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Dear Dr. Chiumente,

Thank you for all of your work in the revision of your manuscript. It is a pleasure to accept your manuscript entitled "First line chemoimmunotherapy versus chemotherapy in PD-L1 negative patients with non-small-cell lung cancer" in its current form for publication in Immunotherapy.
Article title: First line chemoimmunotherapy versus chemotherapy in PD-L1 negative patients with non-small-cell lung cancer: a brief report to compare efficacy evaluated by restricted mean survival time and network meta-analysis

Short running title: RMST in PD-L1 negative patients with non-small-cell lung cancer

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FIRST LINE CHEMOIMMUNOTHERAPY VERSUS CHEMOTHERAPY IN PD-L1 NEGATIVE PATIENTS WITH NON-SMALL-CELL LUNG CANCER

Abstract:

Background: In PD-L1 negative patients with advanced non-small cell lung cancer (NSCLC) conclusive evidence in support of specific treatments is still lacking.

Objectives: The objective of our study was to compare the efficacy of first line chemoimmunotherapy versus chemotherapy alone.

Data sources: PubMed

Study eligibility criteria, participants, and interventions: We identified eligible randomized studies that included patients with advanced NSCLC patients irrespective of PD-L1 status who were treated with chemoimmunotherapy as first line.

Study appraisal and synthesis methods: We extracted the Kaplan-Meier curves and analyzed them using restricted mean survival time (RMST). Patient-level data were reconstructed from progression-free survival (PFS) graphs. A Bayesian network meta-analysis (NETMA) was carried out on the values of RMST.

Results: In 5 trials selected, chemoimmunotherapy regimens, compared with chemotherapy alone, determined an improvement in PFS without statistical significance. In our NETMA, chemoimmunotherapy was found to slightly improve PFS

Limitations: Proportion of PDL-1 negative patients across the trials

Conclusions: Our analysis showed that, in these patients, the incremental benefit of chemoimmunotherapy vs chemotherapy is limited.

Keywords: Non-small-cell lung cancer; Chemoimmunotherapy; Restricted Mean Survival Time; PD-L1 negative; Network meta-analysis

Introduction
Although the standard of care in first-line treatments of patients with advanced non-small cell lung cancer (NSCLC) includes platinum-based chemotherapy, the efficacy of these treatments is limited [1,2]. Inhibitors of programmed death 1 (PD-1) and its ligand PD-L1 have an important role in this disease condition, but their place in therapy still needs to be defined [3-5].

Single-agent pembrolizumab is currently the standard of care in patients with a high level (≥50%) of PD-L1 expression [6]. However, patients with a tumor proportion score of 50% or greater represent a minority of those with NSCLC. The combination of PD-1 or PD-L1 inhibitors with chemotherapy has also improved outcomes in patients with NSCLC [7-11]. These randomized clinical trials (RCTs) have generally stratified patients according to PD-L1 tumor proportion score or PD-L1 expression evaluated by immunohistochemistry. However, the proportion of PD-L1-negative patients varied between cohorts, ranging from 17% to 52% of total patients [9,11]. Across all PD-L1 subgroups, chemoimmunotherapy improves outcomes compared with chemotherapy alone, but there is an association between greater PD-L1 expression and longer progression-free survival (PFS). More recently, the combination of nivolumab with ipilimumab has proved to be superior to chemotherapy alone in advanced NSCLC regardless of PD-L1 expression [12]. Hence, in PD-L1 negative subgroups (PD-L1 score of less than 1%), both immunotherapy (as either a single agent or with two agents combined) and chemoimmunotherapy have been studied as first-line, but their place in therapy remains unsettled.

The objective of the present study was to compare the efficacy of first-line chemoimmunotherapy versus chemotherapy alone in PD-L1 negative patients with advanced NSCLC. RCTs available on this topic were analyzed using restricted mean survival time (RMST) as outcome measure. RMST, which is determined by measuring the area under the Kaplan-Meier survival curve, can be defined the average event-free survival time up to a pre-specified, clinically important time point. A bayesian network meta-analysis (NETMA) was carried out to evaluate direct and indirect comparisons across treatments.

