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286 *The risk of all-cause mortality associated with HT use*

287 On average, ET use was associated with a significant, 20% reduction in mortality risk relative to
288 no ET use (Table 4-a) which translated to 77,401 fewer expected deaths in our large population.
289 All combinations of ET type, route, and dose were also associated with reduced mortality risk.
290 The marginal risk of E2 on mortality was significantly less than that of CEE. Vaginal,
291 transdermal, and oral preparation, in order by size of mortality risk, each had significantly less
292 mortality risk than the one after. The mortality risk of low and medium doses were significantly
293 less than high dose but were not different from each other. Overall, EPT was associated with
294 significant 12% reduction in marginal risk of mortality. Progestin monotherapy had no significant
295 association. Interestingly, oral CEE medium dose, comparable to the drug in the WHI trial of ET,
296 exhibited less risk reduction in mortality (9%) than overall ET (Table 6-a).

297 The 6,405,685 patients (90% of those in the primary analysis) still in the study 12 months after
298 Part D enrollment were the subjects for our ITT analysis. No censoring occurred after the 12-
299 month cut-off in the ITT analysis; so, could not have shaped these results. Yet, marginal
300 association between each type, route and dose level of HT and death from this analysis paralleled
301 and exceeded the mortality reduction observed in the primary analysis (Table 3-b). So, the biases
302 due to informative censoring are unlikely to account for the mortality reduction observed in the
303 primary analysis.

304

305 *The risk of breast, lung, endometrial, colorectal, and ovarian, cancer associated with HT use*

306 During our study period, breast cancer incidence was four times that of any other study cancer
307 (Table 3). ET use was associated with significant *reduction* in marginal risk of breast cancer, 18%
308 overall, as well as within each combination of ET type, route, and dose size (Table 4-c). Oral ET

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exhibited significantly greater reductions than transdermal and transdermal ET significantly greater than vaginal. Furthermore, CEE was associated with significantly greater risk reduction for breast cancer than E2 – a pattern opposite to what we saw with mortality as the outcome. The intervention in WHI's 13-year long-term follow-up postintervention study, oral CEE 0.625 mg, was associated with a 21% % risk reduction for breast cancer (5). Seventy percent of our oral CEE medium dose prescriptions were 0.625 mg, same strength as the WHI dose, 11% were greater (0.9 mg) and 19% less (0.45 mg) than 0.625 mg. In our study, oral CEE medium dose use was associated with a 29% risk reduction and was the second best among all oral ET (Table 6-c). To the negative, both EPT and progestin monotherapy significantly *increased* the risk of breast cancer (Table 3-c).

ET was associated with 13% and 14% decrease in the risks of lung and colorectal cancers, respectively. EPT also exhibited reductions in lung and colorectal cancer risks but by smaller amounts than ET. Progestin monotherapy was also associated with reduced lung cancer risk but had no association with colorectal cancer risk (Table 3-d & f).

With ET use, endometrial, and ovarian cancer risks declined moderately (Table 3-e & g) but this finding is likely an artifact of the selective use of ET in hysterectomized women who lack the organs where such cancers could arise. More than half of hysterectomized women also had bilateral oophorectomy (28). So, these two findings might not be meaningful. On the other hand, 35% risk reduction of endometrial cancer associated with EPT use could be meaningful because it is usually prescribed for women with an intact uterus.

The risk of IHD, CHF, VTE, Stroke, AFM, AMI, and Dementia, risk associated with HT

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IHD occurred in more than a million subjects, twice the size of the next most frequent CV condition, CHF. So, ET's 3% increased risk of IHD is of overweight importance. Most types, routes and dose levels of ET were also associated with increased risk of IHD, up to 19% with the injectables, but less so (3-4%) in other types, route, and doses. Specifically, low dose oral CEE and E2 were associated with significant risk reduction for IHD by 3% and 2%, respectively (Table 7-a). Progestin monotherapy had the same 3% increased risk for IHD as ET. Interestingly, EPT formulations, overall, were associated with a significant 4% risk reduction of IHD (Table 4-a).

To the positive, ET use, overall, was associated with 5% decrease *in* the risk of CHF (Table 4-b). However, among the ET subgroups of high dose and injectable ET were associated with increased risk of CHF. As was true for IHD, all EPT formulations were associated with *reduced* CHF risk.

Overall ET use had small or no, associations with, stroke or dementia, risk (Table 4-d & g). However, high and medium doses of oral E2 and CEE had important increased risk of stroke, reaching 25% with high dose oral CEE (Table 7-d & g). Both estrogen types (CEE and E2) were also associated with increased dementia risk but only with high dose oral preparations. Transdermal and vaginal preparations of ET, that avoid first pass travel through the liver were associated with small *reduce*d or null risk of both conditions, consistent with the results of other studies (29) and with the procoagulant and proinflammation effects of estrogen's liver passage (30,31). EPT use, on average, was also associated with small but significant, *increased* risk of dementia and, decreased risk of all 6 CV conditions.

Discussion

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This study is a population-based retrospective observational study that explored the associations between estrogen types, routes, and dose size and all-cause mortality, dementia, 5 common cancers, and 6 cardiovascular, conditions using an extended Cox regression analysis. Important limitations included that data availability only began at age 65; hysterectomy information was unavailable for most of our subjects.; we depended on claims for encounter diagnoses and could not validate them through chart review; and, as is true for all observational studies, differential influences of unmeasured confounders, such as adherence to healthy behavior among HT users, could have been present.

