OXFORD

JNCI J Natl Cancer Inst (2019) 111(8): djz094

doi: 10.1093/jnci/djz094
First published online May 20, 2019
Review

REVIEW

Sex-Based Heterogeneity in Response to Lung Cancer Immunotherapy: A Systematic Review and Meta-Analysis

Fabio Conforti, Laura Pala, Vincenzo Bagnardi, Giuseppe Viale, Tommaso De Pas, Eleonora Pagan, Elisabetta Pennacchioli, Emilia Cocorocchio, Pier Francesco Ferrucci, Filippo De Marinis, Richard D. Gelber, Aron Goldhirsch

Correspondence to: Fabio Conforti, MD, Division of Medical Oncology for Melanoma, Sarcoma, and Rare Tumors, IEO, European Institute of Oncology IRCCS, Via Ripamonti 435, 20141, Milan, Italy (e-mail: fabio.conforti@ieo.it).

Abstract

Background: We previously showed that therapy with anti–checkpoints T-lymphocyte-associated protein 4 (anti–CTLA-4) or antiprogrammed cell death protein 1 (anti–PD-1) agents was more effective for men as compared with women. However, because the sex-dimorphism of the immune system is complex, involving multiple elements of immune responses, it is possible that women could derive larger benefit than men from strategies other than therapy with immune checkpoint inhibitors (ICIs) alone. Here we investigated whether women could derive larger benefit than men from the combination of chemotherapy and anti-PD-1 or anti-PD-L1.

Methods: We performed two meta-analyses. The first included all randomized controlled trials (RCTs) testing anti-PD1 and anti–PD-L1 plus chemotherapy vs chemotherapy to assess different efficacy between men and women. The second included all RCTs of first-line systemic treatment in advanced non-small cell lung cancer testing anti–PD-1/PD-L1 given either alone or combined with chemotherapy to assess the different efficacy of these two immunotherapeutic strategies according to patients' sex. For each RCT included in the two meta-analyses, first, a trial-specific ratio of hazard ratios (HRs) was calculated from the ratio of the reported hazard ratios in men and in women; second, these trial-specific ratios of hazard ratios were combined across trials using a random-effects model to obtain a pooled hazard ratios ratio. A pooled HRs ratio estimate lower than 1 indicates a greater treatment effect in men, and higher than 1 a greater effect in women.

Results: Eight RCTs were included in the first meta-analysis. The pooled overall survival hazard ratios (OS-HRs) comparing anti–PD-1/PD-L1 plus chemotherapy vs chemotherapy was 0.76 (95% confidence interval [CI] = 0.66 to 0.87) for men and 0.48 (95% CI = 0.35 to 0.67) for women. The pooled ratio of the overall survival hazard ratios reported in men vs women was 1.56 (95% CI = 1.21 to 2.01), indicating a statistically significant greater effect for women. Six RCTs were included in the second meta-analysis: three tested an anti-PD-1 alone, whereas three RCTs tested anti-PD-1/PD-L1 plus chemotherapy. The pooled overall survival hazard ratios were 0.78 (95% CI = 0.60 to 1.00) in men and 0.97 (95% CI = 0.79 to 1.19) in women for anti–PD-1 alone, compared with 0.76 (95% CI = 0.64 to 0.91) in men and 0.44 (95% CI = 0.25 to 0.76) in women for anti–PD-1/PD-L1 plus chemotherapy. The pooled ratio of overall survival hazard ratios was 0.83 (95% CI = 0.65 to 1.06) for anti–PD-1 alone, indicating a greater effect in men, and 1.70 (95% CI = 1.16 to 2.49) for anti–PD-1/PD-L1 plus chemotherapy, indicating a greater effect in women.

Conclusion: Women with advanced lung cancer derived a statistically significantly larger benefit from the addition of chemotherapy to anti–PD-1/PD-L1 as compared with men.

Relevant differences of immune system function and immune responses in men and women are well known. They rely on complex interactions among genetic, hormonal, behavioral features, and commensal microbiome composition (1–3).

Received: December 11, 2018; **Revised:** February 27, 2019; **Accepted:** May 9, 2019

We recently demonstrated that such differences include the modality through which women and men with cancer respond to immunotherapies (4). In a meta-analysis including 20 randomized controlled trials (RCTs), we showed that therapy with anti–checkpoints T-lymphocyte-associated protein 4 (anti–CTLA-4) or antiprogrammed cell death protein 1 (anti–PD-1) agents when compared with standard treatments was more effective for men compared with women for several tumor types (4). However, because the sex dimorphism of the immune system is complex, involving multiple elements of immune responses, it is possible that women could derive a larger benefit than men from strategies other than therapy with immune checkpoint inhibitors (ICIs) alone (1,2). In this paper, we provide evidence that supports this hypothesis.

Methods

We followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for the systematic review and meta-analyses in this study.

Systematic Review and Meta-Analysis of All RCTs Testing the Combination of PD-1 or PD-L1 Inhibitors Plus Chemotherapy

Data Sources and Searches

We searched PubMed, MEDLINE, Embase, and Scopus for phase 2 and 3 RCTs testing the combination of anti-PD-1 or anti-PD-L1 plus chemotherapy in patients with advanced solid tumors, published from the inception of each database to October 22, 2018. We also reviewed abstracts and presentations from all major conference proceedings, including the American Society of Clinical Oncology, the International Association for the Study of Lung Cancer, and the European Society for Medical Oncology, from January 1, 2010, to October 22, 2018.

