5-2019

Do Molecular Subtypes of Breast Cancer Affect Outcomes Following Hypofractionation or Conventional Fractionation Radiation Therapy?

Richard Sleightholm
University of Nebraska Medical Center

Do Molecular Subtypes of Breast Cancer Affect Outcomes Following Hypofractionation or Conventional Fractionation Radiation Therapy? A Single Institute Study of 311 Breast Cancer Patients

Richard Sleightholm

Committee:

Lynette Smith PhD (Chair)

Chi Lin MD/PhD

Stephanie Koraleski PhD

Armando De Alba Rosales MD/MPH

Michael Baine MD/PhD

Department of Radiation Oncology, University of Nebraska Medical Center, Omaha, Nebraska

ABSTRACT

Introduction

Molecular subtypes of breast cancer (BC) are well-established prognostic markers in early-stage BC patients. The addition of radiation therapy (RT) to breast-conserving surgery has improved outcomes in this patient population, with conventional fractionation (CF) and hypofractionation (HF) regimens displaying comparable morbidity and mortality. However, most studies have not taken into account molecular subtype. Thus, it is still unknown if outcomes are similar between CF and HF for each molecular subtype. Herein, the effects of molecular subtype on the efficacy of CF and HF radiotherapy regimens for early-stage BC patients receiving adjuvant RT was investigated.

Methods

A retrospective review of stage I/II BC patients who received surgical intervention (breast conservation surgery or mastectomy) followed by RT at UNMC between 2010 and 2017 was conducted. Demographics, tumor characteristics, treatment data (course and dose of radiotherapy), and outcomes information (progression and survival) were collected. Cumulative incidence function and Kaplan-Meier testing were used to assess recurrence and survival, respectively. Variables were then further analyzed using univariate and multivariable COX proportional hazard models.

Results

In total, 311 patients met the inclusion criteria, including 211 CF and 100 HF patients. Patients undergoing HF were of lower stage and grade, but increased age. Rates of locoregional recurrence, distant recurrence, and survival were similar between cohorts. When stratifying based on molecular subtype, no differences in recurrences or survival were observed. On multivariable analysis, only stage was a significant predictor of distant failure and survival.

Conclusions

Although patient numbers were low, these findings suggest that HF and CF are equally efficacious in controlling locoregional recurrence in early stage BC patients. Thus, a hypofractionated regimen for radiation therapy should be considered an option regardless of molecular subtype in early stage BC following breast conserving surgery.

Introduction

In most women with early-stage breast cancer (BC), whole breast radiation therapy (RT) is recommended after breast conserving surgery.[1] Breast conserving therapy has been shown to improve overall outcomes in BC compared to mastectomy or no surgical intervention, and the addition of RT further decreases the rate of recurrence, signifying an increase in local tumor control.[2, 3] In a meta-analysis of 17 trials of breast conserving therapy, Darby *et al.* showed that RT significantly decreased the risk of local and distant recurrences over 10 years and reduced BC-related mortality rates.[4] Refinement in RT protocols have improved outcomes for patients extending beyond simple survival metrics leading to reductions in both immediate complications and long-term sequelae of RT. These improvements across several health-related metrics including outcomes and quality of life have led to the National Comprehensive Cancer Network (NCCN) recommendation and current standard of care practice of RT for early-staged BC patients in the US.[5]

The likelihood of radiation-related damage to surrounding, healthy tissue increases as dose per fraction increases. This served as the basis for the longer dose schedule in traditional whole breast irradiation treatments, termed conventional fractionation (CF).[6] CF comprises 5 to 7 weeks of daily radiation treatments (1.8 -2 Gy per fraction) with a common regimen consisting of 50-50.4 Gy in 25-28 fractions to the whole breast and an additional 10 Gy in 5 fractions boost to the tumor bed. Although adherence to radiation therapy is good compared to chemotherapy and tamoxifen, a significant percentage of women in the US choose not to undergo CF because of the inconvenience of the long time course and the associated cost.[7, 8] In recent years, hypofractionated (HF) radiation therapy has been explored as a means to increase adherence and,

in some instances, alter the therapeutic window of RT by using larger doses per fraction but an overall lower total dose of radiation, i.e. 39 Gy in 13 fractions.

With the challenges of adherence, expense, and time commitment in mind, and as the momentum in radiation oncology moves toward shorter and cheaper treatment regimens, randomized trials comparing HF to CF have emerged. The Standardization of Breast Radiotherapy Trials (START A and START B) looked at CF versus two different HF regimens in BC patients with completely excised invasive (breast-conservation therapy or mastectomy) BC from several centers across the United Kingdoms. In START A, patients were randomly assigned to one of three groups: CF patients – 50 Gy in 25 fractions over 5 weeks (n=749) versus HF patients that received either 39 Gy (n=750) or 41.6 Gy (n=737) in 13 fractions given over 5 weeks. There was no significant difference in 10-year rates of local-regional relapse between the treatment groups. Furthermore, the rates of radiation-related complications were lower in those who received 39 versus 50 Gy, suggesting that lower total doses of therapy delivered in fewer, larger doses per fraction are at least as safe as CF.[9] Similar to START A, START B investigated a slightly shorter HF regimen (40 Gy in 15 fractions over 3 weeks, n=1110) compared to CF (50 Gy in 25 fractions, over 5 weeks, n=1105). The findings of START B paralleled START A and showed that rates of radiation-related complications were again lower in those who underwent HF.[9] In terms of efficacy, both treatments displayed similar 10-year local-regional control rates (HF - 95.7% v CF – 94.5%), while 10-year distant relapse (12.3% v 16.0%) and all-cause mortality (15.9% v 19.2%) rates were improved in the HF versus CF cohort.[10] In 2002, Whelan *et al.* presented the results from a single-institute, prospective, randomized study of 1,234 women who had node-negative, invasive BC with negative margins after lumpectomy, and received either 42.5 Gy in 16 fractions

(HF, n=622) or 50 Gy in 25 fractions (CF, n=612). The results of this study demonstrated equivalent 5- and 10-year local/regional disease-free and overall survival.[11, 12]

The efficacy of hypofractionation dosing schemes for RT in BC is based on the concept of alpha-beta ratios for radiation-induced cell death. Normal tissues have an alpha-beta ratio of approximately 3. Most tumors have an alpha-beta ratio between 8 - 10; however, slower growing tumors can have much lower rates. For example, BC has a relatively low alpha-beta ratio in the range of 3.5 - 4.[13-15] Applying these alpha-beta ratios to the linear quadratic equation to determine the biologically equivalent dose (BED = N*D[1+D/[ABR]] where N = # of fractions, D = dose per fraction, ABR = alpha-beta ratio) demonstrates that the hypofractionation regimen (higher dose per fraction with fewer total fractions) limits the long-term toxic effects on adjacent healthy tissues while remaining efficacious against the tumors. However, this rationale is potentially challenged when considering the higher-risk, more aggressive molecular subtypes of BC that possess faster growth rates and likely higher alpha-beta ratios.[16] Therefore, we sought to evaluate the efficacy of hypofractionation vs conventional fractionation of radiation therapy in the treatment of different molecular subtypes of BC.

Although several studies have surfaced outlining outcomes in HF compared to CF, there is little data from these studies to describe the effects of molecular subtypes in HF. The histological workup of a tumor's biological characteristics is a well-established prognostic factor, and estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (Her2) receptor expression guides our current target-based therapies in BC.[17] In fact, the combination of these factors have been used to describe subtypes of BC that have prognostic and predictive value: luminal A (ER or PR positive, but lacking Her2), luminal B (ER or PR positive and Her2 expressive), Her2 expressing only, and triple negative.[18] Zhao *et al.* performed a randomized clinical trial exploring long term

outcomes related to CF or HF treatment, in which molecular markers were also analyzed.[19] While HER2+ status was an independent prognostic factor for reduced tumor specific survival, no association was observed in for ER/PR status, and the authors did not describe outcomes stratified by CF or HF treatment as they relate to the molecular subtypes. One previous study has evaluated the effect of HF vs CF regimens of RT in early stage BC with adverse prognostic features, which failed to demonstrate any difference in relapse following HF vs CF RT on luminal vs non-luminal molecular subtypes.[20] With the lack of clinical understanding of the impact these molecular subtypes may have on outcomes in RT, histological markers are not routinely considered when it comes to guiding the decision making of implementing HF versus CF. Thus, we sought to determine if molecular subtypes had any bearing on outcomes inpatients treated with HF or CF RT at our institute.

Methods

A retrospective analysis was performed on BC patients who were consulted for radiation therapy at the University of Nebraska Medical Center between 2010 and 2017. Inclusion criteria included pathologically confirmed disease, surgical intervention, and RT as part of their initial treatment plan, *i.e.* not at recurrence. Those with ductal carcinoma *in situ* or disease greater than stage II were excluded. Those who elected not to undergo radiation therapy, did not finish their planned course, or received intraoperative radiation or brachytherapy were also excluded from the present study. Patients meeting the inclusion criteria were partitioned into those who received conventional fractionation (CF) or hypofractionation (HF) therapy. Treatment for CF consisted of the following dosing regimens (Total Gy in X fractions): 45 in 25, 46 in 23, 50 in 25, or 50.4 in 28. Similarly, HF regimens consisted of: 40.05 in 15 or 42.56 in 16.

