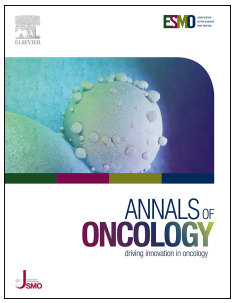
Journal Pre-proof



Event-free survival by residual cancer burden with pembrolizumab in early-stage TNBC: exploratory analysis from KEYNOTE-522

L. Pusztai, C. Denkert, J. O'Shaughnessy, J. Cortes, R. Dent, H. McArthur, S. Kümmel, J. Bergh, Y.H. Park, R. Hui, N. Harbeck, M. Takahashi, M. Untch, P.A. Fasching, F. Cardoso, Y. Zhu, W. Pan, K. Tryfonidis, P. Schmid

PII: S0923-7534(24)00046-2

DOI: https://doi.org/10.1016/j.annonc.2024.02.002

Reference: ANNONC 1442

To appear in: *Annals of Oncology*

Received Date: 30 November 2023

Revised Date: 5 February 2024

Accepted Date: 8 February 2024

Please cite this article as: Pusztai L, Denkert C, O'Shaughnessy J, Cortes J, Dent R, McArthur H, Kümmel S, Bergh J, Park YH, Hui R, Harbeck N, Takahashi M, Untch M, Fasching PA, Cardoso F, Zhu Y, Pan W, Tryfonidis K, Schmid P, Event-free survival by residual cancer burden with pembrolizumab in early-stage TNBC: exploratory analysis from KEYNOTE-522, *Annals of Oncology* (2024), doi: https://doi.org/10.1016/j.annonc.2024.02.002.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2024 European Society for Medical Oncology. Published by Elsevier Ltd. All rights reserved.

Journal Pre-proof

Event-free survival by residual cancer burden with pembrolizumab in early-stage TNBC: exploratory analysis from KEYNOTE-522

L. Pusztai[1], C. Denkert[2], J. O'Shaughnessy[3], J. Cortes[4], R. Dent[5], H. McArthur[6], S. Kümmel[7], J. Bergh[8], Y. H. Park[9], R. Hui[10], N. Harbeck[11], M. Takahashi[12], M. Untch[13], P. A. Fasching[14], F. Cardoso[15], Y. Zhu[16], W. Pan[16], K. Tryfonidis[16], P. Schmid[17]

[1]Yale School of Medicine, Yale Cancer Center, New Haven, CT, USA; [2]Institute of Pathology, Philipps-University Marburg and University Hospital Marburg, Marburg, Germany; [3]Baylor University Medical Center, Texas Oncology, US Oncology Network, Dallas, TX, USA; [4]International Breast Cancer Center, Quironsalud Group, Barcelona, Spain and Universidad Europea de Madrid, Faculty of Biomedical and Health Sciences, Department of Medicine, Madrid, Spain; [5]National Cancer Center Singapore, Duke – National University of Singapore Medical School, Singapore; [6]University of Texas Southwestern Medical Center, Dallas, TX, USA; [7]Breast Unit, Kliniken Essen-Mitte, Essen, Germany and Charité – Universitätsmedizin Berlin, Department of Gynecology with Breast Center, Berlin, Germany; [8]Department of Oncology-Pathology, Karolinska Institutet and Breast Cancer Centre, Cancer Theme, Karolinska University Hospital, Karolinska Comprehensive Cancer Center, Solna, Sweden; [9]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; [10]Westmead Breast Cancer Institute, Westmead Hospital and the University of Sydney, Sydney, NSW, Australia; [11]Breast Center, Dept. OB&GYN, LMU University Hospital, Munich, Germany; [12]Hokkaido University Hospital, Sapporo, Japan; [13]Breast Cancer Center, Helios Klinikum Berlin-Buch, Berlin, Germany; [14]University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany; [15]Breast Unit, Champalimaud Clinical Center/Champalimaud Foundation, Lisbon, Portugal; [16]Oncology, Merck & Co., Inc., Rahway,

Journal Pre-proof

NJ, USA; [17]Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London, London, UK

Corresponding Author: Professor Lajos Pusztai, M.D, D.Phil.
Professor of Medicine
Scientific Co-Director, The Breast Center at Yale Cancer Center
Co-Director, Yale Cancer Center Genetics and Genomics Program
Yale Cancer Center
Yale School of Medicine
333 Cedar St., PO Box 208032
New Haven, CT 05620
Tel: 203-737-8309
e-mail: lajos.pusztai@yale.edu

Journal Pre-proof

Manuscript type: Original article

Previous presentation: These results were presented in part at the 2022 American Society of Clinical Oncology Annual Meeting; Chicago, Illinois, USA; June 3–7, 2022 (abstract 365410)

Target journal: *Annals of Oncology*

Word count: 2540 (limit, 4500)

Tables/figures: 1 table/4 figures

Highlights

- KEYNOTE-522 evaluated neoadjuvant pembrolizumab+chemo/adjuvant pembrolizumab vs neoadjuvant chemo alone in early TNBC.
- Neoadjuvant pembrolizumab+chemo/adjuvant pembrolizumab significantly improved pCR/EFS vs neoadjuvant chemo alone.
- We explored the effect of adding pembrolizumab to chemo on outcomes by RCB category.
- Pembrolizumab not only increased pCR rates, but also improved EFS among many patients who do not have a pCR.
- Findings support neoadjuvant pembrolizumab+chemo/adjuvant pembrolizumab as a standard of care treatment in early TNBC.

