














BRIEF COMMUNICATION OPEN



Tumor infiltrating lymphocyte stratification of prognostic staging of early-stage triple negative breast cancer

Sherene Loi^{1,2}[✉], Roberto Salgado^{1,3}, Sylvia Adams⁴, Giancarlo Pruneri⁵, Prudence A. Francis^{1,2}, Magali Lacroix-Triki⁶, Heikki Joensuu⁷, Maria Vittoria Dieci^{8,9}, Sunil Badve¹⁰, Sandra Demaria¹¹, Robert Gray¹², Elisabetta Munzone¹³, Damien Drubay¹⁴, Jerome Lemonnier¹⁵, Christos Sotiriou¹⁶, Pirkko Liisa Kellokumpu-Lehtinen¹⁷, Andrea Vingiani⁵, Kathryn Gray¹⁸, Fabrice André¹⁹, Carsten Denkert²⁰, Martine Piccart¹⁶, Elvire Roblin¹⁴ and Stefan Michiels¹⁴

The importance of integrating biomarkers into the TNM staging has been emphasized in the 8th Edition of the American Joint Committee on Cancer (AJCC) Staging system. In a pooled analysis of 2148 TNBC-patients in the adjuvant setting, TILs are found to strongly up and downstage traditional pathological-staging in the Pathological and Clinical Prognostic Stage Groups from the AJCC 8th edition Cancer Staging System. This suggest that clinical and research studies on TNBC should take TILs into account in addition to stage, as for example patients with stage II TNBC and high TILs have a better outcome than patients with stage I and low TILs.

npj Breast Cancer (2022)8:3; <https://doi.org/10.1038/s41523-021-00362-1>

INTRODUCTION

The importance of integrating biomarkers into the TNM staging has been emphasized in the 8th Edition of the American Joint Committee on Cancer (AJCC) Staging System (www.cancerstaging.org). Prognostic biomarkers are becoming increasingly important given that many low early-stage breast cancer patients have excellent outcomes with standard of care treatments. The clinical guidelines for systemic treatment in early-stage TNBC are not consistent. The evidence for the integration of biomarkers into the TNM staging system is mostly retrospective and with limited prospective data available¹. In the case of multigene panels, their clinical validity for defining prognosis of particular subgroups of patients had been shown in several retrospective analyses and these were routinely used and incorporated into national (NCCN and ASCO) and international (ESMO, St Gallen) treatment guidelines far before prospective data became available. In the 8th edition of the AJCC, histologic grading, molecular subtype (luminal, HER2-positive, and triple negative [TN]) and multigene panels/genomic signatures were included into staging assessments. Whilst pathological and clinical stage remain a valuable aspect of the staging process and are strongly prognostic, we highlight here the evidence that supports Tumor Infiltrating Lymphocytes (TILs) as a biologic biomarker which improves discrimination over prognostic pathological and clinical staging for early stage TNBC.

A recent pooled analysis provides the relevant work for integrating TIL quantity into clinical staging for early-stage TNBC (JCO 2019)². These data were from 2148 patients enrolled onto eight prospective clinical trials as well as one large institutional cohort. All patients had received anthracyclines with or without taxanes based regimens in the adjuvant setting. The median

follow-up was 6.6 years. In the current study, using the Pathological and Clinical Prognostic Stage Groups from the AJCC 8th edition Cancer Staging Manual (for definitions hereof, see footnote*), three endpoints were calculated for each Stage group: invasive disease-free survival (iDFS), distant disease-free survival (D-DFS) as well as overall survival (OS). At a cut-point of 30% or more, TIL scores are found to strongly up and downstage traditional pathological-staging in this large data set (Fig. 1, Supp Table 1). This suggest that clinical and research studies on TNBC should take TILs into account in addition to stage, as patients with stage II TNBC and high TILs have a better outcome than patients with stage I and low TILs. Importantly, histological grade is not prognostic in this pooled analysis. At the established prognostic cut-off of 30%, trained pathologists have a high pairwise concordance rate up to 0.93 using both core-biopsies as well as full sections, indicating that TILs are a reliable biomarker that can be used in daily practice and in research and clinical trial settings³. This compares favorably to the reproducibility of other commonly used morphological features in breast cancer practice⁴. Similarly, adding TILs to clinical staging is shown to up- and downstage prognostic groups which also take subtype into consideration (Fig. 2; Supp Table 2) though we note the small numbers in some of the subgroups (ie Stage IIA).

