Should tumour infiltrating lymphocytes and PIK3Ca mutation be added as markers to the histology report for breast cancer?

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SUMMARY

Tumour infiltrating lymphocytes are a sign of immune mediated reaction of the host against the tumour. They are considered as a positive prognostic marker and may also have a predictive role for the use of certain therapies. The challenge remains to convert tumours with low tumour infiltrating lymphocytes into tumours with high tumour infiltrating lymphocytes in order to enhance the immune mediated effect of therapies.
PIK3Ca mutation is one of the most frequent mutations encountered in breast cancer, particularly in hormone receptor positive cancer in which it can confer resistance to hormonal therapy. Therefore, a lot of effort has been made to target the PI3K-pathway with drugs, and to find a way to predict their efficacy: some results have been achieved; in particular with the detection of PIK3Ca in circulating DNA, but many questions still remain. This article provides an overview concerning these two biomarkers, and attempts to determine whether they could be used in clinical practice today.
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INTRODUCTION

Research is continuously looking for more and better biomarkers, and a lot of attention has rightly been given to biomarkers that give genetic information.
Finding activating mutations through Next-Generation Sequencing (NGS), such as a PIK3Ca-mutation, may be useful as PI3K inhibitors are available for blocking that pathway.
But could something as 'simple' as the microscopic evaluation of lymphocytes infiltrating a tumour be just as powerful a marker as a full NGS of that very tumour?

TILS

Tumour infiltrating lymphocytes (TILs) are lymphocytes that are trying to attack a tumour, but somehow do not succeed in killing it. When a tumour is surrounded by TILs, there is often a kind of 'ceasefire' going on between the tumour and its lymphocyte assailants. The tumour survives by suppressing the immune response against it.
But it is also possible that some early tumours have not survived because TILs have 'won the battle', thus curing patients without their knowing.

HOW TO EVALUATE TILS

TILs have to be searched for in the stroma that is adjacent to the tumour, situated in-between the tumour beds: this evaluation is far more reproducible for pathologists than evaluating TILs within the tumour beds.
TILs are reported as a percentage of the volume of the

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stroma that they occupy. Lymphocyte predominant breast cancer (LPBC) usually has more than 50% of TILs in its stroma.

TILs can be found in all breast tumour types, but LPBC is mainly found in triple-negative breast cancer (TNBC) or HER2-positive breast cancers, which are usually aggressive tumours creating more immune response.

Obtaining reproducibility between pathologists is very important, and therefore guidelines have been elaborated (*Figure 1*).[1]

TILS AND CLINICAL OUTCOME

Triple Negative Breast Cancer

The first major paper linking TILs with clinical outcome in breast cancer showed that TNBC with LPBC-features has a better prognosis when treated with adjuvant chemotherapy (anthracyclines in particular). The disease free survival (DFS) and overall survival (OS) at five years were both 92% for LPBC patients. These data concerned only a small subgroup of patients: only about 12% of all TNBCs had LPBC features.[2] Considering that TNBCs represent about 10% of all breast cancers, this means that only 1% of all breast cancer patients were concerned.

Recently though, the prognostic value of TILs was also found in a larger TNBC population. In the BEATRICE trial, tumours that had TILS above the median of 7% (accounting for half of the patients), also had a better outcome.[3] Another pooled analysis of 991 TNBC patients out of six clinical trials showed that when putting a cut-off at the average value of 20% of TILs (and higher), the distant disease free survival was excellent.[4]

Although these findings are encouraging and establish TILs as an excellent prognostic biomarker in TNBC, it will for now not change the treatment for patients. Chemotherapy will still be needed in TNBC, and even more so as data emerge showing that chemotherapy is partly immune-inducing. Chemotherapy induces tumour cell death, which releases tumour antigens that can in turn induce a long lasting T-cell response by TILs.

