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# Dose-dense adjuvant chemotherapy in HER2-positive early breast cancer patients before and after the introduction of trastuzumab: exploratory analysis of the GIM2 trial

Matteo Lambertini[1,2], Francesca Poggio[3], Marco Bruzzone[4], Benedetta Conte[3], Claudia Bighin[3], Evandro de Azambuja[5], Mario Giuliano[6], Michele De Laurentiis[7], Francesco Cognetti[8,9], Alessandra Fabi[8,9], Giancarlo Bisagni[10], Antonio Durando[11], Anna Turletti[12], Ylenia Urracci[13], Ornella Garrone[14], Fabio Puglisi[15,16], Filippo Montemurro[17], Marcello Ceppi[4], Lucia Del Mastro[2,3] on behalf of the GIM2 investigators.

[1]Department of Medical Oncology, U.O.C. Clinica di Oncologia Medica, IRCCS Ospedale Policlinico San Martino, Genova, Italy

[2]Department of Internal Medicine and Medical Specialties (DiMI), School of Medicine, University of Genova, Genova, Italy.

[3]Department of Medical Oncology, Breast Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy

[4]Clinical Epidemiology Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy

[5] Department of Medical Oncology, Institut Jules Bordet and l'Université Libre de Bruxelles (U.L.B), Brussels, Belgium

[6]Medical Oncology, Azienda Ospedaliera Universitaria Federico II, Napoli, Italy

[7]Medical Oncology, Istituto Nazionale Tumori-IRCCS Fondazione Pascale, Napoli, Italy

[8]La Sapienza University, Roma, Italy

[9]Department of Medical Oncology 1, Istituto Nazionale Tumori Regina Elena, Roma, Italy

[10] Department of Medical Oncology, Azienda Unità Sanitaria Locale – IRCCS di Reggio Emilia, Reggio Emilia, Italy

[11]Breast Unit, Azienda Ospedaliera Universitaria Città della Salute e delle Scienze, Torino, Italy

[12]Medical Oncology, Martini Hospital, ASL Città' di Torino, Italy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ijc.32789

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[13] Department of Medical Oncology, Hospital Businco, Cagliari, Italy

[14]Breast Unit, Medical Oncology, Azienda Ospedaliera S. Croce e Carle, Ospedale di Insegnamento, Cuneo, Italy

[15]Unit of Medical Oncology and Cancer Prevention, IRCCS Centro di Riferimento Oncologico Aviano-National Cancer Institute, Aviano (Udine), Italy

[16]Medical Oncology, Department of Medicine, University of Udine, Udine, Italy

[17]Investigative Clinical Oncology, Fondazione del Piemonte per l'Oncologia/Candiolo Cancer Center (IRCCS), Candiolo, Torino, Italy

**Correspondence to:** Matteo Lambertini, MD PhD, IRCCS Ospedale Policlinico San Martino, University of Genova, Largo Rosanna Benzi 10, 16132, Genova (Italy), Telephone +39 010 555 4254 – Fax: +39 010 555 8909, E-mail: matteo.lambertini@unige.it

**Keywords:** Breast cancer; adjuvant chemotherapy; dose-dense; HER2; trastuzumab.

**Abbreviations:** Gruppo Italiano Mammella (GIM), fluorouracil, epirubicin and cyclophosphamide (FEC), paclitaxel (P), epirubicin and cyclophosphamide (EC), disease-free survival (DFS), overall survival (OS), confidence intervals (CI), hazard ratios (HRs), Early Breast Cancer Trialists' Collaborative Group (EBCTCG), trastuzumab-emtansine (T-DM1).

**Type article:** research article.

**Novelty and impact:** Dose-dense chemotherapy is standard of care in high-risk early breast cancer; however, its role in HER2-positive patients is still uncertain. In this exploratory analysis of the GIM2 trial, we showed that the benefit of dose-dense chemotherapy appeared to be small (if any) in HER2-positive patients who received trastuzumab raising concerns on the need of chemotherapy



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escalation approaches in this setting. These findings may guide the choice of adjuvant chemotherapy in HER2-positive early breast cancer patients.

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**ABSTRACT**

Dose-dense adjuvant chemotherapy is standard of care in high-risk early breast cancer patients. However, its role in HER2-positive patients is still uncertain. In this exploratory analysis of the GIM2 trial, we investigated the efficacy of dose-dense chemotherapy in HER2-positive breast cancer patients with or without exposure to trastuzumab. In the GIM2 trial, node-positive early breast cancer patients were randomized to receive 4 cycles of (fluorouracil)epirubicin/cyclophosphamide followed by 4 cycles of paclitaxel administered every 2 (dose-dense) or 3 (standard-interval) weeks. After approval of adjuvant trastuzumab, protocol was amended in April 2006 to allow use of trastuzumab for 1 year after chemotherapy completion in HER2-positive patients. The efficacy of dose-dense chemotherapy in terms of disease-free survival (DFS) and overall survival (OS) was assessed according to HER2 status and trastuzumab use. Out of 2,003 breast cancer patients, HER2 status was negative/unknown in 1,551 patients; among the 452 patients with HER2-positive breast cancer, chemotherapy alone or followed by trastuzumab was given to 320 and 132 patients, respectively. Median follow-up was 8.1 years. No significant interaction between HER2 status, trastuzumab use and chemotherapy treatment was observed for both DFS (p=0.698) and OS (p=0.708). Nevertheless, there was no apparent benefit in the HER2-positive group treated with trastuzumab (DFS: HR, 0.99; 95% CI 0.52-1.89; OS: HR, 0.95; 95% CI 0.37-2.41). Although dose-dense chemotherapy was associated with a significant survival improvement in high-risk breast cancer patients, its benefit appeared to be smaller (if any) in patients with HER2-positive disease who received adjuvant trastuzumab.



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**MANUSCRIPT**

## INTRODUCTION

Polychemotherapy following surgical resection remains a mainstay adjuvant treatment for many patients with early breast cancer.[1,2] Although the combination of docetaxel and cyclophosphamide can be considered a valid alternative in some cases,[3] the sequential use of a taxane with anthracycline- and cyclophosphamide-based chemotherapy represents the preferred approach in high-risk patients.[4] In breast cancer patients at higher risk of disease recurrence, dose-dense chemotherapy obtained by shortening the interval between treatment cycles is associated with a significant improvement in survival outcomes.[5]

Before the introduction of adjuvant anti-HER2 targeted therapy, the efficacy of dose-dense chemotherapy was demonstrated also in patients with HER2-positive early breast cancer.[5,6] However, chemotherapy plus anti-HER2 targeted therapy is the current standard of care in this setting.[7] Three studies showed the feasibility (in terms of no increased likelihood of developing cardiac events) of administering dose-dense anthracycline- and taxane-based chemotherapy in combination with adjuvant trastuzumab in patients with HER2-positive early breast cancer.[8–10] However, none of these studies was designed to investigate the superiority of the dose-dense schedule over standard-interval chemotherapy. Therefore, the need for prescribing dose-dense chemotherapy in patients with HER2-positive early breast cancer undergoing adjuvant anti-HER2 targeted therapy remains unknown.[11]

The Gruppo Italiano Mammella (GIM) 2 trial (ClinicalTrials.gov Identifier: NCT00433420) is one of the largest studies that investigated the efficacy of dose-dense chemotherapy as adjuvant treatment of high-risk early breast cancer patients.[12] The trial was conducted at the turn of the years in which adjuvant trastuzumab became standard of care in HER2-positive breast cancer. Therefore, some of the patients included in the trial received adjuvant trastuzumab while others did not



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undergo anti-HER2 targeted therapy. This represented a unique opportunity to explore the efficacy of dose-dense adjuvant chemotherapy in HER2-positive early breast cancer patients before and after the introduction of adjuvant trastuzumab.

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## MATERIALS AND METHODS

### Study design and participants

Details of the GIM2 study design were previously reported.[12,13] Briefly, GIM2 was a multicenter, open-label, randomized phase III study including patients aged 18-70 years who had undergone radical surgery for a histologically confirmed invasive breast cancer with metastasis in at least one axillary lymph node.

According to the criteria in force at the time of study conduction, HER2 status was defined as positive in the presence of at least 10% of cells with HER2 expression assessed by immunohistochemistry or in the presence of gene amplification documented by *in-situ* hybridization assay. The presence of at least 10% of positive cells by immunohistochemical analysis was the criteria to define the tumor positive for estrogen and/or progesterone receptors.

The trial was conducted at 81 Italian centers. The Institutional Review Boards of all participating centers approved the GIM2 protocol and all enrolled patients provided written informed consent before study entry. The present analysis was approved by the members of the GIM2 Steering Committee.

### Study procedures

In the GIM2 trial, eligible patients were randomized in a 1:1:1:1 ratio to one of the following 4 arms: standard-interval fluorouracil, epirubicin and cyclophosphamide (FEC) followed (-) by paclitaxel (P), standard-interval EC-P, dose-dense FEC-P and dose-dense EC-P. The number of



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cycles (4 of anthracycline-based chemotherapy and 4 of single agent taxane) and the dose of chemotherapy agents (fluorouracil at 600 mg/m$^2$, epirubicin at 90 mg/m$^2$, cyclophosphamide at 600 mg/m$^2$ and paclitaxel at 175 mg/m$^2$) were the same in all treatment arms. In the dose-dense arms, administration of subcutaneous pegfilgrastim (6 mg) was mandatory 24-72 hours after chemotherapy.[14]

After approval of adjuvant trastuzumab by the Italian regulatory authorities, an amendment in April 2006 allowed the administration of the anti-HER2 monoclonal antibody for 1 year after chemotherapy in patients with HER2-positive disease. Patients with hormone receptor-positive disease received adjuvant endocrine therapy following completion of chemotherapy according to local guidelines.