Two investigators (FC and LP) independently searched the databases. The search terms were "PD-1", "programmed death receptor 1", "PD-L1", "programmed death ligand 1", "nivolumab", "pembrolizumab", "avelumab", "durvalumab", "atezolizumab." We also reviewed the references of articles included in the final selection. The following inclusion criteria were used: 1) RCT testing of the combination of an anti–PD-1 or anti–PD-L1 with chemotherapy against chemotherapy, and 2) data available on hazard ratio (HR) for progression-free survival (PFS) and/or overall survival (OS), according to patients' sex subgroup. We excluded single-arm phase 1 and 2 trials (ie, nonrandomized trials).

Study Selection and Data Extraction

Two investigators (FC and LP) independently reviewed the list of retrieved articles to choose potentially relevant articles, and disagreements about particular studies were discussed and resolved with the consensus of all investigators.

From each study, the following data were extracted: name of study, first author and year of publication, study design and blinding, study phase, number of patients, age distribution, sex distribution, patients' smoking status distribution, patients' performance status distribution, type of ICI used, hazard ratio for PFS and/or OS in the overall population, and hazard ratios according to patients' sex. We included only the most recent and complete report of controlled trials when duplicate publications were identified.

Risk of Bias Assessment

Study methodological quality was assessed by using the five-point Jadad ranking system, which evaluates quality of randomization, double-blinding, and the flow of patients (withdrawals and dropouts), a practice in agreement with other meta-analyses conducted in this context (5). A clinical trial could receive a Jadad score of between zero (poor methodological quality) and five (optimal methodological quality).

Data Synthesis and Analysis

The primary endpoint was the difference in efficacy of the combination of an anti–PD-1 or anti–PD-L1 with chemotherapy between men and women, measured in terms of the ratio of the hazard ratio for progression or death in the intervention arm compared with those in the control arm reported in men, to the same hazard ratio reported in women.

The hazard ratios for progression or death in the intervention arm compared with those in the control arm, along with their 95% confidence intervals (CIs), were derived from each included study, separately for male and female patients. Hazard ratios and confidence intervals were translated into log-hazard ratios and the corresponding variances. The pooled hazard ratio of progression or death was calculated in men and women, using a random-effects model. Each study log(HR) was weighted by the inverse of its variance. Weights were taken equal to the inverse of the reported within-study variance plus the between-study variance component τ(2). The moment estimator of the between-study variance was used. The Q test was performed to assess between-study heterogeneity, and the I^2 statistics, which express the percentage of the total observed variability due to heterogeneity, were also calculated (6,7).

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 hazard ratios was calculated from the ratio of the reported hazard ratios in men and in women; second, these trial-specific ratios of hazard ratios were combined across trials using a random-effects model (8). A pooled hazard ratio ratio estimate lower than 1 indicates a greater treatment effect in men, and higher than 1 a greater effect in women.

All tests were two-sided, and statistical significance was dependent on the hazard ratios 95% confidence interval not crossing 1.00. For analyses in which the significance cannot be established directly from the hazard ratios confidence intervals, such as analyses in which two ratios of hazard ratios are compared to test whether they are homogeneous, two-sided P values were reported and a P value of less than 0.05 was considered to indicate statistical significance (9). All analyses were performed with R software (version 3.4.0).

Meta-Analysis of RCTs Testing anti-PD-1/PD-L1 Alone or Combined With Chemotherapy

Data Sources and Searches, Study Selections, and Data Synthesis and Analysis

To assess whether outcome differences between the sexes differed according to the immunotherapeutic strategy used, we considered all RCTs testing anti–PD-1 or PD-L1 given either alone or combined with chemotherapy, as first-line systemic treatment for patients with advanced nonsmall-cell lung cancer

(NSCLC). The combination of chemotherapy plus anti–PD-1/PD-L1 has not been tested in second or further lines of treatment for advanced NSCLC, precluding extending this type of analysis beyond the first line of treatment. We used the same search strategy criteria and search terms as in the first meta-analysis.

The following inclusion criteria were used: 1) RCT testing an anti–PD1 or anti–PD-L1 given either alone or combined with chemotherapy, against chemotherapy alone, as first-line systemic treatment for patients with advanced NSCLC; and 2) data available on hazard ratio for overall survival, according to patients' sex subgroup. Risk of bias assessment, data synthesis, and analysis were performed using the same approach described above for the first meta-analysis. Moreover, a z- test was used to test the heterogeneity of the pooled ratios of hazard ratios, measuring the difference in efficacy between men and women, for the two evaluated immunotherapeutic strategies (ie, anti–PD-1/PD-L1 given alone or combined with chemotherapy).

Results

Meta-Analysis of RCTs Testing the Combination of Chemotherapy Plus PD-1 or PD-L1 Inhibitors

We found eight eligible RCTs reporting results of the combination of an anti–PD-1 or anti–PD-L1 plus chemotherapy vs chemotherapy in patients with advanced solid tumors (Figure 1). All eight trials had available data on PFS according to patients' sex subgroups and were included in the analysis for such endpoints (10–17) (Table 1 reports details of trials included). Two trials, KEYNOTE 189 and 407, tested the combination of chemotherapy with the anti–PD-1 pembrolizumab in patients with advanced nonsquamous and squamous NSCLC, respectively (13,14). Two trials, IMpower 130 and 132, tested the combination of chemotherapy plus the anti–PD-L1 atezolizumab in advanced nonsquamous NSCLC, and one trial, IMpower 131, tested the combination of chemotherapy plus atezolizumab in squamous NSCLC (15–17).