The latter was confirmed by a neo-adjuvant study which showed that 10% of TNBCs became LPBCs or increased their amount of TILs on receiving neo-adjuvant chemotherapy. And these patients had an excellent prognosis with 91% OS at five years.[5]

The vast majority of tumours however remained 'low TIL' under chemotherapy, and these are the tumours that probably need additional therapy with immune-enhancing effect.

HER2-positive Breast Cancer

- Prognostic value of TILs

A prognostic effect of TILs in HER2-positive cancer was suggested in the adjuvant FinHer trial. The outcome for 'high TIL' LPBC was superior compared to the 'lower TIL' group but the absolute number of LPBC-tumours was again very small.[6] A larger study investigating this effect was NeoALTTO, comparing trastuzumab versus lapatinib versus both in the neo-adjuvant setting. There was a better outcome when LPBC was found, and patients that had TILs above the median of 12,5% did better compared to those having TILs under the median.[7]

Pathologic complete response (pCR) is also influenced by TILs: pCR in NeoALTTO was significantly better when the percentage of TILs at baseline biopsy was superior to 5%. And even when there was no pCR, but TILs were above the average of 12,5% at baseline, the outcome was just as good for these patients as for those that obtained pCR but had low TILs at baseline. This suggests that TILs can add prognostic information on top of classical pCR-assessment.

- Predictive value of TILs?

In the FinHer trial, the absolute gain in DFS was much higher when trastuzumab was given in tumours that were LPBC compared to tumours that were non-LPBC, suggesting that TILs also can predict trastuzumab efficacy. Trastuzumab has immune mediated effects and TILs may be activated by this mechanism of action. On the other hand, this predictive value or TILs was not confirmed in a larger adjuvant trial - the NCCTG (Alliance) N9831 trial - comparing adding trastuzumab versus no trastuzumab. Provocatively, there was even a trend for a negative effect of the presence of TILs on outcome when treated with trastuzumab.[8] Further analysis is therefore needed to determine the potential predictive value of TILs in HER2-positive patients, in even larger trials, e.g. the ALTTO trial.

CONCLUSION: CLINICAL IMPLICATION OF TILS?

The presence of TILs has positive prognostic value in TNBC treated with chemotherapy, and this may also hold true for HER-2 positive tumours treated with chemotherapy and trastuzumab. However, it is not yet clear whether TILs also have a predictive effect on the efficacy of treatment.

There is a tendency for better response to anthracyclines in TNBC with 'high TIL' score, but more data are needed. There is also more chance of pCR response in TIL-predominant HER2-positive disease treated with

