Pooled analysis of the prognostic value of tumor infiltrating lymphocyte in triple negative breast cancer patients not treated by chemotherapy

Protocol version 1.0

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CONTENTS:

I. SYNOPSIS

Title	Pooled Analysis of the Prognostic value of tumor infiltrating lymphocytes in triple negative breast cancer not treated by chemotherapy
Objective	To conduct an individual patient data analysis of the prognostic significance of stromal tumor infiltrating lymphocytes (sTILs) characterized in patients with triple negative breast cancer
Design	Individual patient data from eligible studies will be pooled together for this analysis. Tumour infiltrating lymphocytes need to have been read following the International TILs Working Group recommendations.
Study eligibility	Eligible studies are those that are randomized clinical trials that have evaluated the prognostic associations of stromal tumour infiltrating lymphocytes in patients diagnosed with early stage TNBC and not-treated with chemotherapy
Data collection and management	The data collection and the overall pooled analysis will be conducted at Gustave Roussy (Villejuif, France)

II. INTRODUCTION

We now have evidence from analyses from previous studies (JH. Park et al Ann Onc, 2019) that host anti-tumor immunity as measured by stromal tumor infiltrating lymphocytes (sTILs) is important for the outcomes of TNBC regardless of adjuvant chemotherapy. It would therefore seem that this finding is robust. Whilst at present, the clinical utility of sTILs in day-to-day management of primary TNBC is limited, TILs could be useful for predicting patients' prognosis and for stratification in future clinical trials enrolling TNBC patients, once the evaluation method has been standardized. We propose in this study to better understand how TILs affect prognosis in the natural history of TNBC with small tumor burden. In the previous Park Ann Onc 2019 publication, it has been suggested that sTILs can identify a subset of stage I TNBC patients with an excellent prognosis without adjuvant chemotherapy: in patients with pathological stage I tumors with sTILs ≥30% (n = 74), 5-year iDFS was 91% (95% CI 84% to 96%), D-DFS was 97% (95% CI 93% to 100%), and OS was 98% (95% CI 95% to 100%). Larger patient numbers are needed to confirm this finding.

A secondary aim is to develop a prognostic model integrating clinico-pathological factors and sTILs in order to estimate a risk of relapse for the individual patient.

III. OBJECTIVES

A. Primary Objective

The objective of this study is to perform an individual patient data pooled analysis of multiple studies exploring the **prognostic impact of tumor infiltrating lymphocytes (sTILs) on invasive disease-free survival patients** diagnosed with triple negative breast cancer (TNBC) non-treated with adjuvant chemotherapy

B. Secondary Objectives

- To evaluate association between STILS and invasive breast cancer–free survival, recurrence free survival, distant-disease free survival and overall survival.
- To evaluate association between sTILs and clinico-pathological features.
- To develop a prognostic model including sTILs as well as clinic-pathological features for patients not treated by chemotherapy.
- To study the association on distant events and second cancers in a competing risk model.

IV. STUDY ELIGIBILITY AND IDENTIFICATION

A. Study Eligibility Criteria

Published or unpublished studies (clinical trials, prospective or retrospective patient cohorts) assessing the prognostic association of sTILs in TNBC patients non-treated with systematic treatments (such as chemotherapy, hormonotherapy) after surgery with available clinical outcome data.

B. Studies Identified (with expected sample size n and study PI)

Studies identified by may 2021 through the TILs International Working Group, ordered by expected sample size are:

1. Nederlands Kanker Instituut, n=481, Sabine Linn, (Paradigm study, ESMO 2020 presentation)
2. British Columbia, n=300, Torsten Nielsen, Olivotto et al JCO 1997
3. Rotterdam, n=300, John Martens
4. Mayo Clinic, n=182, Matthew Goetz, Leon-Ferre et al Breast Cancer Res Treat. 2018
5. IEO Milan, n=159, Guiseppe Curigliano, included in Park et al Ann Onc 2019
6. Institut Curie, n=150, Anne Vincent-Salomon, included in Park et al Ann Onc 2019
7. Japan, n=125, Tatsunori Shimoi
8. Karolinska Institutet, n=100, Barbro Linderholm
9. Centre Léon Bérard, n = 100, Thomas Bachelot
10. Korea, n=142, Sung-Bae Kim, included in Park et al Ann Onc 2019
11. Gustave Roussy, n=95, Fabrice André, included in Park et al Ann Onc 2019
12. Genua, n =50, Matteo Lambertini
13. Padua, n=40, Maria Vittoria Dieci

Provisional eligible total: 2224 TNBC patients

V. CONTACT WITH AUTHORS AND DATA REQUEST

A. Contact with Authors

The secretariat of this project will invite principal investigators (PIs) to participate in this project. Upon agreement, PIs will be requested to provide pseudoanonymous individual patient data, sTILs values and clinical outcome data. A principal contact will be requested per study in case of data clarification needs. A timeframe of 2 months is given for data collection from each PI.

