The effect of metformin vs placebo on sex hormones in CCTG MA.32

Isabel Pimentel MD[1], Bingshu E. Chen PhD[2], Ana Elisa Lohmann PhD[3], Marguerite Ennis PhD[4], Jennifer Ligibel MD[5], Lois Shepherd MD[6], Dawn L. Hershman MD[7], Timothy Whelan MD[8], Vuk Stambolic PhD[9], Ingrid Mayer MD[10], Timothy Hobday MD[11], Julie Lemieux MD[12], Alastair Thompson MD[13], Priya Rastogi MD[14], Karen Gelmon MD[15], Daniel Rea PhD[16], Manuela Rabaglio MD[17], Susan Ellard MD[18], Mihaela Mates MD[19], Philippe Bedard MD[9], Lacey Pitre MD[20], Theodore Vandenberg MD[21], Ryan JO Dowling PhD[9], Wendy Parulekar MD[22], Pamela J. Goodwin MD[23]

Correspondence: Isabel Pimentel

Medical Oncology Department

Vall d`Hebron Institute of Oncology (VHIO)

Paseo Vall d'Hebron 119-129, 08035 Barcelona, Spain

E-mail: ipimentel@vhio.net

Affiliations: [1] Vall d`Hebron Institute of Oncology (VHIO), Barcelona, Spain; [2] Canadian Cancer Trials Group, Queen's University – Cancer Research Institute, Kingston ON, Canada; [3] University of Western Ontario, ON Canada; [4] Applied Statistician, 9227, Kennedy Road, Markham; ON, Canada; [5] Dana-Farber Cancer Institute, Boston, USA; [6] Canadian Cancer Trials Group, Queen's University – Cancer Research Institute, Kingston ON, Canada; [7] Herbert Irving Cancer Center, Columbia University, New York, USA; [8] Juravinski Cancer Centre at Hamilton Health Sciences, Hamilton ON, Canada; [9] University Health Network, Princess Margaret Hospital, Toronto, ON, Canada; [10] Vanderbilt University, Vanderbilt - Ingram Cancer Center,

Nashville TN, USA; [11] Mayo Clinic, Rochester, USA; [12] CHA-Hopital Du St-Sacrement, Hopital Enfant Jesus Site, Quebec City, Canada; [13] Baylor College of Medicine, Houston, Texas, USA; [14] National Surgical Adjuvant Breast and Bowel Project (NSABP), Pittsburgh PA, USA; [15] BCCA – Vancouver Cancer Centre, Vancouver BC, Canada; [16] Institute of Cancer Research, Clinical Trials and Statistics Unit, Sutton, UK; [17] IBCSG Coordinating Centre, Bern, Switzerland; [18] BCCA - Cancer Centre for the Southern Interior, Kelowna BC, Canada; [19] Cancer Centre of Southeastern Ontario, Kingston ON, Canada; [20] Health Sciences North, Sudbury ON, Canada; [21] London Regional Cancer Program, London ON, Canada; [22] Canadian Cancer Trials Group, Queen's University – Cancer Research Institute, Kingston ON, Canada; [23] Lunenfeld Tanenbaum Research Institute at Mount Sinai Hospital, University of Toronto, Canada.

Abstract

Background: Metformin has been associated with lower breast cancer risk and improved outcomes in observational studies. Multiple biologic mechanisms have been proposed, including a recent report of altered sex hormones (SHs). We evaluated the effect of metformin on SHs in MA.32, a phase III trial of nondiabetic BC subjects randomized to metformin or placebo.

Methods: We studied the subgroup of post-menopausal hormone receptor negative BC subjects not receiving endocrine treatment who provided fasting blood at baseline and at 6 months after randomization. Sex hormone binding globulin (SHBG), bioavailable testosterone (BT) and estradiol levels were assayed using ECLIA (electrochemiluminescense immunoassay). Change from baseline to 6 months between study arms was compared using Wilcoxon sum rank tests and regression models.

Results: 312 women were eligible (141 metformin vs 171 placebo); the majority of subjects in each arm had T1/2, N0, HER2 negative BC and had received (neo)adjuvant chemotherapy. Mean age ± SD was 58.1±6.9 vs 57.5±7.9 years, mean BMI was 27.3±5.2 vs 28.9±6.4 kg/m^2 for metformin vs placebo respectively. Median estradiol decreased between baseline and 6 months on metformin vs placebo (-5.7 vs 0 pmol/L; $p<0.001$) in univariable analysis and after controlling for baseline BMI and BMI change ($p<0.001$). There was no change in SHBG or BT.

Conclusion: Metformin lowered estradiol levels, independent of BMI. This observation suggests a new metformin effect that has potential relevance to estrogen sensitive cancers.