One trial, IMpower 150, tested the combination of atezolizumab plus chemotherapy plus bevacizumab vs chemotherapy plus bevacizumab in advanced nonsquamous NSCLC (10). One trial, IMpower 133, tested the combination of chemotherapy plus atezolizumab in advanced small-cell lung cancer (SCLC) (12). Finally, in the PACIFIC trial, patients with locally advanced NSCLC were randomly assigned to chemoradiotherapy plus the anti–PD-L1 durvalumab vs chemoradiotherapy alone (11) (Table 1).

Risk of bias assessment through Jadad score for each trial is reported in Supplementary Table 1 (available online). Randomized treatment allocation sequences were generated in all trials. Four trials were double-blinded. The Jadad mean score was 4 (range 3–5). No trial received a low-quality score (ie, Jadad score of 1–2). All the included studies had a low risk of reporting bias, attrition bias, and other biases (Supplementary Table 1, available online).

The analysis for PFS included 4923 patients, of whom 3345 (67.9%) were men and 1578 (32.1%) were women; 2952 (60.0%) patients had nonsquamous NSCLC, 1568 (31.9%) squamous NSCLC, and 403 (8.2%) SCLC; 4438 (90.1%) patients were former or current smokers, and only 485 (9.9%) were never smokers (Table 1). All these trials enrolled only epidermal growth factor receptor (EGFR) and anaplastic lyphoma kinase (ALK) wild-type tumors, except for the PACIFIC trial, in which 43 EGFR mutated NSCLC were enrolled.

Results showed that men treated with anti–PD-1 or anti–PD-L1 plus chemotherapy had a statistically significant reduced risk of progression or death compared with men treated in control arm (pooled PFS-HR = 0.64, 95% CI = 0.58 to 0.71; Figure 2A). In women, the benefit obtained with anti–PD-1 or PD-L1 plus chemotherapy compared with the control arm was larger (pooled PFS-HR 0.56, 95% CI = 0.49 to 0.65; Figure 2A). No substantial heterogeneity among single-study estimates was observed in either male patients ($Q = 10.9$, $P = .14$, $I^2 = 36.0\%$) or in female patients ($Q = 9.9$, $P = .19$, $I^2 = 29.6\%$).

The pooled ratio of PFS hazard ratios reported in women vs those reported in men in each trial was 1.15 (95% CI = 0.96 to 1.38; Figure 2B). Five of the eight RCTs (ie, KEYNOTE 407, KEYNOTE 189, IMpower 130, IMpower 133, and PACIFIC trial) had available data on overall survival according with patients' sex subgroups and were included in the analysis for such endpoint (11–14,17). The analysis for OS included 2970 patients, of whom 1979 (66.6%) were men and 991 (33.4%) were women; 1682 (56.6%) patients had nonsquamous NSCLC, 885 (29.8%) squamous NSCLC, and 403 (13.6%) SCLC; 2715 (91.4%) patients were former or current smokers, and 255 (8.6%) were never smokers (Table 1).

Men treated with anti–PD-1 or anti–PD-L1 plus chemotherapy had a statistically significant reduced risk of death compared with men treated in the control arm (pooled OS-HR = 0.76, 95% CI = 0.66 to 0.87; Figure 3A). In women, the OS benefit obtained with anti–PD-1 or PD-L1 plus chemotherapy compared with the control arm was larger (pooled OS-HR = 0.48, 95% CI = 0.35 to 0.67; Figure 3A). Heterogeneity among single-study estimates was observed in female patients ($Q = 10.9$, $P = .03$, $I^2 = 63.3\%$), but not in male patients ($Q = 1.55$, $P = .81$, $I^2 = 0\%$). The pooled ratio of OS-HRs reported in women vs those reported in men in each trial was 1.56 (95% CI = 1.21 to 2.01; Figure 3B), indicating a statistically significant larger benefit in women compared with men.

We performed similar analyses to assess the interaction between treatment efficacy and other relevant clinicopathological variables, including patients' age (<65y vs ≥65), smoking status (never vs former or current smoker), performance status (PS 0 vs PS 1), and tumor histology. No statistically significant interaction was found for any of the variables analyzed (data not shown).

We also conducted two sensitivity analyses using as an endpoint the OS. In the first, we assessed sex-based heterogeneity of efficacy of the combination of chemotherapy plus anti–PD-1/PD-L1 excluding the PACIFIC trials because patients also received radiotherapy (11). In the second analysis, we excluded the IMpower 133 trial that enrolled patients with SCLC to assess sex-based heterogeneity of efficacy in trials enrolling only patients with NSCLC (12). The pooled ratio of OS-HRs reported in women vs those reported in men was respectively 1.53 (95% CI = 1.10 to 2.13) for the first sensitivity analysis and 1.68 (95% CI = 1.28 to 2.19) for the second, confirming a statistically significant larger benefit in women compared with men (data not shown).