B. Data Items to be requested from Principal Investigators

Individual data – trial participation and outcomes

The following data items are requested in a tabulated format and should be collected for all individual patients included in all the studies selected for the meta-analysis:

BASIC

- Date of diagnosis (dd/mm/yyyy) (diagnosis)
- Surgery Date (dd/mm/yyyy) (surgery.date)
- Patient age at diagnosis (age_at_diagnosis)
- Menopausal status (0 = unknown, 1 = premenopausal, 3 = postmenopausal, 4 = pregnant, 8 = not applicable (male)) (meno_status)
- Histology (1= ductal, 2= lobular, 3= medullary, 4= tubular, 5= mucinous, 6= comedo, 7= inflammatory, 8= Unknown, 9= other) (histology)
- Grade (Nottingham grade) (grade)
- Size of tumour at diagnosis (in milimetres) (size_lesion)
- The number of pathologically positive axillary lymph nodes at time of diagnosis of breast cancer (num_pos_nod_init_dx)
- ER IHC expression (as a continuum) (ER.LEVEL)
    - (please note if % or Allred score)
- PgR IHC expression (as a continuum) (PR.LEVEL)
    - (please note if % or Allred score)
- **sTILs value per tumor**
    - Stromal TILs (%)

TREATMENT

- Adjuvant Radiation Therapy administered (0 = no initial breast/chest wall or nodal RT, 1 = initial breast/chest wall RT alone, 2 = initial breast/chest wall RT + regional nodal RT, 3 = regional nodal RT alone) (rt)

- Type of surgery (2 = Complete mastectomy [modified radical mastectomy, total mastectomy, complete mastectomy, and simple mastectomy], 1 =partial masectomy [lumpectomy, wedge resection, breast conserving surgery, partial mastectomy, quadrantectomy, and excisional biopsy], 0 =no surgery) (type.surg)

OVERALL SURVIVAL

- Date of last Follow-up or Death (dd/mm/yyyy) (date.last.follup)
- Overall survival status at the date of last observation (0=alive, 1=death, 2=lost to follow-up) (surv.stat)

LOCOREGIONAL RECURRENCE

- Invasive Ipsilateral Breast Tumor Recurrence (0=no, 1 =yes) (inv.ipsi.btr) (please state which)
- Date of Invasive Ipsilateral Breast Tumor Recurrence (dd/mm/yyyy) (date.inv.ipsi.btr)
- Local-Regional Invasive Recurrence (0=no, 1=yes) (lreg.inv.rec)
- Date of Local-Regional Invasive Recurrence (dd/mm/yyyy) (date.lreg.inv.rec)
- Site of Local-Regional Invasive Recurrence (1= ipsilateral axillary lymph nodes, 2= infraclavicular lymph nodes, 3= internal mammary lymph nodes, 4=local, 9=Unknown) (site.lreg.inv.rec)

DISTANT RECURRENCE

- Distant recurrence (0=no, 1=yes) (diststat)
- Date of first positive confirmation of a distant metastasis (a recurrence of tumour in organs beyond the confines of the breast, chest wall, or regional lymph nodes; includes mediastinal and contralateral nodal metastases & contralateral breast). (dd/mm/yyyy) (distdate)
- Site of first distant recurrence (1=Skin or lymph node other than the specified for Regional recurrences, 2=Lung, 3=Liver, 4=Bone, 5=Soft Tissue, 6=Brain, 7=Leptomeningeal, 8=Other, 9=Unknown) (site.dist.rec)

CONTRALATERAL BREAST CANCER

- Invasive Contralateral Breast Cancer (0=no, 1=yes) (INV.CONTR.BC)
- Date of Invasive Contralateral Breast Cancer (dd/mm/yyyy) (date.inv.contr.bc)

2ND CANCER

- Date of Diagnosis of Second Primary Malignancy (dd/mm/yyyy)
- Type of Second Primary Malignancy (1=lung cancer 2=mesothelioma 3=thyroid cancer, 4=lymphoma, 5=leukemia, 6=gynecological cancer, 7=other, 9=unknown)
- if Type of Second Primary Malignancy is gynecological cancer please specify: 1=cervical, 2=ovarian, 3=uterine, 4=vaginal, 5=vulvar, 9=unknown.
- If Type of Second Primary Malignancy is 7=other, please specify which cancer (text)

Data should be sent by secure encryption to the secretariat where they will be held secure and only used for the purposes of this study.

