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Glioblastoma multiforme is a grade IV astrocytoma arising *de novo* or progressing from lower grade II and III gliomas. Currently the etiology of glioma and prediction of disease progression are unknown. Recent epidemiological studies have suggested that hormonal factors, including estrogen treatment, may impact glioma risk however these studies have been limited to case-control trials and retrospective cohort studies. Here, we evaluate results from the Women's Health Initiative, which involved 161,808 women with robust data regarding hormone exposures. During a median of 12.7 years of follow-up, 167 cases of glioma (130 cases of GBM) were ascertained. The relationship between gliomas and hormone therapy (HT; estrogen-alone [E-alone] or estrogen plus progestin [E+P]) was evaluated using Cox proportional hazards models as well as Kaplan-Meier time-to-event analysis. There was no association with gliomas for the E-alone group (HR=0.76, 95% CI=0.43,1.36) but there was an inverse association for E+P (HR=0.48, 95% CI= 0.26, 0.88, p=0.02) after accounting for patient, hormone exposure and reproductive factors. Kaplan-Meier survival analysis demonstrated a significant reduction in time-to-incidence for the E+P group (p=0.0035). Findings from the matched case-control arm of the WHI trial did not demonstrate significant impact of HT on glioma incidence. The results of this study suggest a reduction in glioma risk after treatment with estrogen plus progesterone however a further large scale case-controlled study is warranted to evaluate the impact of HT on this disease.

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Women's Health Initiative Findings on Glioma Incidence after Hormone Therapy in Post-Menopausal Women

Michael Karsy[1], Erol Arslan[2], Nicolas T. Kummer[3], Fred Moy[4]

[1] Department of Neurosurgery, University of Utah, Salt Lake City UT, 84132, USA.

[2] Department of Obstetrics and Gynecology, Derik State Hospital, Mardin, Turkey.

[3] Department of Radiation Oncology, University of California – Los Angeles, CA, 90025, USA.

[4] Department of Pathology, New York Medical College, Valhalla, NY, 10595, USA.

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Corresponding author:
Michael Karsy

Department of Neurosurgery

The University of Utah

175 North Medical Drive East

Salt Lake City, UT 84132, USA.

Michael.Karsy@hsc.utah.edu

Tel: 801-581-6908

Fax: 801-581-4385

Abstract

Glioblastoma multiforme is a grade IV astrocytoma arising *de novo* or progressing from lower grade II and III gliomas. Currently the etiology of glioma and prediction of disease progression are unknown. Recent epidemiological studies have suggested that hormonal factors, including estrogen treatment, may impact glioma risk however these studies have been limited to case-control trials and retrospective cohort studies. Here, we evaluate results from the Women's Health Initiative, which involved 161,808 women with robust data regarding hormone exposures. During a median of 12.7 years of follow-up, 167 cases of glioma (130 cases of GBM) were ascertained. The relationship between gliomas and hormone therapy (HT; estrogen-alone [E-alone] or estrogen plus progestin [E+P]) was evaluated using Cox proportional hazards models as well as Kaplan-Meier time-to-event analysis. There was no association with gliomas for the E-alone group (HR=0.76, 95% CI=0.43,1.36) but there was an inverse association for E+P (HR=0.48, 95% CI= 0.26, 0.88, p=0.02) after accounting for patient, hormone exposure and reproductive factors. Kaplan-Meier survival analysis demonstrated a significant reduction in time-to-incidence for the E+P group (p=0.0035). Findings from the matched case-control arm of the WHI trial did not demonstrate significant impact of HT on glioma incidence. The results of this study suggest a reduction in glioma risk after treatment with estrogen plus progesterone however a further large scale case-controlled study is warranted to evaluate the impact of HT on this disease.

Introduction

Gliomas are astrocyte-derived tumors of the central nervous system, among which glioblastoma multiforme (GBM), a grade IV astrocytoma, is the most common and malignant

variety (Ohgaki and Kleihues 2011; Stupp et al. 2009). Classically, GBM is defined as primary when arising *de novo* or secondary when developing from lower-grade II and III gliomas; however, four genomic subtypes distinguish GBM (Huse and Holland 2010; Ohgaki and Kleihues 2011). Glioma is poorly understood in its etiology, pathogenesis and natural disease course. Radiation exposure and certain familial genetic syndromes have shown to predispose to glioma formation but these factors account for a small proportion of overall tumor burden (Wrensch et al. 2002). Additionally, cancer stem cell research suggests there is marked heterogeneity in resistance to therapy and prognosis because of these specific cell populations, although the effect of these findings is unknown (Karsy et al. 2010; Karsy et al. 2012).

The incidence of glioma and GBM in the United States shows variance by gender with rates that are 1.41 and 1.58 times higher in men, respectively (CBTRUS 2012). Additionally, recent epidemiological and laboratory studies have suggested that hormones may play an important role in GBM pathogenesis (Cowppli-Bony et al. 2011). The reduced incidence rate of GBM in females is first evident at the approximate age of menarche and widens until the age of menopause where the incidence becomes similar, suggesting a protective effect of hormones on glioma formation (McKinley et al. 2000). *In vitro* studies have suggested that estrogen has an impact on GBM due to the presence of estrogen receptors and aromatase in the brain (Kabat et al. 2010). Case-control and cohort studies have shown an equivocal role for estrogens in GBM formation in women (Cowppli-Bony et al. 2011; Kabat et al. 2011). Limitations in study design and statistical power of these investigations have been complicated interpretation of the available data, but overall the findings are suggestive of an association between hormonal exposures and GBM development.

The Woman's Health Initiative (WHI) was a multi-part study of 161,808 women overall in various clinical trial and observation arms over a 15-year period (Stefanick et al. 2003). The impact of estrogen and progesterone on menopause, heart disease, stroke, as well as malignancy

were partially addressed. The purpose of our study was to examine the role of exogenous hormones used after menopause on the incidence of glioma and GBM. The use of a prospectively collected, randomized trial data set, as found in the WHI data set, may provide additional information regarding the impact of female hormones on gliomagenesis. Understanding the impact of these hormones may have broad implications on the pathogenesis and management of gliomas.