A comprehensive evaluation was performed comparing the two cohorts evaluating demographics (age, sex, year of diagnosis and treatment, histology, stage, etc.), tumor pathology, treatment (surgical intervention, chemotherapy, etc.), details of their radiation treatment (dose, fractionation pattern, tumor cavity boost, etc.), and progression and survival outcomes. Molecular subtypes of BC were defined using the following criteria: (1**) Luminal A** – ER or PR positive and Her-2 negative with a ki-67<14; (2) **Luminal B** – ER or PR positive and Her-2 positive or ki67≥14; (3) **Her2 enriched** – those lacking ER and PR staining but expressing Her2; (4) **Triple Negative** – those who do not display ER, PR, and Her2 expression.

All data was analyzed using SAS (version 9.4, SAS/STAT 14.3). Patient and cancer characteristics were reported in Table 1 using descriptive statistics. Continuous variables, i.e. age, were compared between the two groups using a two-tailed student's t-test. Chi-squared testing was used to compare all dichotomous variables between groups. Recurrence was defined as months from the date of diagnosis until radiological or pathological evidence of recurrence, last follow-up visit, or date of death. Patients who were alive and lacking evidence of disease or that were lost to follow-up were treated as censored. Time to local-regional recurrence (LR) and distant recurrence (DR) was assessed both as disease-specific recurrence, treating death without the identification of disease recurrence as censored, as well as in the presence of death as a competing risk in cumulative incidence function (CIF) modeling. Similarly, survival was defined as months from the date of diagnosis until date of death or last date of follow-up and was assessed in three different manners: 1) overall survival (OS) – death from any cause; 2) cause-specific survival (cancer-specific survival, CSS) - death from non-cancer causes were treated as censored; AND 3) competing risk survival – death from non-cancer causes were treated as a competing event in a CIF model. Patients who were alive at the end of the study or lost to follow-up were treated as

censored. LR, DR, and survival were visualized using CIF and Kaplan-Meier (KM) method, and Gray's test or the log rank test was used to evaluate statistically significant differences between cohorts. Further use of Cox proportional hazard (PH) regression was applied to control for variables known to influence survival, i.e. age, stage, etc., and assess any difference between treatment cohorts.

All statistical tests were conducted in a two-sided manner, and statistical results with $p < 0.05$ were considered statistically significant. Review of all medical records and the subsequent analyses were approved by the appropriate institutional review committee and met the guidelines of their responsible governmental agency.

Results

In total, 311 patients were identified, which included 211 CF and 100 HF patients. Median overall follow-up time for all patients was 35.7 months, including 36.6 months for patients who received CF and 30.8 months for patients who received HF. General demographic and tumor characteristics can be found in Table 1. Overall, patients in the HF cohort were of lower T-stage ($p<0.001$), lower grade ($p<0.001$), higher proportion of stage I versus II ($p<0.0001$) and older age ($p=0.001$). For each RT regimen, CF or HF, there were multiple included doses and fractionation schemes that are outlined in Table 2.

Conventional vs Hypofractionation Regimens for Radiation Therapy in Early Stage Breast Cancer

Comparing outcomes in patients with early stage BC treated with RT that was administered using CF or HF (Fig 1) revealed no statistically significant difference in LR ($p=0.6263$) or cancer-specific survival ($p=0.4387$), though distant failure was of borderline significance ($p=0.0763$) in favor of HF. In all three outcomes measures, CF performed slightly worse than HF regimens of RT and had more total failures (Locoregional: 8 CF vs 1 HF; Distant: 14 CF vs 1 HF; cancer-

specific survival: 8 CF vs 1 HF); however, these were not statistically significant differences. Of note, 9 patients died from cancer-related causes (8 CF and 1 HF), while 6 and 1 additional patients within the CF and HF cohorts respectively died without evidence of LR or DR, had cancer-independent causes of death, and were therefore analyzed in accordance with competing risks. Due to the overall low incidence, the effects of competing risks were negligible in all three outcome metrics, LR, DR, and survival, see supplementary section.

Effect of Stage on Conventional vs Hypofractionation Regimens Efficacy

To evaluate the possibility that the difference in stage distribution between CF and HF cohorts (Table 1) was contributing to any efficacy difference between CF and HF cohorts, the effect of stage on LR and DR as well as cancer-specific survival was evaluated. Increased stage did not affect LR ($p = 0.5046$), correlated with an increase in distant failure ($p = 0.0043$), and decreased overall survival ($p = 0.0325$) (Fig. 2 A-C). Cancer-specific deaths were higher in stage II than stage I (7.6% vs 1.8%). However, evaluating the effect of CF vs HF on patient outcomes after isolating individual stage I (Fig. 2 D-F) or stage II (Fig. 2 G-I) patients failed to demonstrate a difference in efficacy between the fractionation regimens for any given stage ($p > 0.05$).

Effect of molecular subtype on Conventional vs Hypofractionation Regimens Efficacy

While the distribution of molecular subtypes between fractionation regimens were largely consistent (Table 1), the possibility that molecular subtypes could be a confounder and independently affect patient outcomes needed to be examined. Molecular subtype did not affect local failure ($p = 0.1815$) (Fig. 3A) or distant failure ($p=0.3399$) (Fig. 4A), but did impact overall survival ($p=0.0452$) (Fig. 5A). Cancer-specific deaths were higher in triple negative patients (12% versus luminal A – 0%, luminal B – 4.8%, and HER-2 enriched – 5.9%).

To evaluate the possibility that the CF vs HF regimens of RT may be more or less efficacious for a given molecular subtype, the effect on patient outcomes following CF or HF RT was evaluated for each molecular subtype individually. When looking at patient outcomes as stratified by CF vs HF for each molecular subtype, no statistically significant difference in efficacy was demonstrated (Fig. 3B-E, Fig. 4B-E, Fig. 5C-F); however, HF regiments appeared to do at least as well if not better than CF.

COX PH Multivariable Analysis of Factors Contributing to Patient Outcomes in Early Stage Breast Cancer

Fractionation regimen, stage, age, and molecular subtype were analyzed for their relationship to patient outcomes via univariate and multivariable analyses using the Cox PH model. On univariate analysis, no factors were associated with LR, and after controlling for the effects of each variable, multivariable analysis of LR failed to reveal any associations (Table 3). Increased stage and the interaction of molecular subtype with stage or fractionation regimen appeared to be of potential significance for increased DR (Table 4). Additionally, stage remained statistically significantly associated with increased DR on multivariable analysis ($p = 0.038$). Lastly, stage and molecular subtype were found to be associated with cancer-specific survival, but upon further evaluation with multivariable analysis, stage was only borderline significant (Table 5).

Discussion and Conclusions

Overall, patients undergoing HF were of lower stage and grade, but older age. There appears to be a clear selection bias for older, but lower-risk, cancer patients to receive HF versus CF. Despite this selection bias, our results do not support a need to select patients with lower stage disease for HF vs CF as controlling for stage revealed that HF regimens were equally efficacious as CF for any given stage. Looking at molecular subtypes, HF appears to be as effective as CF

within each subtype in controlling both local and distant recurrence and produces similar survival outcomes. Thus, these results demonstrate the HF regimens appear equally efficacious as CF in early stage BC patients regardless of stage and molecular subtype. However, a larger, multicenter study should be performed to validate these findings.

Higher stage disease was associated with a higher rate of distant failure and poorer survival. Molecular subtypes were also associated with different rates of survival with Luminal A tumors having the best survival and Luminal B and triple negative tumors demonstrating poorer outcomes. On multivariable analysis, only the effect of increasing stage on higher rates of DR was statistically significant; however, stage and triple negative molecular subtype approached significance in regards to worsening survival. Sensitivity analyses were performed investigating interactions and competing events. Because of the overall low number of competing events, competing risk analyses did not differ from their non-competing event statistical counterpart.

Unfortunately, the conclusions that can be drawn from this study are limited by the relatively small number of patients, patient diversity, and short median follow-up of approximately three years. The small number of patients is especially problematic when attempting to stratify by treatment, molecular subtype, and/or stage where the patient numbers are further reduced. Approximately 80% of patients were Caucasian, and the remaining 20% were distributed over Asian, African American, and Hispanic/Latino ethnicities. Although there is little evidence to date to suggest substantially different outcomes from RT between different racial or ethnic classes, there may exist some differences. Furthermore, socioeconomic status, which is a strong influencer of outcomes, could impact outcomes. However, these two determinants of health were not evaluated in the present study. In regards to the short median follow-up, it is important to consider that the high risk molecular subtypes (HER2-enriched and triple negative) tend to recur both

locally and distantly relatively early in the course of the disease (often by year 2). Thus, median follow up of 3 years should be sufficient to demonstrate if HF regimens of RT were less efficacious than the CF regimens. Moreover, early relapse distantly may suggest spread beyond the area of treatment with RT, and one should consider the goal of RT, which is directed at locoregional control rather than distant. Finally, this study is somewhat limited due to an uneven distribution of cancer stage between the two cohorts. The impact of this limitation was managed by evaluating the efficacy of HF vs CF on patient outcomes within a given cancer stage (either Stage I or Stage II), which demonstrated similar results as compared to the evaluation of the entire cohort of patients. Clearly, despite these limitations, our results demonstrate that the overall rates of LR, DR, and survival appeared comparable in patients who received HF vs CF.

In conclusion, early stage BC patients who received HF following breast conserving surgery displayed similar outcomes to those who received CF. Although stage and molecular subtype impacted recurrence in distant locations and survival, their influence on outcomes appear comparable between RT regimens. These results suggest that HF regimens should be considered in early-stage BC patients and may even be favored for therapy due to the benefits of shorter treatment protocols. However, a larger, prospective study should be conducted that can control for age, stage, and molecular subtype in addition to race/ethnicity and social determinants of health to validate the findings in this study.

