REVIEW

Sex-based differences in response to anti-PD-1 or PD-L1 treatment in patients with non-small-cell lung cancer expressing high PD-L1 levels. A systematic review and meta-analysis of randomized clinical trials

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Background: In our previous works, we demonstrated that patients' sex affects the efficacy of immune checkpoint inhibitors (ICIs) in patients with several advanced solid tumors. Here, we assessed the sex-based heterogeneity of efficacy of anti-programmed cell death protein 1 (anti-PD-1)/anti-programmed death-ligand 1 (anti-PD-L1) given as monotherapy, for advanced non-small-cell lung cancer (NSCLC) expressing high PD-L1 levels, to evaluate if available evidence supports this therapeutic option for both women and men.

Methods: We carried out a systematic review and meta-analysis including all randomized, controlled trials testing anti-PD-1/anti-PD-L1 drugs in monotherapy, as first-line treatment of advanced NSCLC expressing high PD-L1 levels. The primary endpoint was the difference in efficacy of anti-PD-1/anti-PD-L1 drugs versus chemotherapy, between men and women, measured in terms of the difference in overall survival (OS) log [hazard ratio (HR)] reported in male and female study participants.

Results: We analyzed four randomized, controlled trials, including 1672 patients, of whom 1224 (73.2%) were men and 448 (26.8%) were women. The pooled OS-HR comparing anti-PD-1/anti-PD-L1 versus chemotherapy was 0.59 [95% confidence interval (CI), 0.50-0.69] for men and only 0.84 (95% CI, 0.64-1.10) for women. The pooled ratio of the OS-HRs reported in men versus women was 0.71 (95% CI, 0.52-0.98; P-heterogeneity: 0.04), indicating a significantly greater effect for men. No heterogeneity among single-study estimates was observed in either male patients ($Q = 2.39$, $P = 0.50$, $I^2 = 0\%$) or in female patients ($Q = 1.13$, $P = 0.50$, $I^2 = 0\%$).

Conclusion: Evidence available indicates anti-PD-1/anti-PD-L1 monotherapy as highly effective in men but not in women, even in NSCLCs expressing high PD-L1 levels. Prospective trials testing sex-based tailored immunotherapy strategies are needed.

Key words: NSCLC, high PD-L1 expression, immune checkpoint inhibitors

INTRODUCTION

The biological differences between men and women can potentially affect immune responses to both foreign and self-antigens.[1] On average, women mount stronger innate and adaptive immune responses than men.[1] These sex-based differences reflect complex interactions between genes, hormones, environment, and commensal microbiome composition.[2-5]

As a result, the lower severity and prevalence of many infections in women seems to rely on a more rapid clearance of pathogens.[6-8] In addition, women tend to show greater responses to vaccination than men, likely due to an increased production of antibodies.[6,7] As a downside, about 80% of all patients with systemic autoimmune diseases are women.[9,10]

Different immune profiles between the two sexes could also be relevant to the natural course of cancer.[11,12] In fact, men display an almost twofold risk of mortality from all cancers in comparison with women. Specifically, the greatest differences have been described in melanoma, lung, larynx, esophagus, and bladder cancers.[11,12] In this regard, not only diversity in hormonal and, in general, biological factors could account for these differences, but also in the immune system status.[12]

In this sense, immune co-stimulatory/inhibitory pathways such as cytotoxic T-lymphocyte protein 4 (CTLA-4) and

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programmed death receptor-1/programmed death-ligand 1 (PD-1/PD-L1) pathways play a role in the tumor-induced immunosuppression, to the point that they have been exploited as therapeutic targets in several types of advanced malignancies.[13,14] Consistently with previous observations, sex-hormone modulation of the PD-1/PD-L1 pathway has been described in animal studies.[1,15-18] In addition, sex hormones can both regulate the expression of PD-1 and PD-L1 and affect the PD-1/PD-L1 pathway.[15-17]

Besides the mentioned sex dimorphism in cancer-related immunity, a second remark is related to sex dimorphism in cancer biology. A high tumor mutational burden (TMB) is a well-known predictor of benefit from immune checkpoint inhibitors (ICIs) across multiple, yet not all, cancer types.[19,20] Interestingly, a significantly higher TMB has been reported in male patients' tumors of several histotypes, including melanoma and non-small-cell lung cancer (NSCLC), even after adjusting for sex differences in age at diagnosis, stage of disease, smoking status, and other relevant variables that may correlate with TMB.[21-24]