Risk assessment models meeting the AJCC criteria and included in the 8th Edition were Adjuvant Online and PREDICT-Plus. We have developed a prognostic model for early-stage disease that includes age, number of positive lymph nodes, tumor size, grade, and stromal TIL percentage. This prognostic model is available on the website of the International Immuno-Oncology Biomarker Working Group (www.tilsinbreastcancer.org). This prognostic

¹Peter MacCallum Cancer Centre, Melbourne, VIC, Australia. ²Sir Peter MacCallum Cancer Department of Oncology, University of Melbourne, Melbourne, VIC, Australia. ³Department of Pathology, GZA-ZNA, Antwerp, Belgium. ⁴New York University Langone Health, Perlmutter Cancer Center, New York, NY, USA. ⁵Fondazione Istituto di Ricovero e Cura a Carattere Scientifico-Istituto Nazionale dei Tumori, Università degli Studi di Milano, Milan, Italy. ⁶Gustave Roussy, Université Paris-Saclay, Villejuif, France. ⁷Helsinki University Hospital and University of Helsinki, Helsinki, Finland. ⁸Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy. ⁹Medical Oncology 2, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy. ¹⁰Indiana University, Indianapolis, IN, USA. ¹¹Weill-Cornell Medicine, New York, NY, USA. ¹²Dana-Farber Cancer Institute, Boston, MA, USA. ¹³Division of Medical Senology, European Institute of Oncology, IRCCS, Milan, Italy. ¹⁴Service de Biostatistique et d'Epidémiologie, Gustave Roussy, Oncostat U1018, Inserm, Paris-Saclay University, labeled Ligue Contre le Cancer, Villejuif, France. ¹⁵R&D UNICANCER, Paris, France. ¹⁶Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium. ¹⁷Faculty of Medicine and Health Technology Tampere University and Tampere University Hospital Tamper, Tamper, Finland. ¹⁸Frontier Science & Technology Research Foundation, Boston, MA, USA. ¹⁹Gustave Roussy, Université Paris-Saclay, Villejuif, France. ²⁰Institut für Pathologie, Universitätsklinikum Marburg, Philipps-Universität Marburg, Marburg, Germany. ✉email: sherene.loi@petermac.org

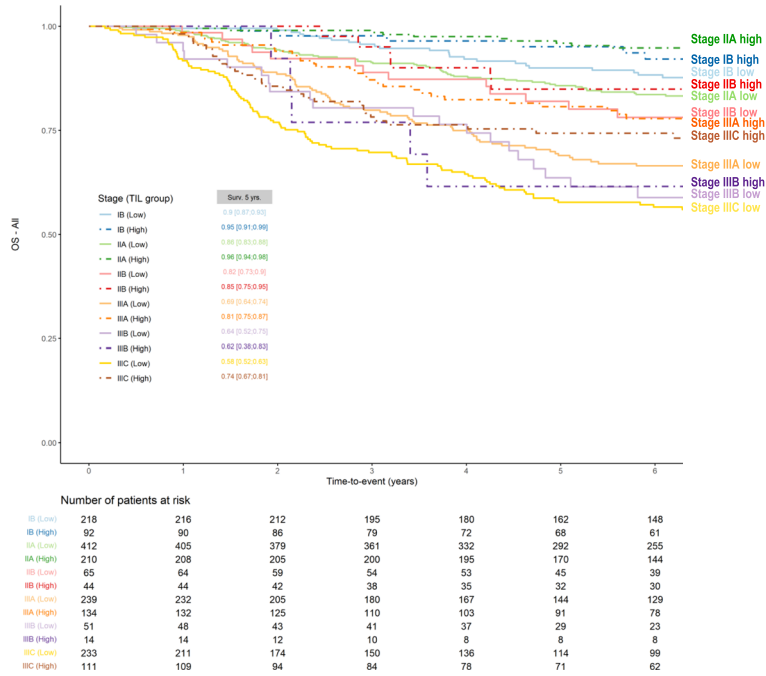


Fig. 1 Kaplan–Meier curves of overall survival of triple-negative breast cancer patients treated with anthracycline-based chemotherapy with or without taxanes, according to pathological prognostic stage and TILs. Pathological Prognostic Stage is assigned stage for patients who have surgical resection as the initial treatment of their cancer before receipt of any systemic or radiation therapy. It is based on clinical information, biomarker data, and findings from surgery and resected tissue. The 5-year estimated overall survival values (5-Yr OS) are provided together with bootstrap confidence intervals.