Metformin has garnered attention as a potential anti-cancer agent across a range of cancers, including breast cancer (BC); potential effects on BC outcomes are being studied in CCTG MA.32, an ongoing Phase 3 adjuvant trial comparing metformin 850 mg bid vs placebo bid (each given for 5 years) in subjects receiving standard breast cancer therapy.[1] It has been postulated that metformin may impact BC directly (for example, via intra-tumoral LKB1 mediated AMPK activation leading to suppression of mTORC1 signaling) and/or indirectly (for example, via inhibition of hepatic gluconeogenesis with subsequent reduction in circulating insulin levels, reducing PI3K/Akt/mTOR signaling in cancer cells expressing the insulin receptor).[2] Data from neoadjuvant clinical trials have provided some support for both direct and indirect mechanisms.[3,4] Recent research has suggested metformin may also act indirectly via an effect on sex hormones (SHs), although findings have been inconsistent. [5-8]

SHs are of relevance to both BC risk and prognosis, particularly in post-menopausal women. [9-12] In a case control study nested in the Women´s Healthy Eating and Living Study (WHEL), BC patients who recurred had higher levels of estrogens than those who did not recur (22.7 vs 10.8 pg/mL; $p = 0.05$).[10] The importance of SHs in hormone receptor positive BC is highlighted by the therapeutic effectiveness of aromatase inhibitors, which reduce estrogen production in postmenopausal women.

Campagnoli et al.[5,6] studied the effect of metformin (1500 mg/day vs 1000 mg/day after a one-month run-in of 1000 mg/day) on SHs in 96 non diabetic post-menopausal BC patients (50% of whom were on tamoxifen) with pre-baseline testosterone above 0.28 ng/ml. Metformin reduced estradiol (-38%; $p< 0.02$) and free testosterone (-29%; $p< 0.01$); these differences remained statistically significant after controlling for baseline BMI and weight change.

Patterson et al.[7] used a 2 X 2 factorial design to randomize 313 overweight or obese postmenopausal BC patients to metformin 1500mg/daily vs placebo and a lifestyle weight loss program vs control. Metformin (vs placebo) lowered estradiol (-10%, 95% CI, -18.5 to -1.5%) and testosterone (-9.5%, 95%CI, -15.2% to -3.8%) and increased sex hormone binding globulin (SHBG) (+7.5%; 95% CI, 2.4-12.6%) levels. However, estradiol appeared to be reduced only in those receiving both metformin and the lifestyle intervention.[8] In a final study conducted in 382 overweight, glucose-intolerant patients without BC enrolled onto the Diabetes Prevention Program (DPP),[13] metformin had no impact on SHBG, estradiol, testosterone or dehydroepiandrosterone.

We investigated the SHs in postmenopausal women with hormone receptor negative BC (selected to avoid use of endocrine therapies that may impact SHs) enrolled onto the MA.32 trial. We also explored effects of the minor allele (C) of the rs11212617 single nucleotide polymorphism (SNP) that has been associated with greater metabolic response to metformin in type 2 diabetes,[14] and increased pathologic response to neoadjuvant metformin in early stage Her2 positive BC.[15]

Methods

Design

We conducted a sub-study of patients enrolled onto the MA.32 randomized phase III clinical trial.[1] The focus on SHs was not part of the original trial protocol; as evidence of an effect of metformin on SHs emerged, this work was approved by the trial steering committee as part of a priori plan to investigate potential mechanisms of metformin action. The primary objective of this sub-study was to compare change in levels of pre-specified SHs (Estradiol, SHBG and Bioavailable Testosterone (BT) from baseline to 6

months between metformin and placebo arms; if a difference between study arms was found, secondary objectives were to explore two possible pathways of metformin action namely BMI and insulin change as well as the impact of the SNP rs11212617 on any SH changes.

Study Population

The Canadian Cancer Trials Group (CCTG) MA.32 Clinical Trial (Clinical Trials.gov identifier: NCT01101438 and EudraCT number: 2011-005230-18)[1] is a phase III, double blind trial that randomly assigned 3649 non-diabetic patients with T1c-3 (any ER, PgR, HER2), N0-3, M0 BC who received standard treatment to receive metformin 850 mg po bid or placebo po bid for 5 years (including a 4-week ramp-up of one tablet per day) between 2010 and 2013. In May 2012, after 2382 women were enrolled, eligibility criteria were amended: those with T1cN0 disease had to have triple negative BC (ER negative, PgR negative, HER2 negative) to enter the trial and those with T2 N0 BC were eligible only if they had at least one of the following risk factors: histologic grade III, presence of lymphovascular invasion, negative estrogen (ER) and progesterone (PgR) receptors, HER2 positivity, Oncotype Recurrence Score ≥ 25 or Ki-67 over 14%. Exclusion criteria included fasting glucose > 7.0 mmol/L (126 mg/dL), known diabetes or current use of diabetes medication, hypersensitivity to or intolerance of metformin, history of lactic acidosis, participation in trials of weight loss or exercise interventions, recurrence of BC or prior BC, excessive alcohol intake, or marked hepatic, kidney, or cardiac dysfunction.