Additionally, exposure to several mutagenic factors, such as ultraviolet light for melanoma and tobacco for NSCLC, share strong co-associations with higher TMB and increased ICIs efficacy.[24] In this regard, in spite of the recent surge of the smoking habit among women, men display a higher prevalence of tobacco use, whereas women tend to show sun-protective behaviors, such as the application of sun-screen skin protection.[25,26]

We previously demonstrated that patients' sex affects efficacy of ICIs in patients with several advanced solid tumors.[1] In a systematic review and meta-analysis of 20 randomized, controlled trials (RCTs) testing anti-CTLA-4 or anti-PD-1 as monotherapy versus control arms not containing immunotherapy, including >11 000 patients with several types of advanced solid tumors (mainly NSCLC and melanoma), we demonstrated that ICIs can improve overall survival (OS) for patients of both sexes, but men have a significantly larger treatment effect from these drugs versus control treatments than women. This magnitude of difference in benefit was clinically relevant: the pooled reduction of risk of death was double the size for males than for female patients. Importantly, the sex-based heterogeneity of ICIs efficacy holds across all the subgroups explored, including cancer histotype, type of ICI tested, and lines of treatments.

Such a relevant role exerted by sex in modulating the response to anticancer immunotherapy has been further demonstrated by Litchfield et al[27]: in a large-scale analysis of individual level data of >1000 patients treated with ICIs, patients' sex showed a significant predictive value of response to ICIs across tumor types, independently of a number of tumor molecular features, including TMB, tumor heterogeneity, abundance of tumor-infiltrating lymphocytes and tumor genetic and epigenetic alterations known to mediate resistance to immunotherapy.

Given the complexity of the sex dimorphism of the immune system function and responses, it is possible that women derive larger benefit than men from immunotherapeutic strategies different than ICI monotherapy.[6] As a matter of fact, in the context of advanced NSCLC we showed that while women derive significantly lower survival benefit than men when treated with anti-PD-1 monotherapy, they experience a much larger survival benefit from the combination of chemotherapy plus anti-PD-1/anti-PD-L1 drugs.[28]

A limit of our previous works is represented by the fact that we did not explore whether the sex-based heterogeneity of ICIs efficacy observed in patients with NSCLC holds true also in the subgroup of tumors expressing high PD-L1 levels. This point is of paramount clinical relevance, since the current guidelines for first-line treatment of NSCLC suggest treatment with anti-PD-1/anti-PD-L1 drugs as monotherapy for patients with tumors expressing high PD-L1 levels—i.e. tumor proportion score (TPS) $\geq$50% or tumor cell 3 (TC3) or tumor-infiltrating immune cell 3 (IC3)—and the combination of chemotherapy plus ICIs for tumors characterized by low or null PD-L1 expression.[29] For these reasons, we assessed the sex-based heterogeneity of efficacy of anti-PD-1 or anti-PD-L1 drugs as monotherapy, specifically in the group of PD-L1-high NSCLCs, to evaluate if available evidence supports such a therapeutic option for both female and male patients.

METHODS

We carried out a systematic review and a meta-analysis of all RCTs testing anti-PD-1/anti-PD-L1 antibodies in monotherapy as first-line treatment in patients with advanced NSCLC expressing high PD-L1 levels. We followed the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) guidelines. We searched PubMed, Embase, and Scopus for RCTs testing anti-PD-1/anti-PD-L1 drugs versus chemotherapy, as first-line therapy in patients with advanced NSCLC, from the inception of each database to 19 February 2021.

We also reviewed abstracts and presentations from all major conference proceedings, including the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), European Lung Cancer Conference, and World Conference on Lung Cancer from 1 January 2010 to 19 February 2021.

Two investigators (FC and LP) independently searched the databases. The search terms were 'PD-1', 'PD-L1', 'nivolumab', 'pembrolizumab', 'avelumab', 'durvalumab', 'atezolizumab', 'cemiplimab', 'spartalizumab'. We included only the most recent and complete report of the RCT when duplicate publications were identified. To be eligible, RCTs had (i) to assess anti-PD-1 or anti-PD-L1 drugs versus chemotherapy as first-line therapy, in patients with advanced NSCLC and (ii) to have data available for assessment of the hazard ratio (HR) for death according to patients' sex for the subgroup of tumors expressing high PD-L1 levels (i.e. TPS $\geq$50% or IC3 or TC3).