Journal Pre-proof

ABSTRACT

Background: KEYNOTE-522 demonstrated statistically significant improvements in pathological complete response (pCR) with neoadjuvant pembrolizumab plus chemotherapy and event-free survival (EFS) with neoadjuvant pembrolizumab plus chemotherapy followed by adjuvant pembrolizumab in patients with high-risk, early-stage triple-negative breast cancer (TNBC). Prior studies have shown the prognostic value of the residual cancer burden (RCB) index to quantify the extent of residual disease after neoadjuvant chemotherapy. In this preplanned exploratory analysis, we assessed RCB distribution and EFS within RCB categories by treatment group.

Patients and Methods: 1174 patients with stage T1c/N1-2 or T2-4/N0-2 TNBC were randomized 2:1 to pembrolizumab 200 mg or placebo Q3W given with 4 cycles of paclitaxel + carboplatin, followed by 4 cycles of doxorubicin or epirubicin + cyclophosphamide. After surgery, patients received pembrolizumab or placebo for 9 cycles or until recurrence or unacceptable toxicity. Primary endpoints are pCR and EFS. RCB is a prespecified exploratory endpoint. The association between EFS and RCB was assessed using a Cox regression model.

Results: Pembrolizumab shifted patients into lower RCB categories across the entire spectrum compared to placebo. There were more patients in the pembrolizumab group with RCB-0 (pCR), and fewer patients in the pembrolizumab group with RCB-1, RCB-2, and RCB-3. The corresponding hazard ratios (95% CIs) for EFS were 0.70 (0.38–1.31), 0.92 (0.39–2.20), 0.52 (0.32–0.82), and 1.24 (0.69–2.23). The most common first EFS events were distant recurrences, with fewer in the pembrolizumab group across all RCB categories. Among patients with RCB-0/1, over half (21/38 [55.3%]) of all events were central nervous system recurrences, with 13/22 (59.1%) in the pembrolizumab group and 8/16 (50.0%) in the placebo group.

Journal Pre-proof

Conclusion: Addition of pembrolizumab to chemotherapy resulted in fewer EFS events in the RCB-0, RCB-1, and RCB-2 categories, with the greatest benefit in RCB-2. These findings demonstrate that pembrolizumab not only increased pCR rates, but also improved EFS among most patients who do not have a pCR.

Trial Registration: Clinicaltrials.gov registration, NCT03036488

Keywords: chemotherapy, event-free survival, immunotherapy, pembrolizumab, residual cancer burden, triple-negative breast cancer

Journal Pre-proof

INTRODUCTION

Triple-negative breast cancer (TNBC) is the most difficult breast cancer subtype to treat due to limited therapeutic options.[1, 2] Neoadjuvant chemotherapy (NAC) is commonly used for the treatment of patients with newly diagnosed TNBC because it allows for pathological response-guided adjuvant therapy.[3-8] Nevertheless, an elevated risk for disease recurrence and death remains, underscoring the importance of additional therapies to augment the effectiveness of NAC.

KEYNOTE-522 is a randomized, placebo-controlled, phase 3 trial of pembrolizumab in early-stage, high-risk TNBC in both the neoadjuvant and adjuvant settings. The primary results showed statistically significant and clinically meaningful improvements in pathological complete response (pCR) with pembrolizumab plus NAC, and in event-free survival (EFS) with pembrolizumab plus NAC followed by adjuvant pembrolizumab. The primary pCR analysis based on the first 602 patients enrolled showed an increase in pCR (defined as pathological stage ypT0/Tis ypN0 at the time of definitive surgery) of 13.6 percentage points (P=0.00055) with the addition of pembrolizumab to NAC.[9] At the definitive EFS analysis based on the entire intention-to-treat population (n=1174), the addition of pembrolizumab to NAC followed by adjuvant pembrolizumab, as compared with NAC alone, resulted in a 37% reduction in the risk of disease progression that precluded definitive surgery, a local or distant recurrence, a second primary tumor, or death from any cause.[10] Based on these results, pembrolizumab combined with chemotherapy as neoadjuvant treatment and then continued as single-agent adjuvant treatment after surgery has been approved for the treatment of patients with high-risk, early-stage TNBC.[11]

Prior studies have shown the prognostic value of the residual cancer burden (RCB) index to quantify the extent of residual disease after NAC.[12] The RCB methodology combines pathologic measurements of primary tumor size, tumor cellularity, the number of positive nodes, and the size of nodal metastases into a single score,[13] which has been validated for its prognostic value across all breast cancer subtypes.[14] The RCB scores can be grouped into 4 categories: RCB-0 is equivalent to pCR, and RCB categories -1, -2, and -3 correspond to increasingly larger residual cancer after NAC, each with distinct survival probabilities. In the current prespecified exploratory analysis, we evaluated EFS within RCB categories for all patients in KEYNOTE-522, and report the distribution of first EFS events by RCB category and treatment group. We also present results for the patient subsets that completed full chemotherapy versus less than full chemotherapy.

Journal Pre-proof

PATIENTS AND METHODS

Study Design and Patients

Detailed methods for the ongoing phase 3, randomized, double-blind, placebo-controlled KEYNOTE-522 study (ClinicalTrials.gov, NCT03036488) were published previously.[9, 10] In brief, adult patients with centrally confirmed, newly diagnosed, previously untreated, nonmetastatic TNBC (tumor stage T1c, nodal stage N1–2 or tumor stage T2–4, nodal stage N0–2 per American Joint Committee on Cancer, 7th edition[15]) as determined by investigator radiologic or clinical assessment were eligible. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1 and provide a tissue sample available for PD-L1 assessment. Patients were eligible for the study regardless of PD-L1 expression.

All patients provided written informed consent before enrollment. The study was conducted in accordance with the protocol and its amendments and with the standards of Good Clinical Practice. An external, independent data monitoring committee oversaw the study, periodically assessed safety, and assessed efficacy at prespecified interim analyses.