All patients provided written informed consent to participate in the MA.32 clinical trial in keeping with approval by relevant institutional human subjects committees.

The SH sub-study was conducted in post-menopausal women (to avoid cyclical changes in endogenous hormone production) with hormone receptor negative BC, who were not receiving endocrine treatment (to avoid effects of hormonal agents used to treat BC). Post-menopausal was defined as prior bilateral oophorectomy or > 12 months since last menses without prior hysterectomy. Baseline blood was obtained before study treatment was initiated; patients were required to be on study treatment at the time of the six-month blood draw.

At baseline, information was collected on age and tumor characteristics (stage, histological type, immunohistochemical profile) and treatment. Height and weight were measured at baseline, and weight at six months. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Blood Assays

The serum samples were collected into heparin tubes, aliquoted and stored at -80°C after an overnight fast of at least 12 hours. Paired specimens from each patient were retrieved, thawed and analyzed in the same batch by technicians blinded to patient treatment. The biologically most active estrogen (17β Estradiol), SHGB and total testosterone levels were determined using competitive ECLIA (electrochemiluminescense immunoassay) on cobas e602 and insulin using Roche ECLIA, catalogue #12017547122, at a CAP/CLIA accredited clinical laboratory (Mount Sinai Services). Intra-assays coefficients of variability were 7%, 2.4%, 4.6.% and 3%, respectively. Albumin was assayed to allow calculation of BT from total testosterone, SHBG and albumin. The automated platform cobas e602 provides a lower detection limit for estradiol of 18.4 pmol/L and for total testosterone of 0.025 ng/mL. The

measurement range for SHBG was 0.350 nmol/L to 200 nmol/L (defined as the limit of detection and the maximum of the master curve).

SNP Analysis

Genomic DNA was extracted from whole blood and samples were genotyped for the rs11212617 SNP (Chr11(GRCh38):g.108412434C>A) at The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada using a QIAsymphony magnetic bead DNA extractor (Qiagen, Germany) and a TaqMan PCR assay with dual-label MGB probes (Applied Biosystems™, ThermoFisher Scientific, Waltham, MA, USA).

Statistical Analysis

Patient and tumor characteristics at diagnosis were summarized. Since the distributions of the SHs were skewed, medians were used as measures of central tendency and 25th and 75th percentiles as measures of dispersion. Descriptive summaries were tabulated for baseline and change in the SH levels, where change was calculated as the six-month value minus the baseline value for each patient. Estradiol, testosterone and SHBG were all considered equally important main outcomes.

The pre-specified method of comparing the degree of change between arms was the Wilcoxon rank-sum test. Ten baseline and seven month 6 SHBG results (3%) were recorded as >200 nmol/L; these were replaced by 200 nmol/L. Overall, 25% of baseline and 33% of month 6 estradiol assay results were below the assay's limit of detection (LOD); change calculations, summary statistics and the Wilcoxon test were performed after replacing these results by half the LOD. Due to criticism of this method,[16] we

performed an additional sensitivity analysis using survival methods for left-censored data. The data can be analyzed using right-censored survival methods (product limit estimator and the log-rank test) by subtracting the left-censored observations from a large constant, a process called "flipping".[17] With only one point of censoring (the LOD), the product-limit method simplifies to the construction of exceedance curves, where for a defined set of levels e* one calculates the proportion of patients in each arm whose estradiol at month 6 was higher than each of the e*.

For SHs with a statistically significant difference between arms, we explored two possible pathways of metformin action by fitting multivariable linear regression models controlling for baseline BMI and BMI change, and insulin change. This was done by log-transforming all continuous variables to reduce skewness and then fitting a model with SH change as dependent variable and with treatment status and baseline assay level as explanatory variables. Baseline and change in BMI, or insulin change, was then added to this model. We back-transformed the coefficient for treatment status to get the percent difference between the metformin and placebo arms. Finally, using a regression model for change that included an interaction term for treatment by SNP, coded as any C vs AA, we examined whether a metformin effect was restricted to patients with the C allele of the rs11212617 SNP. Reported p-values are nominal and not adjusted for multiple comparisons.

