triple-negative (ER negative, PgR negative, HER2 negative) status for patients with T1cN0 disease and at least 1 of the above adverse tumor characteristics for patients with T2N0 tumors. Patients were required to have a fasting glucose of 7.0 mmol/L or lower; those with a history of diabetes, lactic acidosis, current use of diabetes medication, breast cancer recurrence or previous invasive cancer, excessive alcohol intake, or marked hepatic, kidney, or cardiac dysfunction were excluded. Patients were randomly assigned to receive metformin 850 mg caplets po bid or an identical placebo po bid for 5 years; they provided a fasting blood specimen before initiating study drug treatment and at 6 months. Height (baseline) and weight (baseline, 6 months) were measured at study centers. The primary study outcome, IDFS, as well as secondary outcomes, including overall survival and breast cancer–free interval, have not yet been reported.

The study protocol was approved by the Adult Central Institutional Review Board (National Institutes of Health, USA) and the Ontario Cancer Research Ethics Board (Ontario, Canada) and by institutional review boards of the participating institutions. All patients provided written informed consent to participate.

Laboratory Analyses

Blood was drawn into plasma tubes, separated into 1-mL aliquots and frozen at −80°C. Undiluted paired aliquots (baseline, 6 months) were assayed (blinded to treatment allocation) at Mount Sinai Hospital in Toronto in batches with 10% random repeats for: 1) CA 15–3 using the Roche electrochemiluminescence assay (range 1.00-300 U/mL; value <25 U/mL in 88% and 85% of patients with stage I and II breast cancer, respectively), 2) insulin (Roche, electrochemiluminescence assay), 3) leptin (Luminex Milliplex MAP assay), and 4) highly sensitive C-reactive protein (hsCRP) (Roche, particle-based immunoturbidimetric assay). Intra-assay coefficients of variability were 1.2% to 1.5%, 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>A] at The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada, using a QIAsymphony magnetic bead DNA extractor (Qiagen, Germany) and PCR primers (5'ACAAACAGGAAACAATTACAAATACAATAAAT3' and 5'TTAA AGTGGGTTGCTTGTGGATAA3') with TaqMan 100-mM dual-label minor groove binder (MGB) probes AGATCAGAGAC**C**TGTCAGAGC and AGATCA GAGA**A**TGTCAGAGC (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 χ^2 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, insulin, glucose, HOMA, leptin, and hsCRP were calculated. CA 15–3 at 6-month follow-up (F) had a skew distribution; therefore, a log-transformation was used. For change in CA 15–3, the average log-change [log(F)-log(B)] was calculated and the arms compared via a *t* test. An effect size measure was obtained by back-transforming the log-change averages to geometric means F/B and calculating percent relative change as $(F - B)/B \times 100$. Using linear regression with log-change as outcome, a further comparison that adjusted the study drug effect for baseline age, 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 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/m^2 in the metformin arm and 28.5 (6.1) to 28.7 (6.2) kg/m^2 in the placebo arm (Table 1) (change = −0.6 [1.4] vs 0.2 [1.6] kg/m^2, $P < .001$), 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

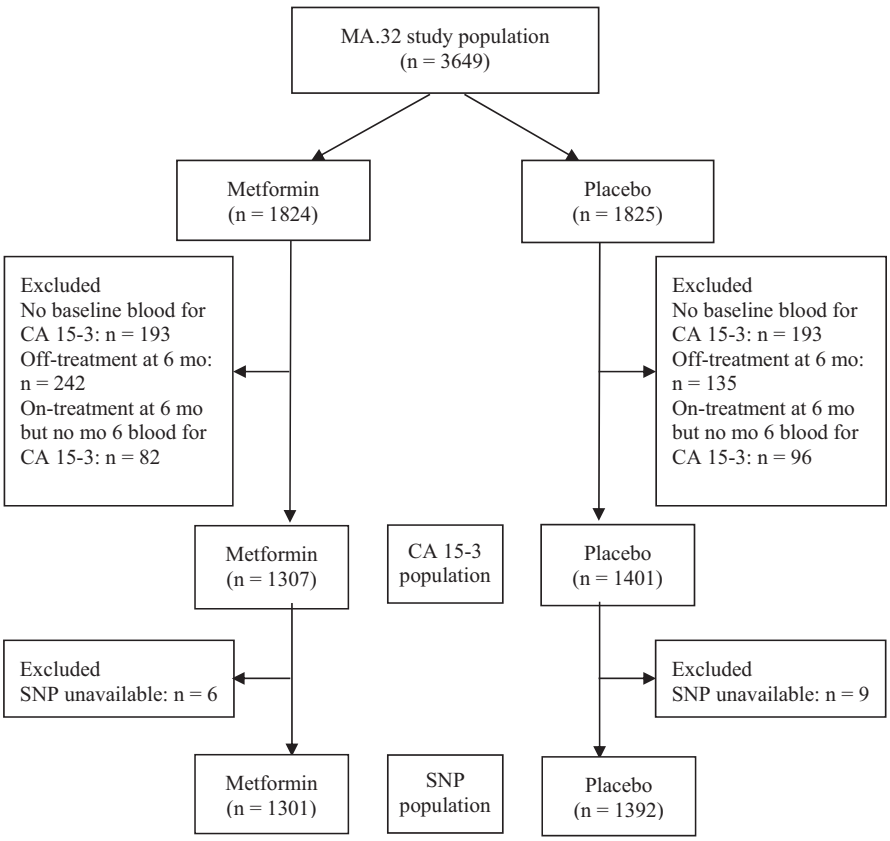

Figure 1. CONSORT diagram. MA32 patients included in cancer antigen (CA) 15–3 and SNP analyses. SNP = single nucleotide polymorphism.