Meta-Analysis of RCTs Testing Anti–PD-1/PD-L1 Alone or Combined With Chemotherapy

Subsequently, we performed a second meta-analysis including all RCTs testing an anti-PD-1/PD-L1 given either alone (KEYNOTE 24 and 42 and CheckMate 026 trials) or combined with chemotherapy (KEYNOTE 189 and 407 and IMpower 130 trials) as first-line systemic treatment for patients with advanced NSCLC, with available OS data according with patients' sex

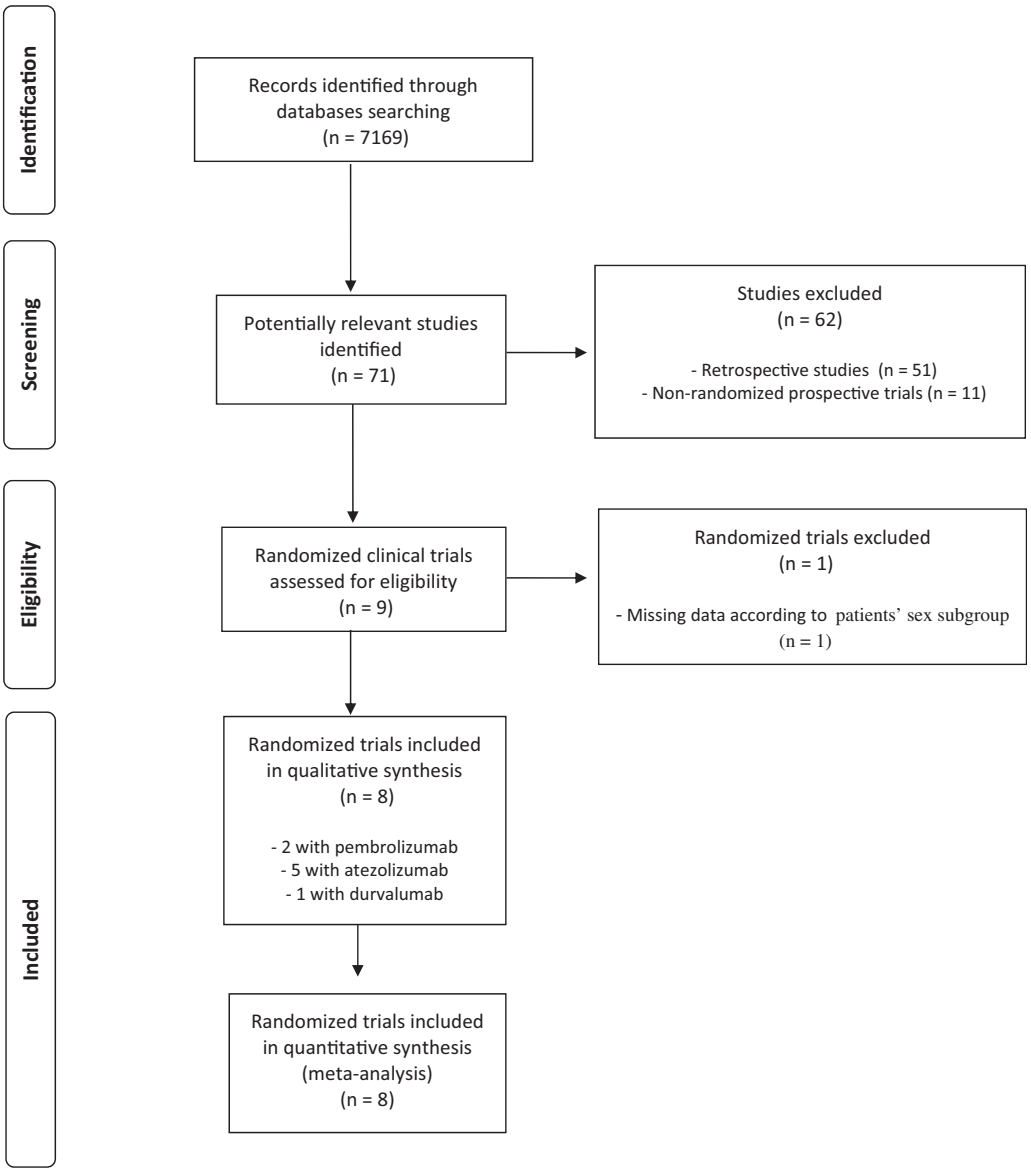

Figure 1. Flow diagram of the search process.

(Table 2) (13,14,17–20). The aim was to assess the interaction between the efficacy of anti–PD-1/PD-L1 given alone or combined with chemotherapy and patients' sex. These six trials had similar designs and enrolled comparable patient populations, in the same setting of disease. All trials enrolled only EGFR and ALK wild-type tumors and had a standard first-line, platinum-based chemotherapy as a control arm (13,14,17–20).

Risk of bias assessment through Jadad score for each trial is reported in Supplementary Table 1 (available online). The mean Jadad score was 4 (range 3–5). No trial received a low-quality score (ie, Jadad score of 1–2). All the included studies had a low risk of reporting bias, attrition bias, and other bias (Supplementary Table 1, available online).

Analysis included 3974 patients, of whom 2639 (66.4%) were men and 1335 (33.6%) were women; 2737 (68.9%) patients had nonsquamous tumors, and 1237 (31.1%) squamous tumors; 2120 patients were treated with anti–PD-1 alone (1579 with pembrolizumab and 541 with nivolumab), and 1854 with anti–PD-1/PD-L1 plus chemotherapy (1175 with pembrolizumab plus chemotherapy and 679 with atezolizumab plus chemotherapy); 3423 (86.1%) patients were former or current smokers, and 544 (13.7%) were never smokers, and for 7 patients, data were unknown (Table 2).

