

ORIGINAL ARTICLE

Association of tumor-infiltrating lymphocytes with distant disease-free survival in the ShortHER randomized adjuvant trial for patients with early HER2+ breast cancer

M. V. Dieci^{1,2}, P. Conte^{1,2*}, G. Bisagni³, A. A. Brandes⁴, A. Frassoldati⁵, L. Cavanna⁶, A. Musolino⁷, F. Giotta⁸, A. Rimanti⁹, O. Garrone¹⁰, E. Bertone¹¹, K. Cagossi¹², S. Sarti¹³, A. Ferro¹⁴, F. Piacentini^{15,16}, A. Maiorana¹⁷, E. Orvieto¹⁸, M. Sanders¹⁹, F. Miglietta¹, S. Balduzzi²⁰, R. D'Amico²⁰ & V. Guarneri^{1,2}

¹Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova; ²Medical Oncology 2, Istituto Oncologico Veneto IRCCS, Padova; ³Oncology Unit, Department of Oncology and Advanced Technologies, Azienda USL-IRCCS, Reggio Emilia; ⁴Medical Oncology, Azienda Unità Sanitaria Locale di Bologna-IRCCS Istituto delle Scienze Neurologiche, Bologna; ⁵Clinical Oncology, Department of Morphology, Surgery and Experimental Medicine, S Anna University Hospital, Ferrara; ⁶Department of Oncology-Hematology, G. da Saliceto Hospital, Piacenza; ⁷Medical Oncology Unit, University Hospital of Parma, Piacenza; ⁸Division of Medical Oncology, IRCCS, Istituto Tumori "Giovanni Paolo II", Bari; ⁹Medical Oncology, Azienda Ospedaliera di Mantova, Mantova; ¹⁰Medical Oncology, A.O. S. Croce and Carle Teaching Hospital, Cuneo; ¹¹Medical Oncology, S. Anna Hospital, Torino; ¹²Breast Unit Ausl Modena, Ramazzini Hospital, Carpi; ¹³Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), IRCCS, Meldola; ¹⁴Rete Clinica Senologica - Oncologia Medica S. Chiara, Trento; ¹⁵Division of Medical Oncology, Department of Medical and Surgical Sciences for Children & Adults, University Hospital of Modena, Modena; ¹⁶Azienda Ospedaliero-Universitaria di Modena, Modena; ¹⁷Department of Diagnostic, Clinical Medicine and Public Health, University Hospital of Modena, Modena; ¹⁸Pathology Unit, Ulss 5 Polesana, Rovigo, Italy; ¹⁹Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, USA; ²⁰Department of Medical and Surgical Sciences for Children & Adults, University Hospital of Modena, Italy

*Correspondence to: Prof. PierFranco Conte, Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy; Division of Medical Oncology 2, Istituto Oncologico Veneto IRCCS, Via Gattamelata 64, 35128 Padova, Italy. Tel: +39-049-821-5290; Fax: +39-049-821-5932; E-mail: pierfranco.conte@unipd.it

Background: There is the need to identify new prognostic markers to refine risk stratification for HER2-positive early breast cancer patients. The aim of this study was to evaluate the association of tumor-infiltrating lymphocytes (TILs) with distant disease-free survival (DDFS) in patients with HER2-positive early breast cancer enrolled in the ShortHER adjuvant trial which compared 9 weeks versus 1-year trastuzumab in addition to chemotherapy, and to test the interaction between TILs and treatment arm.

Patients and methods: Stromal TILs were assessed for 866 cases on centralized hematoxylin and eosin-stained tumor slides. The association of TILs as 10% increments with DDFS was assessed with Cox models. Kaplan–Meier curves were estimated for patients with TILs $\geq 20\%$ and TILs $< 20\%$. Median follow-up was 6.1 years.