A priori power calculations indicated we had 80% power with 150 patients per arm to detect differences in change between the arms of 12%, 14% and 20% for SHBG, testosterone and estradiol respectively, using a two-sided alpha of 0.05 without adjustment for multiple comparisons.

Results

Patient Population

Three hundred and twelve women were eligible based on post-menopausal status, ER/PgR negative invasive BC not receiving hormonal treatment and availability of blood at baseline and 6 months (the latter on study treatment), 141 on metformin and 171 on placebo arm (Fig. 1 - Consort Diagram). Patient and tumor characteristics are shown in Table 1. At baseline mean age (± standard deviation) was 58.1±6.9 vs 57.5±7.9 years, mean weight 72.6±14.1 vs 76.6±17.5 kg and mean BMI 27.3±5.2 vs 28.9±6.4 kg/m^2 in metformin vs placebo arms, respectively. Combining clinical stage (in patients receiving neoadjuvant therapy) and pathologic stage, the majority of patients had T1 or T2 (39.0% T1 and 54.6% T2 in the metformin arm, 42.7% T1 and 46.8% T2 in the placebo arm), and node negative breast cancer (66.7% and 61.4% in the metformin and placebo arms, respectively). HER2 amplification was observed in 22.7% and 15.8% of the women in the metformin and placebo arms respectively. Ninety-eight percent of women had received neoadjuvant or adjuvant chemotherapy.

Baseline SHs measurements

Estradiol levels were below the assay's lower detection limit of 18.4 pmol/L in 24.1% of metformin and 26.2% of placebo patients, Chi-square test p=0.68. As noted above, in the primary analysis, the estradiol levels of these cases were set to half the lower detection limit, 9.2 pmol/L.

Baseline SH measurements are shown in Table 2. At baseline the median estradiol was 32.2 vs 33.3 pmol/L, SHBG 76.4 vs 72.8 nmol/L and BT 0.02 vs 0.03 nmol/L for metformin vs placebo, respectively.

Change in SHs at six months

In the main analysis, the median change between baseline and month 6 in estradiol was 5.7 pmol/L in the metformin arm vs 0 in the placebo arm (Wilcoxon test p<0.001 , Table 2). The supplementary analysis supported this result - Figure 2 shows that the estradiol exceedance probabilities were consistently lower in the metformin than the placebo group (log-rank p<0.001), for example 56.9% vs 71.0% had estradiol levels over 20 pmol/L, 19.7% vs 42.6% had estradiol levels over 40 pmol/L and 8.0% vs 23.1% had estradiol levels over 60 pmol/L, respectively. The multivariable regression model estimated that month 6 estradiol was approximately 30.1% (95% CI, 19.0% to 39.7%) lower in the metformin vs placebo groups. When adjustment for baseline BMI and BMI change was added to the basic model, the reduction in estradiol in metformin vs placebo subjects was 25.7% (95% CI, 13.2% to 36.4%) and when adjustment for change in insulin was added to the basic model this was 30.1% (95% CI, 18.7% to 39.8%). In addition, examination of the interaction term in the regression model did not find evidence that the reduction in estradiol associated with the metformin arm was affected by the C allele of the SNP rs11212617 (Figure 3).

In contrast to estradiol, median changes in SHBG and BT were similar in the metformin vs placebo arms (SHBG -5.9 vs. -5.9 nmol/L, p=0.43; BT 0 vs 0 nmol/L, p=0.24), as observed in Table 2.

Discussion

Our observation of a statistically significant decrease in estradiol between baseline and month 6 in the metformin arm as compared to placebo is consistent with the work published by Campagnoli et al.,[5,6] who studied a selected postmenopausal non-diabetic BC population (50% of whom were receiving tamoxifen) who were required to have

high baseline testosterone levels. In that study, a decrease in estradiol (-38%; p< 0.02) and in testosterone (-29%, p< 0.02) was seen in those receiving metformin 1500 mg/day (close to the 1700 mg/day administered in MA.32) vs metformin 1000 mg/day. The 30% reduction in estradiol we identified was similar to that seen by Campagnoli et al. Our population differs from that studies by Campagnoli et al. in that our subjects were not selected for high baseline levels of testosterone - this difference in entry criteria may account for our failure to identify changes in SHBG or BT with metformin. These observations suggest that the estradiol change we observed is independent of testosterone or of a potential effect of metformin on liver synthesis of SHBG.