Randomization and Study Treatment

Patients were stratified before randomization based on nodal status (positive or negative), tumor size (T1–T2 or T3–T4), and schedule of carboplatin administration (weekly or every 3 weeks). Randomization was done using a central interactive voice-response system with an integrated Web-response system. Patients were randomized 2:1 to receive either pembrolizumab or placebo. In the first neoadjuvant phase (cycles 1–4), patients received intravenous

pembrolizumab (200 mg every 3 weeks) or placebo in combination with carboplatin (area under the concentration–time curve 5 mg/mL/min every 3 weeks or 1.5 mg/mL/min weekly) plus paclitaxel (80 mg/m^2 weekly). In the second neoadjuvant phase (cycles 5–8), patients continued treatment with pembrolizumab or placebo in combination with doxorubicin or epirubicin (60 mg/m^2 or 90 mg/m^2, respectively, every 3 weeks) and cyclophosphamide (600 mg/m^2 every 3 weeks). Following surgery, patients received adjuvant therapy with either intravenous pembrolizumab (200 mg every 3 weeks) or placebo for 9 cycles. Adjuvant capecitabine was not allowed. Patients who completed or discontinued the first neoadjuvant treatment could start the second neoadjuvant treatment or undergo surgery; patients who completed the second neoadjuvant treatment could proceed to surgery. Patients discontinued treatment if they had disease progression, recurrence, or adverse events. Patients who discontinued pembrolizumab or placebo due to adverse events during the neoadjuvant phase were not allowed to receive pembrolizumab or placebo after surgery.

Journal Pre-proof

Endpoints

The study's primary endpoints were pCR (ypT0/Tis ypN0) at the time of definitive surgery and EFS. RCB was a prespecified exploratory endpoint. In the current analysis, EFS within RCB categories was evaluated for all patients, and the distribution of first EFS events was assessed by RCB category and treatment group. Outcomes in the patient subsets that completed full chemotherapy versus less than full chemotherapy were analyzed post hoc.

Assessments

Pathological complete response was assessed after completion of neoadjuvant therapy as determined by a local pathologist who was blinded to treatment group assignment. Follow-up for disease status and survival occurred every 3 months for the first 2 years, every 6 months from years 3 to 5, and yearly thereafter. EFS was assessed by the investigator and defined as the time from randomization to the first occurrence of disease progression that precluded definitive surgery, local or distant recurrence, a second primary cancer, or death from any cause. RCB was assessed by the local pathologist at the time of definitive surgery.[16]

Statistical Analyses

No alpha was assigned to these nonrandomized exploratory analyses, and the results reported herein are descriptive only; instead of statistical significance, only 95% confidence intervals (CIs) are reported. Event-free survival within RCB categories was assessed in the intention-to-treat population, which included all randomized patients. For EFS, the hazard ratios (HRs) and associated 95% CIs were estimated based on a Cox regression model with treatment as a covariate. The impact of full exposure to chemotherapy defined as paclitaxel weekly x 10-12 doses, carboplatin weekly x 10-12 doses or carboplatin Q3W x 4 doses, and doxorubicin or epirubicin concurrent with cyclophosphamide Q3W x 4 doses versus less than full exposure to chemotherapy, regardless of exposure to pembrolizumab, on RCB category distribution and EFS was examined post-hoc. For this analysis, the HRs and associated 95% CIs were based on an unstratified Cox model.

Journal Pre-proof

RESULTS

Patients

Between March 2017 and September 2018, 1174, patients were randomly assigned to treatment, with 784 patients in the pembrolizumab group and 390 patients in the placebo group (**Figure 1**). As reported previously, patient demographics and baseline disease characteristics were generally balanced between the treatment groups.[10] The median age was 48.5 years. Of the 1174 patients, 1019 (86.8%) had an ECOG performance status score of 0. A total of 973 patients (82.9%) had tumors that were PD-L1 positive (CPS ≥1). Overall, 672 patients (57.2%) had received weekly carboplatin in the study, 870 patients (74.1%) had a baseline tumor size of T1/T2, and 605 patients (51%) had nodal involvement. The median duration of follow-up was 39.1 months at the March 23, 2021, data cutoff date.

Efficacy

Pembrolizumab moved patients into lower RCB categories across the entire spectrum compared to placebo. There were more patients in the pembrolizumab group with RCB-0 (pCR) and fewer patients in the pembrolizumab group with residual disease (**Figure 2**). A benefit of pembrolizumab on EFS was observed among patients in the RCB-0, RCB-1, and RCB-2 categories. In the RCB-0 category, 26 of 497 patients (5.2%) in the pembrolizumab group and 16 of 219 patients (7.3%) in the placebo group experienced an EFS event (HR, 0.70 [95% CI, 0.38–1.31]), and the 36-month EFS rates were 94.7% in the pembrolizumab group compared to 92.6% in the placebo group (**Figure 3A**). In the RCB-1 category, 12 of 69 patients (17.4%) in the pembrolizumab group and 9 of 45 patients (20.0%) in the placebo group experienced an EFS event (HR, 0.92 [95% CI, 0.93–2.20]), and the 36-month EFS rates were 84.4% versus 83.8%,

respectively (**Figure 3B**). In the RCB-2 category, 37 of 145 patients (25.5%) in the pembrolizumab group and 35 of 79 patients (44.3%) in the placebo group experienced an EFS event (HR, 0.52 [95% CI, 0.32–0.82]), and the 36-month EFS rates were 75.7% versus 55.9%, respectively (**Figure 3C**). In the RCB-3 category, 29 of 40 patients (72.5%) in the pembrolizumab group and 18 of 26 patients (69.2%) in the placebo group experienced an EFS event (HR, 1.24 [95% CI, 0.69–2.23]), and the 36-month EFS rates were 26.2% in the pembrolizumab group compared to 34.6% in the placebo group (**Figure 3D**).