Materials and Methods

WHI study population and subject groups

The WHI was a multi-part clinical research project that recruited 161,808 women between the ages of 50 and 79 years into three randomized clinical trials (n=68,132) and an observational study (n=93,676), which has been described previously (Stefanick et al. 2003). Briefly, the clinical trials included one hormone therapy clinical trial (HT CT, n=27,347), and two non-hormone therapy clinical trials (non-HT CT), which included a dietary modification (DM, n=48,835) trial and calcium/vitamin D (CaD, n=36,282) trial (Figure 1A). Eligible subjects could enroll in one, two or all three trials and subjects not eligible for the clinical trials were invited to enter the observational study (OS). All entering subjects underwent a careful evaluation of previous hormone use including type and duration. Women with a previous history of breast or brain cancer were excluded. Subjects in the HT CT trial were randomized and received either daily 0.625mg of conjugated equine estrogen (Premarin, Wyeth, Philadelphia), estrogen plus 2.5mg medroxyprogesterone acetate (Prempro, Wyeth, Philadelphia) or placebo. Follow up with the subjects occurred 6 weeks after enrollment and annually thereafter. The HT CT was terminated early in July 2002 and March 2004, respectively, due to increased incidence of cardiovascular complications and breast cancer in the treatment groups. Follow-up continued according to the protocol through March 31, 2005, the original trial completion date, with a first

89 extension phase beginning on 2005 and a second extension phase in 2010. Follow-up was
90 performed by mailed health questionnaires, released medical records, and telephone.
91 All subjects enrolled in the WHI with complete data and follow-up were included in this
92 study. Baseline HT, oral contraceptive and clinical HT treatments determined categorization in
93 this study (Figure 1B). The E-alone group included 1) women who were randomized to the
94 estrogen arm of the CT, 2) reported treatment with estrogen alone (without progesterone) at WHI
95 trial randomization in the non-HT CT, or 3) reported treatment with estrogen at time of
96 enrollment in the OS. Similarly, the E+P group included 1) women who were randomized to the
97 E+P arm of the CT, 2) reported treatment with estrogen and progesterone at WHI trial
98 randomization in the non-HT CT, or 3) reported treatment with estrogen and progesterone at time
99 of enrollment in the OS group. The duration of hormone exposure was factored as a variable.
100 Placebo-controlled subjects from the HT CT were categorized as E-alone or E+P non-users
101 depending on respective groups. Non-HT CT and OS women not using HT and with a prior
102 hysterectomy were categorized in the E-alone non-users group, while those not using HT and
103 without prior hysterectomies were placed in the E+P non-users group to reflect patient allocation
104 in the HT CT trial (Chlebowski and Anderson 2012).

105 ***Glioma and GBM diagnosis***

106 Cases of glioma (ICD-M 9380/3-9451/3) and GBM (ICD-M 9380/3 and 9440/3) were
107 analyzed. Subjects with diagnosed brain tumors presented in a hospital inpatient or outpatient
108 setting, and their clinical treatment and diagnosis were obtained from the medical record
109 (Supplementary table 1). Pathological confirmation of tumor diagnoses was noted in the majority
110 of cases; however, treatment type including surgery, chemotherapy and radiotherapy, was not
111 assessed in the WHI trial.

Statistical analysis

Of the 161,108 women enrolled in the WHI, this analysis includes 126,237 participants reporting Caucasian race/ethnicity at baseline. Women with a previous history of breast or brain cancer were excluded because a large percentage would be placed on anti-estrogen therapies. Participants reporting non-Caucasian race were excluded in the final analysis because of the small number of glioma cases among them (n=11), which might have skewed the hazards modeling; however, inclusion of these subjects did not alter the final results. Subject demographic, reproductive and hormonal exposure characteristics were noted. Follow-up time began at WHI CT randomization or OS enrollment to the most recent follow-up time. Overall, 76.9% of eligible participants consented to the first extension study on March 31, 2005, the original date of WHI completion, and 75% of participants who continued consented to the 2010 extension study.

Results for glioma incidence were assessed using with time-to-event approaches. The total number of events and the annualized percentage are reported (Supplementary table 2). Univariate and multivariate Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the associations among glioma risk and HT use and other demographic, reproductive and hormonal factors. Stratification was based on five-year demographic age groups and WHI DM trial randomization (intervention, control, or non-participant) and CaD trial randomization (active, placebo, or non-participant). Results from univariate models of risks and from a multivariate model of all risk factors are presented. Models with a subset of risk factors (e.g., age, socioeconomic status, and hormone exposure) were also considered during the evaluation of the full multivariate model. Glioma rates over time were assessed by Kaplan-Meier analysis with logrank test from time of enrollment in the WHI trial to time of cancer diagnosis. All significance levels are two-sided, and a p-value of 0.05 was

considered statistically significant. All statistical tests were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Demographic characteristics of the study subjects are shown by HT status (Table 1). Hormonal characteristics including type and duration of hormone use, as well as reproductive characteristics such as age of menarche, age of first parity, number of pregnancies, months breastfeeding, oophorectomy status and years since menopause are shown in Table 2. Tumor diagnoses, localization, morphology, and site characteristics are shown among different treatment groups (Table 3). Overall, 167 cases of glioma, including 130 cases of GBM, were analyzed. Subsequent models were used to evaluate tumor incidence after adjusting for demographic, hormonal, and reproductive characteristics.

Univariate and multivariate Cox proportional hazards modeling were performed for HR of glioma incidence among demographic, hormonal and reproductive characteristics (Table 4). In the univariate analysis, E+P use was associated with a 43% reduction in risk of glioma (HR=0.57, 95% CI: 0.36, 0.90), which was statistically significant (p=0.02). No other comparison of demographic or reproductive characteristics showed a statistically significant difference. After controlling for other variables (Table 1 and 2), the E+P use was associated with a 52% reduction in risk of glioma (HR= 0.48, 95% CI= 0.26, 0.88, p=0.02). For the E+P group, the HRs of 1.73 for age (95% CI: 1.03, 2.89, p=0.04) and 2.06 for age at menarche ≥15 vs. 12-14 years (95% CI: 1.03, 4.15, p=0.04) were statistically significant but the HRs for other variables were not. Univariate and multivariate Cox proportional hazards modeling of HR specifically in GBM cases did not show a statistically significant difference for the other variables evaluated. Evaluation of nested glioma cases restricted only to subjects randomized to the CT arm of the HT compared to

their untreated placebo controls also did not reach statistical significance in the univariate or multivariate analysis.

Kaplan-Meier survival plots for time-to-incidence of glioma from enrollment in the WHI trial among subjects in E-alone and E+P treatment groups was performed. For the E-alone group, no significant difference in incidence was observed between users and non-users (p=0.725) (Figure 2A). However for the E+P group, a significant difference was seen where users showed increased time in glioma incidence compared to non-users (p=0.0035) (Figure 2B). Kaplan-Meier analysis of glioma or GBM case time-to-incidence in subjects randomized in CT HT arm did not demonstrate a significant difference of HT in comparison untreated placebo controls.