Results: Median TILs was 5% (Q1–Q3 1%–15%). Increased TILs were independently associated with better DDFS in multivariable model [hazard ratio (HR) 0.73, 95% confidence interval (CI) 0.59–0.89, $P = 0.006$, for each 10% TILs increment]. Five years DDFS rates were 91.1% for patients with TILs $< 20\%$ and 95.7% for patients with TILs $\geq 20\%$ ($P = 0.025$). The association between 10% TILs increments and DDFS was significant for patients randomized to 9 weeks of trastuzumab (HR 0.60, 95% CI 0.41–0.88) but not for patients treated with 1 year of trastuzumab (HR 0.89, 95% CI 0.71–1.12; test for interaction $P = 0.088$). For patients with TILs $< 20\%$, the HR for the comparison between the short versus the long arm was 1.75 (95% CI 1.09–2.80, $P = 0.021$); whereas, for patients with TILs $\geq 20\%$ the HR for the comparison of short versus long arm was 0.23 (95% CI 0.05–1.09, $P = 0.064$), resulting in a significant interaction ($P = 0.015$).

Conclusions: TILs are an independent prognostic factor for HER2-positive early breast cancer patients treated with adjuvant chemotherapy and trastuzumab and may refine the ability to identify patients at low risk of relapse eligible for de-escalated adjuvant therapy.

Key words: early breast cancer, tumor-infiltrating lymphocytes, HER2-positive breast cancer, trastuzumab, adjuvant

Introduction

The standard adjuvant systemic treatment of patients with HER2-positive early breast cancer includes chemotherapy combined with 1 year of trastuzumab [1].

In this scenario, where many patients experience an excellent prognosis with standard treatment, one major need is to develop de-escalated adjuvant treatment strategies for low-risk patients [2]. Non-inferiority of shorter trastuzumab duration has been tested in five prospective randomized trials [3–7]: although only one succeeded in demonstrating the non-inferiority of <1 year versus 1 year trastuzumab [3], a significant decrease in risk of cardiac toxicity with shorter treatment clearly emerged [3–7].

Therefore, there is the need for new prognostic and predictive biomarkers in order to better refine risk classification and identify HER2-positive patients eligible for de-escalated therapies.

The role of the immune system in breast cancer is being increasingly recognized [8]. Tumor-infiltrating lymphocytes (TILs) are frequently present in HER2-positive breast cancer [9] and the mechanism of action of most anti-HER2 therapies implies an interaction with the immune system [10]. Higher levels of TILs are associated with increased pathological complete response rates after neoadjuvant treatment and improved long-term outcomes for HER2-positive early breast cancer patients [11–16]. However, results of TILs evaluation in the context of two randomized adjuvant trials of chemotherapy with or without trastuzumab showed discordant results for TILs prognostic value according to treatment [14, 16]. No study thus far has explored the role of TILs in a trial comparing shorter versus 1 year adjuvant trastuzumab.

In this study we assessed the association between TILs and long-term outcome for HER2-positive breast cancer patients enrolled in the randomized phase III ShortHER trial which compared 9 weeks versus 1 year of trastuzumab.

Methods

Patients

The ShortHER trial [6] is a phase III multicentric trial of adjuvant therapy that randomized 1253 patients with HER2-positive early breast cancer to anthracycline and taxane-based chemotherapy combined with 1 year (arm A, long) or 9 weeks (arm B, short) of trastuzumab. Chemotherapy schedules were also different between the two arms (supplementary material, available at *Annals of Oncology* online). The study failed to demonstrate the non-inferiority of 9 weeks of trastuzumab: 5-year disease-free survival rates were 88% in the long and 85% in the short arm [HR 1.13, 90% confidence interval (CI) 0.89–1.42, pre-defined non-inferiority margin 1.29] [6].

The present analysis was approved by the competent Ethical Committee in November 2014, patients provided signed informed consent for tumor sample use for research purpose.

TILs and pathology evaluation

TILs were assessed on a single hematoxylin–eosin stained slide. Stromal TILs were scored according to pre-defined criteria [17].