The most common first EFS event in both treatment groups was distant recurrence, which occurred in fewer patients in the pembrolizumab group across all RCB categories, including RCB-3 (**Table 1**). In the pembrolizumab and placebo groups, respectively, distant recurrences were rare among patients in the RCB-0 category (3.2% and 5.5%) and the RCB-1 category (8.7% and 8.9%, respectively), over half (55.3%) were central nervous system (CNS) events, with 13/22 (59.1%) in the pembrolizumab group and 8/16 (50.0%) in the placebo group; by contrast, most first distant recurrences among patients with RCB-2 and RCB-3 were non-CNS events (**Table 1**). The absence of an EFS benefit with pembrolizumab among patients in the RCB-3 category was driven by a higher rate of local recurrence as the first EFS event in the pembrolizumab group (25.0%) compared to the placebo group (7.7%).

In the pembrolizumab and placebo groups, respectively, 75.2% versus 78.9% of patients had full chemotherapy exposure. The pCR rate (RCB-0) was lower in patients who could not complete full chemotherapy in both treatment groups. However, pembrolizumab reduced residual cancer burden and improved the RCB-0 rate even among patients who had less than full chemotherapy

Journal Pre-proof

exposure (**Figure 4A)**. In the subgroup of patients with full chemotherapy exposure, 85 of 585 patients (14.5%) in the pembrolizumab group and 67 of 307 patients (21.8%) in the placebo group experienced an EFS event (0.64 [95% CI, 0.47–0.89]). In the subgroup of patients with less than full chemotherapy exposure, 36 of 193 patients (18.7%) in the pembrolizumab group and 26 of 82 patients (31.7%) in the placebo group experienced an EFS event (0.54 [95% CI, 0.32–0.89]; **Figure 4B**).

Journal Pre-proof

DISCUSSION

KEYNOTE-522 is the first prospective, phase 3, randomized controlled study of pembrolizumab in patients with early-stage TNBC in the neoadjuvant and adjuvant settings. The primary results showed statistically significant and clinically meaningful improvements in pCR with neoadjuvant pembrolizumab plus chemotherapy and in EFS with neoadjuvant pembrolizumab plus chemotherapy followed by adjuvant pembrolizumab, regardless of PD-L1 expression.[9, 10] Conversely, in patients with metastatic TNBC, a greater survival benefit of pembrolizumab is observed with increasing PD-L1 expression.[17-19] This difference may be due to a more immunosuppressive tumor microenvironment in metastatic lesions compared to primary tumors, which may hinder chemotherapy-induced PD-L1 expression in late-stage cancers.[20-23]

The present exploratory analyses demonstrate that the addition of pembrolizumab to neoadjuvant chemotherapy not only increased the pCR rate, but also shifted RCB to lower categories across the entire spectrum of patients with residual disease. This shift to lower residual cancer burden among those with residual disease may explain the higher EFS benefit than would be expected based on the improvement in pCR alone in the overall study population (63.4% vs. 56.2%).[12] Along with the expected association of worse EFS with increased RCB categories in both treatment groups, the addition of pembrolizumab also resulted in fewer EFS events in the RCB-0, RCB-1, and RCB-2 categories, with the most pronounced benefit in RCB-2, which also represents the largest fraction (55%) of patients with residual disease. The small subset of patients in the RCB-3 category had poor prognosis in both treatment groups, indicating a highly treatment-resistant subset of cancers and signaling a need for additional therapies in patients with extensive residual disease. These results indicate that the EFS benefit from the addition of

pembrolizumab extends beyond patients who have a pCR to many of those with residual disease at the time of definitive surgery.

The most common first EFS event in both treatment groups was distant metastatic recurrence. Importantly, the addition of pembrolizumab led to a reduction in distant recurrence as the first EFS event across all RCB categories, including RCB-3. Among patients with RCB-3 in the pembrolizumab group, an unusually high proportion (25.0%) experienced local recurrence as the first EFS event compared to patients in the placebo group (7.7%). This may reflect random chance in this very small subset (*n*=66) of patients, particularly since lower local and distant recurrence rates were observed in all other RCB categories. Although distant recurrences were rare among patients with RCB-0 and RCB-1, nearly half of these were CNS events in both treatment groups; by contrast, the majority of first distant recurrences among patients with RCB-2 and RCB-3 were non-CNS events. Similar findings have been reported by the I-SPY investigators.[24] These results are consistent with the CNS being a treatment sanctuary site and emphasize the importance of additional strategies to eradicate brain micrometastases among patients who have a pCR.

In the post hoc analysis of patient subgroups defined by chemotherapy exposure, the increase in RCB-0 (pCR) response with the addition of pembrolizumab was maintained regardless of full versus less than full exposure to chemotherapy. These findings align with results from the prespecified first interim analysis of KEYNOTE-522, showing a benefit of neoadjuvant pembrolizumab plus chemotherapy on pCR in patients who received less than full chemotherapy.[25] In patients with residual disease and less than full chemotherapy exposure, the

shift to lower RCB categories was generally consistent with that observed in the overall population. Equally important, the addition of pembrolizumab prolonged EFS in patients with full and less than full exposure to chemotherapy.

The present investigation has several limitations. In addition to the inherent weaknesses of exploratory analyses, there were small numbers of patients in some groups, especially the RCB-3 category, which make the findings susceptible to random variation. Further, all the analyses were non-randomized comparisons. The KEYNOTE-522 trial design does not allow distinguishing the EFS benefit derived from the neoadjuvant versus adjuvant administration of pembrolizumab. Finally, although the local pathologists were blinded to treatment assignment and required to complete formal training in pathological staging, interobserver variability could be a source of bias. However, multiple studies have demonstrated good reproducibility for RCB scores across pathologists and consistent prognostic accuracy across data sets, leading to the endorsement of RCB as a secondary endpoint for neoadjuvant trials in the Standardized Definitions for Efficacy End Points in Neoadjuvant Breast Cancer Clinical Trials (NeoSTEEP) guidelines.[26-28] Notwithstanding these considerations, it is encouraging that the survival benefit with pembrolizumab in patients with various extents of residual cancer is consistent with outcomes from the global KEYNOTE-522 study, showing an EFS benefit from pembrolizumab which exceeded that expected by the improvement in pCR alone.[10]

In summary, the present results show that the EFS benefit from pembrolizumab extends beyond patients who have a pCR to many of those with residual disease at the time of definitive surgery

and is observed regardless of exposure to chemotherapy (full vs. < full). These findings support the role of pembrolizumab for improving outcomes in patients with high-risk, early-stage TNBC.