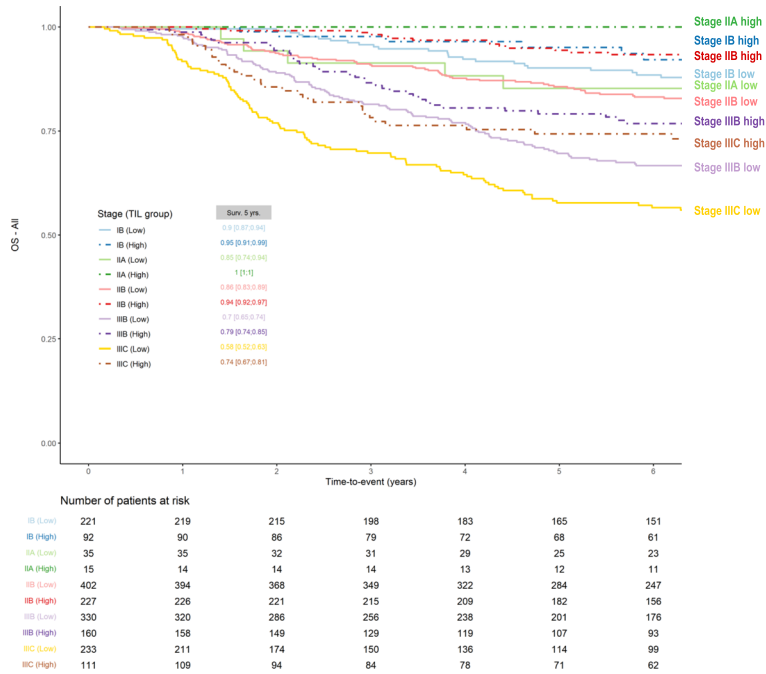


Fig. 2 Kaplan–Meier curves of overall survival of triple-negative breast cancer patients treated with anthracycline-based chemotherapy with or without taxanes, according to clinical prognostic stage and TILs. Clinical prognostic stage assigned stage for all patients based on history, physical examination, imaging studies performed (but not required) and relevant biopsies. Clinical prognostic stage is determined by T, N, M, tumor grade, as well as subtype information using human epidermal growth factor receptor (HER2), estrogen receptor (ER), and progesterone receptor (PR)- status. Supplementary Tables 1 and 2.

model could be used in routine patient care as well as future trial designs.

The inclusion of immune biomarkers is timely given reporting of phase III registration trials of PD-1/PD-L1 targeting agents in

early-stage TNBC. In the recently reported KEYNOTE-522 study (NCT03036488)⁵, pembrolizumab, a PD-1 inhibitor, was found to significantly but moderately increase the rates of pathological complete response (pCR) and significantly improve event-free

survival (EFS) when added to a neoadjuvant regimen of anthracycline, taxanes and carboplatin in patients with stage II and III TNBC. Similar magnitude of EFS improvements have been reported in smaller studies^{6,7}. PD-L1 protein is known to be highly expressed on the breast cancer TIL and correlations between both markers have been observed⁸. In the KEYNOTE-522 study, patients designated as PD-L1 positive by the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, Carpinteria, CA, USA) were reported to have higher rates of pCR in both pembrolizumab and control arms across all TNM stages. An important treatment by PD-L1 interaction was not observed, in contrast to late-stage disease^{9,10}. In any case, the increasing incorporation of PD-1 inhibition into the treatment of both early- and late stage TNBC further reinforces the need for routine evaluation of TIL in all TNBC patients, particularly given discordances in PD-L1 IHC assays. One could also speculate that patients with high expression of both immune biomarkers, i.e. those “immune enriched” could be strong candidates for future treatment optimization (for example, reduced cytotoxic chemotherapy)¹¹.