**Materials & Methods**

We conducted a literature search to identify the eligible RCTs. This search was conducted in PubMed (last query on May 20, 2021) and covered the period from January 2010 to present date. A search string "Carcinoma, Non-Small-Cell Lung"[Majr] AND ("immunotherapy" OR "nivolumab" OR "atezolizumab" OR "pembrolizumab") was employed in combination with filter "randomized controlled trials". We also searched the Cochrane Library and the ClinicalTrials.gov database. Our analysis included trials that met the following criteria: a) patients with untreated NSCLC irrespective of PD-L1 status; b) randomized design; c) chemoimmunotherapy as experimental arm; d) determination of progression-free
survival (PFS) in patients with PD-L1 negative status based on follow-up of at least 15 months and presented through a Kaplan-Meier curve.

The three phases in the analysis of each curve were the following: i) retrieving the published graphs; ii) estimating the survival percentage-vs-time data points with a digitizer [13] iii) reconstructing patient-level data. For this purpose, we used an iterative least squares technique that performs this reconstruction from total number of events, total number of at-risk patients, and distribution of at-risk patients over time. Goodness of fit between the original curve and the reconstructed one was assessed by root mean squared error. After reconstructing each curve, the “survRM2” package was run under the R-platform to determine the value of RMST along with its 95%CI. For this purpose, the so-called milestone was introduced into the R model to indicate the time-point in the follow-up at which the analysis is “restricted” or truncated. To extend the survival analysis over a lifetime horizon, we also determined the mean lifetime survival (MLS) from the same Kaplan-Meier curves examined to determine RMST. MLS was modeled according to the Weibull distribution.

A Bayesian NETMA has the purpose to carry out all direct and indirect comparisons between the treatments. The endpoint was represented by the RMST which was handled as a continuous parameter. Binary comparisons were represented as mean difference (MD) of the two RMST values, along with 95% credible interval [CrI]. The estimation approach implemented by this type of NETMA is iterative and employs Monte Carlo iterations. All the analyses were conducted using the software package WinBUGS 1.4.3 (Cambridge, UK) in combination with the meta-analysis code developed by the National Institute for Health and Care Excellence (NICE).

A Bayesian NETMA can be handled through either the fixed-effect model or the random-effect model. The random-effect model is designed for data sets characterised by some degree of heterogeneity in the clinical material whereas the fixed-effect model is preferred when heterogeneity is low. To decide between these models, we used the deviance information criterion (DIC). To rank effectiveness across the comparators, we used the surface under the cumulative ranking curve (SUCRA) approach. The so-called “simplified figure” is the iconographic method employed in our NETMA; this graphical tool presents the network geometry along with the results of the analysis. Finally, detailed references about the above-mentioned statistical and iconographic methods are reported in a preprint published by Messori [14].
Results

Our literature search extracted a total of 2,704 eligible studies, of which 5 RCTs met our inclusion criteria [7-11]. The PRISMA schematic is reported in Figure 1S of the Supplementary material. In these 5 trials (Table 1), patients with PD-L1-negative tumours constituted a subset of the entire populations and were a quite small minority in particular studies.