**Objectives and endpoints**

This exploratory analysis aimed at investigating a potential interaction between HER2 status, trastuzumab use and chemotherapy by evaluating the efficacy of dose-dense chemotherapy in the subgroup of patients with HER2-positive breast cancer with or without subsequent exposure to adjuvant trastuzumab and in those with HER2-negative/unknown disease.

Outcomes were compared between three groups of patients: HER2-positive group not exposed to adjuvant trastuzumab (HER2-positive no trastuzumab group), HER2-positive group exposed to adjuvant trastuzumab (HER2-positive trastuzumab group) and HER2-negative/unknown group.

As in the main GIM2 trial,[12] disease-free survival (DFS) was the primary endpoint; overall survival (OS) and adverse events were secondary endpoints.

**Statistical analyses**



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Sample size calculation and statistical assumptions of the GIM2 primary objective were previously described.[12] The present analysis focusing on the efficacy of dose-dense chemotherapy according to HER2 status and trastuzumab use was not preplanned in the trial protocol, and the power of the statistical analyses was not prespecified. For the purpose of the present analysis, the two dose-dense arms were considered together as well as the two standard-interval arms. The 88 patients included in the 5 centers that refused randomization to the dose-dense arms were excluded from this analysis (Figure 1).

DFS and OS were defined as previously described.[12] The heterogeneity of treatment effect (dose-dense vs. standard-interval) according to HER2 status and trastuzumab use was investigated by including in each final model (for DFS and OS) an interaction term representing the modification of the effect of dose-dense chemotherapy in patients with HER2-positive disease treated with or without trastuzumab. For descriptive purposes, DFS and OS in patients assigned to dose-dense or standard-interval chemotherapy were also compared separately within each of the three groups of interest (HER2-positive no trastuzumab, HER2-positive trastuzumab and HER2-negative/unknown).

DFS and OS probabilities were computed according to the Kaplan-Meier method and the log-log method was used to calculate confidence intervals (CI) of survival time probabilities. To estimate treatment effect, unadjusted and adjusted hazard ratios (HRs) with 95% CI were calculated with the Cox proportional hazards model. The variables included in the multivariate Cox regression models were age, type of surgery, tumor size, number of lymph nodes, tumor grade, Ki67, hormone receptor status and endocrine therapy. Likelihood ratio test was applied to test the contribution of each variable to the final model.



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As previously described[12], adverse events were assessed clinically as well as by hematological and biochemical measurements throughout chemotherapy and were graded according to the National Cancer Institute common toxicity criteria version 2.0.

All reported statistical analyses were based on the intention-to-treat population. All statistical tests were 2-sided, and p values < 0.05 were considered statistically significant. STATA 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP) was used to perform all statistical analyses.

**Data availability**

Data can be made available upon reasonable request to the corresponding author.

**RESULTS**

Between April 24, 2003 and July 3, 2006, 2,003 breast cancer patients were randomized to receive dose-dense (n=1,002) or standard-interval (n=1,001) chemotherapy (Figure 1).

Among these patients, HER2 status was negative in 1,243 (62.0%), unknown in 308 (15.4%) and positive in 452 (22.6%) cases. Out of 452 patients with HER2-positive breast cancer, 320 (70.8%) received chemotherapy alone without trastuzumab while 132 (29.2%) received trastuzumab at the completion of chemotherapy. Table 1 reports baseline patient and tumor characteristics in the three groups of interest.

Almost 90% of the patients in all treatment groups completed the planned number of chemotherapy cycles (Supporting Information Table S1). Supporting Information Table S2 reports the rate and grade of the adverse events occurring in at least 5% of the patients in the dose-dense and standard-interval arms of the three groups. In terms of grade 3-4 adverse events, neutropenia was more common among patients treated with standard-interval chemotherapy (ranging between 38.9% and



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53.3%) while those in the dose-dense arms tended to developed more often anemia (ranging between 1.2% and 1.7%), myalgia (ranging between 1.7% and 3.3%) and ALT elevation (ranging between 1.2% and 3.4%). The highest rate of grade 3-4 asthenia (10.2%) was observed in the dose-dense arms of the HER2-positive trastuzumab group. There were no cases of treatment-related deaths.

Median follow-up was 8.1 years (IQR, 7.0-9.3). Number and type of survival events in the three cohorts are reported in Table 2. No significant interaction between HER2 status, trastuzumab use and chemotherapy treatment was observed for both DFS (univariate p=0.698 and multivariate p=0.705) and OS (univariate p=0.708 and multivariate p=0.826). Nevertheless, the benefit of dose-dense chemotherapy appeared to be smaller in the HER2-positive trastuzumab group.

In the HER2-positive no trastuzumab group, DFS at 7 years was 72.1% and 64.4% in the dose-dense and standard-interval arms, respectively (HR, 0.78; 95% CI 0.53-1.13; adjusted HR, 0.79; 95% CI 0.53-1.17; Figure 2A). OS at 7 years was 85.2% and 78.6% in the dose-dense and standard-interval arms, respectively (HR, 0.63; 95% CI 0.37-1.07; adjusted HR, 0.59; 95% CI 0.34-1.03; Figure 3A).

In the HER2-positive trastuzumab group, DFS at 7 years was 68.7% and 72.3% in the dose-dense and standard-interval arms, respectively (HR, 0.99; 95% CI 0.52-1.89; adjusted HR, 0.71; 95% CI 0.35-1.42; Figure 2B). OS at 7 years was 84.9% and 86.1% in the dose-dense and standard-interval arms, respectively (HR, 0.95; 95% CI 0.37-2.41; adjusted HR, 0.91; 95% CI, 0.31-2.68; Figure 3B).

In the HER2-negative/unknown group, DFS at 7 years was 78.7% and 72.1% in the dose-dense and standard-interval arms, respectively (HR, 0.74; 95% CI 0.61-0.89; adjusted HR, 0.72; 95% CI 0.59-0.87; Figure 2C). OS at 7 years was 90.9% and 85.3% in the dose-dense and standard-interval arms, respectively (HR, 0.66; 95% CI 0.50-0.86; adjusted HR, 0.64; 95% CI 0.49-0.84; Figure 3C).



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Comparing DFS of the HER2-positive groups with that of the HER2-negative/unknown group among patients that received standard-interval chemotherapy, univariate HRs were 1.27 (95% CI 0.95-1.70) in the HER2-positive no trastuzumab group (adjusted HR, 0.96; 95% CI 0.71-1.30) and 0.93 (95% CI 0.59-1.47) in the HER2-positive trastuzumab group (adjusted HR, 0.81; 95% CI 0.50-1.31) (Figure 4A). For OS, univariate HRs were 1.32 (95% CI 0.90-1.94) in the HER2-positive no trastuzumab group (adjusted HR, 0.95; 95% CI 0.63-1.43) and 0.84 (95% CI 0.44-1.61) in the HER2-positive trastuzumab group (adjusted HR, 0.67; 95% CI 0.34-1.30) (Figure 4B).

Comparing DFS of the HER2-positive groups with that of the HER2-negative/unknown group among patients that received dose-dense chemotherapy, univariate HRs were 1.35 (95% CI 0.99-1.84) in the HER2-positive no trastuzumab group (adjusted HR, 1.20; 95% CI 0.87-1.66) and 1.25 (95% CI 0.76-2.05) in the HER2-positive trastuzumab group (adjusted HR, 0.92; 95% CI 0.55-1.56) (Figure 4C). For OS, univariate HRs were 1.27 (95% CI 0.81-2.00) in the HER2-positive no trastuzumab group (adjusted HR, 1.09; 95% CI 0.68-1.74), and 1.28 (95% CI 0.62-2.63) in the HER2-positive trastuzumab group (adjusted HR, 0.93; 95% CI 0.43-2.00) (Figure 4D).

Results of the Cox regression model for all the variables evaluated in the study are reported as Supporting Information Table S3 for disease-free survival and Supporting Information Table S4 for overall survival.

## DISCUSSION

To our knowledge, this is the first analysis exploring the efficacy of dose-dense adjuvant chemotherapy in patients with HER2-positive disease receiving adjuvant trastuzumab. At a median follow-up of 8.1 years, we showed that dose-dense chemotherapy significantly improved both DFS and OS of patients with HER2-negative/unknown breast cancer. Although no significant interaction between HER2 status, trastuzumab use and chemotherapy was observed, the benefit of dose-dense



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chemotherapy appeared to be smaller (if any) in the subgroup of patients with HER2-positive disease who received adjuvant trastuzumab.

At a longer follow-up than previously reported in other dose-dense adjuvant chemotherapy trials, our analysis confirms the benefit of shortening the interval between treatment cycles in high-risk early breast cancer patients (with a 6.6% and 5.6% absolute improvement in DFS and OS at 7 years, respectively, in the HER2-negative/unknown population). This is in line with the findings from the recent Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis [5] supporting the use of dose-dense chemotherapy as the preferred adjuvant chemotherapy approach in patients deemed at higher risk of disease recurrence. In terms of adverse events, as also confirmed in our analysis, the rate of grade 3-4 neutropenia is lower with the use of dose-dense chemotherapy considering that primary prophylaxis with granulocyte colony-stimulating factor is mandatory in this setting.[5] On the other hand, higher rate of grade 3-4 anemia is expected when shortening the interval between cycles but with no difference in cardiotoxicity or other non-hematological toxicities and neither in treatment compliance.[5] Nevertheless, despite the positive risk-benefit ratio in the overall high-risk breast cancer population, in the era of treatment personalization and de-escalation, it remains crucial to better define the population of patients that really benefit from this approach.