METHODS

This is a follow-up analysis of the Loi et al 2019 study². The study protocol of this project was approved by the local institutional review committee at Gustave Roussy in November 2014 and authorized by the French National Committee on Computing and Liberty. Identified studies were prospective randomized clinical trials or large retrospective hospital series that evaluated the prognostic associations of TILs in patients who were diagnosed with early-stage TNBC treated with anthracycline-based chemotherapy with or without taxane in the adjuvant setting using the same prespecified method for reading TILs (according to International Immuno-Oncology Biomarker Working Group guidelines). TNBC was defined by estrogen receptor (ER), progesterone receptor (PR), and HER2 negativity as outlined in Loi et al. (ref).

The prespecified primary end point was invasive disease-free survival (iDFS), defined as the date of first invasive recurrence, or second primary or death from any cause. Patients still alive without an event of interest were censored at the date of the last visit. Distant disease-free survival (D-DFS) is defined as the date of first distant recurrence or death from any cause. Patients still alive without an event of interest were censored at the date of the last visit. Overall survival (OS) was defined as the date of death from any cause. Patients still alive were censored at the date of the last visit. We used the Kaplan-Meier method to establish survival curves according to AJCC staging categories. The Kaplan-Meier estimates are provided together with bootstrap confidence intervals (1000 samples).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY

Trial data can be made available by contacting the individual trial investigators of the trials included in this project, pending individual sponsors approval. The corresponding author of the paper can redirect requests. A specific data transfer agreement between the individual sponsors of the trials and the researcher may be requested. This data transfer agreement details both parts responsibilities to ensure the required level of data integrity and legal and ethical obligations.

Received: 28 May 2021; Accepted: 3 November 2021;

Published online: 11 January 2022

REFERENCES

- Murthy, R. K. et al. Incorporation of clinical and biological factors improves prognostication and reflects contemporary clinical practice. *NPJ Breast Cancer* **6**, 1–9 (2020).
- Loi, S. et al. Tumor-infiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. *J. Clin. Oncol.* **37**, 559 (2019).
- Kos, Z. et al. Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer. *NPJ Breast Cancer* **6**, 1–6 (2020).
- Gilchrist, K. W. et al. Interobserver reproducibility of histopathological features in stage II breast cancer. *Breast Cancer Res. Treat.* **5**, 3–10 (1985).
- Schmid, P. et al. Pembrolizumab for early triple-negative breast cancer. *N. Engl. J. Med.* **382**, 810–821 (2020).
- Mittendorf, E. et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. *Lancet Oncol.* **396**, 1090–1100 (2020).
- Loibl, S. et al. Durvalumab improves long-term outcome in TNBC: results from the phase II randomized GeparNUEVO study investigating neoadjuvant durvalumab in addition to an anthracycline/taxane based neoadjuvant chemotherapy in early triple-negative breast cancer (TNBC). *J. Clin. Oncol.* **39**, 506–506 (2021).
- Loi, S. et al. Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mTNBC): Results from KEYNOTE-086. *Ann. Oncol.* **28**, p.v608 (2017).
- Loi, S. et al. Abstract PD5-03: relationship between tumor-infiltrating lymphocytes (TILs) and outcomes in the KEYNOTE-119 study of pembrolizumab vs chemotherapy for previously treated metastatic triple-negative breast cancer (mTNBC). *Cancer Res.* **80**, PD5–PD03 (2020).
- Schmid, P. et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N. Engl. J. Med.* **379**, 2108–2121 (2018).
- Gonzalez-Ericsson, P. I. et al. The path to a better biomarker: application of a risk management framework for the implementation of PD-L1 and TILs as immunology biomarkers in breast cancer clinical trials and daily practice. *J. Pathol.* **250**, 667–684 (2020).

ACKNOWLEDGEMENTS

S.L. is supported by National Breast Cancer Foundation of Australia (NBCF) and the Breast Cancer Research Foundation (BCRF), NY, USA. RS is supported by the Breast Cancer Research Foundation (BCRF), NY, US. M.L.-T., J.L., F.A. and S.M. are supported by ANR and CGI (RHU MyPROBE and ANR-17-RHUS-0008).