ACKNOWLEDGEMENTS

We thank the patients and their families and caregivers for participating in this study, along with all investigators and site personnel. Additional support was provided by Gursel Aktan, Vassiliki Karantza, Jing Zhao, Liyi Jia, Yu Ding, Jill Chapman, Ana Viquez, and Shana Hamm of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA. Editorial assistance was provided by Christine McCrary Sisk and Michele McColgan, both of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.

FUNDING

This work was supported by Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD). No grant number is applicable.

DISCLOSURE

All authors' institutions received research funding from Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, for the conduct of this study.
Y.Z., W.P., and K.T. are full-time employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA and hold stock and/or restricted stock units in Merck & Co., Inc., Rahway, NJ, USA.

Data Sharing Statement: Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD) is committed to providing qualified scientific researchers access

to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country- or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

Journal Pre-proof

References

1. Howlader N, Cronin KA, Kurian AW, Andridge R. Differences in breast cancer survival by molecular subtypes in the United States. Cancer Epidemiol Biomarkers Prev 2018; 27: 619-626.

2. Garrido-Castro AC, Lin NU, Polyak K. Insights into molecular classifications of triple-negative breast cancer: improving patient selection for treatment. Cancer Discov 2019; 9: 176-198.

3. Curigliano G, Burstein HJ, Winer EP et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 2017; 28: 1700-1712.

4. Cardoso F, Kyriakides S, Ohno S et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019; 30: 1194-1220.

5. Burstein HJ, Curigliano G, Loibl S et al. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen International Consensus Guidelines for the primary therapy of early breast cancer 2019. Ann Oncol 2019; 30: 1541-1557.

6. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Breast Cancer. Version 3.2020. March 6, 2020.

7. Korde LA, Somerfield MR, Carey LA et al. Neoadjuvant chemotherapy, endocrine therapy, and targeted therapy for breast cancer: ASCO Guideline. J Clin Oncol 2021; 39: 1485-1505.

Journal Pre-proof

8. Pusztai L, Foldi J, Dhawan A et al. Changing frameworks in treatment sequencing of triple-negative and HER2-positive, early-stage breast cancers. Lancet Oncol 2019; 20: e390-e396.

9. Schmid P, Cortes J, Pusztai L et al. Pembrolizumab for early triple-negative breast cancer. N Engl J Med 2020; 382: 810-821.

10. Schmid P, Cortes J, Dent R et al. Event-free survival with pembrolizumab in early triple-negative breast cancer. N Engl J Med 2022; 386: 556-567.

11. KEYTRUDA® (pembrolizumab). Full prescribing information, Merck Sharp & Dohme LLC, Rahway, NJ, USA, 2022.

12. Marczyk M, Mrukwa A, Yau C et al. Treatment Efficacy Score-continuous residual cancer burden-based metric to compare neoadjuvant chemotherapy efficacy between randomized trial arms in breast cancer trials. Ann Oncol 2022; 33: 814-823.

13. Symmans WF, Peintinger F, Hatzis C et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25: 4414-4422.

14. Symmans WF, Wei C, Gould R et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol 2017; 35: 1049-1060.

15. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.

16. University of Texas MD Anderson Cancer Center Residual Cancer Burden Calculator. http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3. Accessed March 20, 2023.

Journal Pre-proof

17. Cortes J, Cescon DW, Rugo HS et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. Lancet 2020; 396: 1817-1828.

18. Winer EP, Lipatov O, Im SA et al. Pembrolizumab versus investigator-choice chemotherapy for metastatic triple-negative breast cancer (KEYNOTE-119): a randomised, open-label, phase 3 trial. Lancet Oncol 2021; 22: 499-511.

19. Cortes J, Rugo HS, Cescon DW et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. N Engl J Med 2022; 387: 217-226.

20. Szekely B, Bossuyt V, Li X et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol 2018; 29: 2232-2239.

21. Rozenblit M, Huang R, Danziger N et al. Comparison of PD-L1 protein expression between primary tumors and metastatic lesions in triple negative breast cancers. J Immunother Cancer 2020; 8.

22. Gilad Y, Eliaz Y, Yu Y et al. Drug-induced PD-L1 expression and cell stress response in breast cancer cells can be balanced by drug combination. Sci Rep 2019; 9: 15099.

23. Boman C, Zerdes I, Martensson K et al. Discordance of PD-L1 status between primary and metastatic breast cancer: A systematic review and meta-analysis. Cancer Treat Rev 2021; 99: 102257.

24. Yau C, DeMichele A, Symmans WF et al. Site of recurrence after neoadjuvant therapy: clues to biology and impact on endpoints. Presented at 2019 San Antonio Breast Cancer Symposium; December 10-14, 2019. Abstract No. P2-20-02.

Journal Pre-proof

25. Schmid P, Park YH, Ferreira M et al. KEYNOTE-522: Phase 3 study of neoadjuvant pembrolizumab + chemotherapy versus placebo + chemotherapy, followed by adjuvant pembrolizumab versus placebo for early triple-negative breast cancer: pathologic complete response in key subgroups and by treatment exposure and residual cancer burden. Presented at San Antonio Breast Cancer Symposium, San Antonio, TX, USA: Dec 10–14, 2019 (presentation GS3-03).