Discussion

Our epidemiological cohort study suggests that glioma risk is significantly reduced in postmenopausal women receiving estrogen and progesterone (E+P) therapy. This secondary analysis of the WHI trial data evaluating 167 cases of glioma (130 cases of GBM) demonstrated a 43% and 52% reduction in glioma risk in subjects in the E+P group by univariate and multivariate Cox proportional hazards analysis, respectively. Moreover, Kaplan-Meier survival analysis also showed a significant reduction in time-to-incidence with the E+P group. No reduction in glioma incidence by Cox proportional hazards or Kaplan-Meier survival analysis was shown for the E-alone group in our analysis and no differences in glioma incidence were also seen between the E-alone or E+P groups and their matched controls in the HT arm of the analysis.

A number of case-control and cohort studies have suggested that estrogens play an important role in GBM incidence but with inconsistent results (Felini et al. 2009; Hatch et al. 2005; Huang et al. 2004; Kabat et al. 2011; Lambe et al. 1997; Michaud et al. 2010; Silvera et al. 2006; Wigertz et al. 2008). Several case-control studies showed statistically significant odds

ratios ranging from 0.66 to 0.56 for glioma incidence in women treated with oral contraceptives or HT (Felini et al. 2009; Hatch et al. 2005; Huang et al. 2004; Ohgaki and Kleihues 2011; Stupp et al. 2009). Furthermore, one study showed similar reduced risk in post-menopausal women treated with HT as seen in our findings (Huang et al. 2004; Huse and Holland 2010; Ohgaki and Kleihues 2011). Various case-controlled and cohort studies suggested the importance of estrogen and progesterone on glioma incidence by reporting the impact of reproductive variables, such as age at parity (Hatch et al. 2005), age at menarche (Felini et al. 2009; Hatch et al. 2005; Huang et al. 2004; Kabat et al. 2011; Silvera et al. 2006; Wrensch et al. 2002), parity (Karsy et al. 2012; Karsy et al. 2010; Lambe et al. 1997; Wigertz et al. 2008), and breastfeeding (Wigertz et al. 2008) on glioma incidence. However, a large study of 276,212 women the European Prospective Investigation into Cancer and Nutrition study showed 193 cases of glioma over an 8.4-year follow-up but failed to find an association with glioma risk and reproductive factors (CBTRUS 2012; Michaud et al. 2010). Despite the significance of these studies, the role of estrogen and progesterone in gliomagenesis continued to be controversial because of the limited study designs, statistical power, and heterogeneous estrogen exposures.

Our analysis suggests a reduced risk of glioma in subjects treated with E+P but has several limitations. The primary strengths of this study are the large sample size, prospective detection of adjudicated glioma cases, and detailed determination of HT exposure as well as type. Interestingly in our study, the E-alone model did not show a significant reduction in glioma risk. This result may have been due to the heterogeneity of reproductive factors relating to hormone exposure in comparison to previous studies. On the other hand, our study suggests that progesterone may play an important role in gliomagenesis, which may not have been account in previous studies. Progesterone has been shown to be neuroprotective independently from estrogen and to affect glioma proliferation *in vitro* (Cabrera-Muñoz et al. 2011; Coughlan et al. 2009; Cowppli-Bony et al. 2011; Inoue et al. 2002). While reproductive characteristics were

evaluated as potential confounders in our models, details regarding hormone type and duration of exposure prior to trial recruitment were obtained at the onset of enrollment. Therefore, recall bias may have been a significant possibility. In addition, the inclusion of subjects into the E-alone and E+P groups not solely from the randomized CT HT may introduce a selection bias. However the duration and type of hormonal exposure along with other reproductive characteristics were adjusted for in the multivariate models and the inclusion of all WHI participants allowed us to achieve sufficient statistical power to test these associations. Furthermore, the effect of estrogen and progesterone on GBM cases may have not been significant because of limited sample size. In addition, limitations of the study include the mean age of participants in the WHI trial being 65 years of age where aggregated hormone exposure may have been after clinically detectable glioma formation.

Various molecular studies also support the role of E+P in the inhibition of glioma. Several estrogen receptors (ER) have been described including, ERα, ERβ, and a G-protein GPR30, which can modulate gene transcription (McKinley et al. 2000; Mhyre and Dorsa 2006). ERβ but not ERα is expressed in gliomas and non-neoplastic astrocytes, with ERβ expression declining in higher-grade gliomas as tumors become more dedifferentiated (Batistatou et al. 2006; Kabat et al. 2010). Aromatase, involved in converting androgens to estrogens, is also expressed in GBM but no studies to date have shown an impact of aromatase inhibitors on glioma or GBM (Cowppli-Bony et al. 2011; Kabat et al. 2011; Yague et al. 2004). GBM cells xenografted into various immunocompromised mice or rodent models show reduced tumor growth and improved survival in female animals as well as females treated with estrogen (Barone et al. 2009; Plunkett et al. 1999; Stefanick et al. 2003; Verzat et al. 1990). While ERs may modulate GBM by the MAPK, AKT or TGFβ1 signaling pathways, complete understanding of how estrogen regulates gliomagenesis is unknown (Behl 2002; Dhandapani et al. 2005; Stefanick et al. 2003). Two progesterone receptor (PR) isoforms also exist, PR-A and PR-B, with multiple

functional isoforms (with PR-A overexpression shown to reduce *in vitro* astrocytoma growth (Cabrera-Muñoz et al. 2009; Felini et al. 2009; Hatch et al. 2005; Huang et al. 2004; Inoue et al. 2002; Kabat et al. 2011; Lambe et al. 1997; Michaud et al. 2010; Silvera et al. 2006; Wigertz et al. 2008). Progesterone treatment alone did not result in improved animal survival in one study (Plunkett et al. 1999). However, the understanding of impact of PRs in gliomas is much more limited than the effect of ERs. Furthermore, the effect of combined E+P on glioma or GBM has not been evaluated with *in vitro*, animal models or clinical trials to date. The presence of these hormone receptors and how they interact with various glioma signaling pathways may indicate their importance in gliomagenesis.

The results of this study suggest that E+P therapy reduces glioma risk but the reproductive variables did not show a significant effect on risk in our study. Studies in parous women have shown lower levels of androgens, prolactin, and free estradiol and higher levels of sex hormone-binding globulin, which may support reduced risk of estrogen-sensitive breast carcinomas in parous women (Chubak et al. 2004). Furthermore, breastfeeding suppresses ovulation and net estrogen exposure while inducing prolactin and oxytocin secretion. Parity, breastfeeding, and oophorectomy would have been expected to increase glioma risk while higher body-mass index and oral contraceptive would have been expected to decrease glioma risk by altering net estrogen exposure. Interestingly, prolactin has been shown to promote glioma cell proliferation (Ducret et al. 2002) while oxytocin can inhibit cell proliferation (Cassoni et al. 1998). It may be possible that estrogen generates distinct regulation in normal and neoplastic tissue suggesting that selective modulation, altered signaling pathways and parallel hormonal signaling may govern the protective effects of estrogen in some cancers (Kabat et al. 2010). Further molecular studies are necessary to identify key hormonal signaling networks involved in gliomagenesis.