References

1. Lee, M.C., et al., *Determinants of Breast Conservation Rates: Reasons for Mastectomy at a Comprehensive Cancer Center.* The Breast Journal, 2009. **15**(1): p. 34-40.
2. Agarwal, S., et al., *Effect of breast conservation therapy vs mastectomy on disease-specific survival for early-stage breast cancer.* JAMA Surg, 2014. **149**(3): p. 267-74.
3. Fisher, B., et al., *Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer.* N Engl J Med, 2002. **347**(16): p. 1233-41.
4. Darby, S., et al., *Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials.* Lancet, 2011. **378**(9804): p. 1707-16.
5. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology. Uterine Neoplasms Version 1.2018. - March 20, N.C.C.N.G.a.a.h.
6. Fisher, C.M. and R. Rabinovitch, *Frontiers in radiotherapy for early-stage invasive breast cancer.* J Clin Oncol, 2014. **32**(26): p. 2894-901.
7. Ma, A.M., et al., *Noncompliance with adjuvant radiation, chemotherapy, or hormonal therapy in breast cancer patients.* Am J Surg, 2008. **196**(4): p. 500-4.
8. Bekelman, J.E., et al., *Uptake and Costs of Hypofractionated vs Conventional Whole Breast Irradiation After Breast Conserving Surgery in the United States, 2008–2013.* JAMA, 2014. **312**(23): p. 2542-2550.
9. Bentzen, S.M., et al., *The UK Standardisation of Breast Radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial.* Lancet, 2008. **371**(9618): p. 1098-107.
10. Haviland, J.S., et al., *The UK Standardisation of Breast Radiotherapy (START) trials of radiotherapy hypofractionation for treatment of early breast cancer: 10-year follow-up results of two randomised controlled trials.* Lancet Oncol, 2013. **14**(11): p. 1086-1094.
11. Whelan, T., et al., *Randomized trial of breast irradiation schedules after lumpectomy for women with lymph node-negative breast cancer.* J Natl Cancer Inst, 2002. **94**(15): p. 1143-50.
12. Whelan, T.J., et al., *Long-term results of hypofractionated radiation therapy for breast cancer.* N Engl J Med, 2010. **362**(6): p. 513-20.
13. Whelan, T.J., D.H. Kim, and J. Sussman, *Clinical experience using hypofractionated radiation schedules in breast cancer.* Semin Radiat Oncol, 2008. **18**(4): p. 257-64.
14. van Leeuwen, C.M., et al., *The alfa and beta of tumours: a review of parameters of the linear-quadratic model, derived from clinical radiotherapy studies.* Radiat Oncol, 2018. **13**(1): p. 96.
15. Qi, X.S., J. White, and X.A. Li, *Is alpha/beta for breast cancer really low?* Radiother Oncol, 2011. **100**(2): p. 282-8.
16. Foulkes, W.D., I.E. Smith, and J.S. Reis-Filho, *Triple-negative breast cancer.* N Engl J Med, 2010. **363**(20): p. 1938-48.
17. Rakha, E.A., et al., *Breast cancer prognostic classification in the molecular era: the role of histological grade.* Breast Cancer Res, 2010. **12**(4): p. 207.

18. Lin, R. and P. Tripuraneni, *Radiation Therapy in Early-Stage Invasive Breast Cancer.* Indian journal of surgical oncology, 2011. **2**(2): p. 101-111.
19. Zhao, S., et al., *The long-term outcome of adjuvant hypofractionated radiotherapy and conventional fractionated radiotherapy after breast-conserving surgery for early breast cancer: a prospective analysis of 107 cases.* J Thorac Dis, 2017. **9**(10): p. 3840-3850.
20. Lee, S.W., et al., *Accelerated whole breast irradiation in early breast cancer patients with adverse prognostic features.* Oncotarget, 2016. **7**(49): p. 81888-81898.

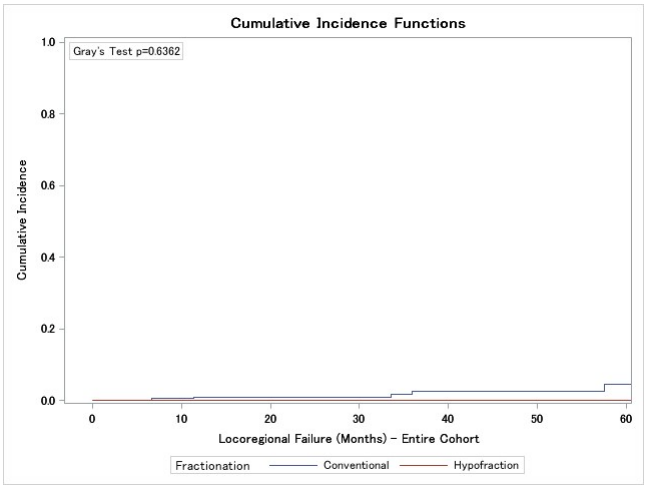
		Conventional (n=211)	Hypofractionated (n=100)	p-value
Age				
	Median (IQR)	56.4 (48.4 - 64.6)	61.9 (52.2 - 67.8)	p=0.001
	Mean (Range)	56.9 (19.8 - 89.4)	61.0 (37.9 - 85.6)	
Sex				
	Female	211 (100%)	100 (100%)	
Year of diagnosis		2014	2015	
Follow up	Median (IQR)	36.6 (23.0 - 54.8)	30.8 (21.8 - 41.1)	p<0.001
	Mean (Range)	44.3 (5.2 - 304.9)	34.5 (4.7 - 124.5)	
Histology				
	invasive ductal carcinoma	185 (87.7%)	88 (88%)	p=0.672
	invasive lobular carcinoma	22 (10.4%)	10 (10%)	
	metaplastic carcinoma	2 (1.0%)	0 (0.0%)	
	mixed	2 (1.0%)	2 (2.0%)	
Grade				
	I	27 (12.8%)	39 (39.0%)	p<0.001
	II	80 (37.9%)	37 (37.0%)	
	III	102 (48.3%)	22 (22.0%)	
	Unknown	2 (1.0%)	2 (2.0%)	
T-Stage				
	1A	13 (6.2%)	15 (15.0%)	p<0.001
	1B	24 (11.4%)	29 (29.0%)	
	1C	82 (38.9%)	36 (36.0%)	
	2	78 (37.0%)	20 (20.0%)	
	3	12 (5.7%)	0 (0.0%)	
	4D/Inflammatory	1 (0.5%)	0 (0.0%)	
	No primary identified	1 (0.5%)	0 (0.0%)	
Positive Nodes				
	Nx	11 (5.2%)	5 (5.0%)	P<0.0001
	0	122 (57.8%)	90 (90.0%)	
	1	76 (36.0%)	5 (5.0%)	
	2	2 (1.0%)	0 (0.0%)	
Overall Stage				p<0.001
	I	85 (40.3%)	81 (81.0%)	
	II	126 (59.7%)	19 (19.0%)	
Subtype				
	Luminal A	47 (22.3%)	52 (52.0%)	p<0.001
	Luminal B	109 (51.7%)	36 (36.0%)	
	Her2 Enriched	15 (7.1%)	2 (2.0%)	
	Triple Negative	40 (19.0%)	10 (10.0%)	

Table 1. Demographics and tumor characteristics of early stage breast cancer patients that underwent either CF or HF regimens of radiation therapy.

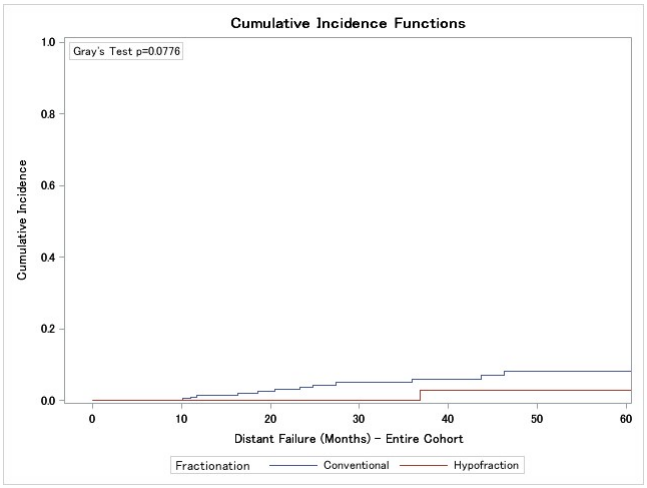
Total Dose (Gy)/ # of Factions	Conventional (n=211)	Hypofractionated (N=100)
4005/15	0 (0%)	5 (5%)
4256/16	0 (0%)	95 (95%)
4500/25	59 (28%)	0 (0%)
4600/23	61 (28.9%)	0 (0%)
5000/25	36 (17.1%)	0 (0%)
5040/28	55 (26.1%)	0 (0%)
Boost Doses (Gy)		
400/600	2 (0.9%)	0 (0%)
795/798	0 (0%)	6 (6%)
1000	40 (19%)	63 (63%)
1200	4 (1.9%)	3 (3%)
1400	57 (27%)	0 (0%)
1500	1 (0.5%)	0 (0%)
1600	59 (28%)	0 (0%)
2000	2 (0.9%)	0 (0%)
No boost	40 (19%)	20 (20%)
Unknown	6 (2.8%)	8 (8%)

Table 2. Radiation doses and fractionation regimens included within the conventional fractionation and hypofractionation cohorts.