Before the introduction of targeted therapies, a retrospective analysis conducted within the phase III MIG1 trial in patients with HER2-positive disease demonstrated that anthracycline-based dose-dense chemotherapy is highly effective in this setting.[6] Similarly, the EBCTCG meta-analysis, in which the majority of patients with HER2-positive disease were not exposed to targeted therapy, showed that dose-dense chemotherapy was effective irrespectively of HER2 status.[5] Consistently with previous findings, our analysis showed apparent similar absolute and relative DFS and OS benefits of administering anthracycline- and taxane-based chemotherapy with a dose-dense



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schedule in patients with HER2-positive tumors not treated with adjuvant trastuzumab and those with HER2-negative/unknown disease. The high proliferative capability and their sensitivity to anthracycline-based chemotherapy [15] may explain the potential predictive value of HER2-positivity in the absence of targeted therapy for the efficacy of dose-dense regimens. Nevertheless, it should be highlighted that trastuzumab has radically changed the prognosis of patients with HER2-positive early breast cancer converting this tumor from an aggressive subtype to one with good outcomes; thus, anti-HER2 targeted therapy remains the cornerstone in the treatment of this disease.[16] In our analysis, we also explored the benefit of introducing adjuvant trastuzumab in patients with HER2-positive disease. Considering that patients receiving anti-HER2 targeted therapy had to survive long enough to be offered trastuzumab, a potential lead-time bias cannot be excluded in this analysis. However, despite this potential bias, our data do not exhibit a severe violation of the proportional hazards assumption to seriously affect our findings when the effect of the treatments within the HER2 / trastuzumab strata is compared (data not shown). As expected, we observed that patients with HER2-positive tumors treated with dose-dense or standard-interval chemotherapy without adjuvant trastuzumab have generally experienced worse DFS and OS than those exposed to adjuvant trastuzumab or patients with HER2-negative/unknown disease. Therefore, chemotherapy escalation approaches should be considered with particular cautious in this setting where anti-HER2 blockade is the mainstay therapy.[16,17]

In the current anti-HER2 targeted therapy era, the benefit of dose-dense chemotherapy remains unknown when trastuzumab is administered.[11] Differently from previous studies in which all patients received dose-dense chemotherapy,[8–10] we could specifically explore whether dose intensification may benefit patients treated with adjuvant trastuzumab. Despite no significant interaction was observed between HER2 status, trastuzumab treatment and the effect of dose-dense chemotherapy, no apparent absolute and relative differences in DFS and OS could be observed between the dose-dense and standard-interval schedules among patients with HER2-positive



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patients who received trastuzumab. Similar conclusions were reached in a recently presented secondary analysis of the PANTHER trial showing no apparent significant benefit of dose-dense chemotherapy in patients with HER2-positive early breast cancer who underwent adjuvant trastuzumab.[18] These data are of particular relevance in the current chemotherapy de-escalation era thanks to the availability of effective anti-HER2 targeted agents.[19] Based on recent findings, patients with stage I HER2-positive breast cancer are candidates to receive adjuvant weekly paclitaxel and trastuzumab.[17] On the contrary, patients with stage II-III disease may benefit from a neoadjuvant approach with taxane-based chemotherapy (with or without anthracyclines) plus dual anti-HER2 blockade with trastuzumab and pertuzumab,[20,21] and the possibility to switch to trastuzumab-emtansine (T-DM1) in the case of residual disease at surgery.[22] In such scenario of increased optimization of anti-HER2 blockade, we may speculate that increasing the dose intensity of chemotherapy in the early setting might not be considered a needed treatment strategy.

Our findings should be considered with caution because of some important limitations. This is an exploratory analysis that was not preplanned in the original protocol. No strong conclusions can be derived considering both the wide 95% CI and that the observed differences within the cohort of patients with HER2-positive disease failed to achieve statistical significance. This may be due also to lack of power based on the small number of patients with HER2-positive disease particularly in the cohort treated with adjuvant trastuzumab. For this same reason, despite our original intent considering the different behavior of HER2-positive tumors according to hormone receptor status,[23] it was not possible to explore potential differences in treatment effect between patients with hormone receptor-positive or negative disease. In addition, HER2 testing was performed locally in an era in which there was no specific recommendation on the type of test to be used. Finally, in terms of safety, cardiotoxicity was not systematically collected in the trial. Despite these limitations, importantly, our analysis was conducted within a large multicenter randomized trial with a median follow-up exceeding 8 years and it allowed for the first time to have some insights on the effect of



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dose-dense chemotherapy in the anti-HER2 targeted therapy era. Notably, it will be difficult to replicate these findings within other dose-dense studies considering that in the older trials most of the patients with HER2-positive disease did not receive anti-HER2 therapy [5] while in the more recent ones all of them received trastuzumab.[18,24] Therefore, this analysis retains a unique value with important clinical implications for the choice of the best adjuvant chemotherapy regimen.

In conclusion, our exploratory analysis of the GIM2 trial showed that dose-dense chemotherapy remains associated with a significant survival improvement in high-risk early breast cancer patients with HER2-negative/unknown disease. Although no significant interaction between HER2 status, trastuzumab use and chemotherapy was observed, the benefit of dose-dense chemotherapy appeared to be smaller (if any) in patients with HER2-positive who received adjuvant trastuzumab. Results of the FeDeriCa trial (NCT03493854) are awaited to better assess the need of chemotherapy escalation approaches in this setting.

**Conflict of interest statement:** Dr. Lambertini served as a consultant for Teva, and received honoraria from Theramex, Takeda and Roche outside the present work. Dr. Bighin served as a consultant for Novartis and Roche, honoraria from Novartis, Roche, Ipsen, Astrazeneca, Pfizer and research funding from Roche outside the present work. Dr. de Azambuja received honoraria and advisory role from Roche/GNE, travel grants from Roche/GNE and GSK Novartis, research grants from Roche/GNE, AstraZeneca, GSK Novartis and Servier (to the Institution) outside the present work. Dr. Giuliano received honoraria from Roche, Pfizer, AstraZeneca, Novartis, Celgene, Eli Lilly, Amgen and Eisai outside the present work. Dr Alessandra Fabi received honoraria and advisory role from Amgen, Celgene, Eli Lilly, Eisai, MSD, Novartis, Pfizer, Roche, Takeda outside the present work. Dr. Anna Turletti received honoraria from Ipsen, EISAI, Novartis, AstraZeneca, Celgene and Pierre Fabre, and travel grants from Roche, Lilly, EISAI and Pierre Fabre outside the present work. Dr. Garrone served as a consultant for Celgene, Eisai, Pfizer, Amgen, honoraria from



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Celgene, Novartis, Eisai, Pfizer, research funding from Eisai, and travel grants from Celgene and Roche/GNE outside the present work. Prof. Puglisi received honoraria and advisory role from Celgene, Eli Lilly, Ipsen, MSD, Novartis, Roche, and Takeda, travel grants from Celgene, Roche and Servier, and research grants from AstraZeneca, EISAI and Roche outside the present work. Dr. Montemurro served as a consultant for Roche and Lilly, and received honoraria from Roche, Novartis, Pfizer and AstraZeneca outside the present work. Dr. Del Mastro received honoraria from Takeda and personal fees from Ipsen and Takeda outside the present work. The other authors declare no conflict of interests.

**Acknowledgments**

Dr. Lambertini acknowledges the European Society for Medical Oncology (ESMO) for an ESMO Merit Award to present these results during the 2019 ESMO Breast Cancer Congress in Berlin (Germany) on May 4, 2019.

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**Figure legend**

**Figure 1.** The Consolidated standards of reporting trials (CONSORT) flow diagram for the GIM2 study.

Abbreviations: FEC, fluorouracil, epirubicin, cyclophosphamide; EC, epirubicin, cyclophosphamide; P, paclitaxel; DFS, disease-free survival; OS, overall survival.

**Figure 2.** Disease-free survival for the comparison between dose-dense and standard-interval chemotherapy: HER2-positive no trastuzumab group (A); HER2-positive trastuzumab group (B); HER2-negative/unknown group (C).

Abbreviations: HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals.

**Figure 3.** Overall survival for the comparison between dose-dense and standard-interval chemotherapy: HER2-positive no trastuzumab group (A); HER2-positive trastuzumab group (B); HER2-negative/unknown group (C).

Abbreviations: HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervals.

**Figure 4.** Disease-free survival in the standard interval cohort A); Overall survival in the standard interval cohort B); Disease-free survival in the dose-dense cohort C); Overall survival in the dose-dense cohort D).



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Abbreviations: HER2+ / No T, HER2-positive no trastuzumab group; HER2+ / T, HER2-positive trastuzumab group; HER2- / Unk, HER2-negative/unknown group; HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence intervalsl.

**Table 1. Baseline patient and tumor characteristics**

| | HER2-positive no trastuzumab (No.=320) No. (%) | HER2-positive trastuzumab (No.=132) No. (%) | HER2-negative/unknown (No.=1551) No. (%) | p value |
|---|---|---|---|---|
| **Median age at study entry** | 52 | 50 | 52 | 0.421 |
| **Age at study entry** | | | | **0.004** |
| ≤40 years | 62 (19.4) | 25 (18.9) | 208 (13.4) | |
| 41-64 years | 214 (66.9) | 98 (74.2) | 1176 (75.8) | |
| ≥65 years | 44 (13.7) | 9 (6.8) | 167 (10.8) | |
| **Menopausal status** | | | | 0.692 |
| Pre-menopausal | 157 (49.1) | 70 (53.0) | 762 (49.1) | |
| Post-menopausal | 163 (50.9) | 62 (47.0) | 789 (50.9) | |
| **Type of surgery** | | | | **0.034** |
| Mastectomy | 141 (44.1) | 62 (47.0) | 575 (37.1) | |
| Lumpectomy | 179 (55.9) | 70 (53.0) | 974 (62.8) | |
| Unknown | 0 (0.0) | 0 (0.0) | 2 (0.1) | |
| **Tumor size** | | | | 0.266 |
| pT1 | 152 (47.5) | 65 (49.2) | 806 (52.0) | |
| pT2 | 135 (42.2) | 55 (41.7) | 634 (40.9) | |
| pT3-T4 | 31 (9.7) | 12 (9.1) | 104 (6.7) | |
| Unknown | 2 (0.6) | 0 (0.0) | 7 (0.4) | |
| **Nodal status** | | | | **<0.001** |
| pN1 (1-3) | 162 (50.6) | 70 (53.0) | 969 (62.5) | |
| pN2 (4-9) - N3 (≥10) | 158 (49.4) | 62 (47.0) | 582 (37.5) | |
| **Tumor grade** | | | | **<0.001** |
| G1 | 12 (3.7) | 2 (1.5) | 106 (6.8) | |
| G2 | 105 (32.8) | 44 (33.3) | 759 (48.9) | |
| G3 | 191 (59.7) | 86 (65.1) | 618 (39.8) | |
| Unknown | 12 (3.7) | 0 (0.0) | 68 (4.4) | |
| **HR status** | | | | **<0.001** |
| ER and/or PR positive | 239 (74.7) | 74 (56.1) | 1198 (77.2) | |
| ER and PR negative | 79 (24.7) | 58 (43.9) | 198 (12.8) | |
| Unknown | 2 (0.6) | 0 (0.0) | 155 (10.0) | |
| **Ki67 value (%)** | | | | **<0.001** |
| 0-14 | 42 (13.1) | 11 (8.3) | 433 (27.9) | |
| 15-20 | 44 (13.7) | 16 (12.1) | 241 (15.5) | |
| >20 | 176 (55.0) | 93 (70.4) | 533 (34.4) | |
| Unknown | 58 (18.1) | 12 (9.1) | 344 (22.2) | |