Male patients treated with anti–PD-1 alone had a statistically significantly reduced risk of death as compared with men treated with standard chemotherapy (pooled OS-HR = 0.78, 95% CI = 0.60 to 1.00; Figure 4A). In women, anti–PD-1 alone was not better than standard chemotherapy (pooled OS-HR = 0.97, 95% CI = 0.79 to 1.19). By contrast, anti–PD-1/PD-L1 administered in combination with chemotherapy was associated with a very large OS advantage compared with chemotherapy alone in women, but a statistically significantly smaller benefit was seen in men (female-pooled OS-HR = 0.44, 95% CI = 0.25 to 0.76; male-pooled OS-HR = 0.76, 95% CI = 0.64 to 0.91).

The pooled ratio of OS-HRs reported in women vs those reported in men in each trial was 0.83 (95% CI = 0.65 to 1.06) for anti-PD-1 alone, indicating a greater effect of anti–PD-1 alone in men (Figure 4B), and 1.70 (95% CI = 1.16 to 2.49) for anti–PD-1/

Table 1. Main features and results of studies included in the first meta-analysis*

Trial	Men	Women	Histotype	PD-L1 status	Experimental arm	Control arm	PFS-HR (95% CI)			OS-HR (95% CI)		
							ITT population	Men	Women	ITT population	Men	Women
KEYNOTE 407, Paz-Ares et al., 2018 (14)	455	104	Only squamous NSCLC	Negative (194) 1%–49% (207) ≥50% (146)	Chemotherapy + pembrolizumab	CBDCA + Txl/nTxl	0.56 (0.45 to 0.7)	0.58 (0.66 to 0.73)	0.49 (0.3 to 0.81)	0.64 (0.49 to 0.85)	0.69 (0.51 to 0.94)	0.42 (0.22 to 0.81)
KEYNOTE 189, Gandhi et al., 2018 (13)	363	253	Only nonsquamous NSCLC	Negative (190) 1%–49% (186) ≥50% (202)	Chemotherapy + pembrolizumab	CDDP/CBDCA + Pem	0.52 (0.43 to 0.64)	0.66 (0.5 to 0.87)	0.40 (0.29 to 0.54)	0.49 (0.38 to 0.64)	0.70 (0.50 to 0.99)	0.29 (0.19 to 0.44)
IMpower 130, Cappuzzo et al., 2018 (17)	400	279	Only nonsquamous NSCLC	Negative (356) Low (TC1/2 or IC1/2) (193) High (TC3 or IC3) (130)	Chemotherapy + atezolizumab	CBDCA + nTxl	0.64 (0.54 to 0.77)	0.67 (0.54 to 0.85)	0.59 (0.45 to 0.78)	0.79 (0.64 to 0.98)	0.87 (0.66 to 1.15)	0.66 (0.46 to 0.93)
IMpower 131, Jotte et al., 2018 (15)	557	126	Only squamous NSCLC	Negative (331) Low (TC1/2 or IC1/2) (250) High (TC3 or IC3) (101)	Chemotherapy + atezolizumab	CBDCA + nTxl	0.71 (0.60 to 0.85)	0.71 (0.59 to 0.85)	0.66 (0.45 to 0.97)	NA	NA	NA
IMpower 132, Papadimitrakopoulou et al., 2018 (16)	384	194	Only nonsquamous NSCLC	Negative (163) Positive (415)	Chemotherapy + atezolizumab	CDDP/CBDCA + Pem	0.60 (0.49 to 0.72)	0.64 (0.51 to 0.79)	0.51 (0.36 to 0.71)	NA	NA	NA
IMpower 150, Socinski et al., 2018 (10)	425	267	Only nonsquamous NSCLC	Negative (338) Low (TC1/2 or IC1/2) (224) High (TC3 or IC3) (135)	Chemotherapy + bevacizumab + atezolizumab	CBDCA + Txl + bevacizumab	0.62 (0.52 to 0.74)	0.55 (0.44 to 0.67)	0.73 (0.54 to 0.96)	0.78 (0.64 to 0.96)	NA	NA
PACIFIC, Antonia et al., 2018 (11)	500	213	Squamous (326) and nonsquamous (387) NSCLC	<25% (292) ≥25% (159) Unknown (262)	Chemoradiotherapy + durvalumab	Chemoradiotherapy	0.55 (0.45 to 0.68)	0.56 (0.44 to 0.71)	0.54 (0.37 to 0.79)	0.68 (0.47 to 0.99)	0.78 (0.59 to 1.03)	0.46 (0.30 to 0.73)
IMpower 133, Horn et al., 2018 (12)	261	142	SCLC	NA	Chemotherapy + atezolizumab	CBDCA + VP16	0.77 (0.62 to 0.96)	0.87 (0.67 to 1.13)	0.59 (0.41 to 0.85)	0.70 (0.54 to 0.91)	0.74 (0.54 to 1.02)	0.65 (0.42 to 1.00)

*Numbers in parentheses in the PD-L1 status and histotype columns indicate the number of patients within each subgroup: Total number of patients with squamous tumor and number of patients with non-squamous tumors, etc. CBDCA = carboplatin; CDDP = cisplatin; CI = confidence interval; HR = hazard ratio; ITT = intention to treat; NA = not available; NSCLC = nonsmall-cell lung cancer; nTxl = nab-pacltitaxel; OS = overall survival; PD-L1 = programmed death-ligand 1; Pem = pemetrexed; PFS = progression-free survival; SCLC = small-cell lung cancer; Txl = paclitaxel; VP16 = etoposide.