A better understanding of estrogen and progesterone may aid in the design of rational, targeted therapies in glioma. The use of selective estrogen receptor modulators (SERMs), which

are selective receptor agonists and/or antagonists depending on receptor and site, may play a role in the treatment of this disease. Tibolone, an ER antagonist, has been shown to reduce proliferation of human and rat GBM cells (Altinoz et al. 2009). In another study, genistein, an isoflavone that binds to ERβ, showed reduced DNA synthesis in glioma cells (Yakisich et al. 2009). In several studies, the SERM tamoxifen showed inhibition of glioma cell proliferation and increased apoptosis (Hui et al. 2004; Kim et al. 2005; Liu et al. 2001; Pollack et al. 2010; Zhang et al. 2000), and in several other studies, RU486 (mifepristone), a progestin receptor antagonist, has also been shown to suppress GBM proliferation and tumor volume in a xenografted GBM cell line (Pinski et al. 1993; Ramaswamy et al. 2012). Currently clinical trials of SERM and other hormonal agents in glioma have been limited (Patel et al. 2012; Sankar et al. 2008).

Conclusion

In conclusion, the results of this study suggest a reduced glioma risk for subjects in association with E+P therapy. Furthermore, these results were constant after controlling for demographic, reproductive and hormonal characteristics. Limitations in patient selection and hormone exposure limit the findings and support the need for well-designed, prospective studies for evaluating the impact of E+P treatment on glioma incidence.

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Figure 1

Allocation strategy for Women's Health Initiative (WHI) subjects in this study

A schematic of WHI subjects allocated in this current study as well as the number of glioma and GBM cases per group is shown. A) Eligible subjects from the WHI trial were allocated either to the non-hormone therapy clinical trial (non-HT CT) consisting of a calcium/vitamin D trial (CaD) and dietary modification trial (DM) or the hormone trial therapy clinical trial (HT CT). Subjects were allowed to enroll in one, two or all three clinical trials. Subjects who were eligible but declined to be included in any trial were followed as part of the observation study (OS). Furthermore, subjects in the HT CT were randomized to the estrogen alone (E-alone) or estrogen plus progesterone (E+P) treatment and placebo groups. B) Our study allocated E-alone (square) and E+P (circle) users based on current or previous hormone exposure. In addition, women not using HT and with a prior hysterectomy were categorized in the E-alone non-users group while those without prior hysterectomy were categorized in the E+P non-users group. Subjects with previous histories of breast or brain cancer along with incomplete hormonal exposure characteristics were excluded.

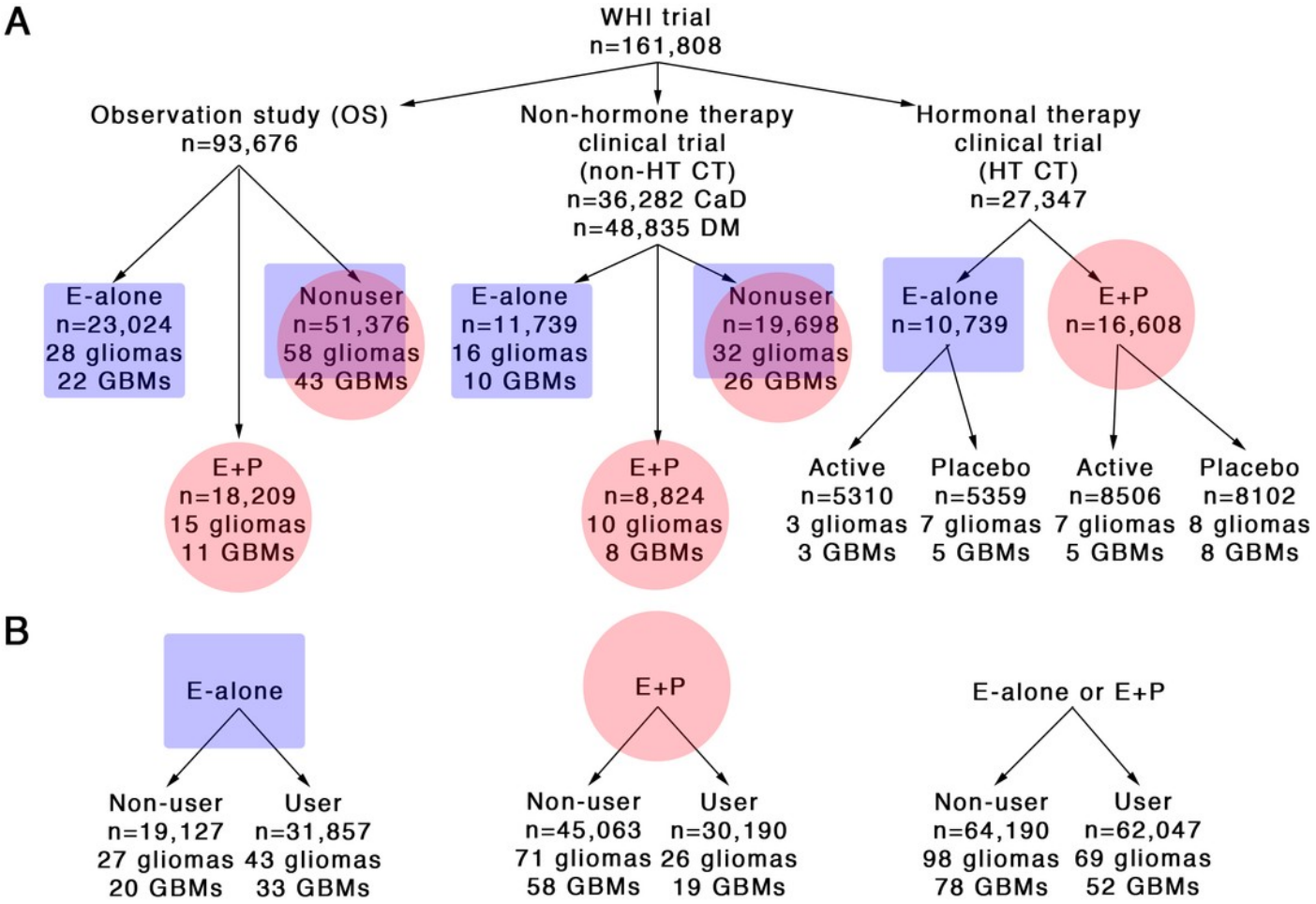

Figure 2

Kaplan-Meier plots of incidence for subjects receiving hormone therapy (HT)

Kaplan-Meier survival analysis and logrank test of glioma survival for Caucasian participants in the E-alone or E+P groups. A) No statistically significant difference in incidence was seen for E-alone users compared with non-users. B) A statistically significant decreased incidence was seen for E+P users compared with non-users (p=0.0035).