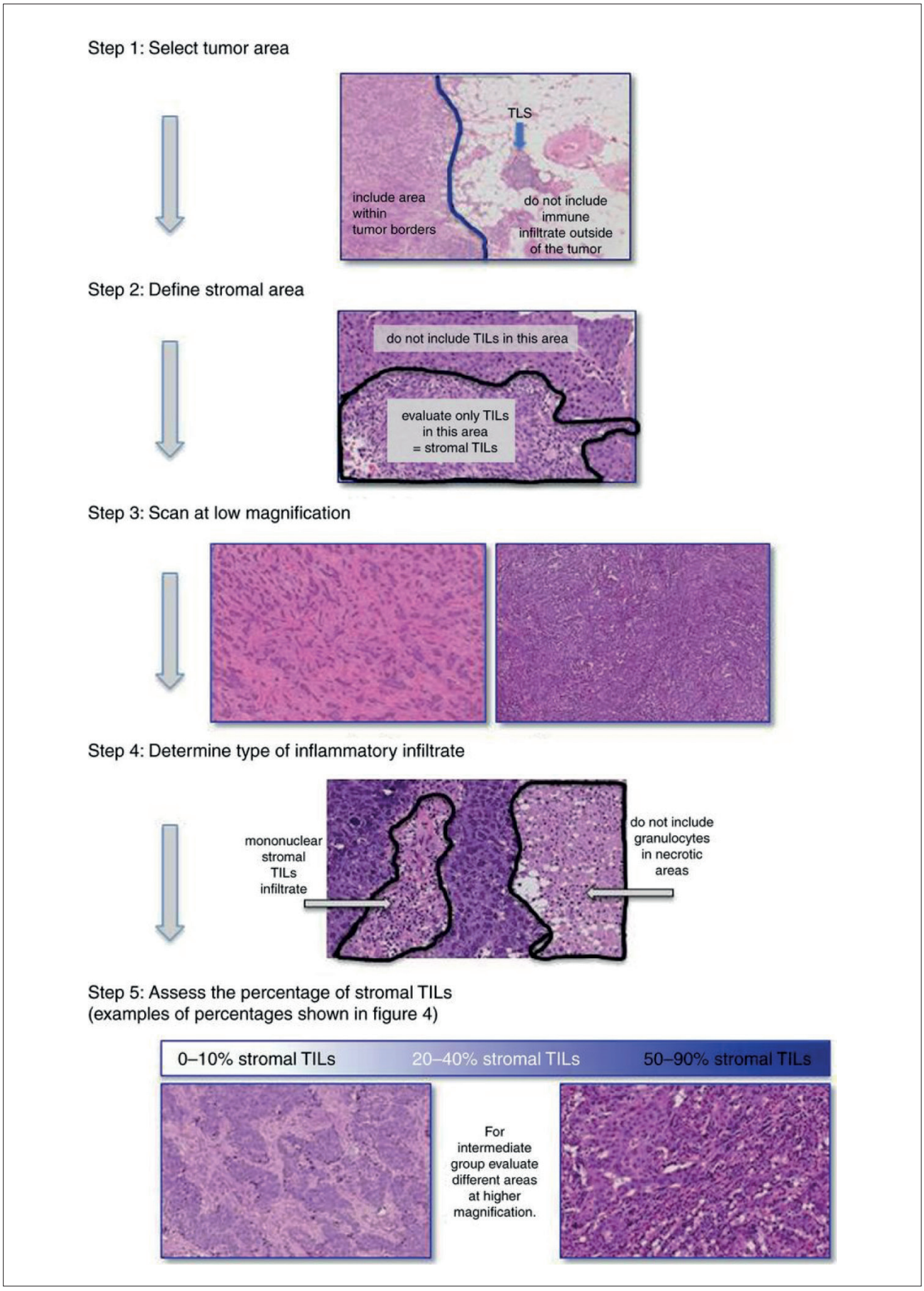

FIGURE 1 Standardised approach for TILs evaluation in breast cancer. R. Salgado et al. Ann Oncol 2015;26:259-271.

chemotherapy and trastuzumab. But it is unlikely that these findings will lead to a change in treatment.

The most interesting clinical implication would probably be to convert 'low-TIL' tumours into 'high- TIL' tumours. This could possibly be achieved by adding checkpoint-inhibitors, 'immunogenic' chemotherapy, or targeted radiotherapy (the so-called 'abscopal' effect of radiotherapy is probably due to TIL invasion in non-irradiated lesions). Clinical trials investigating this 'TIL-conversion' are ongoing.

PIK3CA

The PI3K pathway (implicating PI3K but also other enzymes such as AKT and mTOR) is an important signalling pathway in breast cancer. It is often overactivated, and this can lead to resistance to HER2- and Hormone receptor (HR)-based therapies. Therefore, inhibiting this pathway may have therapeutic implications: e.g. dual blockade with a PI3K inhibitor and endocrine therapy may act synergistically and overcome endocrine resistance.

The most frequent cause of PI3K dysregulation in breast cancer is a mutation of the alpha-isoform of the PI3K–protein, called a PIK3Ca mutation. It is present in up to 40% of ER-positive tumours.

Determining PIK3Ca in a pathology block is feasible through NGS (the cost is high and only reimbursed to a certain extent in Belgium). But is there enough proof to promote the use of NGS-platforms to orientate our therapeutic choices?