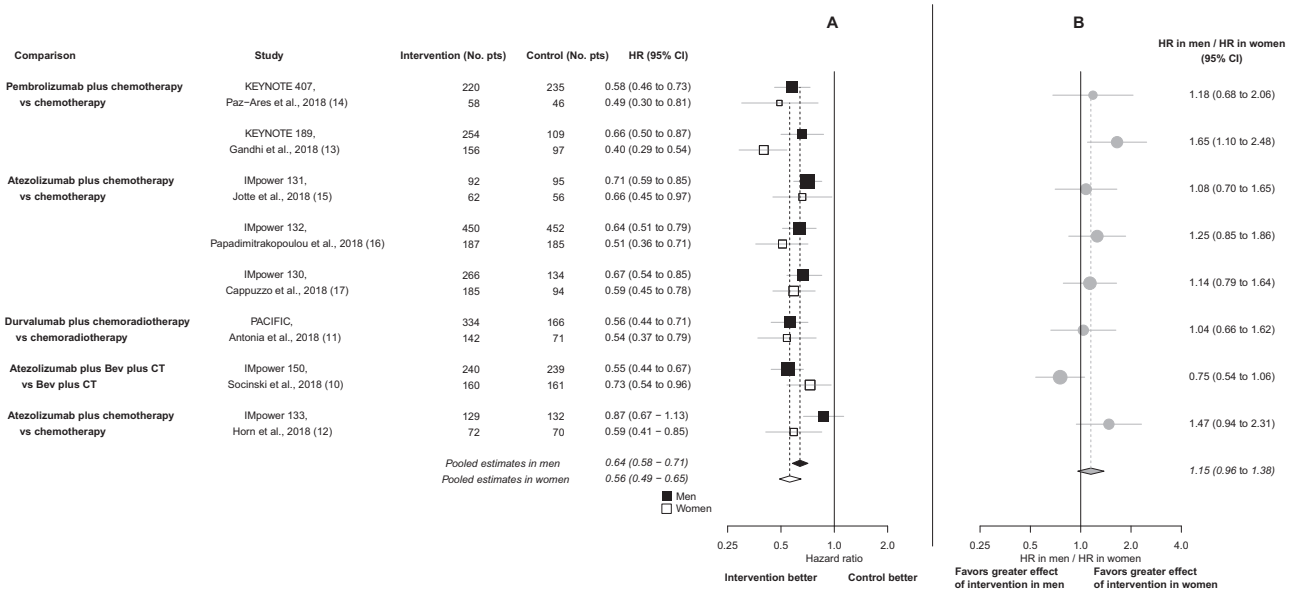

Figure 2. Hazard ratios of progression or death according to patients' sex. **A)** The hazard ratios (HRs) of progression or death for patients assigned to intervention treatment (ie, chemotherapy plus anti–PD-1 or anti–PD-L1) compared with those assigned to control treatment (ie, chemotherapy), according to sex. **Squares** indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval (CI). **Diamonds** indicate the meta-analytic pooled hazard ratios, calculated separately in women and men, with their corresponding 95% confidence intervals. The **dashed vertical lines** indicate the sex-specific pooled hazard ratios, and the **solid vertical line** indicates a hazard ratio of 1, which is the null-hypothesis value (ie, no association between type of treatment and risk of progression or death). **B)** The interaction between treatment efficacy and sex. Each **filled circle** indicates the study-specific ratio of hazard ratios, that is, the ratio of the reported hazard ratios in men and in women. Values greater than 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 (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval. The **diamond** indicates the meta-analytic pooled ratio of hazard ratios, with its corresponding 95% **CI**. The **dashed vertical line** indicates the pooled ratio of hazard ratios, and the **solid vertical line** indicates a pooled hazard ratio of 1, which is the null-hypothesis value (ie, no difference between men and women regarding the efficacy of the combination of chemotherapy plus anti–PD-1/PD-L1).

PD-L1 plus chemotherapy, indicating a greater effect for such a therapeutic strategy in women. Thus, the difference in efficacy for men vs women was statistically significantly different for the two immunotherapeutic strategies (ie, anti–PD-1/PD-L1 alone or combined with chemotherapy; $P = .002$).

In a previous meta-analysis, we demonstrated a statistically significantly larger OS benefit in men treated with anti–PD-1 or anti–CTLA4 drugs compared with women across a large spectrum of advanced solid tumors (4).

To confirm that men derive a statistically significantly larger OS benefit than women when treated with immunotherapeutic approaches other than the combination of chemotherapy plus anti–PD-1/PD-L1, we updated our previous meta-analysis, including all RCTs with anti–PD1, anti–PD-L1, or anti–CTLA-4 published after its publication, but excluding RCTs testing the combination of anti–PD1/PD-L1 plus chemotherapy.

We included in this analysis 25 RCTs: Six RCTs tested CTLA-4 inhibitors, 14 tested PD-1 inhibitors, three PD-L1 inhibitors, and two the combination of anti–CTLA-4 plus anti–PD-1 (Supplementary Figure 1, available online). Seven RCTs were performed in patients with melanoma, nine in NSCLC, two head and neck cancer, two renal cell carcinoma, two gastric cancer, and one each in SCLC, urothelial tumors, and mesothelioma. All RCTs were conducted in advanced or metastatic settings.

The pooled OS-HR was 0.86 (95% CI = 0.78 to 0.94) in women vs 0.74 (95% CI = 0.68 to 0.80) in men. The pooled ratio of OS-HRs reported in men vs those reported in women in each trial was 0.88 (95% CI = 0.80 to 0.96), confirming a statistically significant larger OS benefit in men compared with women, when treated with immunotherapies other than the combination of chemotherapy plus anti–PD-1/PD-L1.