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VI. STATISTICAL METHODS

Survival endpoints (invasive disease-free survival, recurrence free survival, distant-disease free survival and overall survival) will be defined using STEEP2.0 definitions (Tolaney JCO 2021). The primary end point is iDFS, defined as the time from surgery to the occurrence of first invasive (local or regional) or distant event, contralateral, or second primary tumors or death from any cause. Patients still alive without an event of interest will be censored at the date of the last visit.

Invasive breast cancer–free survival (IBCFS) is similar to iDFS but with second nonbreast primary cancers excluded.

Recurrence-free survival (RFS) is defined as the time from surgery to the occurrence of first invasive (local or regional), distant event or death from any cause. Patients still alive without an event of interest will be censored at the date of the last visit.

Distant disease-free survival (D-DFS) is defined as the time to the first distant recurrence, second primary tumors, or death from any cause. Patients still alive without an event of interest are censored at the date of the last visit. OS is defined as the time from surgery to the date of death from any cause.
Based on the Park et al publication, if we expect a 91% IDFS for stage I TNBC tumours with sTILS >=30%, and if among the entire pooled data set there 400 of such patients, we would expect a confidence interval of the survival probability of a width of 5% (assuming a number of 30 IDFS events).

Subgroup survival analysis are planned according to AJCC stage, radiotherapy administration, histological subtypes, according to the previously proposed 30% cutoff (Loi et al JCO 2019) and also a higher 50% cut-off. We will use the Kaplan−Meier method to establish survival curves. Confidence intervals for survival probabilities will be calculated using a percentile bootstrap method. All analysis will be carried out using R (https://www.R-project.org/).

Survival analyses will be based on a Cox regression model stratified by study. The primary analysis will use stromal TILS as continuous variable in the Cox model (per 10% increments). Forest plots will be used with assessment of heterogeneity (chi-squared test).

Associations of these end points with clinicopathologic variables will be carried out using Spearman's correlation for continuous variables and Kendall's τ for categorical variables. We will use pairwise complete observations for the handling of missing data. Correlation coefficient values and confidence intervals (CIs) will be obtained with bootstrap method.

The Cox regression models will be used to evaluate the added independent prognostic value of sTILs to standard clinicopathologic factors [continuous age; continuous tumor size; continuous number of lymph nodes (LNs); tumor grade: well differentiated, moderately differentiated and poorly differentiated; and radiotherapy treatment: yes/no] through the use of likelihood ratio tests. We will evaluate the proportional hazard assumption using trend tests and graphical diagnoses based on Schoenfeld residuals as well as the log-linearity assumption using fitting linear tail-restricted cubic splines. To estimate the discrimination and calibration of the multivariable prognostic models, we will use a leave-one-study-out cross-validation approach.

A competing risk model will be fitted with distant events (including deaths) and second cancers as competing events (Fine & Gray model stratified by study as in Meddis et al Biom J 2020).

VII. DATA CONFIDENTIALITY AND TIMELINE

Data will be provided by principal investigators of the eligible studies in confidence. These data will be used solely for the purpose of this study which will be conducted according to local French regulations.
The proposed timeline for project from the receipt of all data, database assembly and cleaning, statistical analysis of the overall pooled analysis to first results is 6 months.

VIII. WORKING PARTIES

Two groups with specific functions have been created:

1) the Secretariat
2) the Collaborative Group

The Secretariat is in charge of the coordination of the study. It is responsible for and in charge of checking, processing and analyzing the data. Finally, the Secretariat is responsible for preparing reports and publications.

The secretariat consists of:
-Stefan Michiels, Sarah-Flora Jonas: Gustave Roussy (data analysis center of current project)
-Roberto Salgado, Sherene Loi: International TILs Working Group
-Roberto Leon-Ferre, Matthew Goetz: Mayo Clinic

Membership to the Collaborative Group will be granted to a number of individuals from each of the participating studies designated by the principal investigator of each study. This may also include the data manager(s), statistician(s) and all other significant contributors to this project (research fellows included). These members may or not include the PI of the study and must be available to respond to data queries, if required. The number of members is based on the amount

of patient data provided: <=100 pts: 1 member, 101-200: 2 members, 201-300: 3 members, 301-300: 4 members, 401-500: 5 members, >500: 6 members.

Regular emails, teleconferences and/or face-to-face meetings will be organized to discuss progress of the data analysis.