In conducting our NETMA, firstly we separately ran the fixed-effect and the random-effect models. According to DIC analysis, the random-effect model showed a better fit (DIC 20.810 for the random-effect model versus 31.204 for the fixed-effect model; Figure 2S of the Supplementary material). The values of RMST estimated from the 5 trials (with milestone set at 15 months) are shown in Table 1. The graphical results of our network meta-analysis are shown in Figure 1 along with the geometry of direct and indirect comparisons. According to our results, both pembrolizumab plus chemotherapy and atezolizumab plus chemotherapy did not determine a significant improvement in PFS compared with chemotherapy alone (mean difference: 1.65 mos, 95%CI -1.76 to 5.16, and 1.32 mos, 95%CI -1.50 to 4.22, respectively). The trial-specific gains in PFS determined by chemoimmunotherapy vs chemotherapy alone ranged from 0.08 months for the trial by Paz-Ares et al.[9] to 3.25 months for the trial by Gandhi et al.[7]. Some differences instead emerged across the PFS values for the 5 control groups (Table 1).

The values of MLS offer another perspective about the expected survival gains on the long term. However, variability in MLS was wide, likely because the follow-up of these patients was limited. Ranking the values of RMST generated the results shown in Table 1 and Figure 2. In the comparison between the treatment that ranked best (pembrolizumab plus chemotherapy from KEYNOTE189 trial) and the one that ranked worst (chemotherapy alone from IMPOWER-130), the difference was 4.26 months.

Discussion

Our NETMA in previously untreated patients with PDL-negative advanced NSCLC examined 5 randomized trials and found that chemoimmunotherapy improved PFS compared with chemotherapy alone; however, the magnitude of incremental benefit was small. The statistical significance of this improvement was borderline in our analysis, because significance was reached using the fixed effect meta-analytic model, but not using the random effect one. Since this latter model showed a better fit based on the DIC criterion, it was considered more reliable. Indeed, in the 5 original trials significance (determined according to the hazard ratio[HR] from primary patient-level data) was reached in three cases [8,9,11], and not in the remaining two [7,10]. To better interpret these puzzling results, one important point is that the HR is known to over-estimate significance in comparison with the RMST [15-17]. The results of our NETMA based on RMSTs, on the one hand, were not exposed to biases due to different lengths of
follow-up and, importantly, were expressed in terms of an absolute outcome measure (PFS survival gain), and not a relative one (HR). On the other hand, since our results were more conservative than those suggested by the 5 trials, this finding from our analysis is consistent with the above-mentioned tendency of HR to over-estimate the incremental benefit. Based on the evidence emerging from a growing literature [15-17], the opposite conclusion (i.e. the tendency of RMST to under-estimate incremental benefits) seems to be unlikely.

In summary, while significance was at limits depending on the statistical approach employed, the values of PFS gain conveyed a clear message in that the magnitude of the incremental benefit was small (with trial-specific improvements ranging from 0.08 to 3.25 months). Therefore, the clinical relevance of incremental benefit of chemoimmunotherapy in this disease condition seems to be questionable when the PD-L1 status is negative.

Our study had several limitations. For example, those related to the differences in the proportion of PDL-1 negative patients across the trials included in our analysis. Likewise, there were differences in TPS score across the trials. Another issue is the difficulty in comparing studies that included different PDL-1 assays.

Conclusion

Although chemoimmunotherapy is generally considered the current standard for these patients, our analysis based on a more conservative statistics (RMST as opposed to HR) showed that the incremental benefit of chemoimmunotherapy vs chemotherapy is limited, especially in terms of clinical relevance. Our findings are in line with those of a very recent NETMA conducted irrespective of PDL status of the patients [18].

Interestingly enough, in another recent meta-analysis by Herbst et al. [19] that included patients with high PD-L1 expression, chemoimmunotherapy showed significantly longer expected PFS versus chemotherapy in the non-squamous histology, whereas the increases were not significant in the mixed or squamous disease. Our results are in substantial agreement with Herbst et al. [19] because the PFS gain that we found in non-squamous histology (1.55 and 2.17 months according to IMPOWER-130 [8] and IMPOWER-132 [11], respectively; see Table 1) was more pronounced than in squamous histology (0.08 and 0.38 months according to KEYNOTE-407 [9] and IMPOWER-131 [10], respectively; see Table 1). Despite the small magnitude of these gains, our results however confirm that non-squamous histology has a better response to chemoimmunotherapy than squamous histology.