One strength was the use of filled prescription records, rather than patient recall, to ascertain HT use. Another strength was its size —a study population of more than 7 million menopausal women, nearly an order of magnitude larger than any previous HT study (32). Its large size let us estimate differences in associations between each of 22 HT drug type, route and dose combinations on the one hand and the many study outcomes on the other.

Association with all-cause mortality

ET use, overall, was associated with a 20% reduction in mortality risk considering all ET preparations together. The sole estrogen preparation studied in the WHI trials was 0.625 mg of oral CEE, and it exhibited a close to significant, 6% (HR=0.94; 95% CI 0.88-1.01) mortality reduction in the 18-year long-term follow-up of the WHI trails (33). This result gives some plausibility to the 9% mortality reduction we observed in association with the use of our medium dose, oral CEE which was almost identical to the drug randomized in WHI's estrogen only trial. In our study, vaginal E2 in low and medium doses were associated with greatest reduction of mortality risk (29-40%) (Table 6-a). Our overall mortality results are consistent with the mortality results from an meta-analysis of 31 observational and RCT studies that reported reduced mortality among HT users (34) and with the re-analyses of the Prostate, Lung, Colorectal, and

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Ovarian (PLCO) Cancer Screening RTC, which reported a 23% decrease in all-cause mortality among current users of any HT (35).

Associations with cancer

In our study, overall ET use and oral CEE medium dose use were associated with an 18% and 29% reduction of breast cancer risk, respectively. The long-term follow-up of the WHI study reported a significant, and similarly sized, 21% reduction in breast cancer incidence (36) giving credence to this result.

In our study, ET use was also associated with significantly reduced colorectal (14%) and lung (13%) cancer risk as was EPT, but to a lesser degree. A few observational studies support our observations of reduced lung cancer rates in association with HT use (37,38), though there is controversy. Two observational studies (39,40) and a re-analysis of the PLCO trial data (35) support our protective associations between HT use and colorectal cancer. The greater incidence of colorectal (+79%) (41) and lung cancer (+26%) (42) among patients with lower level of estrogen due to oophorectomy as part of their hysterectomy compared to hysterectomized patients without ovary removal also support our results by implying that estrogen protects against these two cancers. On the downside, both EPT and progestin alone were associated with significant *increases* (11% and 9% respectively) in breast cancer risk.

Associations with CV disease and dementia

Our data revealed a 3% increase in risk for IHD with ET use compared to no use. However, this increased risk is concentrated in the use of high (9%) and medium (2%) dose levels. Low dose

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ET, used by one third of ET users, exhibited a salubrious, 2%, d*ecreased* risk for IHD. The associations between ET use and other CV conditions was also to the good—4-11% risk reductions. E2 and vaginal/transdermal compared to CEE and oral preparation exhibited numerically lower risks for all CV conditions. It further suggests that a similar decrease in dementia risk would accrue when low dose, topical (vaginal, transdermal) and E2, were prescribed and in stroke risk when low dose topicals and low dose oral E2 were prescribed. Topical ET preparations in our study, that would avoid the procoagulant and proinflammatory effects ascribed to liver passage (30,31), were associated with *reduced* risk of both conditions, consistent with the results of other studies (29).

Overall considerations

The risk reduction associated with various HT we saw generally favor less use of EPT, more use of the topical (vaginal or transdermal) rather than the oral route, use E2 rather than CEE, and low or medium dose rather than high dose for menopausal care. These advantages have been emphasized by others (43) and our trend data suggest prescribers have adopted them to some degree. However, our results on breast cancer are less consistent with patterns we saw with mortality as the outcome. ET use overall exhibited a decreased risk of breast cancer, but the reduction was significantly greater among CEE, oral route, and high dose, uses, than their counter parts. Further our EPT was used only by small subset of HT users (12%) and in that subset its use was associated with a reduction in the risk of endometrial cancer.

We did not include fractures as an outcome in our study. However, among the outcomes we included, we saw significant association with the use of specific HT medications that paralleled effects seen in each of the *significant* WHI end points including, the decreased incidence of colorectal cancer with both ET and EPT use (35), the decreased incidence of breast cancer with

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ET use, the increased incidence of breast cancer with EPT respectively and the decreased incidence of endometrial cancer with EPT use (36). These parallels give credence to our observational results.

Our follow-up began when women entered Medicare at about age 65, but it is likely that many of them started taking HT closer to the time of their menopausal symptoms and continued it into their Medicare years. If so, our positive results align with the timing hypotheses (44) that asserts HT use early in menopauses is better than later, but extend it by reporting positive effects with usage continued into Medicare years. Such use is not a rarity per our data.

The FDA's black box warning about HT (45) extrapolates from the deleterious effect of EPT on breast cancer (1) and dementia (46) to all forms of ET, because no studies to the contrary existed then. Now, such studies do exist. The long-term WHI studies reported a significant decrease in breast cancer incidence (5) and death due to Alzheimer's disease (33), among patient in the ET study arm. Furthermore, many studies suggest that E2, topical routes, and low doses may produce better outcomes than CEE, oral and medium dose (0.625 mg) studied in the WHI's ET study (47,48).

Menopausal HT of all types has been maligned by the results of earliest WHI study (1), long public memory of the frightening media reports about it (2) and lack of attention to recent more positive studies. Our study though purely observational raises the *possibility* of important improvements in survival and reduction in the risk of three cancers. We hope our results stimulate, interest in hormone therapy and further observational studies using other large national database and biologic research, to support them.