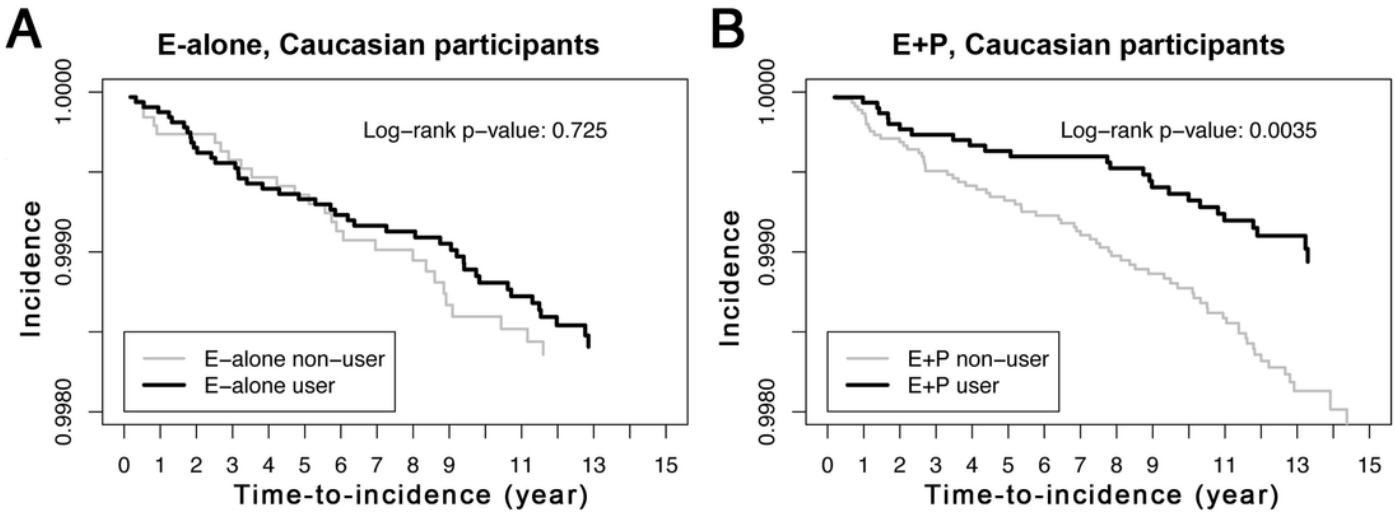

Table 1(on next page)

Demographic characteristics of HT users and non-users

Table 1: Demographic characteristics of HT users and non-users

	E-alone		E+P	
	Non-user N = 19127	**User N = 31857**	**Non-user N = 45063**	**User N = 30190**
Age (years)				
50-59	4285 (22.4%)	10312 (32.4%)	11922 (26.5%)	13055 (43.2%)
60-69	8835 (46.2%)	14889 (46.7%)	20730 (46.0%)	13098 (43.4%)
70-79	6007 (31.4%)	6656 (20.9%)	12411 (27.5%)	4037 (13.4%)
Education				
Missing	130 (0.7%)	199 (0.6%)	313 (0.7%)	174 (0.6%)
≤8 years	205 (1.1%)	204 (0.6%)	303 (0.7%)	117 (0.4%)
Some high school	895 (4.7%)	946 (3.0%)	1256 (2.8%)	495 (1.6%)
High school diploma/GED	4213 (22.0%)	5894 (18.5%)	8232 (18.3%)	3936 (13.0%)
School after high school	7863 (41.1%)	13271 (41.7%)	16316 (36.2%)	10308 (34.1%)
College degree or higher	5821 (30.4%)	11343 (35.6%)	18643 (41.4%)	15160 (50.2%)
Annual family income				
Missing	1296 (6.8%)	1807 (5.7%)	3280 (7.3%)	1621 (5.4%)
< $10,000	855 (4.5%)	812 (2.5%)	1458 (3.2%)	492 (1.6%)
$10,000 - $19,999	3026 (15.8%)	3088 (9.7%)	5223 (11.6%)	2083 (6.9%)
$20,000 - $34,999	5112 (26.7%)	7324 (23.0%)	10915 (24.2%)	5659 (18.7%)
$35,000 - $49,999	3615 (18.9%)	6543 (20.5%)	8866 (19.7%)	5883 (19.5%)
$50,000 - $74,999	2991 (15.6%)	6342 (19.9%)	8045 (17.9%)	6735 (22.3%)
> $75,000	2232 (11.7%)	5941 (18.6%)	7276 (16.1%)	7717 (25.6%)
Smoking status				
Missing	257 (1.3%)	337 (1.1%)	561 (1.2%)	340 (1.1%)
Never	9749 (51.0%)	15989 (50.2%)	22406 (49.7%)	14146 (46.9%)
Past	7696 (40.2%)	13700 (43.0%)	19014 (42.2%)	13777 (45.6%)
Current	1425 (7.5%)	1831 (5.7%)	3082 (6.8%)	1927 (6.4%)
Smoking pack years				
Missing	686 (3.6%)	1045 (3.3%)	1548 (3.4%)	1122 (3.7%)
None	9749 (51.0%)	15989 (50.2%)	22406 (49.7%)	14146 (46.9%)
≤10 years	3279 (17.1%)	6129 (19.2%)	8205 (18.2%)	6298 (20.9%)
10-20 years	1472 (7.7%)	2683 (8.4%)	3868 (8.6%)	2731 (9.0%)
20-30 years	1192 (6.2%)	1987 (6.2%)	2937 (6.5%)	2047 (6.8%)
> 30 years	2749 (14.4%)	4024 (12.6%)	6099 (13.5%)	3846 (12.7%)

Table 1: Demographic characteristics of HT users and non-users

	E-alone		E+P	
	Non-user N = 19127	**User N = 31857**	**Non-user N = 45063**	**User N = 30190**
Alcohol consumption				
Missing	142 (0.7%)	196 (0.6%)	297 (0.7%)	149 (0.5%)
Non drinker	2206 (11.5%)	2965 (9.3%)	4054 (9.0%)	2025 (6.7%)
Past drinker	4126 (21.6%)	5583 (17.5%)	7339 (16.3%)	4009 (13.3%)
<1 drink per month	2532 (13.2%)	3899 (12.2%)	5617 (12.5%)	3414 (11.3%)
<1 drink per week	3852 (20.1%)	6724 (21.1%)	9281 (20.6%)	6400 (21.2%)
1 - <7 drinks per week	4365 (22.8%)	8656 (27.2%)	12388 (27.5%)	9619 (31.9%)
7+ drinks per week	1904 (10.0%)	3834 (12.0%)	6087 (13.5%)	4574 (15.2%)
Previous cancer diagnosis				
Missing	176 (0.9%)	275 (0.9%)	360 (0.8%)	216 (0.7%)
No	16372 (85.6%)	28798 (90.4%)	42759 (94.9%)	29044 (96.2%)
Yes	2579 (13.5%)	2784 (8.7%)	1944 (4.3%)	930 (3.1%)
Family history of cancer				
Missing	759 (4.0%)	1269 (4.0%)	1790 (4.0%)	1083 (3.6%)
No	5516 (28.8%)	9461 (29.7%)	13993 (31.1%)	9886 (32.7%)
Yes	12852 (67.2%)	21127 (66.3%)	29280 (65.0%)	19221 (63.7%)