From each study, two investigators (FC and LP) extracted the name of the study, first author and year of publication,

study design and blinding, study phase, number of patients, patients' age, patients' smoking status and Eastern Cooperative Oncology Group (ECOG) score, sex distribution, median follow-up time, study drugs, HR for death in overall population, and HR according to patients' sex. The primary endpoint was the difference in efficacy of anti-PD-1/anti-PD-L1 drugs versus chemotherapy, between men and women, measured in terms of the difference in OS log(HR) reported in male and female study participants.

We derived the HRs for death in the intervention group and the control group, and their 95% confidence intervals (CIs) from each study, separately for male and female patients. We calculated the pooled HR of death in men and women using a fixed-effects model. We assessed the heterogeneity between the two estimates using an interaction test, to give the P-heterogeneity. We did the Q test to assess between-study heterogeneity, and calculated the I^2 statistic, which expresses the percentage of the total observed variability due to study heterogeneity.

To avoid the risk of ecological bias, the null hypothesis that the difference of treatment effect between women and men is zero was tested using the following approach: first, a trial-specific ratio of OS-HRs was calculated from the ratio of the reported OS-HRs in men and in women; second, these trial-specific ratios of OS-HRs were combined across trials using a fixed-effects model.[1] A pooled estimate of the ratios of OS-HRs <1 indicates a greater treatment effect in men, and >1 a greater effect in women. All reported P values are two-sided. We did all analyses using R (version 3.4.0).

RESULTS

Four phase III RCTs fulfilled all the inclusion criteria and were analyzed (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2021.100251).[30-33] Two RCTs (i.e. KEYNOTE-024 and EMPOWER-Lung 1) enrolled exclusively patients with tumors expressing high PD-L1 levels, while the other two (i.e. KEYNOTE-042 and IMpower110) enrolled patients irrespective of PD-L1 tumor levels, but OS in the subgroup of tumors with high PD-L1 levels was a prespecified primary endpoint.[30-33] The trial design and features of populations enrolled across the four RCTs were very similar, as were the final results reported (Table 1). Our analysis included 1672 patients, of whom 1224 (73.2%) were men and 448 (26.8%) were women; 1102 (65.9%) patients had non-squamous, and 570 (34.1%) squamous NSCLC; 1493 (89.3%) patients were former or current cigarette smokers, and only 179 (10.7%) were never cigarette smokers (Table 1). All these trials enrolled only epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) wild-type tumors.

Men treated with anti-PD-1/anti-PD-L1 drugs had a significant reduced risk of death compared with men treated with chemotherapy in the control arm (pooled OS-HR = 0.59, 95% CI 0.50-0.69; Figure 1A). In women, the benefit obtained with anti-PD-1/anti-PD-L1 drugs compared with the control arm was smaller and not statistically significant

Table 1. Characteristics of trials included in the analysis

Trial	Experimental arm	Control arm	Men	Women	Non-squamous tumor	Squamous tumor	Current or former smokers	Never smokers	ECOG 0	ECOG 1	Age <65 years	Age ≥65 years	HR-OS (95% CI)	Median FUP in months (IQR)	PD-L1 high definition	IHC Assay
KEYNOTE-042	Pembrolizumab 200 mg flat dose	Platinum-based chemotherapy	415	184	378	221	468	131	187	412	328	271	0.69 (0.56-0.85)	12.8 (6.0-20.0)	TPS ≥50%	22C3 pharmDx
KEYNOTE-024	Pembrolizumab 200 mg flat dose	Platinum-based chemotherapy	187	118	249	56	281	24	107	198	NA	NA	0.63 (0.47-0.86)	25.2 (20.4-33.7)	TPS ≥50%	22C3 pharmDx
IMpower110	Atezolizumab 1200 mg flat dose	Platinum-based chemotherapy	143	62	155	50	181	24	73	132	102	103	0.59 (0.40-0.89)	15.7 (0-35)	TC3 and/or IC3	SP142 Ventana
EMPOWER-Lung 1	Cemiplimab 350 mg flat dose	Platinum-based chemotherapy	479	84	320	243	563	excluded	152	411	304	259	0.57 (0.42-0.77)	10.8 (7.6-15.8)	TPS ≥50%	22C3 pharmDx