Discussion

An individual's sex is one of the most important factors that influence risk of a number of diseases and can modulate pharmacokinetics, pharmacodynamics, and toxicities of drugs (3). Relevant differences both of innate and adaptive immune responses between men and women explain different prevalence and mortality from autoimmune and infectious diseases and from several types of cancers (1,2). These sex-based differences of immune responses reflect complex interactions among genes, hormones, and the environment (1–3). The X chromosome contains a large number of immune-related genes (1,2,21). These genes code for proteins involved in the regulation of the innate immunity, like pattern recognition receptors (eg, Toll-like receptor 7 and Toll-like receptor 8), as well as in the regulation of adaptive immunity, including cytokine receptors (eg, IL2RG and IL13RA2) and key transcriptional factors (eg, FOXP3) (1,2,21). Immune-related genes encoded on the X chromosome may escape X inactivation, resulting in higher expression levels in women than men (1,2,21).

Sex hormones constitute another major determinant of sex differences in immunity (1,2,22). They modulate the development and function of multiple immune cell populations (1,2,22). Putative androgen response elements (AREs) and estrogen response elements (EREs) are present in the promoters of several innate and adaptive immune genes, suggesting that sex steroids may directly regulate their expression (1,2,22). Preclinical studies suggest that sex hormones regulate the expression and function of PD-1 and that the hormone-mediated effects on PD-1 pathway is important in mediating autoimmunity (23). The expression of PD-L1 has also been shown to

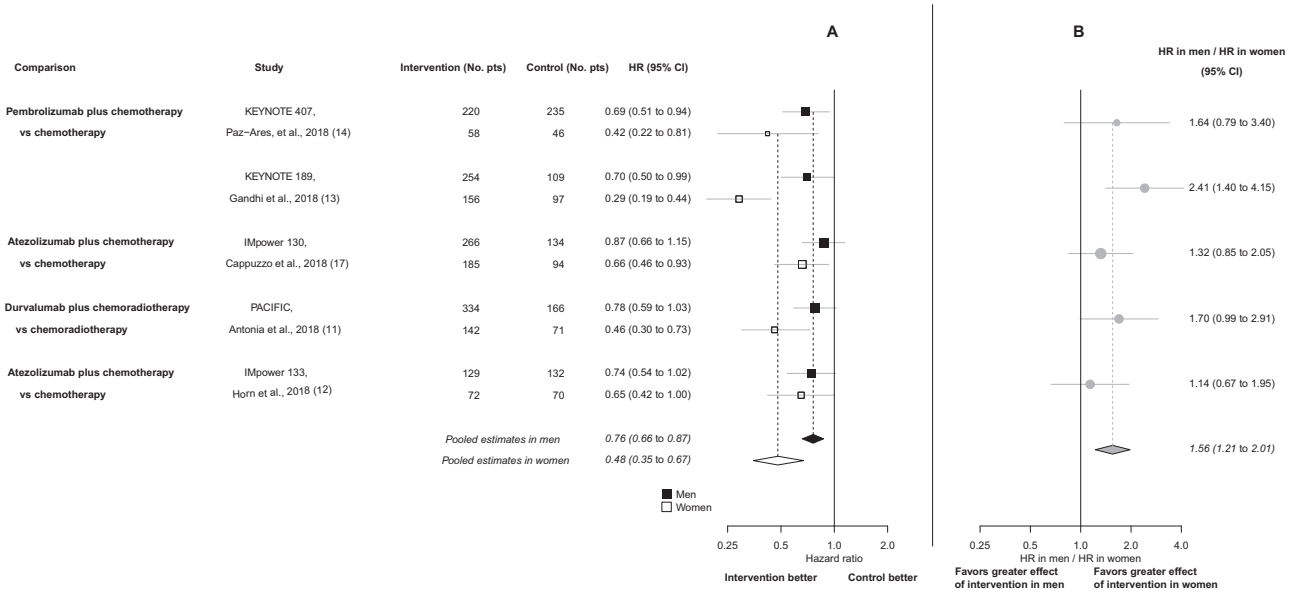

Figure 3. Hazard ratios of death according to patients' sex. **A)** The hazard ratios (HRs) of death for patients assigned to intervention treatment (ie, chemotherapy plus anti–PD-1 or anti–PD-L1) compared with those assigned to control treatment (ie, chemotherapy), according to sex. **Squares** indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval (CI). **Diamonds** indicate the meta-analytic pooled hazard ratios, calculated separately in women and men, with their corresponding 95% confidence intervals. The **dashed vertical lines** indicate the sex-specific pooled hazard ratios, and the **solid vertical line** indicates a hazard ratio of 1, which is the null-hypothesis value (ie, no association between type of treatment and risk of death). **B)** The interaction between treatment efficacy and sex. Each **filled circle** indicates the study-specific ratio of hazard ratios, that is, the ratio of the reported hazard ratios in men and in women. Values greater than 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 (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval. The **diamond** indicates the meta-analytic pooled ratio of hazard ratios, with its corresponding 95% confidence interval. The **dashed vertical line** indicates the pooled ratio of hazard ratios, and the **solid vertical line** indicates a pooled hazard ratios ratio of 1, which is the null-hypothesis value (ie, no difference between men and women regarding the efficacy of the combination of chemotherapy plus anti–PD-1/PD-L1).

be modulated in an estrogen-dependent and sex-dependent manner (1,2,23).