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	
	Included (n = 2708)	Excluded (n = 941)	P[a]	Metformin (n = 1307)	Placebo (n = 1401)
Treatment arm, No. (%)			<.001		
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/m^2	28.7 (6.3)	28.6 (6.6)	.42	28.8 (6.6)	28.5 (6.1)
Race, No. (%)			<.001		
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)
America Indian, Alaska Native, 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)
Total	2708 (100)	941 (100)		1307 (100)	1401 (100)
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 (33.2)	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 (38.8)	457 (41.3)
T2	1125 (52.1)	384 (53.6)		555 (52.7)	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)	620 (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		
No	1005 (37.1)	404 (42.9)		475 (36.3)	530 (37.8)
Yes neoadjuvant and/or yes postoperative	1706 (62.9)	538 (57.1)		832 (63.7)	871 (62.2)
Total	2711 (100)	942 (100)		1307 (100)	1401 (100)
Adjuvant trastuzumab, No. (%)			.85		
No	2240 (82.7)	781 (83)		1077 (82.4)	1163 (83)
Yes	468 (17.3)	160 (17)		230 (17.6)	238 (17)
Total	2708 (100)	941 (100)		1307 (100)	1401 (100)

(continued)

Table 1. (continued)

Characteristics	Included vs excluded from CA 15–3 population			CA 15–3 population by study arm	
	Included (n = 2708)	Excluded (n = 941)	P[a]	Metformin (n = 1307)	Placebo (n = 1401)
Sample for rs11212617 SNP, No. (%)			<.001		
Available	2693 (99.4)	633 (67.3)		1301 (99.5)	1392 (99.4)
Unavailable	15 (0.6)	308 (32.7)		6 (0.5)	9 (0.6)
Total	2708 (100)	941 (100)		1307 (100)	1401 (100)
rs11212617 SNP, No. (%)			.32		
AA	808 (30.0)	194 (30.6)		392 (30.1)	416 (29.9)
CA	1322 (49.1)	292 (46.1)		645 (49.6)	677 (48.6)
CC	563 (20.9)	147 (23.2)		264 (20.3)	299 (21.5)
Total, No. (%)	2693 (100)	633 (100)		1301 (100)	1392 (100)

[a]Statistical tests: χ^2 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.

Table 2. CA 15–3 (U/mL) levels at baseline by study arm, T- and N-stage, receptor status, adjuvant treatment, and SNP status[a]

Description	Group	No.	Mean (SD)	Median (Q1, Q3)	P[b]
Study arm	Metformin	1307	17.7 (7.6)	17 (12, 22)	.33
	Placebo	1401	18.0 (8.1)	17 (12, 22)	
T-stage clinical	1	54	17.8 (8.0)	17 (12, 22)	.47
	2,3	493	16.8 (6.9)	16 (12, 21)	
T-stage pathologic	1	866	17.7 (7.6)	17 (12, 22)	.29
	2,3,4	1295	18.3 (8.4)	17 (12, 22)	
N-stage clinical	0	179	17.2 (7.2)	16 (12, 21)	.56
	1,2,3	368	16.7 (6.9)	16 (11, 21)	
N-stage pathologic	0	1035	18.2 (8.0)	17 (12, 22)	.50
	1,2,3	1126	18.0 (8.2)	17 (12, 22)	
ER or PgR status	Either positive	795	18.5 (8.5)	17 (12, 23)	.03
	Both negative	1913	17.6 (7.6)	17 (12, 22)	
HER2 status	Negative	2244	18.0 (8.0)	17 (12, 22)	.28
	Positive	464	17.3 (7.2)	17 (12, 21)	
(Neo)Adjuvant hormones	None	1012	18.4 (8.7)	17 (12, 23)	.08
	Any	1696	17.5 (7.4)	17 (12, 21)	
(Neo)Adjuvant chemotherapy	None	289	16.6 (6.5)	16 (12, 21)	.03
	Any	2419	18.0 (8.0)	17 (12, 22)	
(Neo)Adjuvant trastuzumab	None	2240	18.0 (8.0)	17 (12, 22)	.32
	Any	468	17.4 (7.3)	17 (12, 21)	
SNP rs11212617	AA	808	17.4 (7.5)	17 (12, 21)	.11
	Any C	1885	18.0 (8.1)	17 (12, 22)	

[a]For stage, patients were split by whether they received neo-adjuvant treatment (n = 547, clinical stage) or not (n = 2161, pathologic stage). AA = 2 A alleles of the rs11212617 SNP; C = at least one 1 C allele of the rs11212617 SNP; CA = cancer antigen; ER = estrogen receptor; PgR = progesterone receptor; Q1 = 25th percentile; Q3 = 75th percentile; SNP = single nucleotide polymorphism.
[b]P values are from Wilcoxon rank sum tests with null hypothesis that the distributions of the 2 groups are equal. All statistical tests were 2-sided.