A



B



C

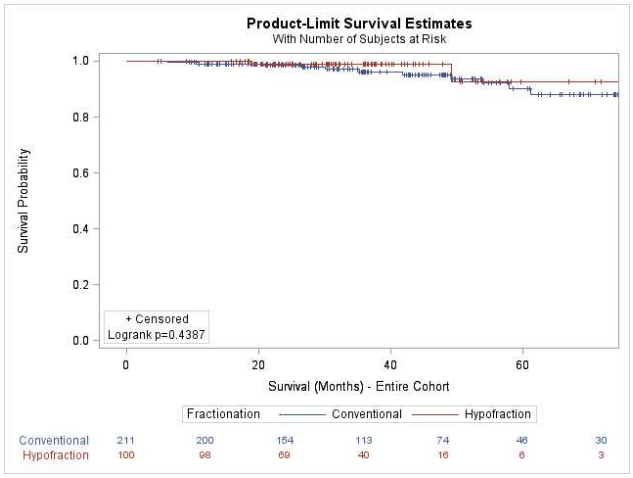


Fig. 1: Patient outcomes in early stage breast cancer patients stratified by conventional or hypofractionated radiation therapy (RT) regimens. (A-C) Cumulative incidence functions of local (A) and distant failure (B) as well as Kaplan-Meier curves of cancer-specific survival (C) in early stage breast cancer patients stratified by RT fractionation protocol.

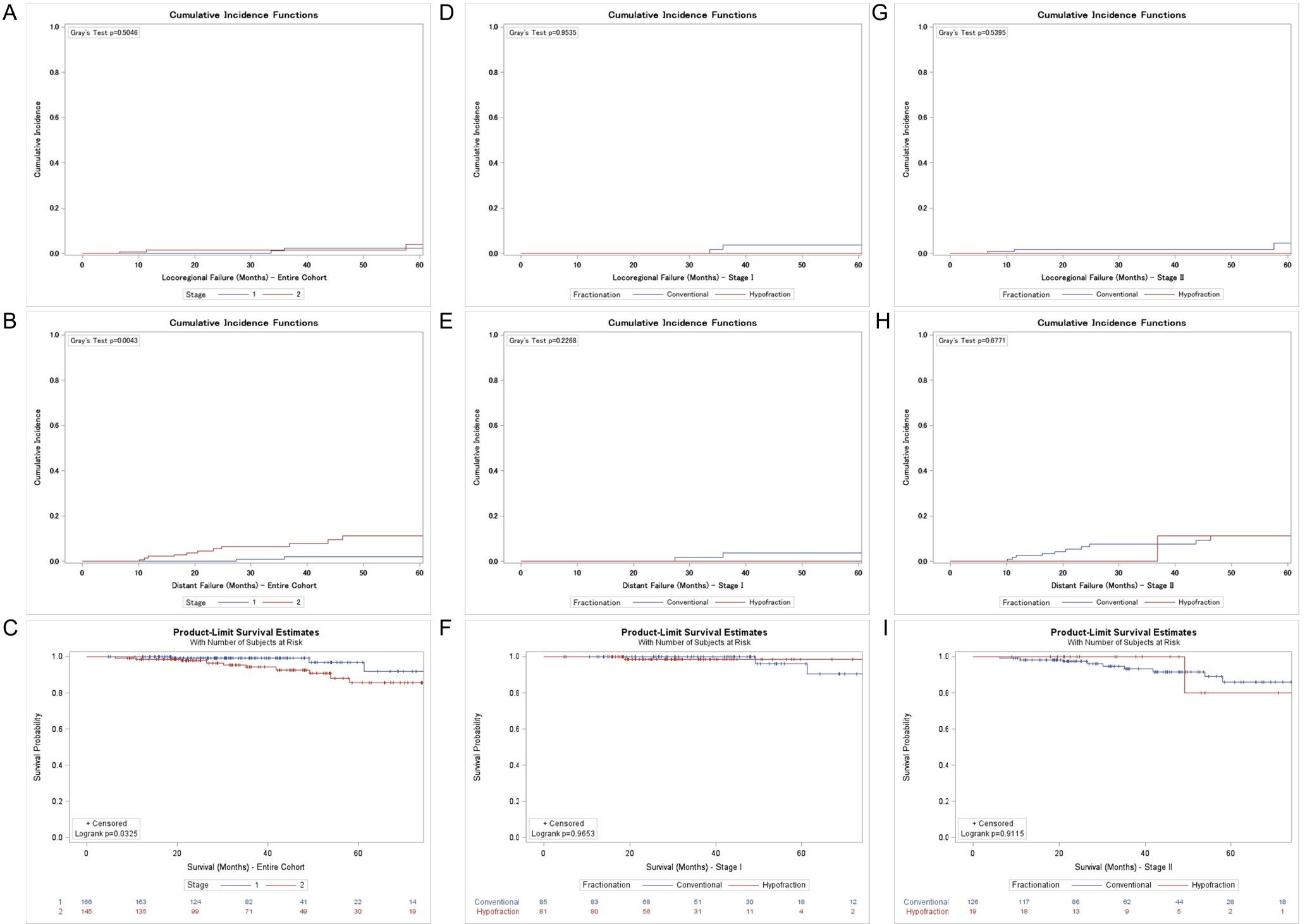


Fig. 2: Patient outcomes in early stage breast cancer patients relative to stage in conjunction with fractionation regimens of RT. Cumulative incidences of local (A) and distant failure (B) as well as cancer-specific survival (C) in early stage breast cancer patients stratified by stage. Cumulative incidences of local (D) and distant failure (E) as well as cancer-specific survival (F) in stage I breast cancer patients stratified by fractionation regimen of RT. Cumulative incidences of local (G) and distant failure (H) as well as cancer-specific survival (I) in stage II breast cancer patients stratified by fractionation regimen of RT.

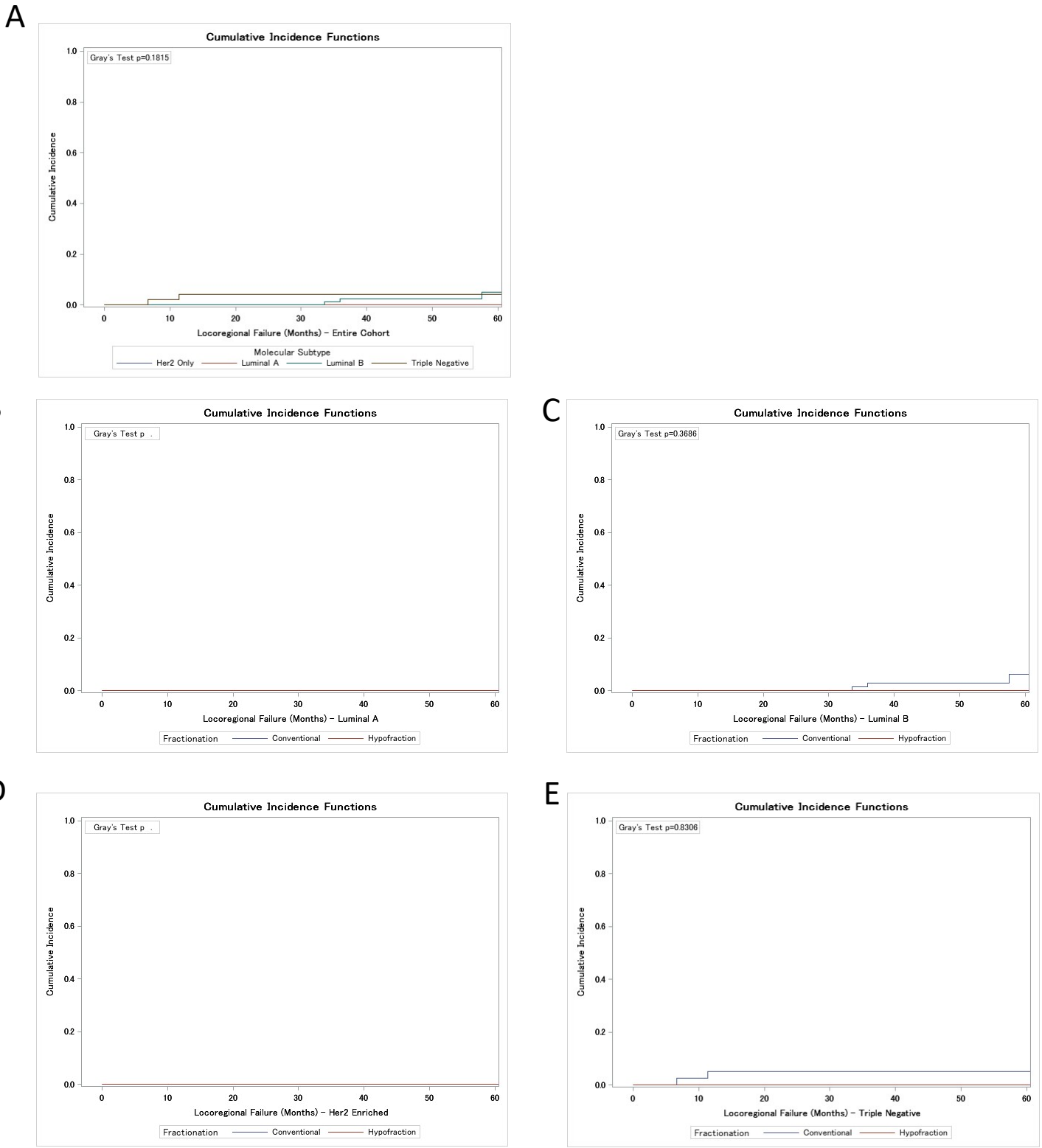


Fig. 3: Local failure in early stage breast cancer patients relative to fractionation regimens of RT within different molecular subtypes of breast cancer. (A) Cumulative incidence of local failure in early stage breast cancer patients stratified by molecular subtype. (B-E) Cumulative incidence of local failure in early stage breast cancer patients with Luminal A (B), Luminal B (C), HER2 Enriched (D) or Triple Negative (E) tumors stratified by fractionation regimen of RT.