26. Peintinger F, Sinn B, Hatzis C et al. Reproducibility of residual cancer burden for prognostic assessment of breast cancer after neoadjuvant chemotherapy. Mod Pathol 2015; 28: 913-920.

27. Yau C, Osdoit M, van der Noordaa M et al. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. Lancet Oncol 2022; 23: 149-160.

28. Litton JK, Regan MM, Pusztai L et al. Standardized Definitions for Efficacy End Points in Neoadjuvant Breast Cancer Clinical Trials: NeoSTEEP. J Clin Oncol 2023; 41: 4433-4442.

Journal Pre-proof

FIGURE LEGENDS

Figure 1. Trial Enrollment. Shown are data until the data-cutoff date of March 23, 2021. Patients did not have to complete all neoadjuvant therapy to undergo surgery. No patients remained on treatment at this interim analysis. ITT, intention-to-treat.

Figure 2. Residual Cancer Burden (RCB) category distribution.

Figure 3. Kaplan–Meier estimates of event-free survival in Residual Cancer Burden (RCB) categories: (A) RCB-0 (equivalent to pCR); (B) RCB-1; (C) RCB-2; and (D) RCB-3. Tick marks indicate censored data.

Figure 4. Post-hoc analysis of full chemotherapy versus less than full chemotherapy on (A) Residual Cancer Burden (RCB) category distribution, and (B) Event-free survival. Full chemotherapy exposure = (Paclitaxel Weekly 10-12 doses) and (Carboplatin Weekly 10-12 doses or Carboplatin Q3W 4 doses) and (Doxorubicin Q3W 4 doses or Epirubicin Q3W 4 doses) and (Cyclophosphamide Q3W 4 doses). [*]Regardless of exposure to pembrolizumab. [†]HRs and 95% CIs are based on an unstratified Cox model.