Table 2(on next page)

Hormonal and reproductive characteristics of HT users and non-users

Table 2: Hormonal and reproductive characteristics of HT users and non-users

	E-alone		E+P	
	Non-user N = 19127	**User N = 31857**	**Non-user N = 45063**	**User N = 30190**
Current hormone use				
Missing	2 (<0.1%)	0 (0.0%)	2 (<0.1%)	3 (<0.1%)
Never used	10782 (56.4%)	1985 (6.2%)	35149 (78.0%)	5224 (17.3%)
Past user	7781 (40.7%)	1495 (4.7%)	9501 (21.1%)	1445 (4.8%)
Current user	562 (2.9%)	28377 (89.1%)	411 (0.9%)	23518 (77.9%)
Type of hormone use				
Missing	2 (<0.1%)	0 (0.0%)	2 (<0.1%)	3 (<0.1%)
Never	10782 (56.4%)	1985 (6.2%)	35149 (78.0%)	5224 (17.3%)
E-alone only	7314 (38.2%)	26641 (83.6%)	3535 (7.8%)	626 (2.1%)
E+P only	591 (3.1%)	78 (0.2%)	5537 (12.3%)	21655 (71.7%)
Both	438 (2.3%)	3153 (9.9%)	840 (1.9%)	2682 (8.9%)
Duration of hormone use				
Missing	2 (<0.1%)	0 (0.0%)	2 (<0.1%)	3 (<0.1%)
None	10782 (56.4%)	1985 (6.2%)	35149 (78.0%)	5224 (17.3%)
< 5 years	4400 (23.0%)	5717 (17.9%)	7071 (15.7%)	10237 (33.9%)
5-10 years	1484 (7.8%)	5963 (18.7%)	1637 (3.6%)	7447 (24.7%)
≥10 years	2459 (12.9%)	18192 (57.1%)	1204 (2.7%)	7279 (24.1%)
Oral contraceptive use ever				
No	12706 (66.4%)	17394 (54.6%)	28558 (63.4%)	14351 (47.5%)
Yes	6421 (33.6%)	14463 (45.4%)	16505 (36.6%)	15839 (52.5%)
BMI				
Missing	176 (0.9%)	226 (0.7%)	461 (1.0%)	223 (0.7%)
<25	5452 (28.5%)	11322 (35.5%)	16081 (35.7%)	13132 (43.5%)
25 - <30	6691 (35.0%)	11496 (36.1%)	15534 (34.5%)	10074 (33.4%)
≥30	6808 (35.6%)	8813 (27.7%)	12987 (28.8%)	6761 (22.4%)
Treated diabetes				
Missing	13 (0.1%)	25 (0.1%)	31 (0.1%)	11 (<0.1%)
No	18041 (94.3%)	30824 (96.8%)	43502 (96.5%)	29565 (97.9%)

Table 2: Hormonal and reproductive characteristics of HT users and non-users

	E-alone		E+P	
	Non-user N = 19127	**User N = 31857**	**Non-user N = 45063**	**User N = 30190**
Yes	1073 (5.6%)	1008 (3.2%)	1530 (3.4%)	614 (2.0%)
Age at menarche (years)				
Missing	74 (0.4%)	106 (0.3%)	175 (0.4%)	84 (0.3%)
≤9	307 (1.6%)	428 (1.3%)	452 (1.0%)	300 (1.0%)
10	1102 (5.8%)	1764 (5.5%)	2155 (4.8%)	1448 (4.8%)
11	3029 (15.8%)	5071 (15.9%)	6586 (14.6%)	4503 (14.9%)
12	5104 (26.7%)	8497 (26.7%)	11697 (26.0%)	7801 (25.8%)
13	5278 (27.6%)	9314 (29.2%)	13428 (29.8%)	9274 (30.7%)
14	2411 (12.6%)	3886 (12.2%)	6253 (13.9%)	4109 (13.6%)
15	1030 (5.4%)	1645 (5.2%)	2546 (5.6%)	1543 (5.1%)
16	603 (3.2%)	899 (2.8%)	1370 (3.0%)	906 (3.0%)
≥17	189 (1.0%)	247 (0.8%)	401 (0.9%)	222 (0.7%)
Age at first birth (years)				
Missing	1782 (9.3%)	2368 (7.4%)	4055 (9.0%)	2037 (6.7%)
Never preg/No term preg	1792 (9.4%)	3062 (9.6%)	5890 (13.1%)	3734 (12.4%)
<20	2887 (15.1%)	4946 (15.5%)	3715 (8.2%)	2749 (9.1%)
20 - 29	11581 (60.5%)	19894 (62.4%)	27347 (60.7%)	19159 (63.5%)
30+	1085 (5.7%)	1587 (5.0%)	4056 (9.0%)	2511 (8.3%)
Number of times pregnant				
Missing	70 (0.4%)	129 (0.4%)	162 (0.4%)	96 (0.3%)
None	1458 (7.6%)	2450 (7.7%)	4838 (10.7%)	2901 (9.6%)
1	1177 (6.2%)	1956 (6.1%)	2966 (6.6%)	2175 (7.2%)
2	3510 (18.4%)	6387 (20.0%)	8336 (18.5%)	6550 (21.7%)
3	4100 (21.4%)	7523 (23.6%)	9936 (22.0%)	7190 (23.8%)
4	3383 (17.7%)	5739 (18.0%)	7818 (17.3%)	5169 (17.1%)
5	2228 (11.6%)	3547 (11.1%)	4908 (10.9%)	2930 (9.7%)
6	1356 (7.1%)	1950 (6.1%)	2724 (6.0%)	1523 (5.0%)
7	749 (3.9%)	1037 (3.3%)	1500 (3.3%)	780 (2.6%)
≥8	1096 (5.7%)	1139 (3.6%)	1875 (4.2%)	876 (2.9%)
Number of months breastfeeding (months)				
Missing	263 (1.4%)	420 (1.3%)	556 (1.2%)	311 (1.0%)
Never breastfed	9494 (49.6%)	15363 (48.2%)	22280 (49.4%)	13917 (46.1%)
1-6	5044 (26.4%)	8787 (27.6%)	10729 (23.8%)	7415 (24.6%)