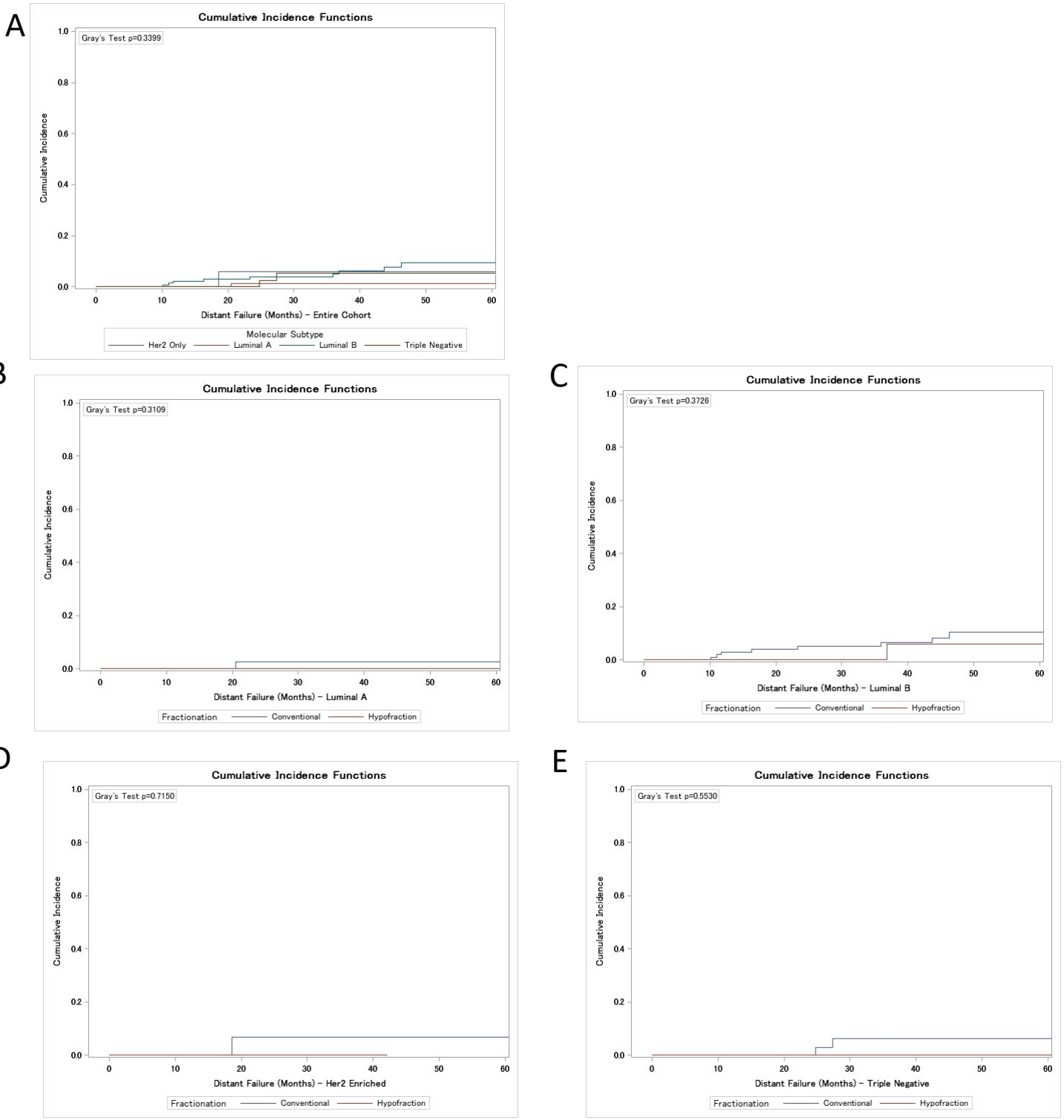


Fig. 4: Distant failure in early stage breast cancer relative to fractionation regimens of RT within different molecular subtypes of breast cancer. (A) Cumulative incidence of distant failure in early stage breast cancer patients stratified by molecular subtype. (B-E) Cumulative incidence of distant failure in early stage breast cancer patients with Luminal A (B), Luminal B (C), HER2 Enriched (D) or Triple Negative (E) tumors stratified by fractionation regimen of RT.

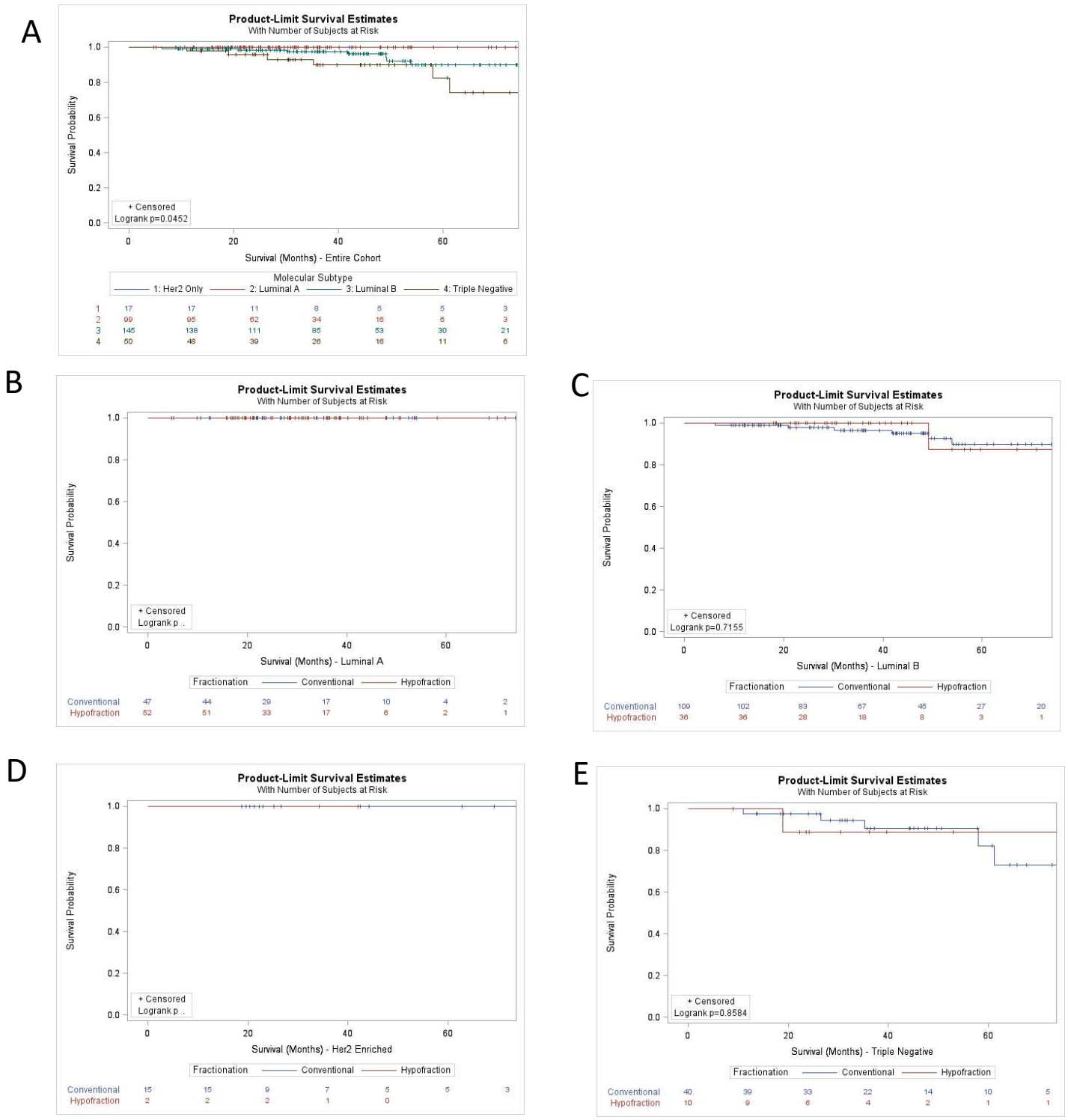


Fig. 5: Cancer-specific survival in early stage breast cancer relative to fractionation regimens of RT within different molecular subtypes of breast cancer. (A) Kaplan-Meier curves of survival in early stage breast cancer patients stratified by molecular subtype. (B-E) Kaplan-Meier curves of survival in early stage breast cancer patients with Luminal A (B), Luminal B (C), HER2 Enriched (D) or Triple Negative (E) tumors stratified by fractionation regimen of RT.

