Editorial

Companion and complementary diagnostics for first-line immune checkpoint inhibitor treatment in non-small cell lung cancer

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Introduction

Within the last few years, the programmed death 1 (PD-1) and the programmed death ligand 1 (PD-L1) immune checkpoint inhibitors have dramatically changed the treatment of cancer. By blocking the interaction between PD-L1 and its receptor PD-1 the host immunity can be restored resulting in enhanced T-cell response and increased antitumor activity (1). For advanced non-small cell lung cancer (NSCLC) these inhibitors have demonstrated clinical superiority over docetaxel as second-line treatment with an improvement in overall survival (OS) (2-5). So far, three immune checkpoint inhibitors have obtained regulatory approval for this indication: nivolumab (Opdivo, Bristol-Myers Squibb); pembrolizumab (Keytruda, Merck Sharp & Dohme); and atezolizumab (Tecentriq, Roche/Genentech). Along with the approval of these inhibitors their companion or complementary diagnostic assays were approved simultaneously. These assays are based on an immunohistochemical (IHC) detection of PD-L1 expression in the tumor tissue by the use different antibody clones (1).

When it comes to first-line treatment of advanced NSCLC the picture is somewhat different, as only pembrolizumab has shown superiority over platinum-based chemotherapy so far. Based on data from the KEYNOTE-024 trial, pembrolizumab has recently obtained regulatory approval for this indication in the US, the EU, and in a number of other countries (6). More or less at the same time as the KEYNOTE-024 trial was conducted with pembrolizumab the corresponding CheckMate 026 trial was performed with nivolumab (7). However, in contrast to the pembrolizumab trial, the CheckMate 026 trial did not show superiority of nivolumab over platinum-based chemotherapy neither with respect to progression-free survival (PFS) nor to OS. In this short editorial we will discuss the possible reasons for the failure of the CheckMate 026 trial with emphasize on the differences between the complementary diagnostic linked to the use of nivolumab and the companion diagnostic linked to the use of pembrolizumab, which is an aspect that has not been discussed as intensively as other trial related issues so far.

Nivolumab—CheckMate 026

In the CheckMate 026 trial, 541 stage IV NSCLC patients with a PD-L1 tumor expression ≥1%, measured by the PD-L1 IHC 28-8 pharmDx assay (Dako), were randomized to receive nivolumab or platinum-based chemotherapy (7). The primary endpoint was PFS, assessed in the 423 patients with a PD-L1 expression ≥5%, and here, nivolumab failed to demonstrate superiority over chemotherapy. The median PFS was 4.2 months in the group receiving nivolumab and 5.9 months in the group of patients who received chemotherapy (HR =1.15; 95% CI, 0.9–1.45; P=0.25). Similar results were obtained for OS, here, the group who received nivolumab achieved 14.4 months versus 13.2 months for the chemotherapy group (HR =1.02; 95% CI, 0.80–1.30).

Furthermore, the response rates (RR) were similar for the two groups, with 26% for nivolumab and 33% for chemotherapy. Based on the results from the CheckMate 026 trial the investigators concluded that nivolumab was not associated with a significantly longer PFS than chemotherapy in the selected group of NSCLC patients with a PD-L1 expression level of ≥5%. Likewise, OS was similar for the two treatment groups. However, with regard to safety the profile of nivolumab was more favorable than that of platinum-based chemotherapy.

The failure of the CheckMate 026 trial has been discussed in several articles, including the recent publication of the trial results in *New England Journal of Medicine* (1,7-9). As a main reason for the failure, the authors mention the imbalance in the baseline patient characteristics that favored chemotherapy; such as fewer liver metastases, smaller tumor burden, and a higher proportion of women in this group. Furthermore, a lower proportion of patients with high PD-L1 expression (≥50%) as well as high tumor mutation burden in the nivolumab group is likely important factors that have disfavored the efficacy of the immune checkpoint inhibitor. With regard to the data on OS, it is important to mention that 60% of the chemotherapy patients subsequently received nivolumab, which is likely to have influenced the relatively long survival for this group of patients. Another likely contributing factor to the negative study result compared to the KEYNOTE-024 trial with pembrolizumab is that a relatively larger proportion of the patients in the CheckMate 026 trial had previously received radiotherapy (7-9).