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; FUP, follow up; HR, hazard ratio; IC3, immune cell 3; IHC, immunohistochemistry; IQR, interquartile range; OS, overall survival; PD-L1, programmed death-ligand 1; TC3, tumor cell 3; TPS, tumor proportion score.
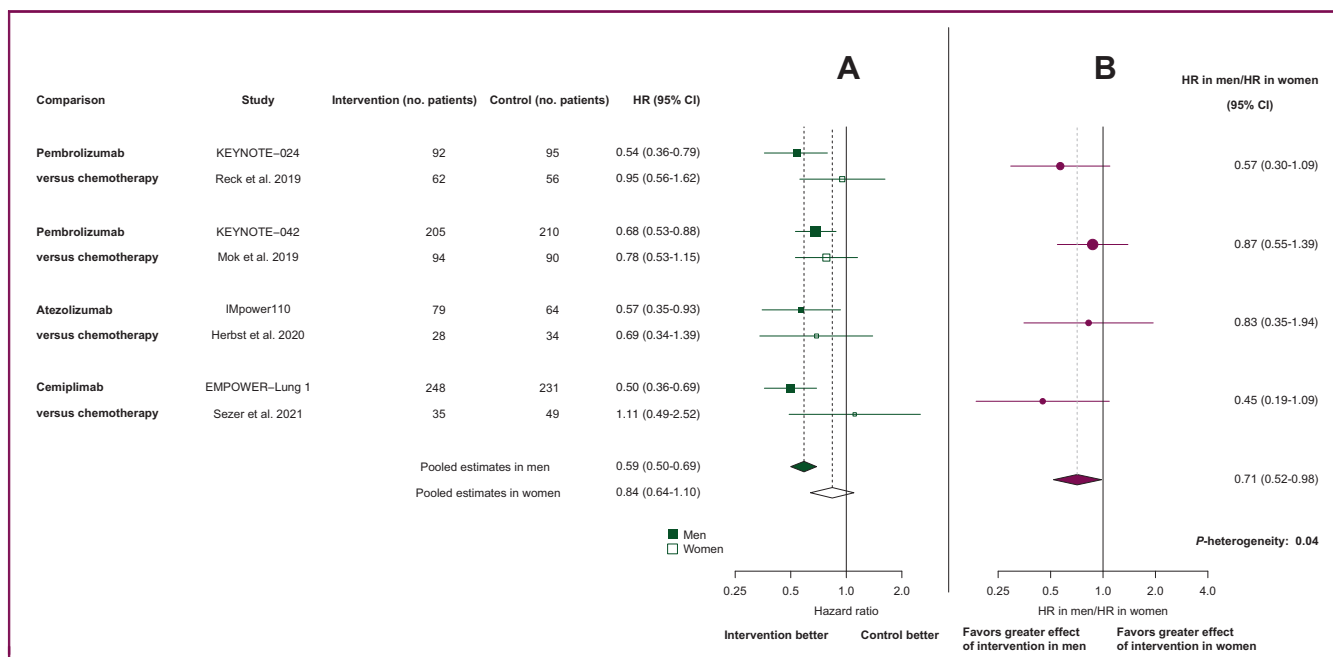
Figure 1. Hazard ratios of death according to patients' sex.