Journal Pre-proof

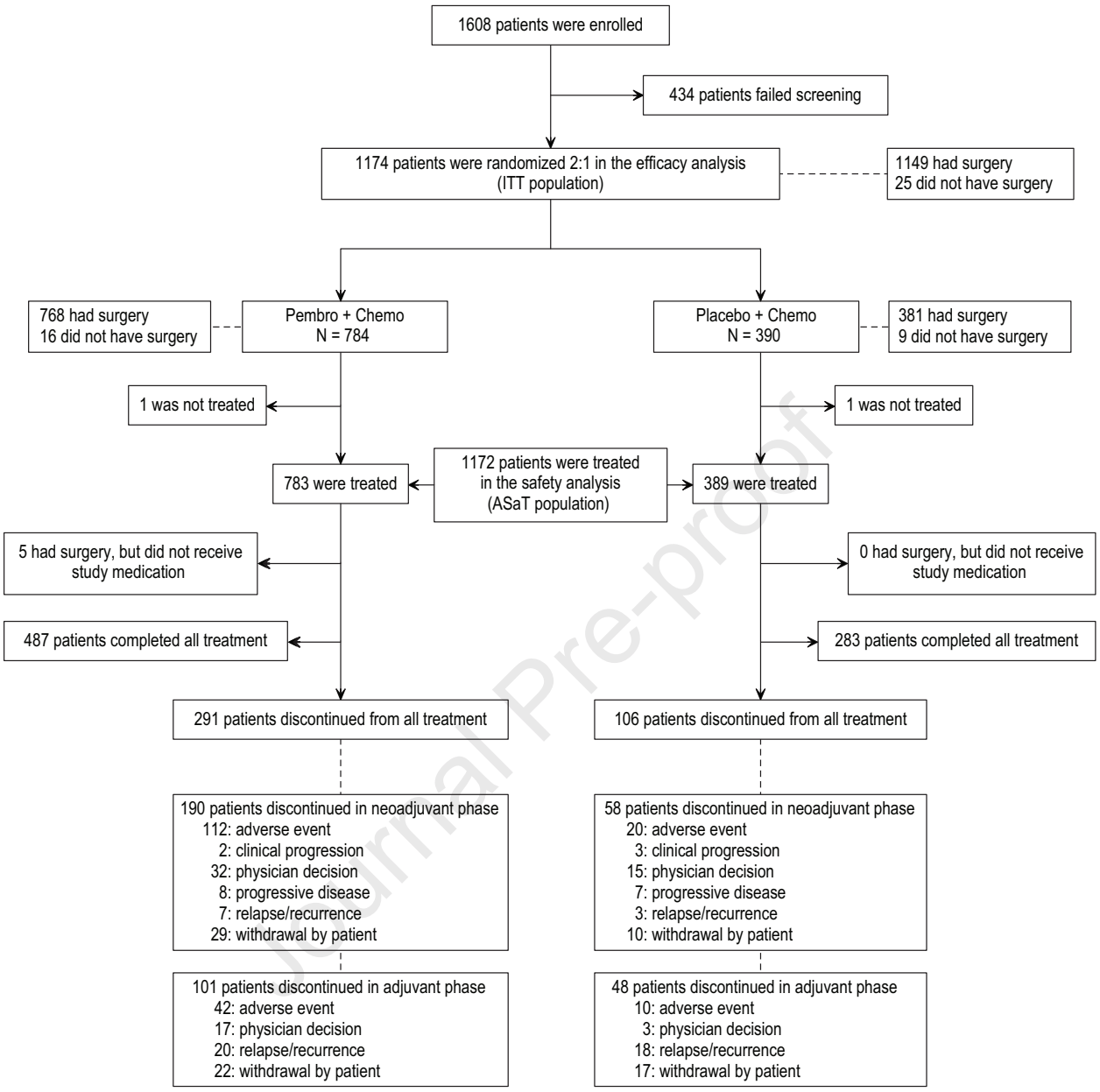
Journal Pre-proof

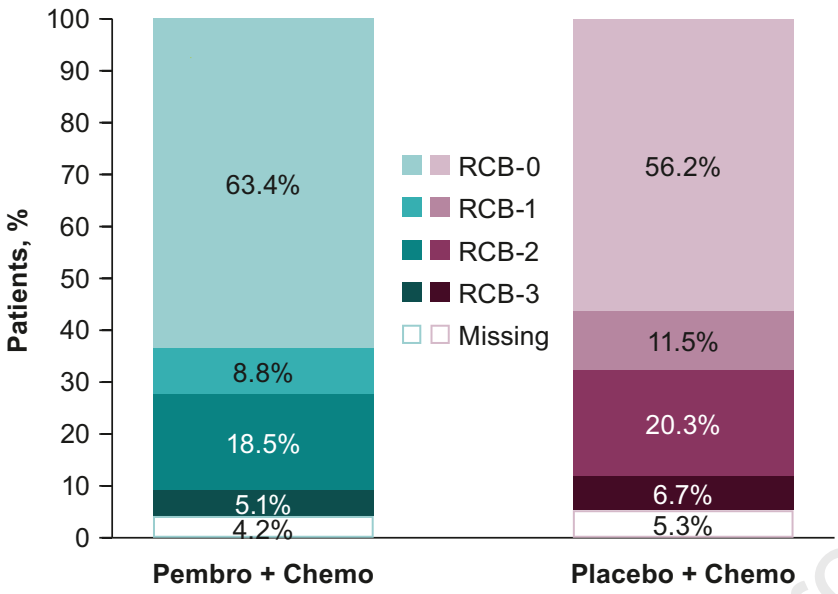
Table 1. Summary of First EFS Events by RCB Category

Event	RCB-0		RCB-1		RCB-2		RCB-3		Overall[a]	
	Pembro + Chemo *N* = 497	Placebo + Chemo *N* = 219	Pembro + Chemo *N* = 69	Placebo + Chemo *N* = 45	Pembro + Chemo *N* = 145	Placebo + Chemo *N* = 79	Pembro + Chemo *N* = 40	Placebo + Chemo *N* = 26	Pembro + Chemo *N* = 784	Placebo + Chemo *N* = 390
Any EFS event	5.2%	7.3%	17.4%	20.0%	25.5%	44.3%	72.5%	69.2%	15.7%	23.8%
Secondary primary malignancy	0.2%	0	1.4%	2.2%	1.4%	3.8%	2.5%	0	0.8%	1.0%
PD precluded definitive surgery	0	0	1.4%	2.2%	1.4%	5.1%	10.0%	7.7%	1.4%	3.8%
Local recurrence	0.6%	1.4%	4.3%	6.7%	6.9%	8.9%	25.0%	7.7%	3.6%	4.4%
Distant recurrence	3.2%	5.5%	8.7%	8.9%	15.2%	22.8%	35.0%	53.8%	7.7%	13.1%
Brain only	1.8%	3.2%	4.3%	2.2%	2.1%	3.8%	2.5%	3.8%	2.0%	3.3%
Other only[b]	1.2%	2.3%	4.3%	6.7%	13.1%	19.0%	32.5%	46.2%	5.5%	9.5%
Brain and other[b]	0.2%	0	0	0	0	0	0	3.8%	0.1%	0.3%
Death	1.2%	0.5%	1.4%	0	0.7%	3.8%	0	0	1.9%	1.5%

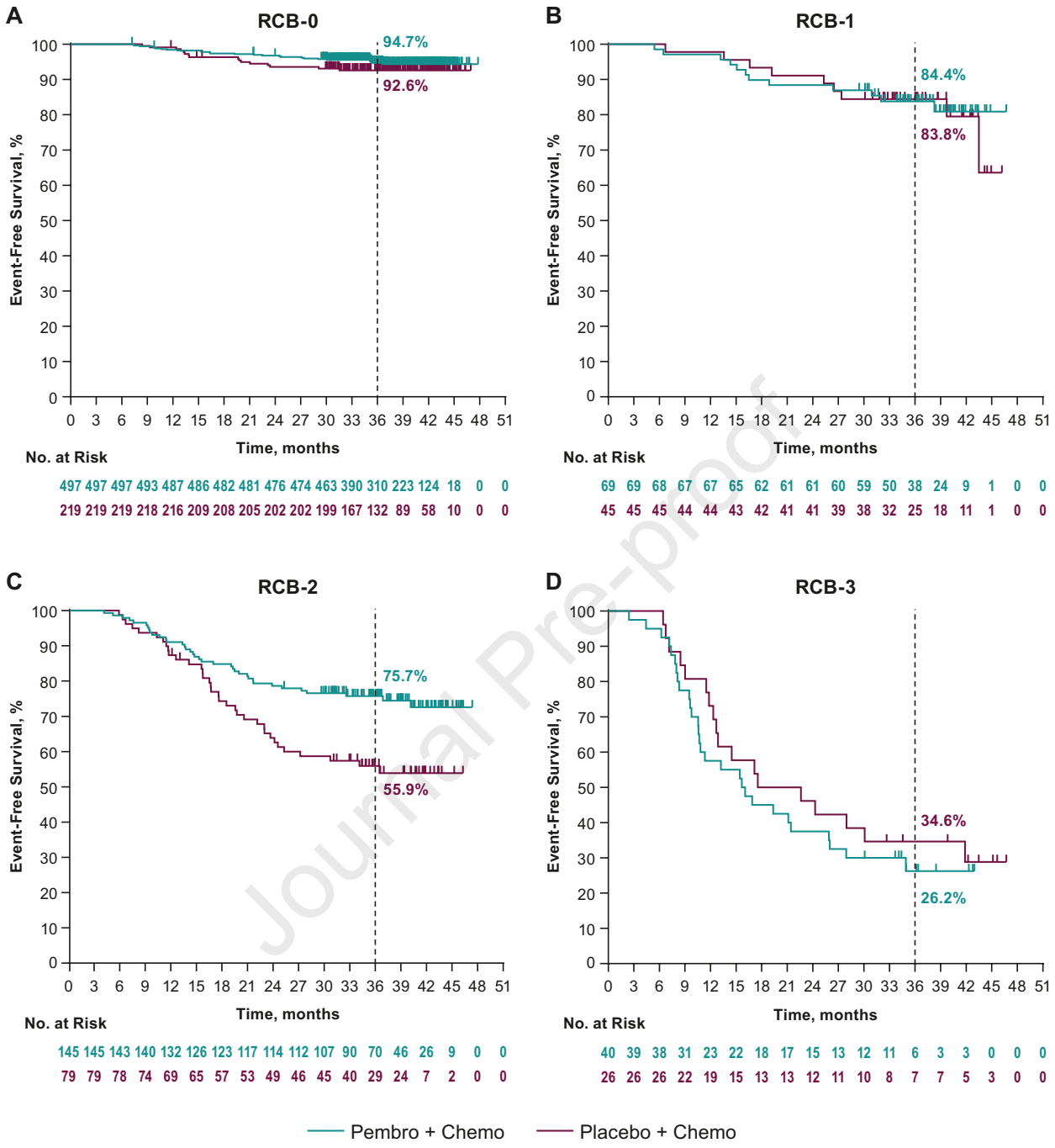
[a]Includes patients with missing RCB data; among all patients (*n*=1174), 54 patients (4.6%) had missing RCB categorical data: 33 (4.2%) in the pembrolizumab group and 21 (5.4%) in the placebo group. [b]Other refers to non-brain distant recurrence sites, which were classified per clinical identification.
EFS, event free survival; RCB, residual cancer burden; PD, progressive disease.