Other treatment options urgently need to be investigated. Among these, the combination of two immunotherapy agents without chemotherapy has recently been studied in two trials [12, 20], but their results are conflicting. In fact, while the combination of nivolumab plus ipilimumab [12] determined a remarkable gain in PFS of 4.03 months compared with chemotherapy alone (gain estimated according to RMST difference), the combination of durvalumab plus tremelimumab gave the opposite result because no gain in PFS was observed [19].
We conclude that the role of chemoimmunotherapy or immunotherapy is very limited or absent in patients with advanced NSCLC with PD-L1 negative status. In contrast, the effectiveness of these agents remains unquestionable in patients whose PDL expression is greater than 50% [1-3], and particularly if their histology is non-squamous [19]. On the other hand, nivolumab plus ipilimumab [12] might be an adequate option in patients with PDL-negative tumors, but further confirmation based on new trials is still needed.

**Future Perspective**

For patients with negative PD-L1 expression, no definite optimal therapeutic strategy has been defined. The combination of two immune checkpoint inhibitor with anti-angiogenesis drugs or chemotherapy might be an effective therapeutic strategies for this patient population. Further research is needed, particularly phase III RCTs comparing different treatment options in PD-L1–negative patients.

**Summary Points**

- The combination of PD-1 or PD-L1 inhibitors with chemotherapy or two immunotherapy agents has proved to be superior to chemotherapy alone in advanced NSCLC. Hence, in PD-L1 negative subgroups (PD-L1 score of less than 1%), both immunotherapy (as either a single agent or with two agents combined) and chemoimmunotherapy have been studied as first-line, but their place in therapy remains unsettled.
- The objective of the present study was to compare the efficacy of first-line chemoimmunotherapy versus chemotherapy alone in PD-L1 negative patients with advanced NSCLC. RCTs available on this topic were analyzed using restricted mean survival time (RMST) as outcome measure.

**Methods**

We used the RMST for analyzing survival curves. A bayesian network meta-analysis (NETMA) was carried out to evaluate direct and indirect comparisons across treatments.

**Results**

Our NETMA in previously untreated patients with PDL-negative advanced NSCLC examined 5 randomized trials and found that chemoimmunotherapy improved PFS compared with chemotherapy alone; however, the magnitude of incremental benefit was small.

**Discussion /Conclusion**
The effectiveness of chemoimmunotherapy or immunotherapy is well documented in patients with advanced PDL-positive NSCLC (PDL expression>50%). On the other hand, nivolumab + ipilimumab might be an adequate option in patients with PDL-negative tumors, but further confirmation based on new trials is still needed.

**Figure/Table legends**

Figure 1. Iconographic representation of the results of our NETMA: two trials were analyzed for pembrolizumab plus chemotherapy, and three for atezolizumab plus chemotherapy

Figure 2. Graphical presentation of RMST values estimated from included trials. All values in months. Treatment arms, in blue; control arms, in green. Vertical lines with tick marks (in black) indicate 95% confidence intervals.

Table 1. Characteristics of the 10 cohorts and values of RMST estimated from the time-to-event curves. All survival data refer to freedom from progression. See also Figure 2.

**References:**


Reference annotations:


The above 5 references are of particular significance because the data on PFS were extracted from these trials.


This reference reported the statistical and iconographic methods used in our study.
pembrolizumab+ chemotherapy

ND(t)

ND

MD=0.33 (95%Crl -4.18 to 4.81)

ND(t)

化疗

MD=1.85 (95%Crl -1.76 to 5.16)

MD=1.32 (95%Crl -0.30 to 4.22)

atezolizumab+ chemotherapy

ND(t)

ND

Legend

- direct comparison
- indirect comparison
- ND: no difference
- ND(t): no difference (favoured by a trend)
- Crl: credible interval
- MD: mean difference