(A) Figure shows the hazard ratios (HRs) of death for patients assigned to intervention treatment [i.e. anti-programmed cell death protein 1 (anti-PD-1) or anti-programmed death-ligand 1 (anti-PD-L1 drugs)], compared with those assigned to control treatment (i.e. platinum-based chemotherapy), according to sex. Squares indicate study-specific HR. Values <1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate (i.e. the inverse of the variance). Horizontal lines indicate the 95% confidence interval (CI). Diamonds indicate the meta-analytic pooled HRs, calculated separately in women and men, with their corresponding 95% CIs. The dashed vertical lines indicate the sex-specific pooled HRs, and the solid vertical line indicates an HR of 1, which is the null-hypothesis value (i.e. no association between type of treatment and risk of death). (B) Figure shows the interaction between treatment efficacy and sex. Each filled circle indicates the study-specific ratio of HRs, that is, the ratio of the reported HRs in men and in women. Values >1 indicate that the effect of the intervention compared with control is greater for women than for men. Size of the circle is proportional to the precision of the estimate (i.e. the inverse of the variance). Horizontal lines indicate the 95% CI. The diamond indicates the meta-analytic pooled ratio of HRs, with its corresponding 95% CI. The dashed vertical line indicates the pooled ratio of HRs, and the solid vertical line indicates a pooled HR of 1, which is the null-hypothesis value (i.e. no difference between men and women regarding the efficacy of anti-PD-1 or PD-L1 drugs).

(pooled OS-HR = 0.84, 95% CI 0.64-1.10; Figure 1A). No heterogeneity among single-study estimates was observed in either male patients ($Q = 2.39$, $P = 0.50$, $I^2 = 0\%$) or in female patients ($Q = 1.13$, $P = 0.50$, $I^2 = 0\%$). In each of the four RCTs, the survival benefit observed in men treated with immunotherapy was larger than that observed in women. The pooled ratio of OS-HRs reported in men versus those reported in women in each trial was 0.71 (95% CI, 0.52-0.98, P-heterogeneity: 0.04; Figure 1B), indicating a statistically significant larger benefit in men compared with women.

DISCUSSION

These results demonstrate a statistically significant and clinically meaningful sex-based heterogeneity of efficacy of anti-PD-1/anti-PD-L1 antibodies even in patients selected for tumors highly responsive to ICIs. Such heterogeneity of efficacy strongly penalizes female patients, who obtained a very limited benefit compared with a poor control arm, such as platinum-based chemotherapy.

Previously, we showed that women with advanced NSCLC derived impressive survival benefit when treated with the combination of chemotherapy and anti-PD-1/anti-PD-L1 drugs, that represents a current available first-line treatment option for patients with advanced NSCLC regardless of PD-L1 expression levels.[28,29]

Taken together, the results reported here and in our previous work[28] support sex as a relevant variable that should be taken into account to choose the best treatment option for patients with advanced NSCLC expressing high PD-L1 levels: while anti-PD-1/anti-PD-L1 drugs given in monotherapy were confirmed as highly effective in men, the modest results consistently reported for women in all the RCTs induce consideration of their combination with chemotherapy as the best treatment option for female patients.

Strength points of our analysis are that data analyzed derived from large RCTs with similar trial design and populations enrolled, and the consistent pattern of results observed in the patients' sex subgroups across all trials, led to a complete absence of heterogeneity among single-study estimates (i.e. $I^2 = 0$ for both male and female subgroups). Furthermore, our results are based on the data from randomized comparisons from RCTs. The issue of confounding in subgroup analyses from RCTs is subtle. As reported by VanderWeele and Knol[34]: 'the effect of treatment within subgroups will not be confounded because treatment is randomized; but the effect of the secondary factor defining subgroups might be confounded since it is not randomized'. This means that we can safely say that the effect of ICIs is different between males and females. On the contrary, we cannot conclude that the observed difference is due to a

causal effect of sex *per se*, but relevant biological and physiological differences between men and women strongly support such a hypothesis. It is well known that sex (i.e. the biological differences between men and women) and gender (i.e. behavioral differences associated with being male or female) are variables that affect immune responses to both foreign and self-antigens.[2,6]

The aforementioned sex-based dimorphism of immune system function could account for our results.[6] For example, the X chromosome contains a large number of immune-related genes.[2] These genes encode proteins involved in the regulation of the innate immunity, like pattern recognition receptors [e.g. Toll-like receptor 7 and 8 (TLR7 and TLR8)], as well as in the regulation of adaptive immunity, including cytokine receptors [e.g. interleukin 2 receptor subunit gamma, (IL-2RG) and interleukin 13 receptor subunit alpha-2 (IL-13RA2)] and key transcriptional factors [e.g. forkhead box P3 (FOXP3)].[2] Immune-related genes encoded on the X chromosome may escape X inactivation resulting in higher expression levels in immune cells of females than males.[2] Sex hormones constitute another major determinant of sex difference in immunity.[6] They modulate the development and function of multiple immune cell populations, shaping innate and adaptive immune responses.[6] Indeed, sexual dimorphism in immune responsiveness is most accentuated after puberty. Hormonal fluctuations accompanying the menstrual cycle, pregnancy, and menopause have a deep impact on susceptibility to infectious disease and autoimmunity.[6,35] As anticipated, sex hormones exert potent effects on the regulation of a broad number of immune-related genes in multiple immune cell subsets and, recently, a role for sex hormone modulation of the PD-1/PD-L1 pathway has emerged.[15,17]