All cases were scored by a single investigator (MVD) blinded for clinicopathological, treatment and follow-up data. A second investigator (MS) scored 383 cases in order to assess inter-rater agreement. HER-2 and hormone receptors status were assessed locally [6].

Statistical analysis

Statistical analysis was carried out using IBM SPSS Version 24.

Association between variables was evaluated by χ^2 test or Mann–Whitney test. Inter-rater agreement was evaluated with weighted κ statistic.

The survival end point was distant disease-free survival (DDFS), calculated from the time from randomization until distant relapse or death, whichever first, as defined by DATECAN [18]. The choice of DDFS instead of disease-free survival (which was the primary end point of the ShortHER trial [6]) was driven by the fact that DDFS includes lethal events with the strongest impact on overall survival and is considered a preferred end point to uncover the prognostic value of biomarkers in ancillary studies [19].

For survival analysis, TILs were primarily considered as semi-continuous variable (10% increments). Kaplan–Meier curves were estimated for patients with low and high TILs by adopting the $\geq 20\%$ cut-off. The cut-off point was arbitrarily chosen as the one able to identify a group of patients with a DDFS rate at 5 years of at least 95%, which was considered a clinically acceptable definition of low-risk patients. The previously proposed cut-off of TILs $\geq 50\%$ [14] was not applicable in our study because too few patients would have been classified as having high TILs (7.8%, with 1 event only).

Cox proportional regression models were used to calculate hazard ratio (HR) and 95% CI. Multivariable model included TILs (per 10% increments), age (continuous), stage (I versus II versus III), hormone receptor status (positive versus negative) and histologic grade (1–2 versus 3). To study the interaction between treatment arm and TILs, a Cox model including arm, TILs and the interaction term was used. The Kaplan–Meier method was used to estimate survival curves and the log-rank test compared between groups. Level of significance was $P < 0.05$.

No formal sample size calculation was carried out, since the sample population was based on the number of cases with centralized tumor samples suitable for the analysis.

Results

Patients characteristics

A total of 866 cases (69% of all randomized patients) were included in this analysis (TILs cohort, supplementary Figure S1, available at *Annals of Oncology* online). Table 1 shows characteristics of the TILs cohort patients and of patients not included in the TILs analysis (no-TILs cohort). The majority of TILs cohort patients were postmenopausal (65%), with stage I or II (85%), node negative (53%), hormone receptor positive (69%) and grade 3 tumors (71%). Characteristics were similar in the TILs cohort and in the no-TILs cohort, with the exception of age: TILs cohort patients were older than no-TILs cohort patients (median age 56 versus 54, $P = 0.014$).

The HR for the DDFS comparison between the short versus long arms was 1.28 (95% CI 0.90–1.81) in all randomized patients. The HR for DDFS in the TILs cohort was similar (1.37, 95% CI 0.89–2.12).

Tumor-infiltrating lymphocytes

Inter-rater agreement between two investigators, assessed on a subset of 383 cases, was good for the classification in TILs categories defined by 10% increments (78% concordance; weighted Cohen's $\kappa = 0.75$, 95% CI 0.69–0.81). For further analyses, the scores of the investigator who reviewed all cases were used.

Table 1. Baseline characteristics of the patients included (TILs cohort) or not (no-TILs cohort) in the TILs analysis

Characteristics		TILs cohort N (%)	No-TILs cohort N (%)	All randomized N (%)	P value
Age (years)	Randomized	866 (69)	387 (31)	1253 (100)	
	<60	536 (62)	266 (69)	802 (64)	
	≥60	330 (38)	121 (31)	451 (36)	0.020
Menopausal status	Median (Q1–Q3)	56 (48–64)	54 (46–62)	55 (48–64)	0.014
	Premenopausal	300 (38)	148 (38)	448 (36)	
	Postmenopausal	565 (62)	237 (62)	802 (64)	0.201
AJCC Stage	I		155 (40)	509 (41)	
	II	383 (44)	166 (43)	549 (44)	
	III	127 (15)	64 (17)	191 (15)	0.681
N stage	N0	460 (53)	212 (55)	672 (54)	
	N1–N2	274 (32)	109 (28)	383 (30)	
	N3	132 (15)	64 (17)	198 (16)	0.417
Hormone receptors	Negative	266 (31)	128 (34)	400 (32)	
	Positive	600 (69)	251 (66)	853 (68)	0.170
Histological grade					
	Grade 1–2	246 (29)	128 (34)	374 (30)	
	Grade 3	607 (71)	251 (66)	858 (70)	0.082