Parameter	Comparison vs Reference Group	Hazard Ratio	95% Hazard Ratio		P-value
		Univariate			
Age	Every 1 year increase	1.004	0.950	1.061	0.8818
Stage	II vs I	0.684	0.176	2.660	0.5834
Grade	2 vs 1	4.126	0.476	541.003	0.3578
Grade	3 vs 1	8.484	1.107	1090.059	0.1570
Ki-67	***Every 1% increase***	***1.022***	***1.006***	***1.039***	***0.0091***
Fractionation	Conventional vs Hypofraction	1.699	0.197	14.679	0.6301
Subtype	Luminal B vs Luminal A	4.313	0.172	108.155	0.3739
Subtype	Her2 Only vs Luminal A	1.819	0.022	148.045	0.7899
Subtype	Triple Negative vs Luminal A	9.825	0.356	271.044	0.1770
		Multivariable – Molecular Subtype			
Stage	II vs I	1.584	0.663	4.0240	0.3220
Fractionation	Conventional vs Hypofraction	0.807	0.271	3.180	0.7320
Subtype	Luminal B vs Luminal A	8.71	1.101	1126.51	0.1527
Subtype	Her2 Only vs Luminal A	10.254	0.778	1447.94	0.1569
Subtype	***Triple Negative vs Luminal A***	***22.132***	***2.644***	***2888.92***	***0.0426***
		Multivariable – Ki-67			
Stage	II vs I	0.708	0.270	1.937	0.4972
Fractionation	Conventional vs Hypofraction	1.433	0.457	5.817	0.5796
Ki-67	***Every 1% increase***	***1.022***	***1.006***	***1.040***	***0.0110***

Table 3. Cox PH univariate and multivariate analysis for age, stage, fractionation and molecular subtype for local failure. Interactions were additionally tested but found not to be significant.

		Univariate			
Parameter	**Comparison vs Reference Group**	**Hazard Ratio**	**95% Hazard Ratio**		**P-value**
Age	Every 1 year increase	0.968	0.924	1.013	0.1577
Stage	***II vs I***	***6.816***	***1.522***	***30.521***	***0.0121***
Grade	2 vs 1	*4.018*	*0.427*	*532.383*	*0.3671*
Grade	**3 vs 1**	***17.088***	***2.339***	***2173.920***	***0.0554***
Ki-67	***Every 1% increase***	***1.027***	***1.012***	***1.043***	***0.0006***
Fractionation	Conventional vs Hypofraction	5.223	0.679	40.160	0.1122
Subtype	Luminal B vs Luminal A	0.193	0.025	1.477	0.1131
Subtype	Her2 Only vs Luminal A	0.931	0.120	7.215	0.9454
Subtype	Triple Negative vs Luminal A	0.558	0.123	2.522	0.4481
		Multivariable – Molecular Subtype			
Stage	***II vs I***	***3.232***	***1.309***	***9.438***	***0.0196***
Frac	Conventional vs Hypofraction	1.621	0.498	8.275	0.4894
Subtype	Luminal B vs Luminal A	2.925	0.693	27.135	0.2330
Subtype	Her2 Only vs Luminal A	4.857	0.630	54.204	0.1460
Subtype	***Triple Negative vs Luminal A***	***7.302***	***1.650***	***68.834***	***0.0299***
		Multivariable – Ki-67			
Stage	II vs I	2.35	0.857	7.936	0.1307
Fractionation	Conventional vs Hypofraction	1.812	0.546	9.350	0.4016
Ki-67	***Every 1% increase***	***1.022***	***1.006***	***1.038***	***0.0079***

Table 4. Cox PH univariate and multivariate for age, stage, fractionation and molecular subtype for distant failure. All interactions were tested (see supplementary section).

Parameter	Comparison vs Reference Group	Hazard Ratio	95% Hazard Ratio		P-value
		Univariate			
Age	Every 1 year increase	1.013	0.971	1.058	0.5433
Stage	***II vs I***	***3.686***	***1.025***	***13.262***	***0.0458***
Grade	2 vs 1	0.339	0.002	63.062	0.6216
Grade	3 vs 1	5.302	0.6335	690.460	0.2999
Ki-67	***Every 1% increase***	***1.031***	***1.006***	***1.061***	***0.0263***
Fractionation	Conventional vs Hypofraction	1.807	0.396	8.242	0.4450
Subtype	Luminal B vs Luminal A	6.125	0.733	798.255	0.2494
Subtype	Her2 Only vs Luminal A	9.235	0.478	1362.759	0.2080
Subtype	***Triple Negative vs Luminal A***	***16.041***	***1.87***	***2098.196***	***0.0790***
		Multivariable – Molecular Subtype			
Stage	***II vs I***	***5.355***	***1.135***	***52.377***	***0.0759***
Fractionation	Conventional vs Hypofraction	0.669	0.137	6.583	0.6786
Subtype	Luminal B vs Luminal A	4.940	0.239	755.016	0.3868
Subtype	Her2 Only vs Luminal A	2.887	0.315	383.529	0.5131
Subtype	Triple Negative vs Luminal A	6.800	0.634	925.288	0.2480
		Multivariable – Ki-67			
Stage	II vs I	2.536	0.469	26.933	0.3616
Fractionation	Conventional vs Hypofraction	0.961	0.191	9.570	0.9681
Ki-67	***Every 1% increase***	***1.025***	***0.999***	***1.056***	***0.0845***

Table 5. Cox PH univariate and multivariate for age, stage, fractionation and molecular subtype for cancer-specific survival. Interactions were additionally tested but found not to be significant.

		Conventional	Hypofractionated
		% (# at risk)	% (# at risk)
LR			
	1-yr	1.0% (198)	0.0% (98)
	2-yr	1.0% (152)	0.0% (69)
	3-yr	2.6% (111)	0.0% (39)
	4-yr	2.6% (72)	0.0% (16)
	5-yr	4.5% (44)	0.0% (6)
Total Events		*8 (12 comp.)*	*1 (2 comp.)*
DR			
	1-yr	1.5% (197)	0.0% (98)
	2-yr	3.8% (150)	0.0% (69)
	3-yr	6.0% (109)	0.0% (39)
	4-yr	8.3% (69)	2.8% (15)
	5-yr	8.3% (43)	2.8% (6)
Total Events		*14 (6 comp.)*	*1 (1 comp.)*
Survival			
	1-yr	99.0% (200)	100.0% (98)
	2-yr	98.5% (154)	98.9% (69)
	3-yr	96.2% (112)	98.9% (39)
	4-yr	95.3% (74)	98.9% (16)
	5-yr	90.3% (46)	92.7% (6)
Total Events		*14 (6 comp.)*	*2 (1 comp.)*

Table 6. Yearly failure (LR – Locoregional Recurrence, DR – Distant Recurrence) and survival rates stratified by conventional vs hypofractionation of RT. In LR and DR, competing event (comp.) includes death of any cause. In survival, event includes cancer-related mortality, while competing event is death from any other cause. Of note, one patient experience both a LR and DR as their initial recurrence.