The PD-L1 IHC 28-8 pharmDx assay used to select the patients in the CheckMate 026 trial is a qualitative IHC assay using the monoclonal rabbit anti-PD-L1 clone 28-8 (1). In the USA, the assay has regulatory status as a complementary diagnostic, which is an assay that is not essential for the safe and effective use of the corresponding therapeutic product, but is able to identify a subset of patients that responds particularly well and can aid the risk/benefit assessment of the treatment (10). The PD-L1 IHC 28-8 pharmDx assay has likewise been used in both the CheckMate 017 and the CheckMate 057 trials, where nivolumab demonstrated to be superior to docetaxel in both squamous and non-squamous advanced NSCLC (2,3). In the CheckMate 017 trial, PD-L1 expression did not show to be predictive for the outcome of nivolumab. Here, the RRs range from 15% to 21%, more or less independent of the different levels of PD-L1 expression. However, in this study the tumor samples were not available from all enrolled patients, and for the group of patients where the samples could not be evaluated the RR was 39%. So, based on data from this study alone it might be difficult to conclude on the predictive value of PD-L1 expression in squamous NSCLC using the PD-L1 IHC 28-8 pharmDx assay (1,3). In the CheckMate 057 trial, patients with non-squamous NSCLC, who had progressed following treatment with platinum-based chemotherapy, were randomized to either nivolumab or docetaxel (2). Nivolumab demonstrated superiority over docetaxel for both OS and RR, and in contrast to the CheckMate 017 trial a statistically significant association between PD-L1 expression and clinical outcome was shown using the PD-L1 IHC 28-8 pharmDx assay. In the CheckMate 057 trial, this relationship was investigated for three expression levels; 1%, 5%, and 10%; however, in this interval the results showed only a modest increase in RR with an increase in the PD-L1 expression. For the PD-L1 expression level of ≥1% the RR was 31%, which increased to 36% and 37%, respectively, for the expression levels of ≥5% and ≥10%. In relation to the evaluation of the clinical cut-off for the assay it would have been desirable if the investigators had looked at higher PD-L1 expression levels than 10%, as approximately 80% of the responding patients where to be found here (1,2).

Pembrolizumab—KEYNOTE-024

So far, pembrolizumab is the only immune checkpoint inhibitor that has been approved for first-line treatment of advanced NSCLC, which was based on the results from the KEYNOTE-024 trial (6). In this study, 305 stage IV NSCLC patients with a PD-L1 tumor expression ≥50% measured by the PD-L1 IHC 22C3 pharmDx assay were randomized to receive pembrolizumab or platinum-based chemotherapy. The primary study endpoint was as in the CheckMate 026 trial PFS, and here, pembrolizumab showed to be superior to platinum-based chemotherapy. In the KEYNOTE-024 trial, the median PFS was 10.3 months in the pembrolizumab group and 6.0 months in the group of patients who received chemotherapy (HR =0.50; 95% CI, 0.37–0.68; P<0.001). The estimated OS at 6 months was 80.2% for pembrolizumab versus 72.8% for the chemotherapy group (HR =0.60; 95% CI, 0.41–0.89; P=0.005). A similar positive result was achieved for RR with 44.8% in the pembrolizumab group and 27.8% for the patients who received platinum-based chemotherapy. Based on the results of the KEYNOTE-024 trial the investigators concluded that pembrolizumab in patients with advanced

NSCLC and a PD-L1 expression ≥50% was associated with significantly longer PFS and OS. Furthermore, pembrolizumab was associated with fewer adverse events than with platinum-based chemotherapy (6).