TILs, tumor-infiltrating lymphocytes; AJCC, American Joint Committee on Cancer.

Median TILs was 5% (interquartile range 1%–15%). TILs were significantly higher in younger (<60 years) patients ($P = 0.018$), and in case of hormone receptor negative ($P < 0.001$) and Grade 3 tumors ($P < 0.001$; supplementary Figure S2, available at *Annals of Oncology* online). The association between lower TILs and older age was unlikely confounded by other factors, since the proportion of hormone receptor positive and grade 1–2 patients was similar in the two age categories (supplementary Table S1, available at *Annals of Oncology* online).

TILs and DDFS

At a median follow-up of 6.1 years, 83 DDFS events occurred. Increased TILs were associated with better DDFS: the HR for each 10% TILs increment was 0.76 (95% CI 0.62–0.92, $P = 0.006$).

As shown in Figure 1, 5 years DDFS rates were 91.1% for patients with TILs <20% and 95.7% for patients with TILs ≥20% (log-rank $P = 0.025$).

In multivariable model (Table 2) TILs 10% increments showed an independent prognostic value for DDFS (HR 0.73, 95% CI 0.59–0.89, $P = 0.002$). Stage was the other parameter that was independently associated with DDFS. TILs retained an independent prognostic value in both hormone receptor positive and negative patients (Table 2).

To further explore the independent prognostic information provided by TILs and stage, we evaluated the association between TILs and DDFS in stage-defined subgroups. Results showed a significant impact of TILs only for patients with stage II disease (supplementary Figure S3, available at *Annals of Oncology* online).

Interaction between TILs and treatment arm

The prognostic value of 10% TILs increments was significant in the short (HR 0.60, 95% CI 0.41–0.88, $P = 0.009$) but not in the

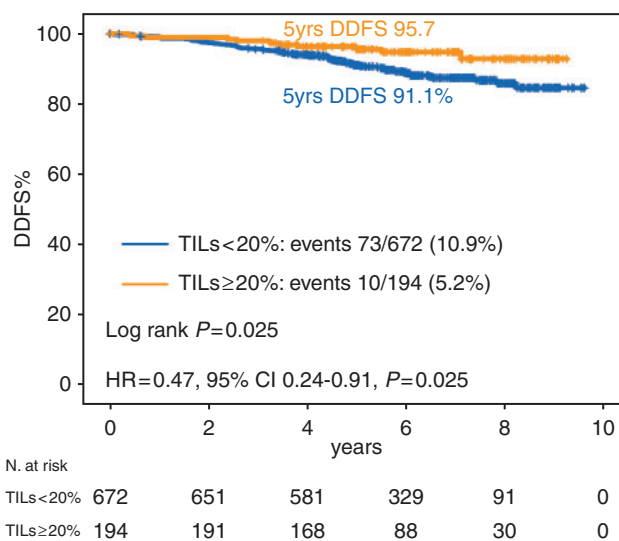


Figure 1. Kaplan–Meier curves for distant disease-free survival according to TILs level.

long arm (HR 0.89, 95% CI 0.71–1.12, $P = 0.317$). The interaction test showed $P = 0.088$ (Figure 2A).