PIK3CA AS A PROGNOSTIC MARKER?

PIK3Ca does not seem to be a good prognostic biomarker: there are conflicting data on its value in early hormone receptor (HR)-positive disease (where it seems to confer a better prognosis) and advanced HR-positive disease (where it can be a factor of endocrine resistance and worse prognosis).[9] There are also indications that other PI3K-pathway activations (like PTEN-loss) may override the PIK3Ca mutation and influence prognosis.[10]

PIK3CA AS A PREDICTIVE MARKER?

In HER2-negative HR-positive disease, mTOR-inhibitors (acting through the PI3K-pathway) have been used in the clinic for some time now, and a good predictive biomarker would be useful given the tolerability issues. PIK3Ca was therefore analysed in the BOLERO-2 study evaluating the effect of everolimus and exemestane over exemestane alone. Unfortunately, PIK3Ca was not predictive for benefit.[11]

PIK3Ca has also been analysed in trials evaluating the effect of a pan-PI3K-inhibitor in metastatic breast cancer (MBC). No difference in outcome was seen between the PIK3Ca wild-type tumours and the PIK3Ca-mutated tumours.[12,13] PIK3Ca mutation in these trials was mainly determined on archival tumour blocks. Most of these blocks came from the primary tumour, and this may be of importance as tumour mutation load evolves over time.

The only trial to date that demonstrated a differential effect according to PIK3Ca was the BELLE-2 trial, comparing letrozole alone to letrozole with a PI3K inhibitor: more benefit was seen in the latter group when a PIK3Ca mutation was detected in the circulating tumour-DNA, measured in the blood of the patient at the initiation of the treatment.[14] This could mean that when targeting a certain tumour pathway with treatment, one should verify whether that pathway is still active enough (e.g. PIK3Ca-mutation) at the moment of treatment. Analysing circulating tumour-DNA (ctDNA) sounds very promising as an easy way to obtain that information.

Also, PIK3Ca may not yet be a good predictive biomarker because the drugs used until now were not selectively targeting the PIK3Ca mutation. Therefore, alpha-selective PI3K blockers, such as taselisib and alpelisib, hold more promise for response in PIK3Ca mutated tumours. The first results of phase Ib trials with these 'second generation' compounds are indeed very promising, and phase III trials are ongoing.[15]

In HER2-positive neo-adjuvant trials, PIK3Ca status of the tumour rather seems to be a 'predictive' factor of resistance to anti-HER2-directed therapy (single or dual blockade): pCR–rate was lower in PIK3Ca-mutated tumours compared to the wild-type tumours.[16]

CONCLUSION

Assessment of TILs is almost ready for use in the clinic. It has a prognostic role and could be used as a dynamic biomarker reflecting the immunogenic capacity of a certain therapeutic intervention: this may be of interest in clinical trials in particular. Moreover, it is a 'low cost' biomarker needing only a pathology assessment. It may also become a predictive marker for future immune-modulating treatments.

PIK3Ca is a more complex biomarker, and also a more expensive one. It has no clear prognostic value, and its predictive role is still to be determined given its unconvincing results with 'first-generation' drugs targeting the PIK3-pathway. A more pragmatic approach with as-

KEY MESSAGES FOR CLINICAL PRACTICE

1. **Presence of TILs is a positive prognostic marker, particularly in TNBC and HER2-positive breast cancer.**
2. **Reporting of TILs in pathology needs to be standardised: guidelines exist.**
3. **PIK3Ca from archival tissue is not yet ready for validation as a marker.**
4. **'Real-time' PIK3Ca assessments in circulating DNA may prove to be helpful as a marker in the near future.**

sessment of 'real-time' PIK3Ca in ctDNA in blood may identify patient populations who are likely to benefit. The results of this marker in the phase III trials using 'second generation' PIK3- inhibitors are eagerly awaited.

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