Table 2: Hormonal and reproductive characteristics of HT users and non-users

	E-alone		E+P	
	Non-user N = 19127	**User N = 31857**	**Non-user N = 45063**	**User N = 30190**
7-12	1993 (10.4%)	3379 (10.6%)	4692 (10.4%)	3647 (12.1%)
13-23	1404 (7.3%)	2594 (8.1%)	4086 (9.1%)	3062 (10.1%)
≥24	929 (4.9%)	1314 (4.1%)	2720 (6.0%)	1838 (6.1%)
Oophorectomy status				
Missing	543 (2.8%)	547 (1.7%)	211 (0.5%)	109 (0.4%)
No	7320 (38.3%)	11234 (35.3%)	42752 (94.9%)	8692 (95.0%)
Yes	11264 (58.9%)	20076 (63.0%)	2077 (4.6%)	1389 (4.6%)
Unilateral	2714 (24.1%)	3905 (19.5%)	1855 (89.3%)	1247 (89.8%)
Bilateral	8127 (72.2%)	15738 (78.4%)	205 (9.9%)	134 (9.6%)
Unknown	423 (3.8%)	433 (2.2%)	17 (0.8%)	8 (0.6%)
Years since menopause (years)				
Missing	2953 (15.4%)	4630 (14.5%)	4640 (10.3%)	3858 (12.8%)
≤10	1386 (7.2%)	4026 (12.6%)	13435 (29.8%)	14019 (46.4%)
10-20	5116 (26.7%)	9808 (30.8%)	16672 (37.0%)	8980 (29.7%)
20-30	6988 (36.5%)	10510 (33.0%)	9064 (20.1%)	3023 (10.0%)
30-40	2429 (12.7%)	2698 (8.5%)	1212 (2.7%)	300 (1.0%)
> 40	255 (1.3%)	185 (0.6%)	40 (0.1%)	10 (<0.1%)

Table 3(on next page)

Glioma characteristics among HT users and non-users

Table 3: Glioma characteristics among HT users and non-users

	E-alone		E+P	
	Non-user	**User**	**Non-user**	**User**
Brain cancer	30	44	79	29
Glioma	27 (90.0%)	43 (97.7%)	71 (89.9%)	26 (89.7%)
GBM	20 (66.7%)	33 (75.0%)	58 (73.4%)	19 (65.5%)
Localization				
Localized	20 (74.1%)	30 (69.8%)	57 (80.3%)	17 (65.4%)
Regional	5 (18.5%)	8 (18.6%)	12 (16.9%)	7 (26.9%)
Distant	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Unknown	2 (7.4%)	4 (9.3%)	2 (2.8%)	2 (7.7%)
Morphology description				
Astrocytoma anaplastic	6 (22.2%)	4 (9.3%)	4 (5.6%)	3 (11.5%)
Astrocytoma NOS	1 (3.7%)	4 (9.3%)	3 (4.2%)	1 (3.8%)
Ependymoma NOS	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Gemistocytic astrocytoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.8%)
Glioblastoma NOS	19 (70.4%)	31 (72.1%)	53 (74.6%)	19 (73.1%)
Glioma - malignant	1 (3.7%)	2 (4.7%)	3 (4.2%)	0 (0.0%)
Gliosarcoma	0 (0.0%)	0 (0.0%)	2 (2.8%)	0 (0.0%)
Mixed glioma	0 (0.0%)	0 (0.0%)	1 (1.4%)	2 (7.7%)
Oligodendroglioma, anaplastic	0 (0.0%)	1 (2.3%)	2 (2.8%)	0 (0.0%)
Oligodendroglioma NOS	0 (0.0%)	0 (0.0%)	3 (4.2%)	0 (0.0%)
Site description				
Brain NOS	3 (11.1%)	3 (7.0%)	4 (5.6%)	4 (15.4%)
Brain stem	0 (0.0%)	2 (4.7%)	0 (0.0%)	0 (0.0%)
Cerebrum	1 (3.7%)	3 (7.0%)	5 (7.0%)	1 (3.8%)
Frontal lobe	9 (33.3%)	15 (34.9%)	25 (35.2%)	8 (30.8%)
Occipital lobe	0 (0.0%)	1 (2.3%)	3 (4.2%)	1 (3.8%)
Overlapping lesion of brain	4 (14.8%)	3 (7.0%)	8 (11.3%)	1 (3.8%)
Parietal lobe	3 (11.1%)	8 (18.6%)	10 (14.1%)	5 (19.2%)
Temporal lobe	7 (25.9%)	8 (18.6%)	16 (22.5%)	6 (23.1%)

Table 4(on next page)

Cox proportional regression modeling of hazard risk in subjects receiving HT

Table 4: Cox proportional regression modeling of hazard risk in subjects receiving HT

			E-alone group		E+P group	
	Univariate HR (95% CI)*	Univariate p-value	Multivariate HR (95% CI)*	Multivariate p-value	Multivariate HR (95% CI)*	Multivariate p-value
User vs. non-user HR						
E-alone group: User vs. non-user	0.91 (0.56, 1.48)	0.69	0.76 (0.43, 1.36)	0.36		
E+P group: User vs. non-user	**0.57 (0.36, 0.90)**	**0.02**			**0.48 (0.26, 0.88)**	**0.02**
Age: +5 years	1.19 (0.90, 1.57)	0.22	1.03 (0.60, 1.74)	0.93	**1.73 (1.03, 2.89)**	**0.04**
Education						
College degree or higher vs. School after high school	1.02 (0.73, 1.43)	0.91	1.29 (0.69, 2.42)	0.42	0.96 (0.54, 1.73)	0.90
High school or less vs. School after high school	0.82 (0.53, 1.27)	0.38	0.68 (0.31, 1.50)	0.34	0.92 (0.43, 1.97)	0.82
Annual family income						
$50,000 vs. $20,000 - $49,999	0.98 (0.69, 1.38)	0.90	0.73 (0.37, 1.43)	0.36	1.49 (0.85, 2.61)	0.17
< $20,000 vs. $20,000 - $49,999	0.91 (0.55, 1.49)	0.71	1.35 (0.64, 2.86)	0.44	0.81 (0.33, 1.99)	0.64
Smoking status						
Current vs. never	0.73 (0.34, 1.58)	0.42	0.87 (0.20, 3.74)	0.85	1.13 (0.39, 3.25)	0.82
Past vs. never	1.07 (0.78, 1.46)	0.69	1.44 (0.81, 2.55)	0.21	0.99 (0.58, 1.68)	0.97
Alcohol consumption						
≤1 drink/wk vs. none	1.19 (0.80, 1.78)	0.39	1.16 (0.58, 2.32)	0.67	1.18 (0.59, 2.35)	0.64
>1 drink/wk vs. none	0.99 (0.67, 1.47)	0.96	0.85 (0.40, 1.79)	0.67	0.98 (0.49, 1.96)	0.95
Previous cancer diagnosis: Yes vs. no	1.00 (0.54, 1.86)	0.99	0.86 (0.34, 2.18)	0.74	0.81 (0.20, 3.33)	0.77
Family history of cancer: Yes vs. no	1.14 (0.81, 1.61)	0.44	1.40 (0.73, 2.68)	0.31	0.88 (0.52, 1.47)	0.62