The PD-L1 IHC 22C3 pharmDx assay used to select the patients in the KEYNOTE-024 trial is a qualitative IHC assay using the monoclonal mouse anti-PD-L1 clone 22C3 (1). In the USA, the assay has regulatory status as a companion diagnostic, which is an assay that provides information that is essential for the safe and effective use of a corresponding therapeutic product (10). According to the labeling for pembrolizumab it is mandatory to use the PD-L1 IHC 22C3 pharmDx assay to determine the level of PD-L1 expression before the drug is prescribed to the patients. When pembrolizumab obtained regulatory approval for treatment of second-line treatment of advanced NSCLC, the PD-L1 IHC 22C3 pharmDx assay was approved simultaneously. The clinical validation of this assay was performed during the KEYNOTE-001 trial, which was a large-scale "seamless" phase Ib trial (11). This trial enrolled 495 patients of whom approximately one third was assigned to a training group and two thirds to a validation group. The data from the training group was used to select the clinical cut-off for the assay. Based on receiver operating characteristic analysis (ROC) a tumor proportion score (TPS) ≥50% was selected, which corresponds to a PD-L1 membrane expression in at least 50% of the tumor cells. When the treatment outcome data from the validation group was analyzed, applying the selected clinical cut-off, the results showed that patients with a TPS ≥50% had a higher RR and longer PFS compared to patients with a TPS <50% (11,12). With the selected 50% cut-off value for the assay the area under the ROC curve was 0.743 corresponding to a sensitivity of 70.4% and a specificity of 79.0%. Furthermore, the Youden Index was calculated to be 0.494 (13). The data from the KEYNOTE-001 trial showed a clear positive correlation between PD-L1 expression and the efficacy of pembrolizumab with regard to RR (11,13). The clinical utility of the PD-L1 IHC 22C3 pharmDx assay has further been demonstrated in the second-line setting. In the KEYNOTE-010 trial pembrolizumab demonstrated superiority over docetaxel in NSCLC patients who had progressed after platinum-based chemotherapy (4). Also in this trial, the patients with the highest levels of PD-L1 expression (TPS ≥50%) experienced the best treatment outcome compared to the group of patients with a lower level of PD-L1 expression (TPS of 1–49%) (1).

Selection of the cut-off for the PD-L1 assays

The selection of the right clinical cut-off value for a companion or complementary diagnostic assay is a critical exercise in any drug-diagnostic co-development project (14). Through this process, the biomarker status is linked to the outcome of the drug, and looking at the way that the cut-offs have been selected for the PD-L1 expression assays linked to nivolumab and pembrolizumab, respectively, we find striking differences (1). For the PD-L1 IHC 22C3 pharmDx assay, linked to the use of pembrolizumab, the cut-off was selected based on well-described methodology using ROC analysis, whereas for the PD-L1 IHC 28-8 pharmDx assay, linked to the use of nivolumab, the methodology used is somewhat more unclear (1,10). However, comparing the cut-off levels for the two PD-L1 expression assays used in the CheckMate 026 trial and the KEYNOTE-024 trial it is important to remember that we are talking about difference assays and different drugs. The PD-L1 IHC 22C3 pharmDx, assay linked to pembrolizumab, has a cut-off of ≥50%, whereas for the PD-L1 IHC 28-8 pharmDx assay, linked to nivolumab, it is much lower; only 5%. A ten times higher cut-off value for the PD-L1 IHC 22C3 pharmDx assay compared to the PD-L1 IHC 28-8 pharmDx sounds quite dramatical, however, the two assays cannot be directly compared across the studies. Despite the assays using the same staining platform and visualization system the antibody clones in the two assays are different as are the drugs they are meant to guide. That being said, it would have been desirable if the investigators of the preceding Checkmate 057 trial had analyzed the outcome data with respect to cut-offs above the 10% PD-L1 expression level. Selection of the clinical cut-off for a companion diagnostic assay must be performed with great care, as mistakes can be a question of success or failure for the drug it is meant to guide, which might have been the situation for nivolumab in relation to the first-line NSCLC indication (1).