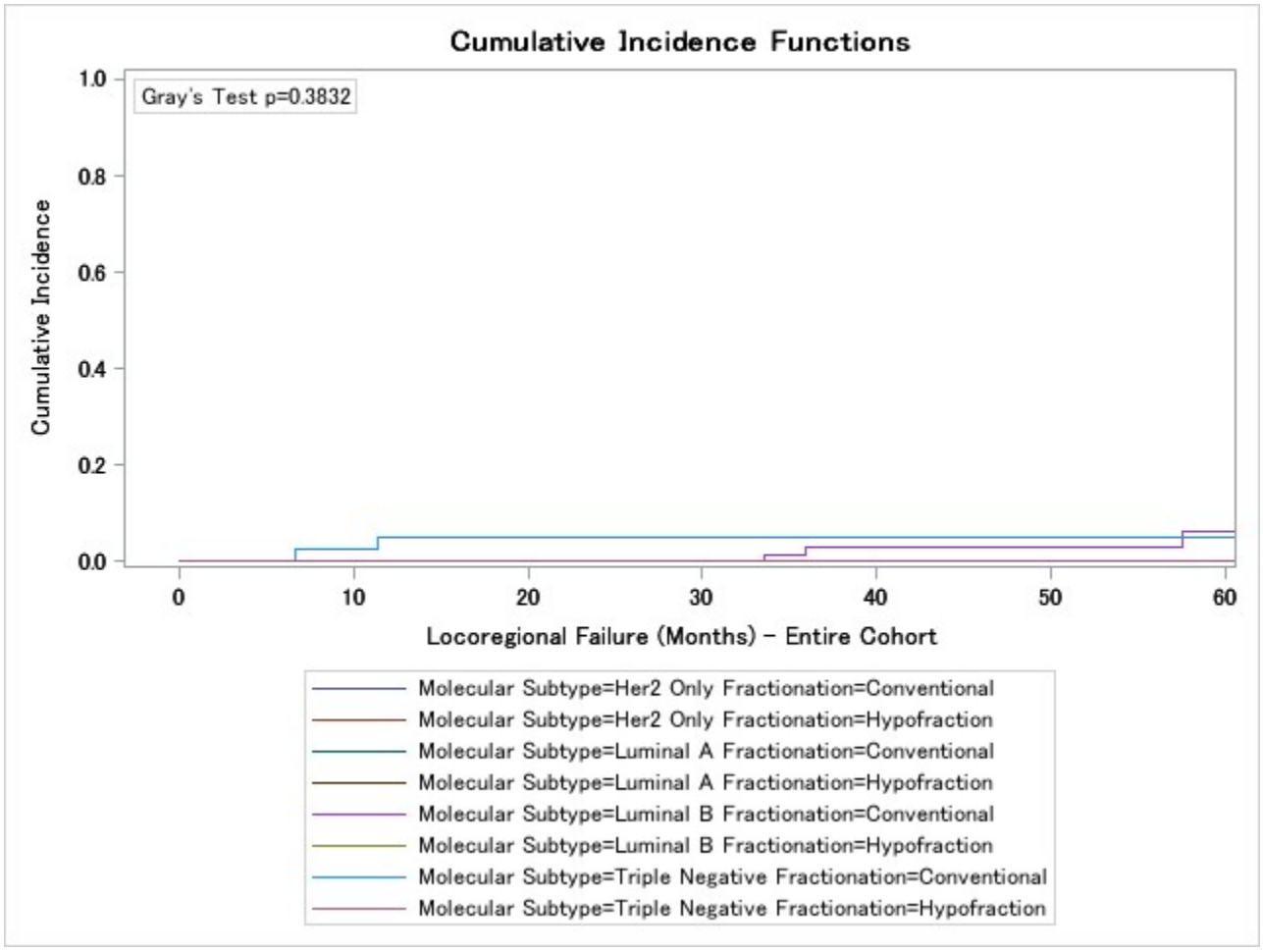
Supplementary

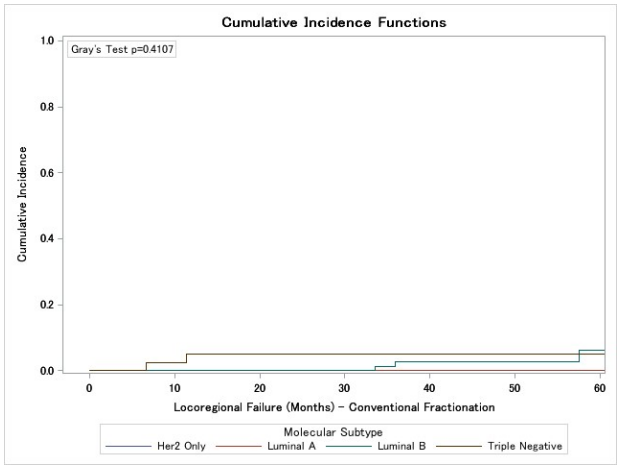
Race/Ethnicity	Conventional (n=211)	Hypofractionated (N=100)
Asian	1 (0.5%)	3 (3.0%)
Black/ African American	19 (9.0%)	4 (4.0%)
White	149 (70.6%)	80 (80.0%)
Histpanic/ Latino	12 (5.7%)	2 (2.0%)
Unknown	30 (14.2%)	11 (11.0%)

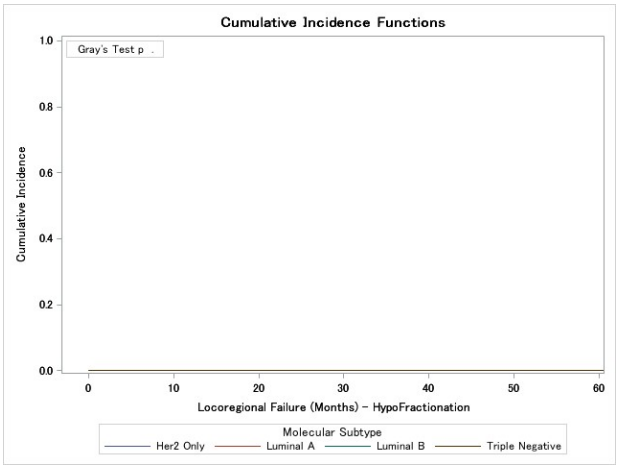
Supplementary Table 1. Race/ethnicity of early stage breast cancer patients that underwent either CF or HF regimens of radiation therapy.

		Ki-67			
		Median (Mean)	**IQR (SD)**	**Range**	**P-value**
Molecular Subtype					
	Luminal A	6.0 (6.8)	5.0 - 10.0 (3.0)	0.0 - 13.0	< 0.001
	Luminal B	27.0 (35.6)	17.0 - 49.5 (22.6)	2.0 - 91.0	
	Her2 Only	40.0 (38.2)	23.0 - 50.0 (18.2)	12.0 - 75.0	
	Triple Negative	70.0 (61.5)	40.0 - 80.0 (23.2)	5.0 - 90.0	
Fractionation					
	Hypofraction	10.0 (20.0)	6.0 - 25.0 (21.2)	0.0 - 91.0	< 0.001
	Conventional	27.0 (34.8)	11.0 - 55.0 (27.1)	1.0 - 91.0	
Grade					
	1	7.0 (10.2)	5.0 - 10.0 (9.9)	1.0 - 66.7	< 0.001
	2	13.0 (16.0)	7.0 - 20.0 (13.5)	0.0 - 80.0	
	3	50.0 (53.2)	36.0 - 70.0 (22.8)	2.0 - 91.0	

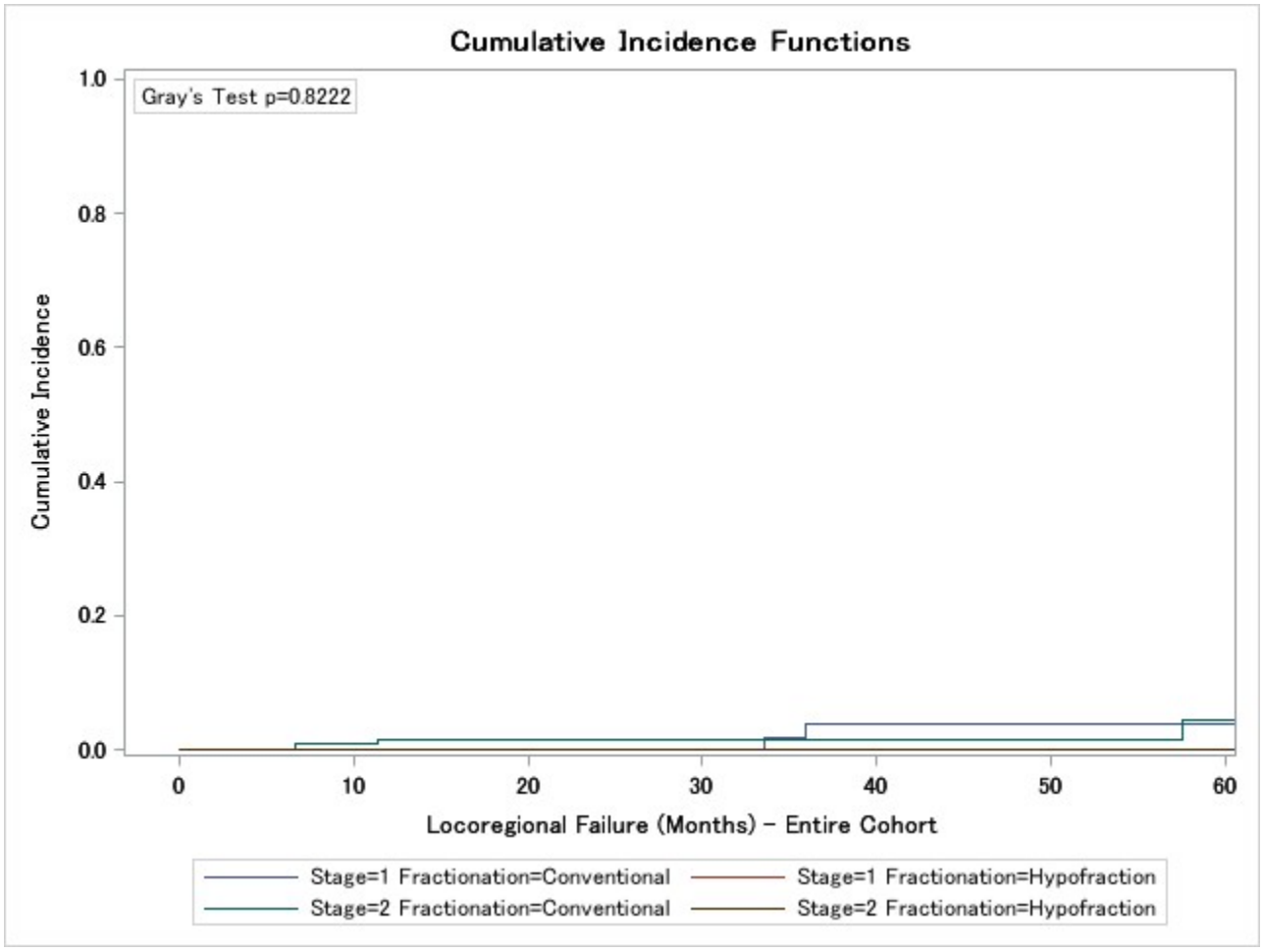
Supplementary Table 2. Ki-67 of early stage breast cancer patients that underwent either CF or HF regimens of radiation therapy as it relates to molecular subtype, fractionation regimen, and grade.