Table 4: Cox proportional regression modeling of hazard risk in subjects receiving HT

	E-alone group				E+P group	
	Univariate HR (95% CI)*	Univariate p-value	Multivariate HR (95% CI)*	Multivariate p-value	Multivariate HR (95% CI)*	Multivariate p-value
Previous hormone use: Yes vs. no	1.21 (0.89, 1.64)	0.23	1.21 (0.70, 2.11)	0.50	1.48 (0.87, 2.52)	0.15
Oral contraceptive use ever: Yes vs. no	0.90 (0.65, 1.26)	0.55	0.67 (0.36, 1.27)	0.22	1.14 (0.66, 1.99)	0.63
BMI						
25 - <30 vs. <25	1.15 (0.80, 1.66)	0.44	1.22 (0.63, 2.39)	0.56	1.11 (0.61, 2.03)	0.73
≥30 vs. <25	1.25 (0.85, 1.83)	0.26	0.99 (0.47, 2.11)	0.99	1.40 (0.74, 2.66)	0.30
Treated diabetes: Yes vs. no	1.01 (0.41, 2.46)	0.99	0.98 (0.23, 4.17)	0.98	0.59 (0.08, 4.32)	0.60
Age at menarche (years)						
≤11 vs. 12-14	1.28 (0.89, 1.82)	0.18	1.02 (0.53, 1.98)	0.95	1.12 (0.60, 2.11)	0.72
≥15 vs. 12-14	1.27 (0.77, 2.10)	0.34	0.89 (0.31, 2.51)	0.82	**2.06 (1.03, 4.15)**	**0.04**
Age at first birth (years)						
20-29 vs. None	0.96 (0.59, 1.56)	0.86			0.43 (0.12, 1.52)	0.19
30+ vs. None	1.20 (0.61, 2.37)	0.59			0.60 (0.15, 2.40)	0.47
<20 vs. None	0.93 (0.48, 1.79)	0.83			0.55 (0.12, 2.49)	0.44
Number of pregnancies						
1-2 vs. None	0.81 (0.44, 1.49)	0.50			2.13 (0.53, 8.63)	0.29
3-4 vs. None	1.15 (0.66, 2.01)	0.61			2.56 (0.57, 11.49)	0.22
≥5 vs. None	1.03 (0.57, 1.86)	0.93			1.86 (0.38, 8.97)	0.44
Number of months breastfed (months)						

Table 4: Cox proportional regression modeling of hazard risk in subjects receiving HT

			E-alone group		E+P group	
	Univariate HR (95% CI)*	Univariate p-value	Multivariate HR (95% CI)*	Multivariate p-value	Multivariate HR (95% CI)*	Multivariate p-value
1-12 vs. None	1.17 (0.84, 1.63)	0.36	1.46 (0.83, 2.59)	0.19	0.82 (0.44, 1.54)	0.54
>12 vs. None	1.12 (0.71, 1.76)	0.63	0.48 (0.14, 1.63)	0.24	1.38 (0.68, 2.79)	0.37
Oopherectomy status: Yes vs. no	1.19 (0.86, 1.65)	0.29	1.15 (0.63, 2.11)	0.64	1.01 (0.32, 3.25)	0.98
Years since menopause (years)						
10-20 vs. <10	0.86 (0.55, 1.36)	0.52	1.09 (0.39, 3.06)	0.87	0.72 (0.33, 1.56)	0.41
20-30 vs. <10	0.86 (0.50, 1.46)	0.57	0.65 (0.21, 1.99)	0.45	1.06 (0.38, 3.00)	0.91
>30 vs. <10	0.45 (0.16, 1.23)	0.12	0.39 (0.09, 1.74)	0.22	0.54 (0.06, 5.06)	0.59

* Stratified by 10 year age intervals and HT trial participation.
** Stratified by 10 year age intervals, HT trial participation and hysterectomy at baseline.

Table 5(on next page)

GBM hospital and diagnosis characteristics among subjects receiving HT

Supplementary table 1: GBM hospital and diagnosis characteristics among subjects receiving HT

	E-alone		E+P	
	Non-user	**User**	**Non-user**	**User**
Brain cancer	30	44	79	29
Glioma	27 (90.0%)	43 (97.7%)	71 (89.9%)	26 (89.7%)
GBM	20 (66.7%)	33 (75.0%)	58 (73.4%)	19 (65.5%)
Reporting source				
Hospital inpatient	25 (92.6%)	38 (88.4%)	67 (94.4%)	24 (92.3%)
Hospital outpatient/radiation/chemo, surgical center, clinic	1 (3.7%)	2 (4.7%)	3 (4.2%)	1 (3.8%)
Laboratory only including pathology office	0 (0.0%)	2 (4.7%)	1 (1.4%)	1 (3.8%)
Physician's office/private medical practitioner	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Death certificate only	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Diagnostic confirmation				
Positive histology (pathology)	25 (92.6%)	39 (90.7%)	62 (87.3%)	25 (96.2%)
Radiography & other imaging techniques w/o micro confirm	1 (3.7%)	3 (7.0%)	9 (12.7%)	1 (3.8%)
Clinical diagnosis only (other than 5, 6, 7)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Unknown if microscopically confirmed	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Missing	2 (7.4%)	3 (7.0%)	9 (12.7%)	4 (15.4%)
Unknown cancer site	0 (0.0%)	0 (0.0%)	1 (1.4%)	0 (0.0%)
Other known cause	0 (0.0%)	1 (2.3%)	1 (1.4%)	0 (0.0%)

Table 6(on next page)

Annualized rates of brain cancer (glioma) by race and treatment group

Supplementary table 2: Annualized rates of brain cancer (glioma) by race and treatment group

	White N (% per year)	Non-white N (% per year)	All N (% per year)
E+P user	26 (0.0072)	4 (0.0081)	30 (0.0073)
E+P non-user	71 (0.017)	1 (0.0010)	72 (0.012)
E-alone user	43 (0.012)	3 (0.0045)	46 (0.010)
E-alone non-user	27 (0.013)	3 (0.0045)	30 (0.011)