Journal Pre-proof

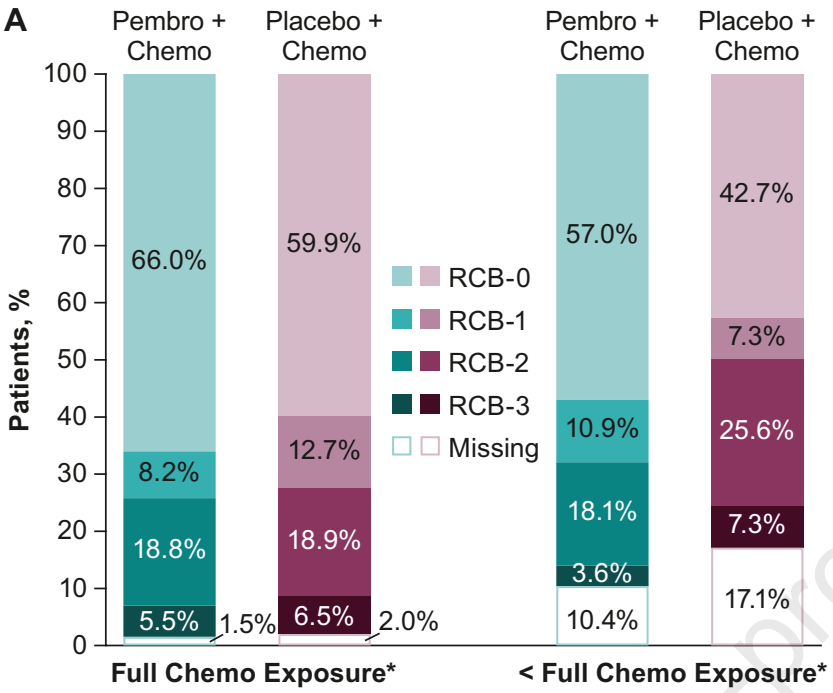


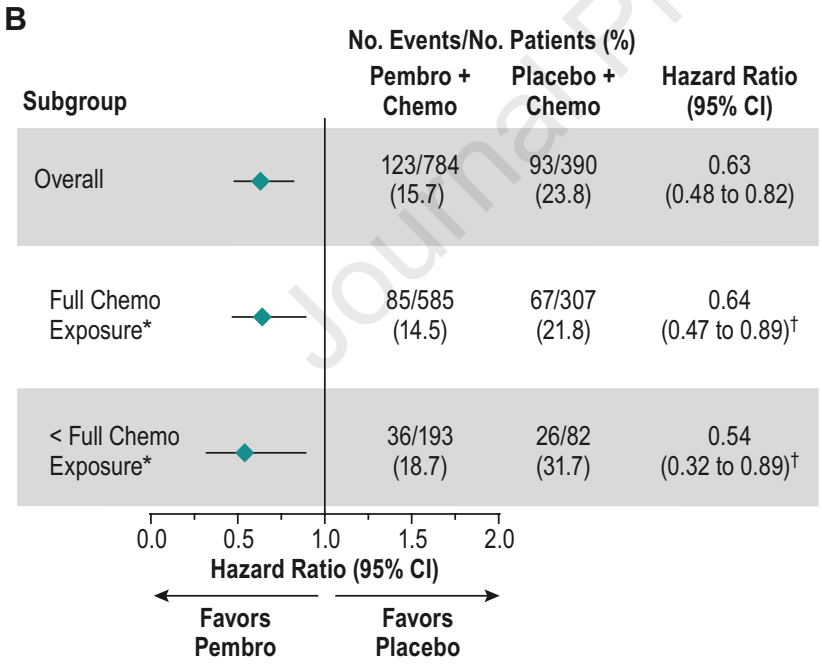


Journal Pre-proof



Journal Pre-proof





Journal Pre-proof