IX. PUBLICATION POLICY

A manuscript summarizing the results of the pooled analysis will be prepared for submission to and publication by a peer-reviewed scientific journal. The manuscript will be prepared by the Secretariat and submitted to the Collaborative Group for review and agreement. The Collaborative Group is defined as all collaborators of each partner that have contributed according to section VIII. Any publication arising from this project will be made on behalf of the Collaborative Group, which will be listed as Collaborators in the publication, and consequently, all will be included on Pubmed. A Writing Committee will be listed as co-authors in the following author order: Roberto Leon-Ferre (Mayo Clinic), Sarah Jonas (GR data center), Roberto Salgado (TILS WG), Sherene Loi (TILs WG), Vincent De Jonghe (NKI), Jodi Carter (Mayo Clinic)…., authors from participating studies,…., Marleen Kok (NKI), Sabine Linn (NKI), Matthew Goetz (Mayo Clinic), Stefan Michiels (GR data center). Depending on the number of co-authors allowed by the target journal, the Writing Committee may need to include nominatively a reduced number of Collaborative Group members. The following scenario is aimed for: <=100 pts: 1 co-author, 100-200: 2 co-authors, >200: 3 co-authors, while all other collaborators will be listed as Collaborative Group members only. If the Journal allow a larger number of co-authors, the inclusion of more authors might be considered. If the maximum allowed nominative co-authors is not reached by the collaborative groups, the author-position can be proposed to another collaborative group member. All authors will be given sufficient time to provide comments and attempts will be made to come to mutual agreement. No financial benefits will be pursued or derived by the study and the corresponding results.

X. FUNDING

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XI. XI.1 References

Park JH, Jonas SF, Bataillon G, Criscitiello C, Salgado R, Loi S, Viale G, Lee HJ, Dieci MV, Kim SB, Vincent-Salomon A, Curigliano G, André F, Michiels S. Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy. Ann Oncol. 2019 Dec 1;30(12):1941-1949. doi: 10.1093/annonc/mdz395. PMID: 31566659.

Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, Joensuu H, Dieci MV, Badve S, Demaria S, Gray R, Munzone E, Lemonnier J, Sotiriou C, Piccart MJ, Kellokumpu-Lehtinen PL, Vingiani A, Gray K, Andre F, Denkert C, Salgado R, Michiels S. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. J Clin Oncol. 2019 Mar 1;37(7):559-569. doi: 10.1200/JCO.18.01010. Epub 2019 Jan 16. PMID: 30650045; PMCID: PMC7010425.

Tolaney SM, Garrett-Mayer E, White J, Blinder VS, Foster JC, Amiri-Kordestani L, Hwang ES, Bliss JM, Rakovitch E, Perlmutter J, Spears PA, Frank E, Tung NM, Elias AD, Cameron D, Denduluri N, Best AF, DiLeo A, Baizer L, Butler LP, Schwartz E, Winer EP, Korde LA. Updated Standardized Definitions for Efficacy End Points (STEEP) in Adjuvant Breast Cancer Clinical Trials: STEEP Version 2.0. J Clin Oncol. 2021 May 18:JCO2003613. doi: 10.1200/JCO.20.03613. Epub ahead of print. PMID: 34003702.

Leon-Ferre RA, Polley MY, Liu H, Gilbert JA, Cafourek V, Hillman DW, Elkhanany A, Akinhanmi M, Lilyquist J, Thomas A, Negron V, Boughey JC, Liu MC, Ingle JN, Kalari KR, Couch FJ, Visscher DW, Goetz MP. Impact of histopathology, tumor-infiltrating lymphocytes, and adjuvant chemotherapy on prognosis of triple-negative breast cancer. Breast Cancer Res Treat. 2018 Jan;167(1):89-99. doi: 10.1007/s10549-017-4499-7. Epub 2017 Sep 14. PMID: 28913760; PMCID: PMC5790598.

Olivotto A, Coldman AJ, Hislop TG, Trevisan CH, Kula J, Goel V, Sawka C. Compliance with practice guidelines for node-negative breast cancer. J Clin Oncol. 1997 Jan;15(1):216-22. doi: 10.1200/JCO.1997.15.1.216. PMID: 8996145.

Hudis CA, Barlow WE, Costantino JP, Gray RJ, Pritchard KI, Chapman JA, Sparano JA, Hunsberger S, Enos RA, Gelber RD, Zujewski JA. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. J Clin Oncol. 2007 May 20;25(15):2127-32. doi: 10.1200/JCO.2006.10.3523. PMID: 17513820.

Meddis A, Latouche A, Zhou B, Michiels S, Fine J. Meta-analysis of clinical trials with competing time-to-event endpoints. Biom J. 2020 May;62(3):712-723. doi: 10.1002/bimj.201900103. Epub 2019 Dec 9. PMID: 31815321.