AUTHOR CONTRIBUTIONS

Conception and design: S.L., S.M. and R.S. Provision of study materials or patients: all. Collection and assembly of data: all. Data analysis and interpretation: all. Manuscript writing and final approval: all.

COMPETING INTERESTS

S.L. receives research funding to her institution from Novartis, Bristol Meyers Squibb, Merck, Puma Biotechnology, Eli Lilly, Nektar Therapeutics Astra Zeneca, Roche-Genentech and Seattle Genetics. S.L. has acted as consultant (not compensated) to Seattle Genetics, Novartis, Bristol Meyers Squibb, Merck, AstraZeneca and Roche-Genentech. S.L. has acted as consultant (paid to her institution) to Aduro Biotech, Novartis, GlaxoSmithKline, Roche-Genentech, Astra Zeneca, Silverback Therapeutics, G1 Therapeutics, Daiichi Sankyo, PUMA Biotechnologies, Seattle Genetics and Bristol Meyers Squibb. RS reports non-financial support from Merck and Bristol Myers Squibb; research support from Merck, Puma Biotechnology, and Roche; and personal fees from Roche for an advisory board related to a trial-research project. S.D. has received honorarium as a member of the scientific advisory board of Lytix Biopharma, and ad hoc consulting for Ono Pharmaceuticals. M.P. is a Scientific Board Member of Oncolytics. M.P. has acted as a consultant (compensated) to AstraZeneca, Camel-IDS/Precirix, Gilead, Immunomedics, Lilly, Menarini, MSD, Novartis, Odonate, Pfizer, Roche-Genentech, Seattle Genetics, Immuteq, Seagen, NBE Therapeutics, Frame Therapeutics. M.P. receives research funding to her institution from AstraZeneca, Immunomedics, Lilly, Menarini, MSD, Novartis, Pfizer, Radius, Roche-Genentech, Servier, Synthon. E.M. has acted as a consultant (compensated) to Pierre Fabre, Genomic Health and Eisai. E.M. has received funding for travel expenses and/or accommodation from Celgene, Roche, Eisai, Mylan and Pfizer. C.D. reports stock and other ownership interests from Sividon Diagnostics (unitl 2016), honoraria from Novartis and Roche. C.D. has acted as a consultant (compensated) to MSD Oncology, Daiichi Sankyo, Molecular Health, Astra Zeneca, merck. C.D. receives research funding (to institution) from Myriad Genetics and Roche. C.D. reports patents, royalties and/or other intellectual property from VMScope digital pathology software, patent applications WO2015114146A1 and WO2010076322A1(therapy response) and WO2020109570A1 (cancer immunotherapy). C.D. has received funding for travel expenses and/or accommodation from Roche. H.J. is the Chair of the Scientific Advisory Board at Orion Pharma and at Neutron Therapeutics Ltd. S.M. reports fees

outside the submitted work from statistical advice (IDDI, Janssen Cilag, Amaris, Roche) and from data and safety monitoring membership of clinical trials (Hexal, Steba, IQVIA, Sensorion, Biophytis, Servier, Yuhan. F.A. received research funding and served as speaker / advisor (compensated to the hospital) for Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Novartis, Lilly. S.A. has uncompensated consulting or advisory roles with Bristol Meyers Squibb, Genentech, and Merck as well as research funding to her institution from Amgen, Bristol Meyers Squibb, Celgene, Genentech, Merck and Novartis. M.V.D. reports personal fees for consultancy/advisory from Eli Lilly, Pfizer, Novartis, Genomic Health. MLT reports honorarium/expertise from Roche, Roche Diagnostics, MSD, AstraZeneca, Daiichi Sankyo. P.L.K.L. has received honorarium as member of advisory board of Bristol Myers Squibb and has received compensation for traveling from Sanofi. PAF reports honoraria from AstraZeneca and Novartis and travel support from Pfizer and Ipsen. The remaining authors declare competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41523-021-00362-1>.

Correspondence and requests for materials should be addressed to Sherene Loi.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022