A significant interaction between TILs binary variable and treatment duration was observed (Figure 2B). In the group with TILs <20%, patients in the long arm experienced a better DDFS when compared with patients in the short arm: 5 years DDFS rates were 88.8% for the short and 93.3% for the long arm (HR short versus long=1.75, 95% CI 1.09–2.80, $P = 0.021$). To the opposite, in the group of patients with TILs ≥20%, the DDFS was excellent in both arms and numerically superior for patients treated with short trastuzumab (5 years DDFS 97.6% and 93.7%

Table 2. Multivariate cox models for distant disease-free survival in all patients and in hormone receptor-negative and hormone receptor-positive groups

Factors	All patients		Hormone receptor negative		Hormone receptor positive		
	HR 95% CI	P	HR 95% CI	P	HR 95% CI	P	
TILs 10% increments	0.73 (0.59–0.89)	0.002	0.73 (0.54–0.98)	0.036	0.73 (0.55–0.98)	0.034	
Age (continuous)	1.00 (0.98–1.02)	0.827	1.04 (0.99–1.08)	0.093	0.99 (0.97–1.02)	0.409	
AJCC stage	III	Ref	Ref		Ref		
	II	0.41 (0.25–0.67)	<0.001	0.30 (0.12–0.72)	0.007	0.47 (0.26–0.85)	0.013
	I	0.22 (0.12–0.40)	<0.001	0.23 (0.09–0.60)	0.003	0.19 (0.09–0.42)	<0.001
Hormone receptors	Negative	Ref	–		–		
	Positive	0.75 (0.47–1.20)	0.231				
Grade	G3	Ref	Ref		Ref		
	G1–2	0.61 (0.36–1.04)	0.069	1.33 (0.52–3.40)	0.551	0.47 (0.24–0.89)	0.021

TILs, tumor-infiltrating lymphocytes; HR, hazard ratio; CI, confidence interval; G, grade.