Patterson et al.,[7] reported reductions in estradiol, testosterone and SHBG in overweight or obese BC patients receiving metformin, however, it was not clear whether the small reductions in estradiol that were observed (-10%, 95% CI -18.5 to -1.5%) in those receiving metformin were due to co-administration of the lifestyle-based weight loss intervention. Small changes were also seen in testosterone and SHBG. As noted above, metformin had no impact on SHBG, estradiol, testosterone and dehydroepiandrosterone in 382 overweight, glucose-intolerant patients enrolled onto the Diabetes Prevention Program.[13]

Our study is the first to report the independent effect of metformin on estradiol in a placebo-controlled trial without co-intervention (tamoxifen or lifestyle intervention). The reduction we observed (approximately 30%) is substantial and of potential clinical relevance in breast cancer, and possibly in women with hormone receptor positive BC, although we did not study whether similar effects would have occurred in women receiving hormonal therapies for their BC. Our findings are also potentially relevant to other estrogen-sensitive cancers, notably endometrial cancer for which observational data suggest strong associations of metformin with both risk and

prognosis. Should beneficial effects of metformin be seen in our primary efficacy analysis in hormone receptor positive BC, we plan to investigate effects of metformin on SHs, including estradiol, in this population. The independence of the observed reduction in estradiol from baseline BMI, BMI change and insulin change suggests it did not occur as a result of loss of fat mass, nor is it associated with an insulin effect.

The mechanism by which metformin lowered estradiol remains unclear. Pre-clinical data have suggested that metformin may inhibit aromatase activity, potentially accounting for our observed reduction and also suggesting an additional mechanism of anti-cancer action of metformin. Both ER positive breast cancer cells and breast adipose stromal cells exhibited reductions in aromatase mRNA levels in response to metformin treatment via mechanisms involving the suppression of promoter (PII) and P1.3 specific transcripts as well as activation of AMPK.[18,19]

Strengths of our study include its conduct in the setting of a placebo controlled randomized trial in carefully selected postmenopausal women (thereby excluding menstrual cycle variability in sex hormones) who were not receiving hormonal therapy (thereby excluding potential confounding by these treatments). Limitations include the use of a non-highly sensitive estradiol assay – just under 30% of estradiol assays yielded results below the lower detection limit. In our primary analyses we assigned an estradiol level of half the lower detection limit. We performed an additional sensitivity analysis to generate exceedance curves that provided results similar to those obtained in our primary analysis. The similarity of our findings using two different methods of analysis, one designed specifically for censored values, reduces the likelihood that use of a more sensitive assay we would have led to different findings. Furthermore, the selection criteria used in this sub-study, particularly the requirement that subjects be on study medication for the 6 month blood draw, may have led to some imbalances

between study arms. Additionally, the p-values reported are not adjusted for multiple comparisons, thus the possibility of false positives cannot be excluded.

In conclusion, metformin lowered estradiol levels, independent of BMI and insulin in non-diabetic post-menopausal women with ER and PR negative BC enrolled onto MA.32 trial. This observation suggests a new mechanism of metformin action that may be relevant in breast and other estrogen mediated cancers.

Funding

This work was supported by the Canadian Cancer Society Research Institute, National Cancer Institute (US), Breast Cancer Research Foundation (New York), Canadian Breast Cancer Foundation, Hold'Em for Life and Apotex Canada (in kind donation of placebo and metformin).

Notes

Role of the funder: The funders had 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.

Acknowledgements: Canadian Clinical Trials Group.

Disclosures: Dr. Goodwin reports research funding from the Breast Cancer Research Foundation, Epic Sciences and Hold'Em for Life Charities. Dr Mayer reports research funding from Genentech/Roche, Novartis, Pfizer; consulting with Genentech/Roche, Novartis, Pfizer, Lilly, Abvie, GSK, Immunomedics, Macrogenics, Seattle Genetics, PUMA. Dr Rastogi reports travel and accommodation support from Roche, AstraZeneca and Lilly. Dr Rea reports research funding from Roche, Biotheranostics,

RNA diagnostics and Celgene; honoraria from Roche, Novartis, Pfizer, Diachii-Sanyo; travel support from Diachii-Sanhyo, Novartis and Eisai. Dr. Bedard reports research funding from Bristol-Myers Squibb, Sanofi, AstraZeneca, Genentech/Roche, Servier, GSK, Novartis, SignalChem, PTC Therapeutics, Nektar, Merck, Seattle Genetics, Mersana, Immunomedics, Lilly; consulting with BristolMyersSquibb, Sanofi, Pfizer.