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| | | | | |
|---|---|---|---|---|
| **CT treatment** | | | | 0.327 |
| FEC | 172 (53.7) | 65 (49.2) | 763 (49.2) | |
| EC | 148 (46.3) | 67 (50.8) | 788 (50.8) | |
| **Treatment arm** | | | | 0.471 |
| DD | 166 (51.9) | 60 (45.5) | 776 (50.0) | |
| SI | 154 (48.1) | 72 (54.5) | 775 (50.0) | |
| **Endocrine therapy*** | | | | **<0.001** |
| Tamoxifen (± GnRHa) | 98 (41.0) | 20 (27.0) | 532 (44.4) | |
| Tamoxifen (± GnRHa)→AI | 54 (22.6) | 18 (24.3) | 347 (29.0) | |
| AI | 65 (27.2) | 32 (43.2) | 233 (19.4) | |
| None | 11 (4.6) | 3 (4.1) | 30 (2.5) | |
| Unknown | 11 (4.6) | 1 (1.4) | 56 (4.7) | |

*The percentages were calculated on the total number of patients with hormone receptor-positive breast cancer

Abbreviations: pT, pathologic tumor stage; pN, pathologic nodal stage; G, tumor grade; HR, hormone receptor; ER, estrogen receptor; PR, progesteron receptor; CT, chemotherapy; FEC, fluorouracil, epirubicin, and cyclophosphamide; EC, epirubicin and cyclophosphamide; DD, dose dense; SI, standard interval; GnRHa, gonadotropin-releasing hormone agonist; AI, aromatase inhibitor.

**Table 2. Disease-free survival and overall survival events in the intention-to-treat population**

| | **HER2-positive no trastuzumab (No.=320) No. (%)** | **HER2-positive trastuzumab (No.=132) No. (%)** | **HER2-negative/unknown (No.=1,551) No. (%)** | **p value** |
|---|---|---|---|---|
| Follow-up, median (IQR) years | 8.0 (6.0-9.0) | 7.3 (6.1-8.8) | 7.9 (6.4-9.2) | **0.032** |
| No events | 212 (66.3) | 95 (72.0) | 1,117 (72.0) | 0.116 |
| Loco-regional recurrence only | 31 (9.7) | 10 (7.6) | 89 (5.7) | **0.032** |
| Distant recurrence | 61 (19.1) | 25 (18.9) | 245 (15.8) | 0.251 |
| Second primary malignancy | 7 (2.2) | 0 (-) | 41 (2.6) | 0.136 |
| Second primary breast cancer | 5 (1.6) | 1 (0.8) | 33 (2.1) | 0.632 |
| Death without any disease-free survival event | 4 (1.3) | 1 (0.8) | 26 (1.7) | 0.841 |
| Death with some disease-free survival event | 53 (16.6) | 17 (12.9) | 194 (12.5) | 0.153 |

Abbreviations: IQR, interquartile range.



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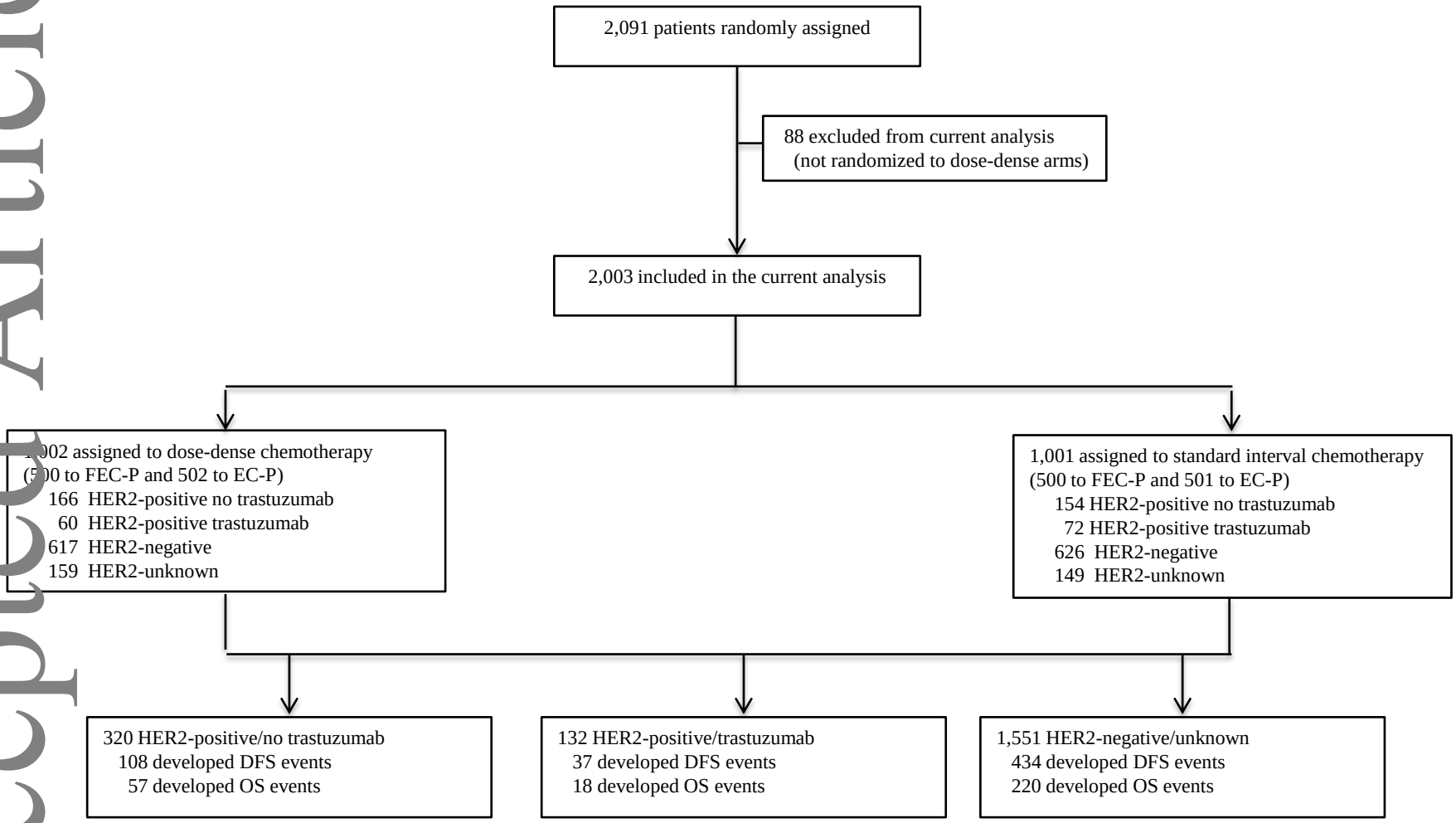