(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). Interaction models showed that this outcome was not affected differentially depending on baseline stage, receptor status, adjuvant treatment, SNP status, BMI, or insulin level (see Figure 2).

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

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

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 (1.6)	—
Change (follow-up-baseline)/baseline, %	−7.7	−2.0	<.001[b]
Metformin:placebo standardized-ratio (95% confidence interval)	0.94 (0.92 to 0.96)		<.001[c]

[a]Geometric 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.
[b]Percent change: study arms compared using a *t* test applied to log-change, adjusted for baseline differences in the variable, body mass index, and age.
[c]Standardized ratio: study arms compared using a regression model for log-change, adjusted for baseline differences in the variable, body mass index, and age.

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 solamargine) 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 phosphatidylinosytol 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

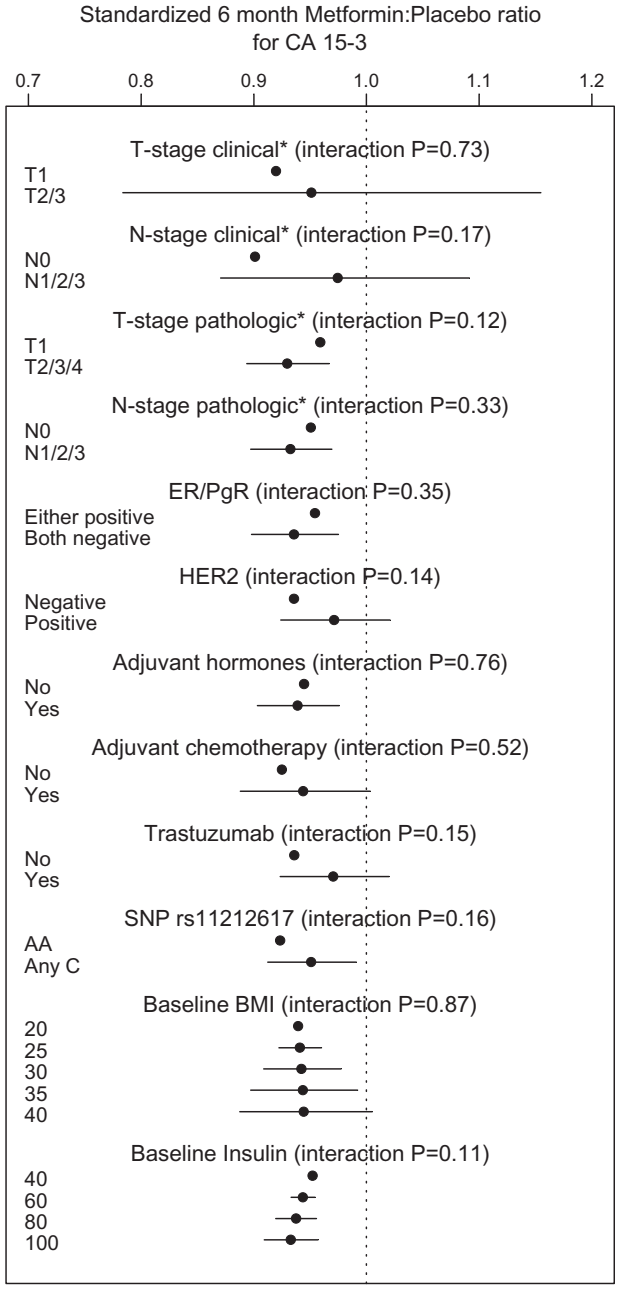

Figure 2. Assessing whether the study drug had a differential effect on cancer antigen (CA) 15–3 depending on baseline stage, receptor status, adjuvant treatment, single nucleotide polymorphism (SNP) status, body mass index (BMI) (kg/m^2) or insulin (U/mL) level. Depicted is the standardized metformin to placebo ratio of CA 15–3 at 6 months, with 95% confidence intervals, obtained from adjusted regression models that included interaction terms to model the differential effect. Clinical stage was used for 547 patients who received neo-adjuvant treatment. Pathologic stage was used for 2161 patients who did not receive neo-adjuvant treatment. ER = estrogen receptor; HER 2 = human epidermal growth factor receptor 2; N = nodal stage; PgR = progesterone receptor; T = tumor stage.

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

2.5 U/mL of CA 15–3 during 12 months were statistically significant predictors of recurrence ($P = .01$) (21). 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.

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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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