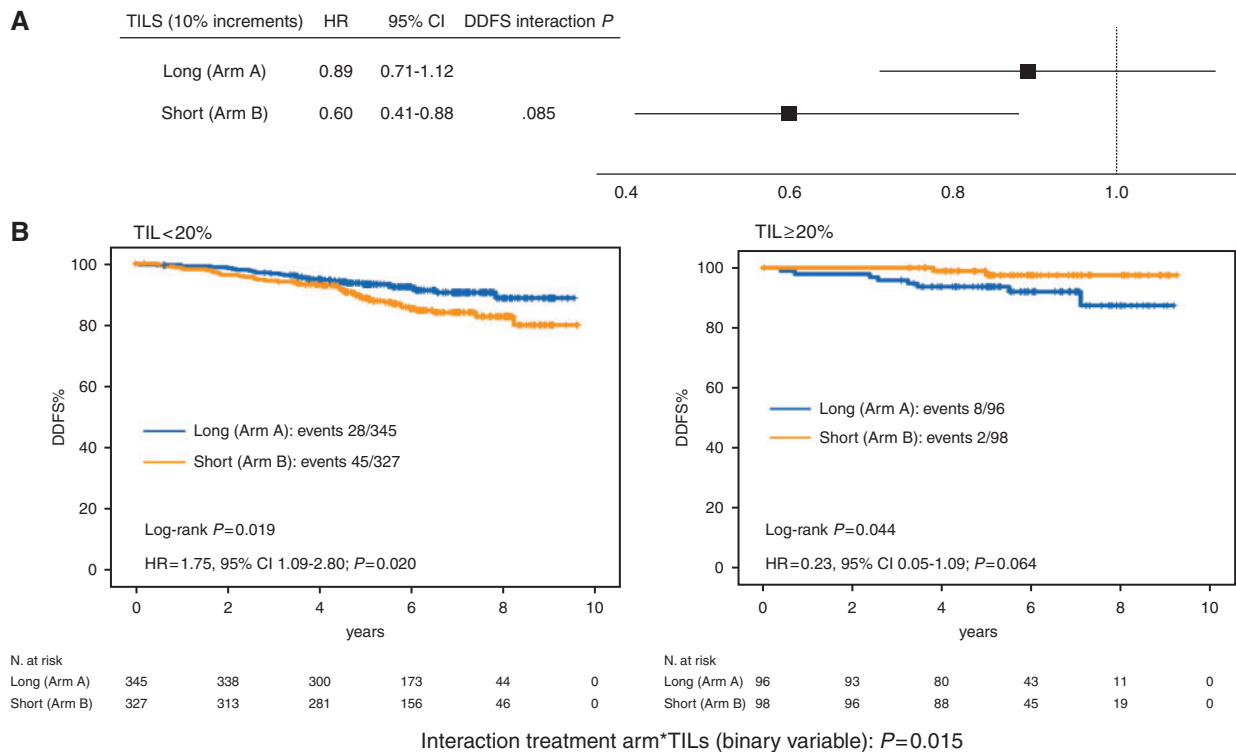


Figure 2. Interaction between TILs and treatment arm. Forest plot shows the prognostic effect on distant disease-free survival of TILs 10% increments according to trastuzumab duration (A). Kaplan–Meier curves illustrate distant disease-free according to treatment arm (short-versus long-trastuzumab duration) in patients with high and low TILs separately (B).

for the short and long arm, respectively, HR short versus long=0.23, 95% CI 0.05–1.09, $P=0.064$). Interaction test showed $P=0.015$.

Discussion

This is the first study assessing TILs in patients enrolled in a randomized adjuvant trial comparing shorter versus 1-year trastuzumab. The results, beyond confirming TILs as an independent

prognostic marker for HER2-positive early breast cancer patients, suggest that higher TILs may discriminate patients at good prognosis who may be optimally treated with shorter trastuzumab. If further validated in other studies, this may represent the first evidence of potential clinical utility in this setting.

In our study TILs were an independent favorable prognostic factor for patients treated with adjuvant standard chemotherapy combined with trastuzumab, both in case of hormone receptor positive and hormone receptor negative disease. Two previous studies of adjuvant trastuzumab showed conflicting results: in

the FinHER study, a trend for a better DDFS with increased TILs was reported for patients treated with chemotherapy plus trastuzumab [14], whereas in the N9831 trial, a significant association between high TILs and better recurrence-free survival was observed only for patients treated with chemotherapy without trastuzumab [16]. However, another analysis of the N9831 trial suggested that increased expression of immune-related genes was associated with improved outcome after trastuzumab treatment [20]. More recently, Kim et al. [15] reported TILs data from the NSABP B-31 trial showing an association between increased TILs and improved outcome that was significant both in the trastuzumab-treated and trastuzumab-untreated groups.

Overall, these data support the concept that pre-existing immune activation is a determinant of improved outcome for HER2-positive breast cancer patients treated with chemotherapy and anti-HER2 therapy. Although general TILs evaluation does not allow to capture the complexity of the tumor immune milieu, it constitutes a simple, inexpensive and reproducible [21] biomarker able to discriminate patients at different prognosis independently from classic clinicopathological features.

The development of new prognostic biomarkers for HER2-positive breast cancer patients is of particular relevance in the actual clinical and research context focused on de-escalated therapies for low-risk patients.

In our study, the prognostic value of semi-continuous TILs appeared significant only for patients treated with 9-weeks trastuzumab. When comparing the short versus long arm in low and high-TILs patients, the results suggested that low-TILs patients benefit from standard 1 year trastuzumab, whereas high-TILs patients experience an excellent outcome irrespectively of trastuzumab duration, with a significant test for interaction. The rate of events was even lower for patients in the short arm, however the total number of events was very low. These data indicate that high TILs may discriminate patients at very good prognosis following treatment with de-escalated trastuzumab duration. These results need to be further validated. Importantly, decreasing trastuzumab duration is associated with a lower risk of cardiac toxicities [3–6] and this is of particular relevance for real-world patients, often presenting with higher risk of cardiac events when compared with patients in clinical trials [22].