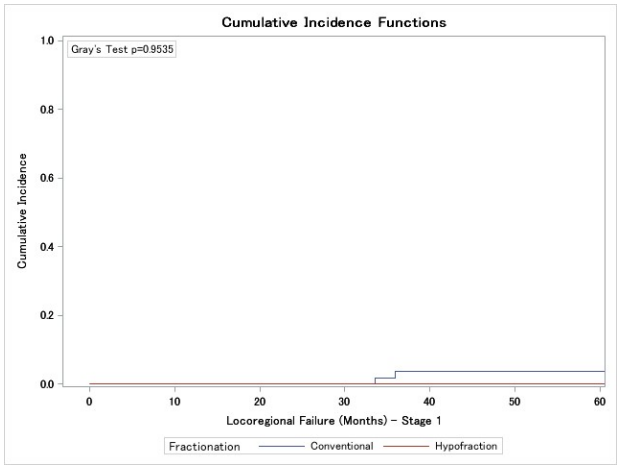


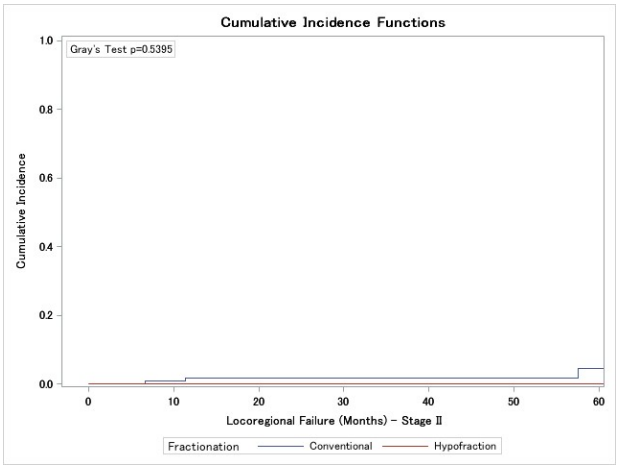




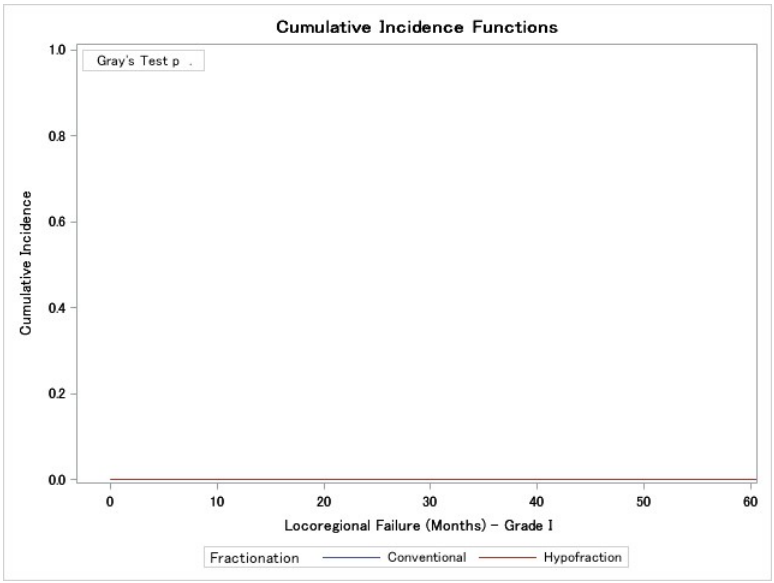
Supplementary Fig. 1: Locoregional recurrence of early-stage breast cancer patients as it relates to molecular subtype and fractionation regimen.

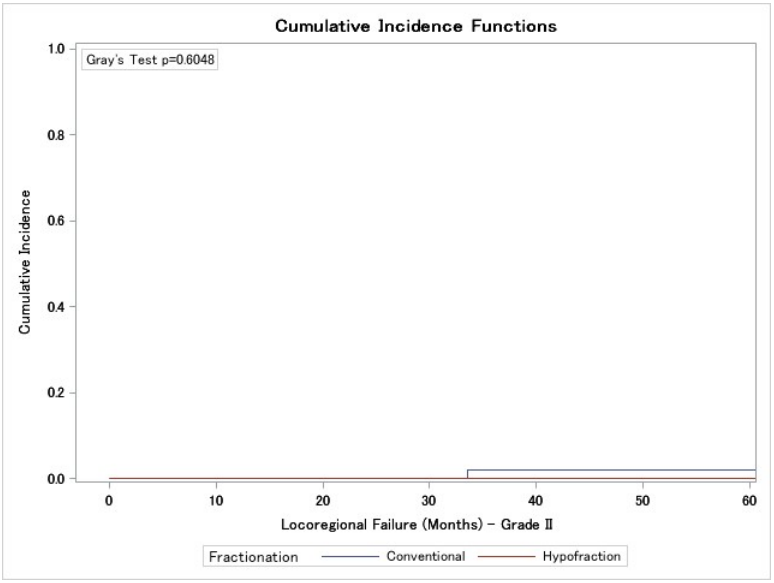


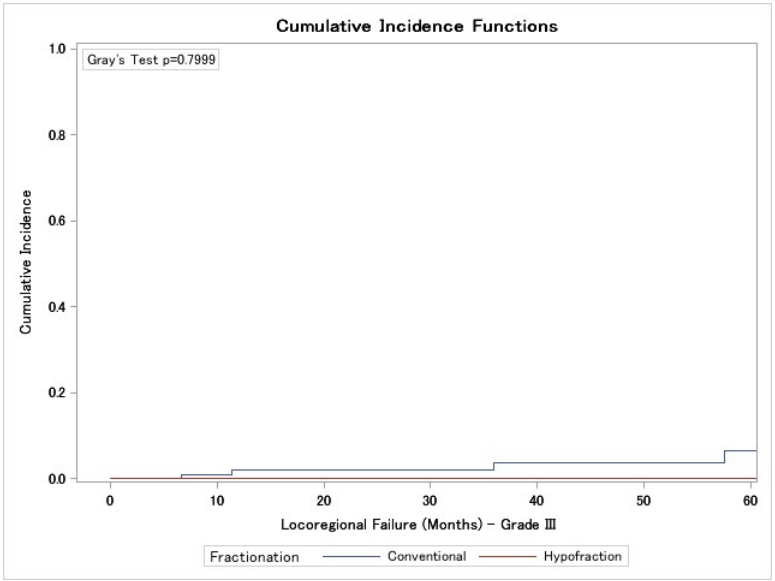




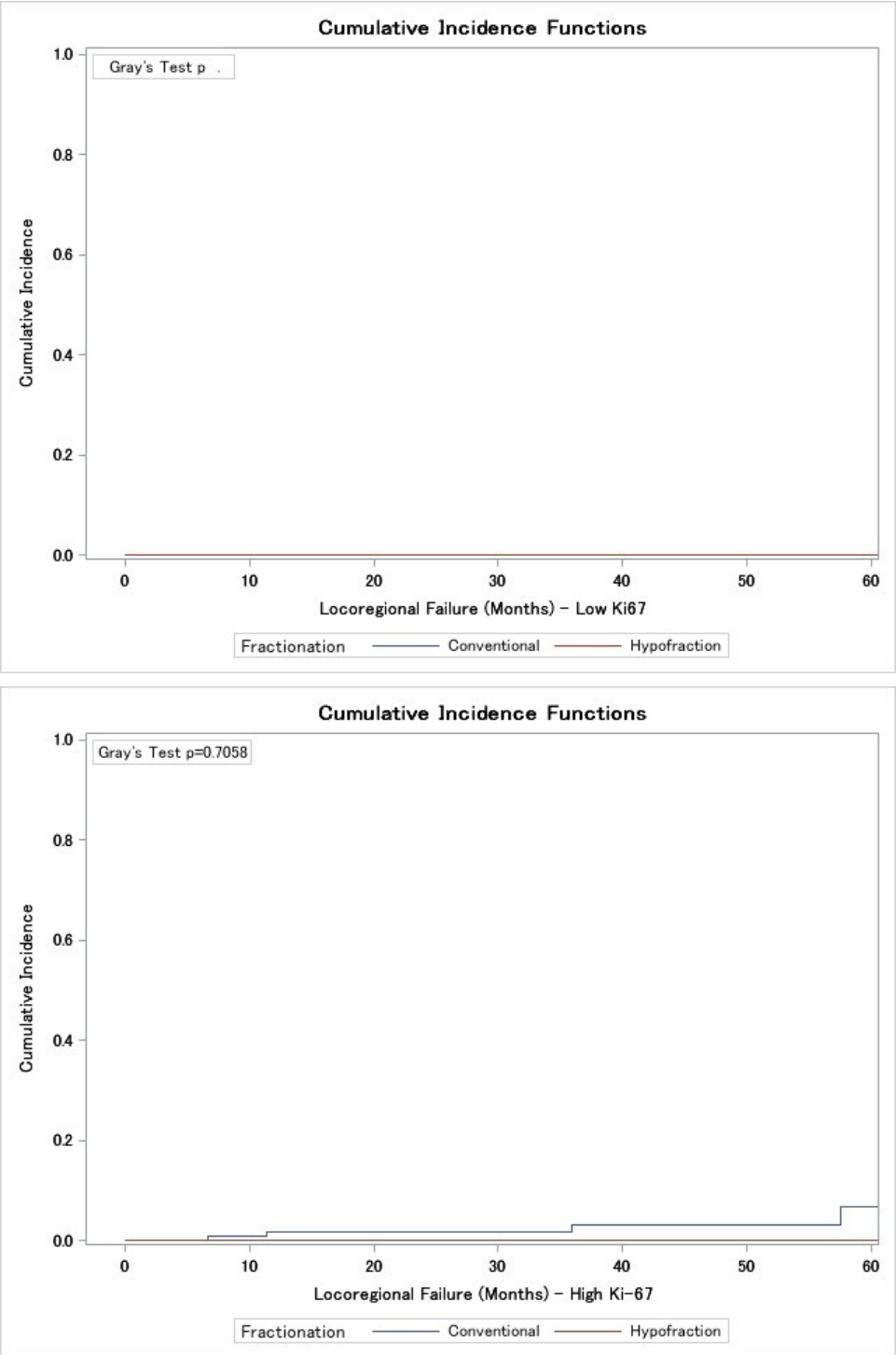
Supplementary Fig. 2: Locoregional recurrence of early-stage breast cancer patients as it relates to stage and fractionation regimen.







Supplementary Fig. 3: Locoregional recurrence of early-stage breast cancer patients as it relates to grade and fractionation regimen.



Supplementary Fig. 4: Locoregional recurrence of early-stage breast cancer patients as it relates to Ki-67 level (high: >14 or low: ≤14).