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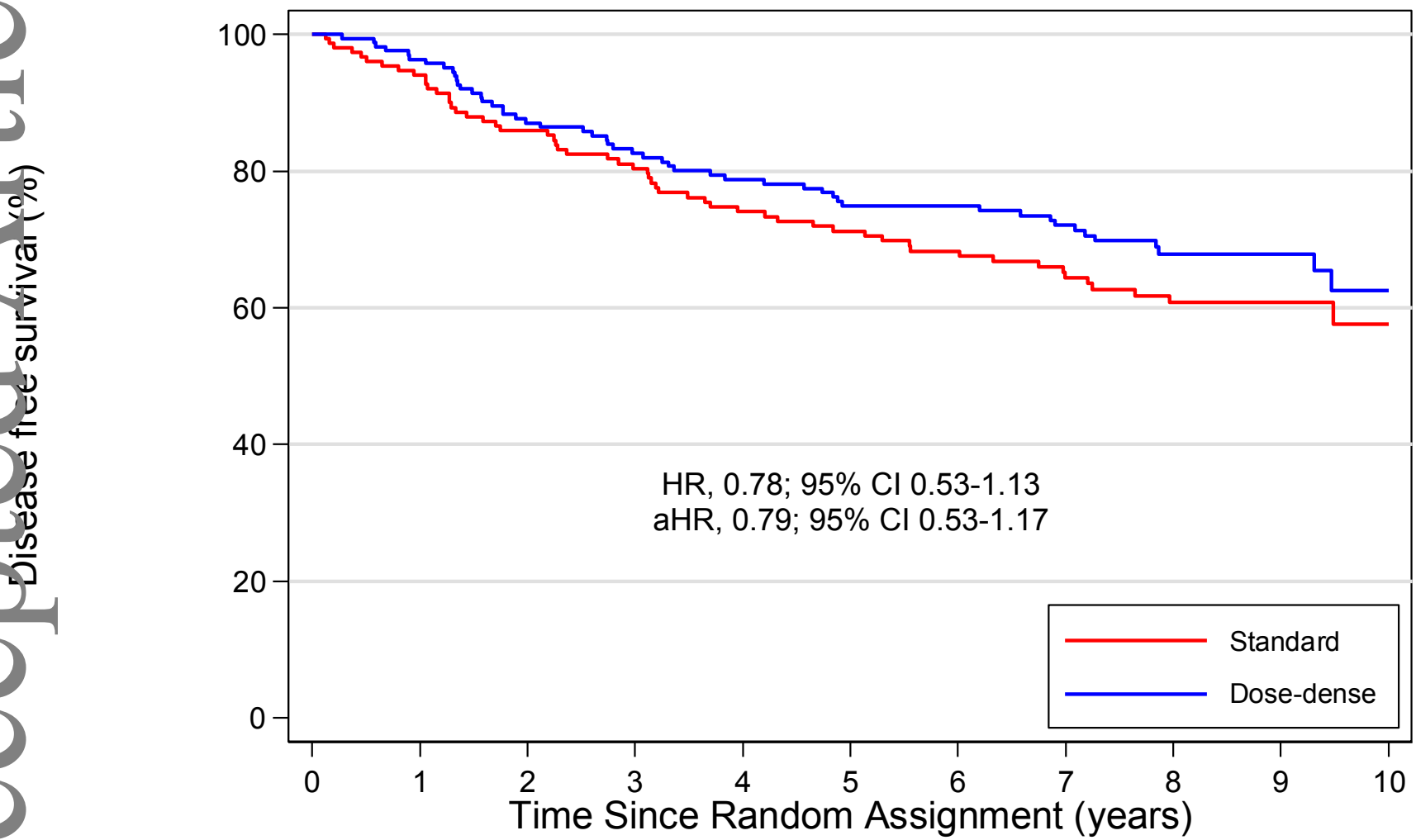
A

# HER2-positive no trastuzumab




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard | 154 | 138 | 125 | 115 | 105 | 100 | 91 | 80 | 60 | 30 | 13 |
| Dose-dense | 166 | 156 | 139 | 130 | 123 | 115 | 108 | 99 | 65 | 35 | 11 |

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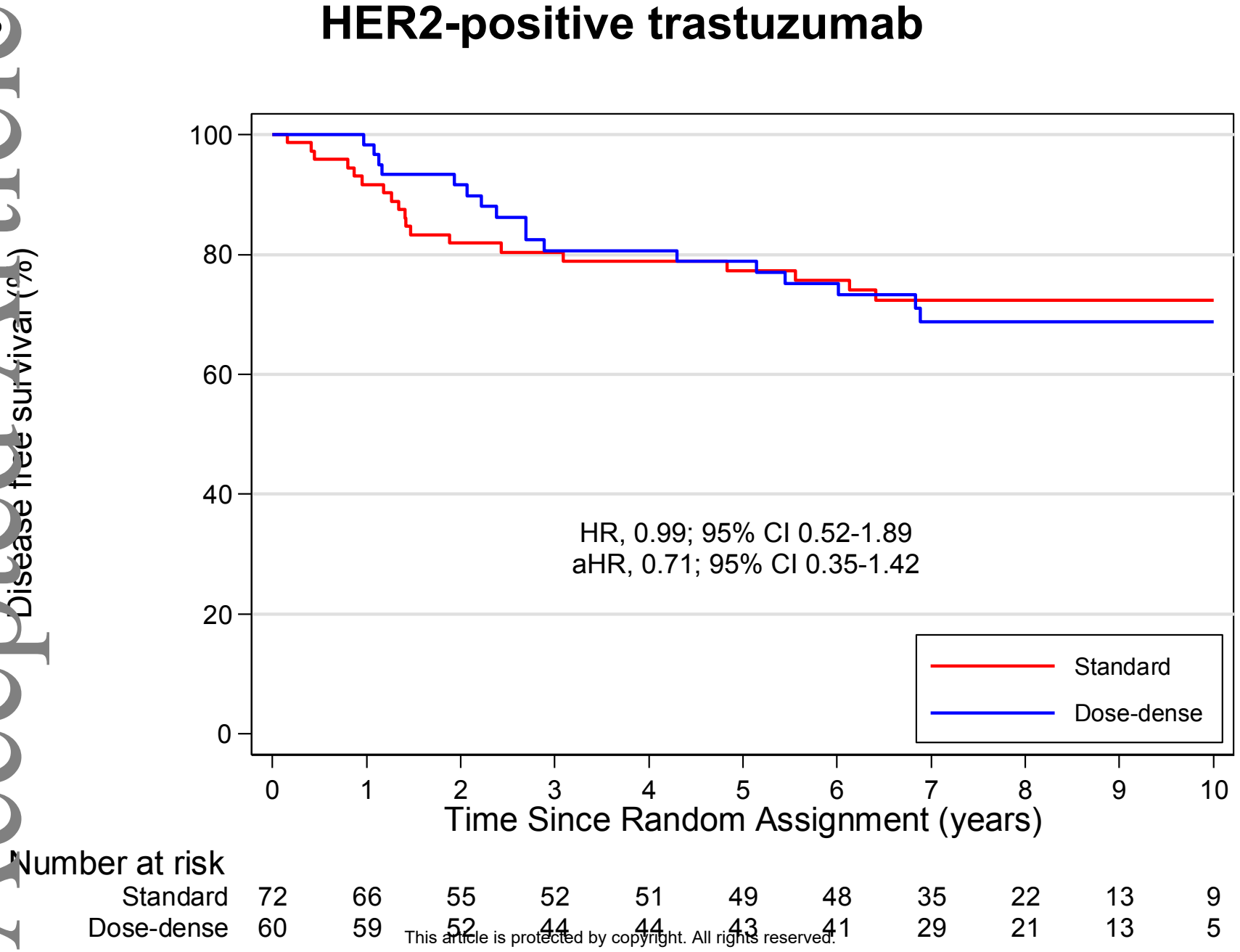


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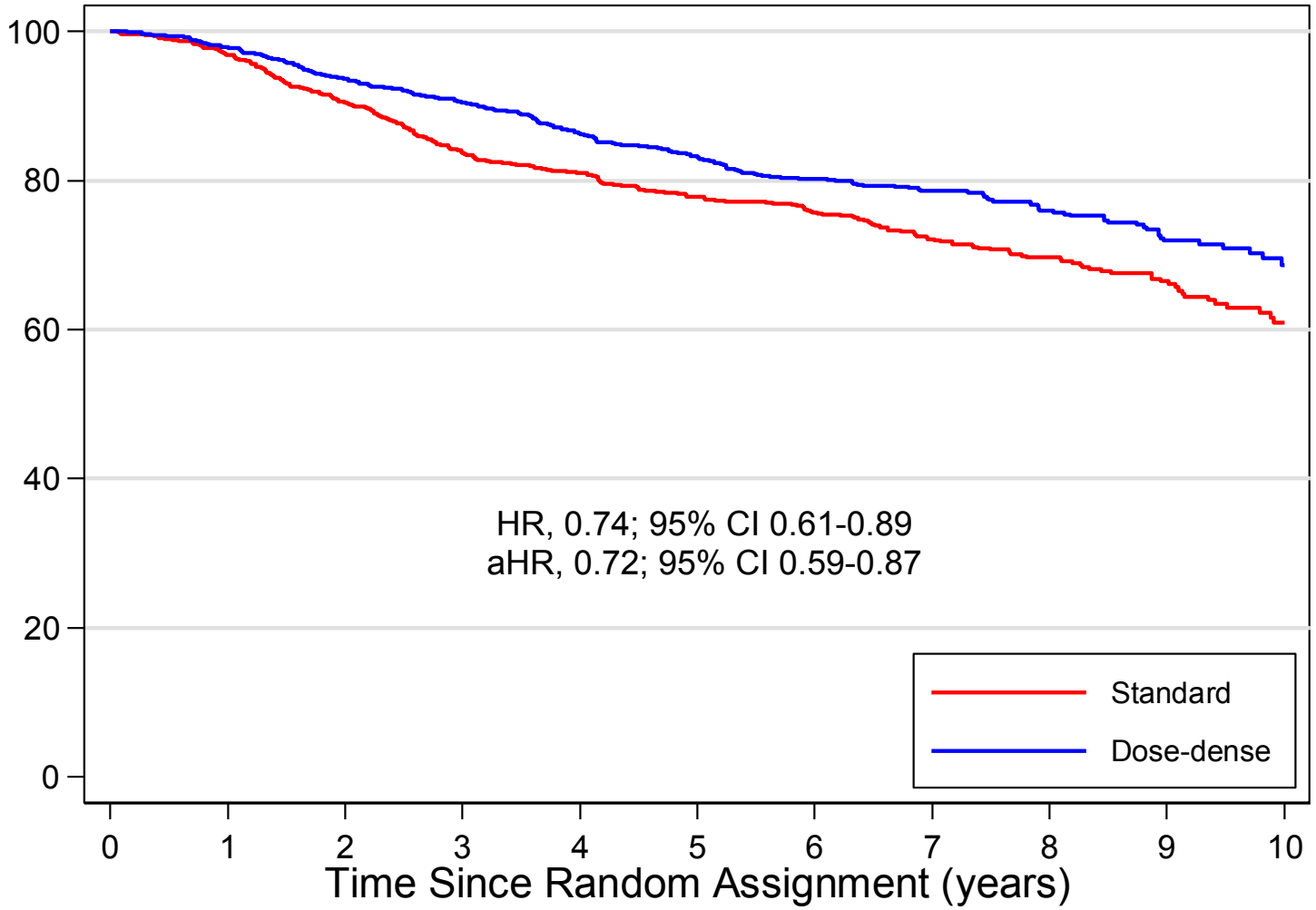
## HER2-negative/unknown