How to improve the predictability of the PD-1/PD-L1 checkpoint inhibitors

When it come to the sensitivity of the different IHC PD-L1 assays, it has been shown that some NSCLC patients with no or low PD-L1 expression also respond to treatment with the PD-1/PD-L1 immune checkpoint inhibitors. In the KEYNOTE-001 trial, approximately 10% of the patients with a TPS <1% responded to treatment with

pembrolizumab (11). Some of these test negative cases might be explained by PD-L1 expression heterogeneity in the tumor tissue. Another contributing factor could be the dynamic of PD-L1 expression and the fact that the IHC testing is performed on the original diagnostic tumor samples (15). Despite these possible shortcomings with regard to the sensitivity of the assays, most clinical studies conducted in patients with advanced stage NSCLC have shown a relatively clear link between PD-L1 expression and a positive outcome following treatment with the different PD-1/PD-L1 checkpoint inhibitors, which has also been confirmed in a recent meta-analysis (16).

Currently, the use of the PD-L1 expression assays for selection of NSCLC patients for treatment with a PD-1/PD-L1 immune checkpoint inhibitor is probably the best option available. However, we have to realize that these assays are not perfect companion diagnostics for this type of therapy and the predictive properties need to be improved, which could be through a combination with other types of biomarkers. One such biomarker could be tumor mutation burden, and in fact, data from the CheckMate 026 trial indicates that this might be feasible (7,17). As part of this trial, an exploratory analysis was conducted in 312 randomized patients, in order to assess the effect of tumor mutation burden on treatment outcome. The result of this analysis showed that patients with high tumor mutation burden had a higher RR when treated with nivolumab compared to chemotherapy and also a longer PFS. Furthermore, when the tumor mutation burden was combined with PD-L1 expression, some interesting results emerged for the patients treated with nivolumab. For the groups of patients with a high mutation-burden and a PD-L1 expression ≥50% the RR was as high as 75%, which was much higher than those with only one of these factors present. Patients with a high mutation-burden had a RR of 32% and for the patients with a PD-L1 expression ≥50% the RR was 34% so the combination resulted in a clear additive effect. Despite these interesting results, it should be emphasized that the data is exploratory and according to the investigators they are not powered for statistical analysis, so confirmations from other clinical trials are needed (7). Predictive biomarkers for the immune checkpoint inhibitors may also be found outside the tumor. A couple of recent articles published in *Science* indicate that the gut microbiome composition can influence and modulate the efficacy of the PD-1 immune checkpoint inhibitors (18,19). In the search of accurate predictive biomarkers for the immune checkpoint inhibitors several other approaches have been suggested, such as immune gene signatures, T-cell receptor clonality, as well as several plasma biomarkers, so the possibility of combining different modalities will increase in the future (20).

Until now, the drug-diagnostic co-development model has, more or less, been built, on a "one drug one biomarker" scenario; however, when it comes to the different types of immuno-oncology treatments a multiplex approach integrating information from different molecular sources will most likely be needed in order to develop companion diagnostics with higher predictive properties.

Despite our editorial has discussed aspects in relation to drug development, companion diagnostics are not only important during this phase but likewise an essential treatment decision tools after the approval of the drug. The companion diagnostic assay has the individual patient as a point of reference and they are decisive for the move toward a more individualized pharmacotherapy and an important element in the realization of precision medicine (21).

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Footnote

Conflicts of Interest: JT Jørgensen has worked as a consultant for Agilent, Euro Diagnostica, Oncology Venture and Azanta and has given lectures at meetings sponsored by AstraZeneca, Merck Sharp & Dohme, and Roche. KB Nielsen is a former employee of Agilent.

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