The observation that the prognostic value of TILs was mostly evident for patients with stage II disease is also of potential clinical interest. Indeed, for stage I patients, generally considered at low risk, effective de-escalated treatments are already available [23], whereas stage III patients are considered at high risk and deserve escalated therapies. Stage II patients represent a ‘grey area’ of heterogeneous prognosis where a more precise risk stratification is needed.

We reported in our study median TILs of 5%, which is lower when compared with other HER2-positive patients cohorts from adjuvant studies (15% in the FinHER and between 10% and 19% in the N9831) [14–16, 24]. It is unlikely that this difference is due to methodological issues. All these studies applied the same established methodology for TILs evaluation [17], which has shown good reproducibility [21], as also confirmed by our study. One possible explanation may be a higher prevalence of older and hormone receptor positive patients in our study. Median age was 56 years in our cohort and 50 years in N9831 [16]; prevalence of hormone receptor positive patients was 69% in our cohort, 54%

in N9831 [16] and 49% in FinHER [14]. Hormone receptor positive status is known to be associated with lower TILs [11, 12, 14–16], whereas the correlation between low TILs and older age observed in our study had not been previously reported. A hypothesis may be a higher prevalence of luminal intrinsic subtypes (typically associated with low TILs) in older HER2-positive/hormone receptor positive patients, as previously suggested [25].

Strengths of this study include: the adoption of a validated methodology for TILs assessment, the good level of agreement between two raters, the study design, being the first assessing TILs in a randomized non-inferiority trial of short adjuvant trastuzumab and the large sample size.

Our study has limitations. First, 69% of cases from the overall ShortHER population were evaluated for TILs, however this figure is consistent with other studies [15, 16]. Second, the analysis was not pre-specified in the protocol. Third, the 20% cut-off value was arbitrarily chosen, therefore all analyses of TILs as a binary variable should be considered as exploratory.

In conclusion, TILs are an independent prognostic factor for patients treated with chemotherapy and adjuvant trastuzumab. Individual pooled analysis of large studies will be required in order to draw definitive conclusions on the potential clinical utility of TILs in selecting patients for de-escalated therapies.

Funding

This work was supported by research grants from Agenzia Italiana del Farmaco (AIFA, grant FARM62MC97), Italian Association for Cancer Research (AIRC, project MFAG 2014-15938); Veneto Institute of Oncology (5×1000 program).

Disclosure

MVD has received: fees from EliLilly for consultancy role and participation on advisory boards; fees from Genomic Health for consultancy role; fees from Celgene for participation on advisory boards. PFC has received lecture fees and honoraria for participation on advisory boards from EliLilly, Novartis, Roche, AstraZeneca. VG has received honoraria from EliLilly and Roche for participation on advisory boards, and honoraria from AstraZeneca and Novartis. All these activities are outside the submitted work. All remaining authors have declared no conflicts of interest.

References

- Moja L, Tagliabue L, Balduzzi S et al. Trastuzumab containing regimens for early breast cancer. *Cochrane Database Syst Rev* 2012; 18: CD006243.
- Dieci MV, Vernaci G, Guarneri V. Escalation and de-escalation in HER2 positive early breast cancer. *Curr Opin Oncol* 2018; 35: 35–42.
- Earl HM, Hiller L, Vallier A-L et al. PERSEPHONE: 6 versus 12 months (m) of adjuvant trastuzumab in patients (pts) with HER2 positive (+) early breast cancer (EBC): randomised phase 3 non-inferiority trial with definitive 4-year (yr) disease-free survival (DFS) results. *J Clin Oncol* 2018; 36(Suppl); Abstr 506.
- Pivot X, Romieu G, Debled M et al. PHARE trial investigators. 6 months versus 12 months of adjuvant trastuzumab for patients with HER2-positive early breast cancer (PHARE): a randomised phase 3 trial. *Lancet Oncol* 2013; 14(8): 741–748.