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard | 775 | 725 | 662 | 606 | 578 | 548 | 520 | 430 | 300 | 170 | 80 |
| Dose-dense | 776 | 742 | 692 | 659 | 622 | 590 | 558 | 488 | 344 | 189 | 82 |

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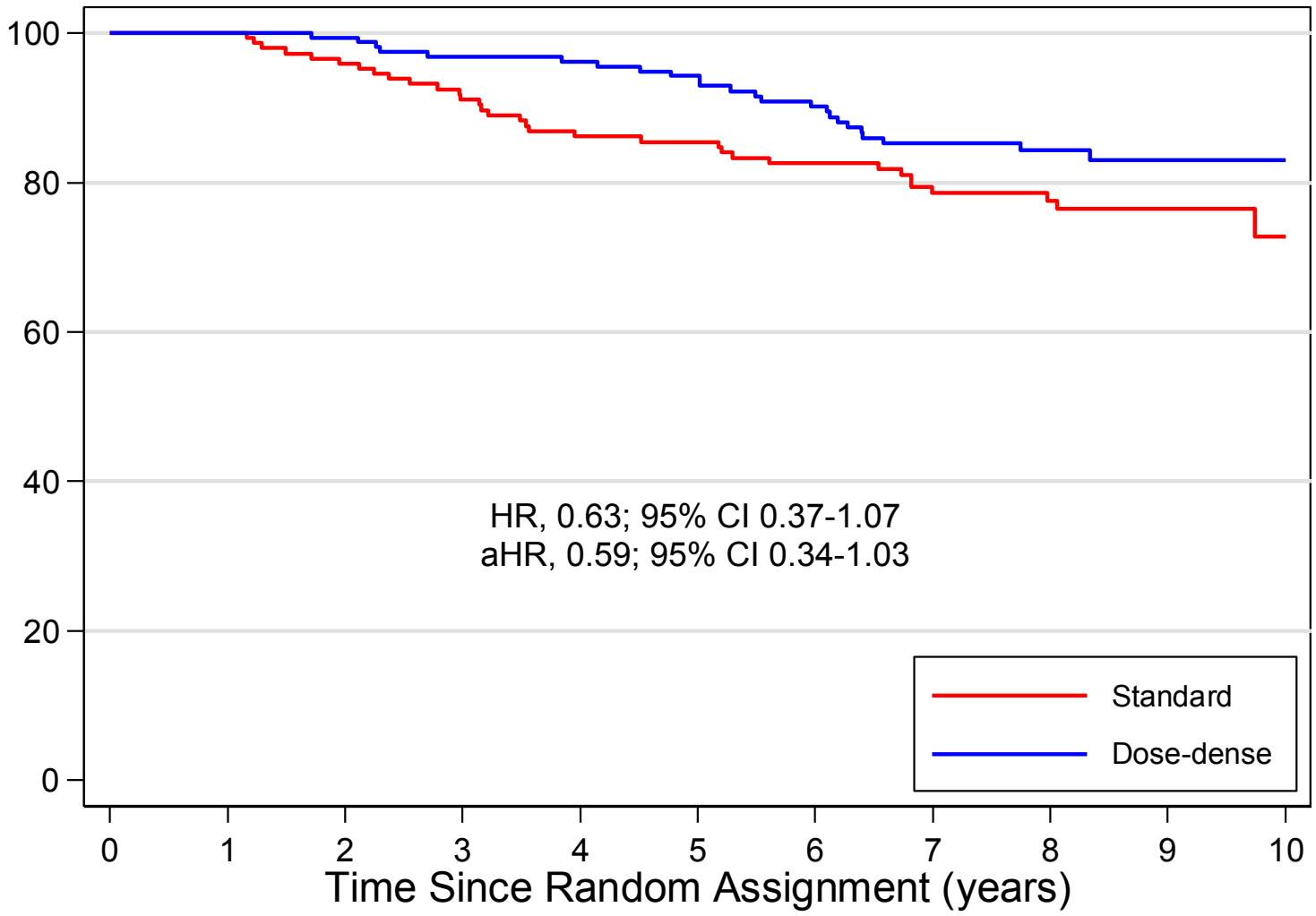
A

# HER2-positive no trastuzumab




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard | 154 | 147 | 140 | 131 | 122 | 120 | 110 | 98 | 76 | 40 | 17 |
| Dose-dense | 166 | 162 | 158 | 152 | 148 | 143 | 129 | 116 | 82 | 43 | 13 |

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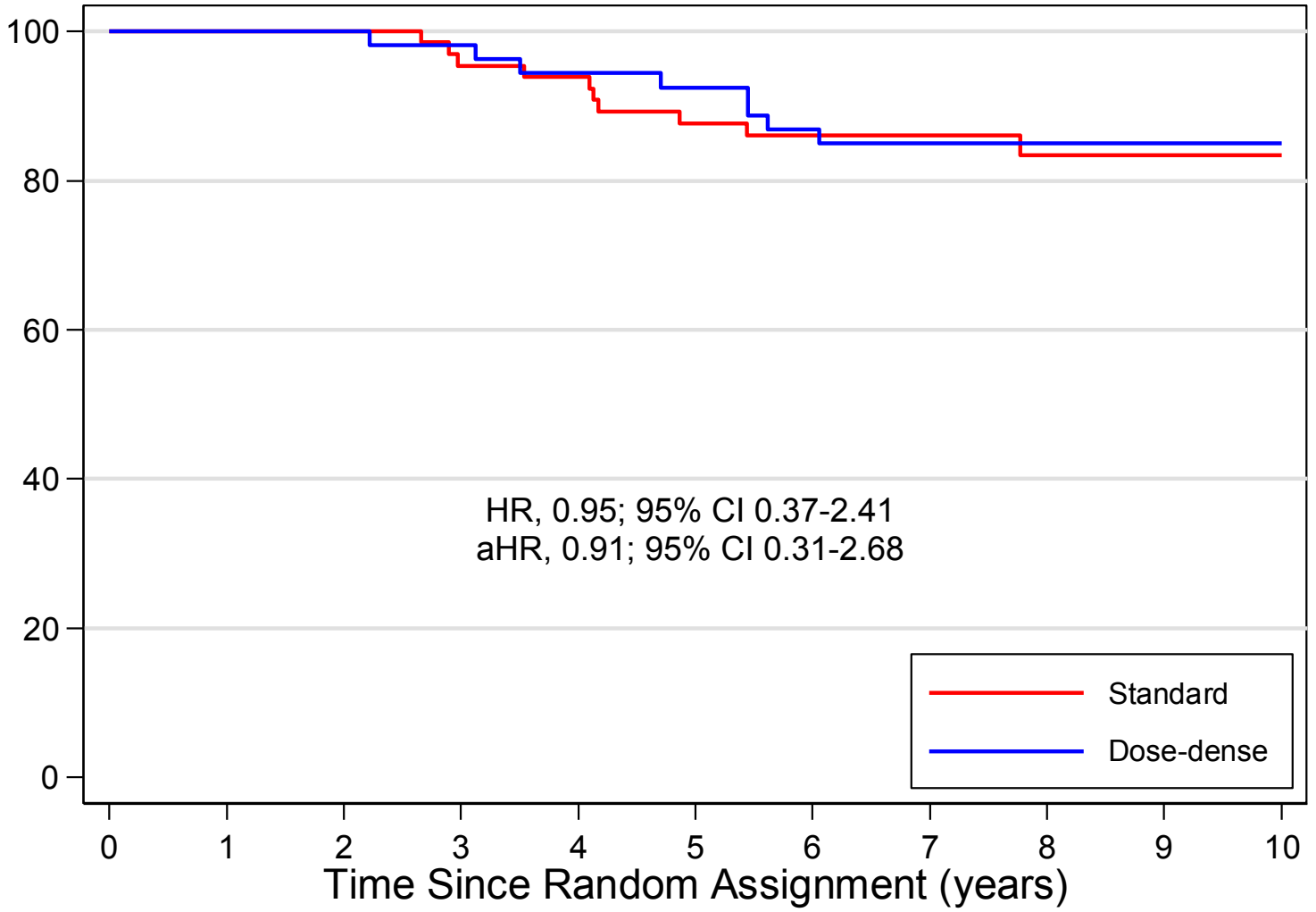
B

# HER2-positive trastuzumab




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard | 72 | 72 | 68 | 62 | 61 | 55 | 54 | 43 | 26 | 15 | 10 |
| Dose-dense | 60 | 60 | 56 | 52 | 50 | 49 | 46 | 33 | 21 | 13 | 5 |