References:

1. Canadian Cancer Trials Group (CCTG) MA.32 Clinical Trial: A Phase III Randomized Trial of Metformin vs Placebo in Early Stage Breast Cancer, https://clinicaltrials.gov/ct2/show/NCT01101438
2. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res. 2007 Nov 15;67(22):10804-12
3. Niraula S, Dowling RJ, Ennis M, et al. Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res Treat. Oct 2012, Vol 135, Issue 3, pg 821–830
4. Dowling RJ, Niraula S, Chang MC, et al. Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res. 2015; 17(1): 32
5. Campagnoli C, Pasanisi P, Abbà C, et al.: Effect of different doses of metformin on serum testosterone and insulin in non-diabetic women with breast cancer: A randomized study. Clinical Breast Cancer 2012, 12:175-182

6. Campagnoli C, Berrino F, Venturelli E, et al.: Metformin decreases circulating androgen and estrogen levels in nondiabetic women with breast cancer. Clinical Breast Cancer 2013, 13:433-438

7. Patterson RE, Marinac CR, Sears D, et al.: The effects of metformin and weight-loss on biomarkers associated with breast cancer, JNCI Nov 2018, Vol 110, Issue 11, Pag 1239–1247

8. Lohmann AE, Pimentel I, Goodwin PJ. Novel Insights into the Impact of Lifestyle-Based Weight Loss and Metformin on Obesity-Associated Biomarkers in Breast Cancer. JNCI Nov 2018, Vol 110, Issue 11, Pages 1161–1162

9. Folkerd E, Dowsett M. Sex hormones and breast cancer risk and prognosis. Breast. 2013 Aug;22 Suppl 2: S38-43

10. Rock CL, Flatt SW, Laughlin GA, et al. Reproductive Steroid Hormones and Recurrence-Free Survival in Women with a History of Breast Cancer. Cancer Epidemiol Biomarkers Mar 2008;17(3)

11. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, et al. Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study. Br J Cancer. 2004 Jan 12;90(1):153-9

12. Hetemäki N, Savolainen-Peltonen H, Tikkanen MJ, et al.: Estrogen Metabolism in abdominal subcutaneous and visceral adipose tissue in postmenopausal women. J Clin Endocrinol Metabol 2017 Dec 1;102(12):4588-4595

13. Kim C, Kong S, Laughlin GA, et al.: Endogenous sex hormone changes in postmenopausal women in the diabetes prevention program. J Clin Endocrinol Metab 2012, 97:2853-2861

14. Zhou K, Bellenguez C, Spencer CC, et al. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. Nat Genet 2011 Feb; 43(2): 117-120.

15. Cuyàs E, Buxó M, Ferri Iglesias MJ, et al. The *C* Allele of *ATM* rs11212617 Associates with Higher Pathological Complete Remission Rate in Breast Cancer Patients Treated with Neoadjuvant Metformin. Front Oncol. 2019 Mar 28; 9:193.

16. Helsel DR. Fabricating data: how substituting values for nondetects can ruin results, and what can be done about it. Chemosphere. 2006 Dec;65(11):2434-9

17. Helsel DR. Nondetects and data analysis: statistics for censored environmental data. Hoboken, N.J.: Wiley-Interscience, 2005.

18. Samarajeewa NU, Ham S, Yang F, Simpson ER, Brown KA. Promoter-specific effects of metformin on aromatase transcript expression. Steroids. 2011 Jul;76(8):768-71

19. Brown KA, Hunger NI, Docanto M, Simpson ER. Metformin inhibits aromatase expression in human breast adipose stromal cells via stimulation of AMP-activated protein kinase. Breast Cancer Res Treat. 2010 Sep;123(2):591-6

20. Goodwin PJ, Stambolic V, Lemieux J, et al. Evaluation of metformin in early breast cancer: a modification of the traditional paradigm for clinical testing of anti-cancer agents. Breast Cancer Res Treat 2011; 126:215-220

21. Goodwin PJ, Parulekar WR, Gelmon KA, et al.: Effect of metformin vs placebo on weight and metabolic factors in NCIC CTG MA.32. JNCI. 2015 Mar 4;107(3)

22. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early stage breast cancer: results of a prospective cohort study. J Clin Oncol 2002; 20:42-51

23. Lohmann AE, Goodwin PJ, Chlebowski RT, Pan K, Stambolic V and Dowling RJ. Association of Obesity-Related Metabolic Disruptions with Cancer Risk and Outcome. J Clin Oncol. 2016, Vol 34: 4249- 4255

Table 1: Patient and tumor characteristics at baseline

Characteristics	Metformin n=141	Placebo n=171
Age, years mean (SD)	58.1 (± 6.9)	57.5 (± 7.9)
Weight, kg mean (SD)	72.6 (± 14.1)	76.6 (± 17.5)
BMI, kg/m^2 mean (SD)	27.3 (± 5.2)	28.9 (± 6.4)
Post-menopausal, n (%)	141 (100)	171 (100)
Receptor status, n (%)		
ER/PR negative	141 (100)	171 (100)
Her2 status, n (%)		
Her2 positive	32 (22.7)	27 (15.8)
Her2 negative	109 (77.3)	144 (84.2)
Any (neo)adjuvant chemotherapy, n (%)		
Yes	139 (98.6)	168 (98.2)
No	2 (1.4)	3 (1.8)
T stage, n (%)		
T1	55 (39.0)	73 (42.7)
T2	77 (54.6)	80 (46.8)
T3	9 (6.4)	18 (10.5)
N stage, n (%)		
N0	94 (66.7)	105 (61.4)
N1	34 (24.1)	40 (23.4)
N2	8 (5.7)	18 (10.5)
N3	5 (3.5)	8 (4.7)