5. Joensuu H, Fraser J, Wildiers H et al. Effect of adjuvant trastuzumab for a duration of 9 weeks vs 1 year with concomitant chemotherapy for early human epidermal growth factor receptor 2-positive breast cancer: the SOLD randomized clinical trial. *JAMA Oncol* 2018; 4(9): 1199–1206.
6. Conte PF, Bisagni G, Frassoldati A et al. 9 weeks vs 1 year adjuvant trastuzumab in combination with chemotherapy: Results of the phase III multicentric Italian study Short-HER. *Ann Oncol* 2018; 29: 2328–2333.
7. Mavroudis D, Saloustros E, Malamos N et al. Six versus 12 months of adjuvant trastuzumab in combination with dose-dense chemotherapy for women with HER2-positive breast cancer: a multicenter randomized study by the Hellenic Oncology Research Group (HORG). *Ann Oncol* 2015; 26: 1333–1340.
8. Savas P, Salgado R, Denkert C et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat Rev Clin Oncol* 2016; 13(4): 228–241.
9. Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review. *JAMA Oncol* 2016; 2(10): 1354–1360.
10. Bianchini G, Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. *Lancet Oncol* 2014; 15(2): e58–e68.
11. Salgado R, Denkert C, Campbell C et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. *JAMA Oncol* 2015; 1(4): 448–454.
12. Dieci MV, Prat A, Tagliafico E et al. Integrated evaluation of PAM50 subtypes and immune modulation of pCR in HER2-positive breast cancer patients treated with chemotherapy and HER2-targeted agents in the CherLOB trial. *Ann Oncol* 2016; 27(10): 1867–1873.
13. Denkert C, von Minckwitz G, Darb-Esfahani S et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 2018; 19(1): 40–50.
14. Loi S, Michiels S, Salgado R et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol* 2014; 25(8): 1544–1550.
15. Kim RS, Song N, Gavin PG et al. NRG Oncology/NSABP B-31: stromal tumor infiltrating lymphocytes (sTILs) and outcomes in early-stage HER2-positive breast cancer (BC). *J Clin Oncol* 2018; 36(Suppl); Abstr 12010.
16. Perez EA, Ballman KV, Tenner KS et al. Association of stromal tumor-infiltrating lymphocytes with recurrence-free survival in the N9831 adjuvant trial in patients with early-stage HER2-positive breast cancer. *JAMA Oncol* 2016; 2(1): 56–64.
17. Salgado R, Denkert C, Demaria S et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015; 26(2): 259–271.
18. Gourgou-Bourgade S, Cameron D, Poortmans P et al. Guidelines for time-to-event end point definitions in breast cancer trials: results of the DATECAN initiative (Definition for the Assessment of Time-to-event Endpoints in CANcer trials). *Ann Oncol* 2015; 26(5): 873–879.
19. Hudis CA, Barlow WE, Costantino JP et al. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. *J Clin Oncol* 2007; 25(15): 2127–2132.
20. Perez EA, Thompson EA, Ballman KV et al. Genomic analysis reveals that immune function genes are strongly linked to clinical outcome in the North Central Cancer Treatment Group n9831 Adjuvant Trastuzumab Trial. *J Clin Oncol* 2015; 33(7): 701–708.
21. Denkert C, Wienert S, Poterie A et al. Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: results of the ring studies of the international immuno-oncology biomarker working group. *Mod Pathol* 2016; 29(10): 1155–1164.
22. Mantarro S, Rossi M, Bonifazi M et al. Risk of severe cardiotoxicity following treatment with trastuzumab: a meta-analysis of randomized and cohort studies of 29,000 women with breast cancer. *Intern Emerg Med* 2016; 11(1): 123–140.
23. Tolane SM, Barry WT, Dang CT et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. *N Engl J Med* 2015; 372(2): 134–141.
24. Dieci MV, Mathieu MC, Guarneri V et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials. *Ann Oncol* 2015; 26(8): 1698–1704.
25. Jenkins EO, Deal AM, Anders CK et al. Age-specific changes in intrinsic breast cancer subtypes: a focus on older women. *Oncologist* 2014; 19(10): 1076–1083.