Details on the molecular mechanisms underlying sex-based differences in responsiveness to ICIs have been recently characterized in mice models, such as an estrogen-mediated recruitment of myeloid derived suppressive cells (MDSCs), known to be involved in resistance to ICIs, in the tumor microenvironment (TME) of liver metastases of several tumor types, including colorectal, lung, and pancreatic carcinoma.[36] Importantly, such sex-based differences disappeared in ovariectomized mice, were reconstituted by estradiol supplementation and modulated by tamoxifen.[36]

Recently, we reported the results of a large-scale analysis of genome-wide transcriptome data of 2575 early-stage NSCLCs from seven different datasets and 327 multiregion tumor samples extensively characterized at the molecular level from the TRACERx lung study.[37] We found that NSCLCs of men and women exploited different mechanisms of immune evasion.[37] The TME of females was characterized by a significantly greater T-cell dysfunction status, higher expression of inhibitory immune checkpoint molecules, and higher abundance of immune-suppressive cells, including cancer associated fibroblasts, MDSCs, and regulatory T cells.[37]

Importantly, among the inhibitory immune checkpoint molecules that we showed to be expressed at significantly higher levels in the TME of NSCLCs arising in women compared with men were T cell immunoglobulin and mucin-domain containing-3, Lymphocyte-activation gene 3, T cell immunoreceptor with Ig and ITIM domains, and B- and T-lymphocyte attenuator. The overexpression of such molecules in the microenvironment of females' tumors can account for the lower efficacy of anti-PD-L1 drugs given as monotherapy, shown here even in the context of high PD-L1 tumor levels.[37] Furthermore, for this reason we also hypothesize that a significant sex-based heterogeneity of efficacy will be observed for the new immunotherapeutic combinations currently under investigation, such as anti-PD-L1 combined with anti-T cell immunoglobulin and mucin-domain containing-3, anti-T cell immunoreceptor with Ig and ITIM domains, or anti-Lymphocyte-activation gene 3 drugs.

Finally, two observations are noteworthy, because they strongly oppose the hypothesis that the different prevalence of smoking habits between males and females can explain the different efficacy of ICIs observed. The first is that the Empower-Lung 1 trial, testing cemiplimab versus chemotherapy in high PD-L1 NSCLC, excluded never smokers per protocol. The difference in OS-HRs observed between male patients (OS-HR 0.5, 95% CI 0.36-0.69) and female patients (OS-HR 1.1, 95% CI 0.49-2.52) enrolled in the Empower-Lung 1 trial was the largest among all the RCTs analyzed. The second is that among all patients included in our meta-analysis, only 10.7% (179 out of 1672) were never smokers: it is hard to sustain that such a small subgroup of patients can account for the large difference reported.

Similarly, all patients enrolled in trials analyzed had EGFR and ALK wild-type tumors, demonstrating that sex-based heterogeneity of ICI efficacy observed in patients with NSCLC does not rely on a different prevalence of molecular alterations in such genes among men and women.

A limitation of our meta-analysis is that it relies on published results rather than on individual patients' data. This precludes the possibility of exploring relevant issues, such as the menopausal status of female patients on the efficacy of immunotherapeutic treatments, which deserves to be investigated given the key role exerted by sex hormones in the regulation of the immune system and for the potential therapeutic implications.[6]

Our findings are hypothesis generating because they are based on a meta-analysis of aggregate published results derived from RCTs and, as such require further validation in prospective future trials.

In conclusion, although our results should be considered as hypothesis generating, they corroborate our previous findings and highlight the urgent need to prospectively test the hypothesis that women and men with advanced NSCLC need to be treated with different and personalized immunotherapy strategies to further improve the prognosis of both.

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