Table 2: Baseline Sex Hormones measurements and change from baseline to month 6 on metformin vs placebo arms.

Sex Hormones	Baseline Median (Q1, Q3)		Change[1] Median (Q1, Q3)		
	Metformin n=140	Placebo n=170	Metformin n=136	Placebo N=168	P[2]
Estradiol pmol/L	32.2 (19.0, 45.5)	33.3 (9.2, 56.4)	-5.7 (-18.6,0)	0 (-12.6, 14.7)	<0.001
BT nmol/L	0.02 (0.01, 0.05)	0.03 (0.02,0.06)	0 (-0.01, 0.01)	0 (-0.01, 0.02)	0.02
SHBG nmol/L	76.4 (60.9, 112)	72.8 (48.8, 105)	-5.9 (-15.6, 3.0)	-5.9 (-17.4, 1.5)	0.43

*Q1 and Q3 are the 25th and 75th percentiles, SHBG - Sex Hormone Binding Globulin, BT - Bioavailable Testosterone

[1] Change calculated with-in patient as month 6 value minus baseline value

[2] P-value from two-sided Wilcoxon rank sum test comparing change between the two arms

Figure titles and legends

Figure1: Consort Diagram.

Figure 2: Exceedance curves for estradiol at 6 months, by study arm. The curves give the proportion of patients whose estradiol was higher than any chosen cut-off on the x-axis, for example, 20% of metformin patients versus 43% of placebo patients had estradiol scores over 40 pmol/L.

Figure 3: Linear regression model for change in estradiol, adjusted for baseline estradiol, with treatment (metformin vs placebo) and SNP (Any C vs AA) as explanatory variables. [1] E_{AnyC}/E_{AA} denotes the ratio of estradiol levels at month 6 for the two SNP levels, adjusted to the same baseline estradiol. [2] Main effects model: log(month 6 estradiol) - log(baseline estradiol) as outcome and treatment status, SNP status and baseline assay level as covariates. The p-value is for the null hypothesis of no SNP effect, which in terms of back-transformed results is that the ratio $E_{AnyC}/E_{AA}=1$. [3] Interaction model: A treatment status-by-SNP status interaction was added to the main effects model. The p-value is for the null hypothesis of no interaction effect, which in terms of back-transformed results is that E_{AnyC}/E_{AA} in the metformin arm equals E_{AnyC}/E_{AA} in the placebo arm.