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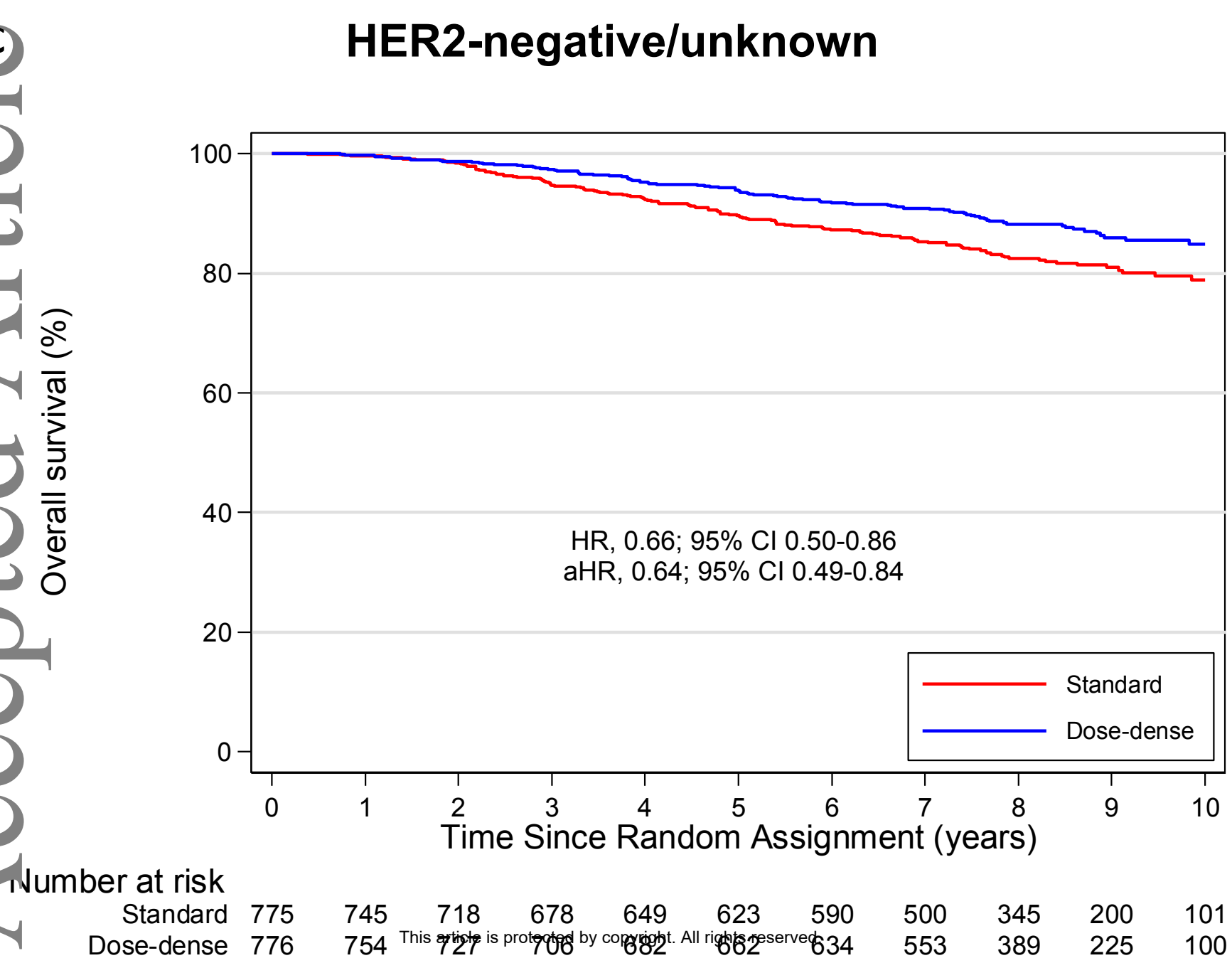


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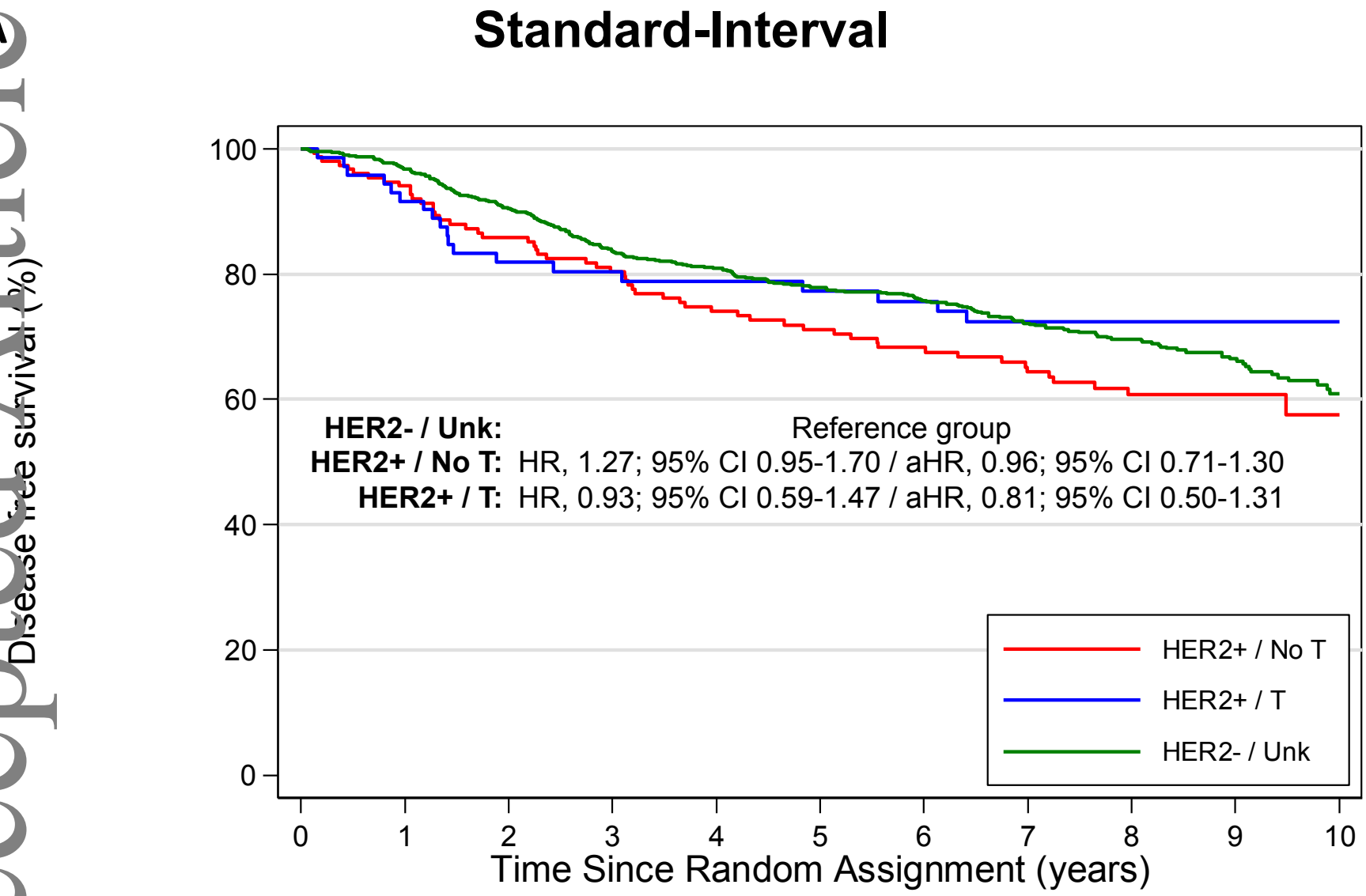


Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HER2+ / No T | 154 | 138 | 125 | 115 | 105 | 100 | 91 | 80 | 60 | 30 | 13 |
| HER2+ / T | 72 | 66 | 55 | 52 | 51 | 49 | 48 | 35 | 22 | 13 | 9 |
| HER2- / Unk | 775 | 725 | 662 | 606 | 578 | 548 | 520 | 430 | 300 | 170 | 80 |

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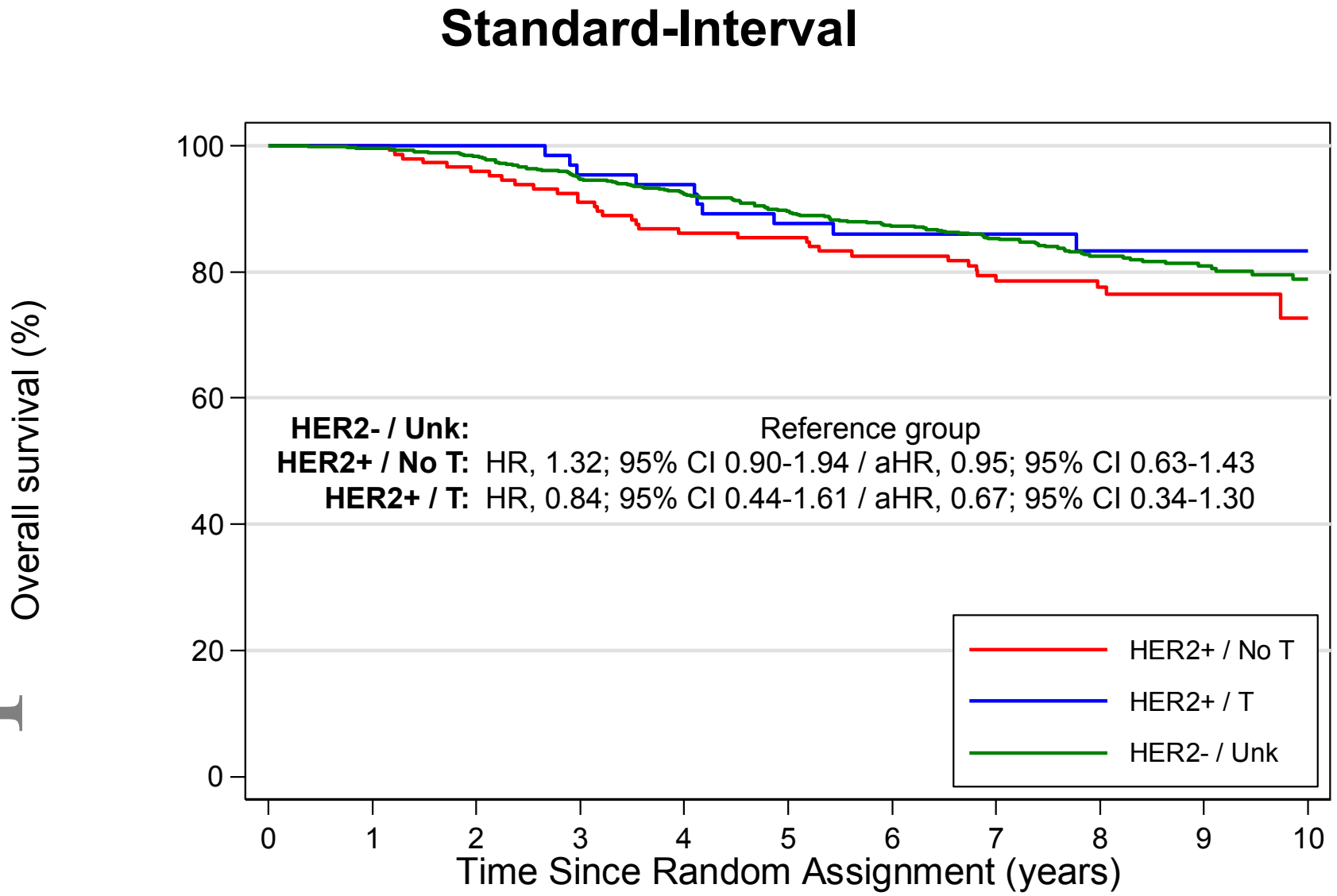


Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HER2+ / No T | 154 | 147 | 140 | 131 | 122 | 120 | 110 | 98 | 76 | 40 | 17 |
| HER2+ / T | 72 | 72 | 68 | 62 | 61 | 55 | 54 | 43 | 26 | 15 | 10 |
| HER2- / Unk | 775 | 745 | 718 | 678 | 649 | 623 | 590 | 500 | 345 | 200 | 101 |

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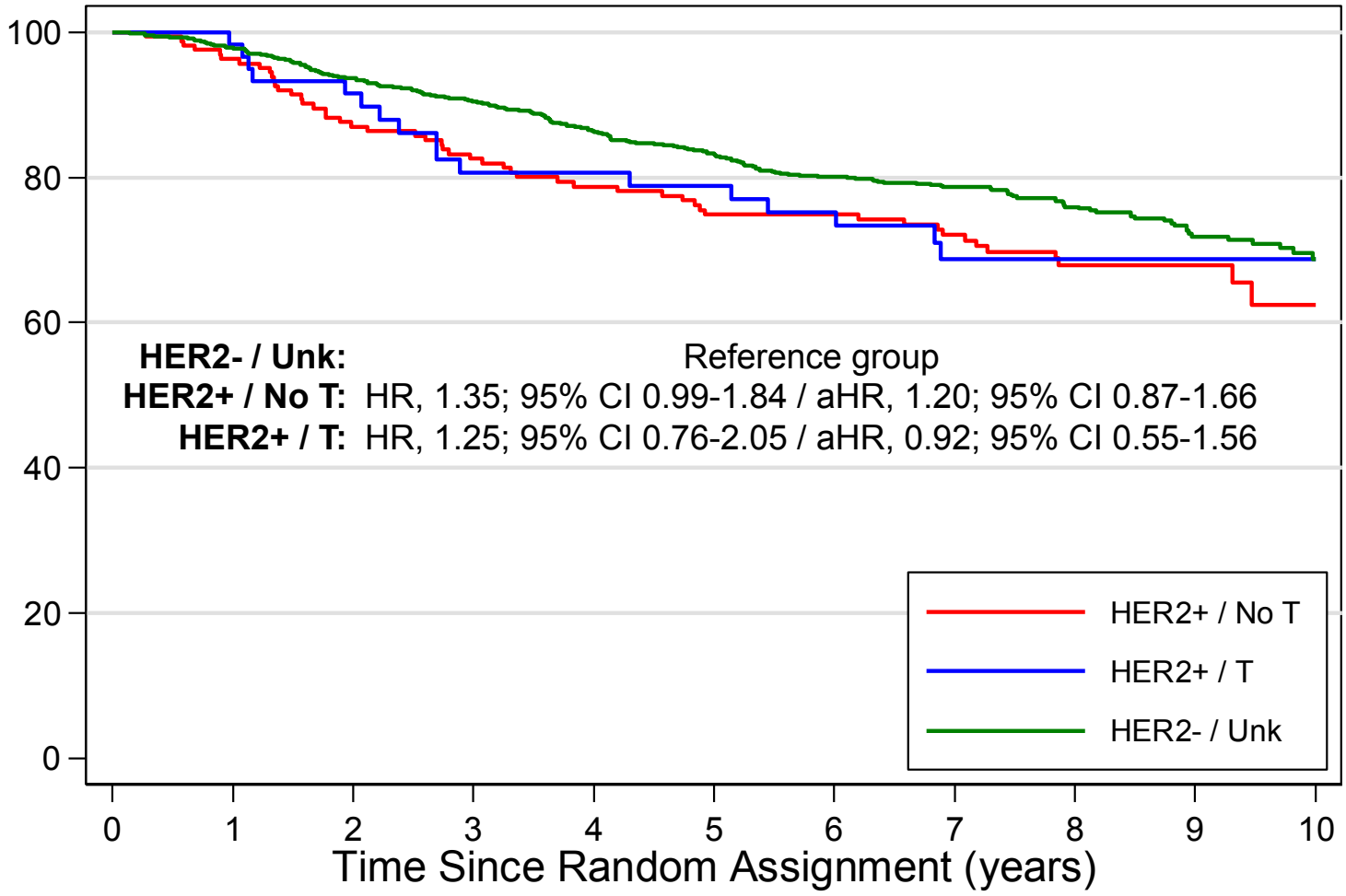
## Dose-Dense




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HER2+ / No T | 166 | 156 | 139 | 130 | 123 | 115 | 108 | 99 | 65 | 35 | 11 |
| HER2+ / T | 60 | 59 | 52 | 44 | 44 | 43 | 41 | 29 | 21 | 13 | 5 |
| HER2- / Unk | 776 | 742 | 692 | 659 | 622 | 590 | 558 | 488 | 344 | 189 | 82 |

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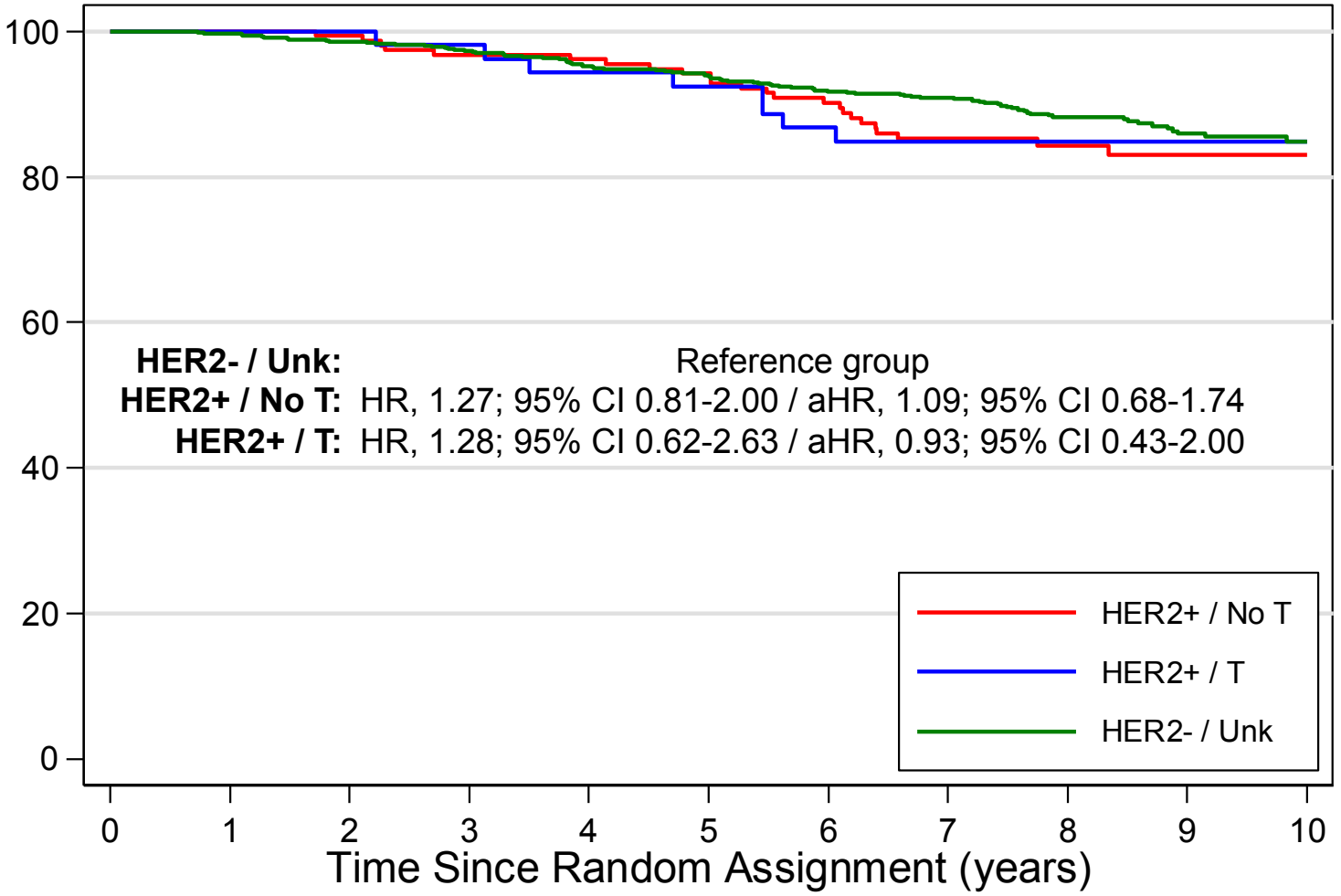
D

## Dose-Dense




Number at risk

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HER2+ / No T | 166 | 162 | 158 | 152 | 148 | 143 | 129 | 116 | 82 | 43 | 13 |
| HER2+ / T | 60 | 60 | 56 | 52 | 50 | 49 | 46 | 33 | 21 | 13 | 5 |
| HER2- / Unk | 776 | 754 | 727 | 706 | 682 | 662 | 634 | 553 | 389 | 225 | 100 |

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