Figure 1

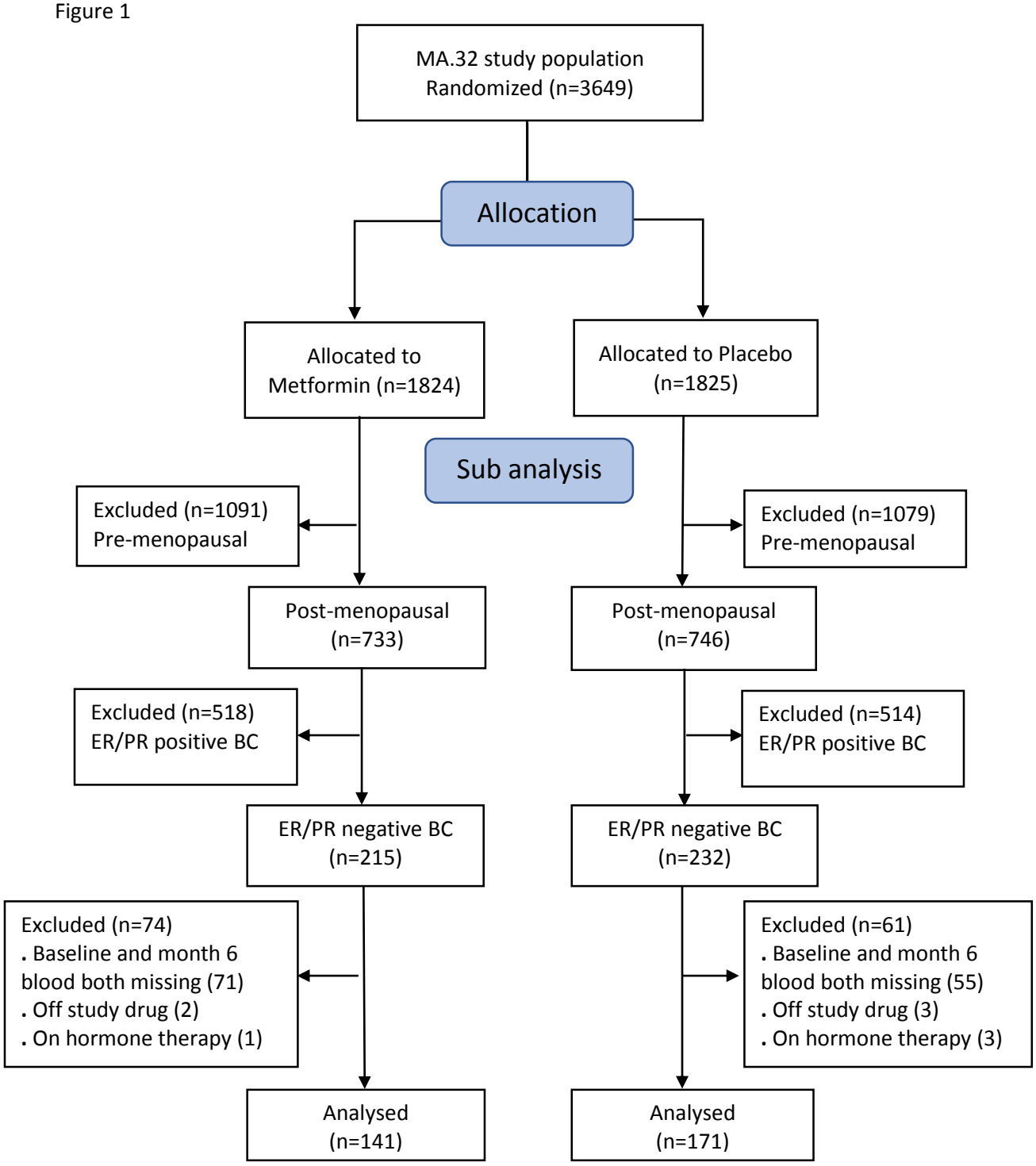

Figure 2

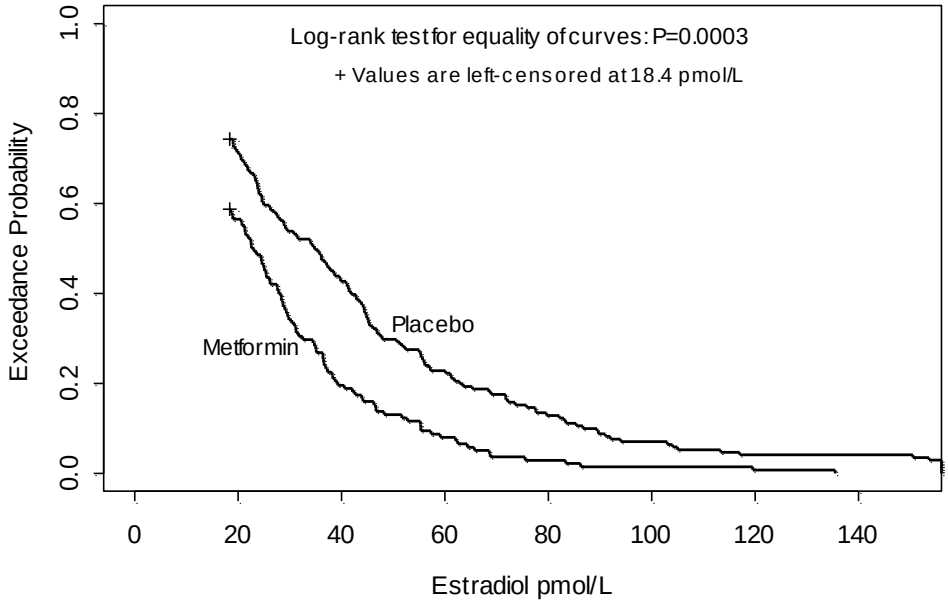

Figure 3

Regression model for estradiol with an overall SNP effect[2]			
E_{AnyC}/E_{AA} ratio[2] in both arms combined			P[2]
1.00 (0.85 – 1.18)			0.98
Regression model for estradiol that allows a different SNP effect by study arm[3]			
E_{AnyC}/E_{AA} ratio[1] in metformin arm	E_{AnyC}/E_{AA} ratio in placebo arm	Ratio of two treatment arm ratios	P[3]
0.94	1.05	0.90 (0.64 - 1.25)	0.51