Sex-related differences in anticancer immune response has been described in the amount and composition of intratumoral immune infiltrates as well as in tumor expression levels of PD-L1 across a large spectrum of tumors including NSCLC (24–26). An intratumoral immune infiltrate enriched with partially exhausted cytotoxic T lymphocytes (ie, tumor-infiltrating CD8+ T cells expressing high levels of CTLA-4 and PD-1) strongly correlate with higher response to anti–PD-1 monotherapy (25). It has been shown that intratumoral infiltrates of male patients with melanoma had a statistically significantly larger proportion of these cells compared with those of women (25). PD-L1 expression levels have been found to be statistically significantly higher in a number of solid tumors of female patients compared with those of men, including NSCLC (24).

In a previous work, we demonstrated that male patients with several solid tumors, treated with anti–CTLA-4 or anti–PD-1 drugs, obtain statistically significantly larger benefit than women (4). We anticipated in the discussion of that previous work that, given the complexity of the sex–dimorphism of the immune system function and responses, it would be possible that women derive larger benefit than men from different immunotherapeutic strategies (4).

Here, we showed that women with advanced lung cancer experience a statistically significantly larger benefit from the addition of chemotherapy to an anti–PD-1 or PD-L1 than men. It could be hypothesized that such heterogeneity of response is due to the ability of chemotherapy to increase the mutational burden and neoantigenic load of female lung cancer tumors that are statistically significantly lower than those of male tumors, with this being also a potential biological rationale to explain the lower efficacy of anti–PD-1 alone in women (26–29). Furthermore, different efficacy of chemotherapy in modulating the anticancer immune responses of men and women could be speculated, given the sex-related differences in the amount and composition of intratumoral immune infiltrates reported (24,25,30).

We also provided data suggesting that the interaction between patients' sex and the efficacy of different immunotherapeutic strategies could be important when choosing therapeutic options for female and male patients with NSCLC. We meta-analyzed six RCTs testing two different immunotherapeutic approaches (ie, anti–PD-1/PD-L1 alone or in combination with chemotherapy) in comparable populations, and we found a statistically significant interaction between patients' sex and the efficacy of both these therapeutic strategies, with opposite-direction interaction in men and women.

Historically, results from immunotherapy trials for advanced breast cancer have been disappointing (31,32). However, these prior studies have primarily tested immunotherapy alone. Recently, the results of the IMpassion 130 study were reported showing that the combination of atezolizumab plus chemotherapy improved results compared with chemotherapy alone for women with advanced triple-negative breast cancer, especially if their tumors expressed PD-L1 (33). This provides additional support for the need to use combination of immunotherapy plus chemotherapy to improve outcomes for female patients (32).

The results reported here highlight a relevant methodological issue: Given the complexity of the sex-based dimorphism of the immune system, involving multiple elements of innate and adaptive immune responses, the proper way to assess the

Table 2. Main features and results of studies included in the second meta-analysis*

Trial	Men	Women	NSCLC histotype	PD-L1 status	Experimental arm	Control arm	Overall survival HR (95% CI)				
							ITT population	PD-L1 1%–49%	PD-L1 ≥ 50%	Men	Women
KEYNOTE 407, Paz-Ares et al., 2018 (14)	455	104	Only squamous	Negative (194) 1%–49% (207) ≥50% (146)	Chemotherapy + pembrolizumab	CBDCA + Txl/ nTxl	0.64 (0.49 to 0.85)	0.57 (0.36 to 0.9)	0.64 (0.37 to 1, 10)	0.69 (0.51 to 0.94)	0.42 (0.22 to 0.81)
KEYNOTE 189, Gandhi et al., 2018 (13)	363	253	Only nonsquamous	Negative (190) 1%–49% (186) ≥50% (202)	Chemotherapy + pembrolizumab	CDDP/CBDCA + Pem	0.49 (0.38 to 0.64)	0.55 (0.34 to 0.90)	0.42 (0.26 to 0.68)	0.70 (0.50 to 0.99)	0.29 (0.19 to 0.44)
IMpower 130, Cappuzzo et al., 2018 (17)	400	279	Only nonsquamous	Negative (356) Low (TC1/2 or IC1/2) (193) High (TC3 or IC3) (130)	Chemotherapy + atezolizumab	CBDCA + nTxl	0.79 (0.64 to 0.98)	0.70 (0.45–1.08)	0.84 (0.51–1.39)	0, 87 (0.66 to 1.15)	0.66 (0.46 to 0.93)
KEYNOTE 24, Reck et al., 2016 (18)	187	118	Squamous (56) Non-squamous (249)	Only >50%	Pembrolizumab	CBDCA + TXL/ GEM/PEM or CDDP + GEM/ PEM	0.60 (0.41 to 0.89)	NA	0.60 (0.41 to 0.89)	0.54 (0.36 to 0.80)	0.96 (0.56 to 1.64)
KEYNOTE 42, Lopes et al., 2018 (19)	902	372	Squamous (492) Nonsquamous (782)	1%–49% (675) ≥50% (599)	Pembrolizumab	CBDCA + TXL or CBDCA + PEM	0.81 (0.71 to 0.93)	0.92 (0.77 to 1.11)	0.69(0.56 to 0.85)	0.80(0.68 to 0.94)	0.89 (0.68 to 1.17)
CheckMate 026, Carbone et al., 2017 (20)	332	209	Squamous (130) Nonsquamous (411)	1%–49% (327) ≥50% (214)	Nivolumab	CBDCA + TXL/ GEM/PEM or CDDP + GEM/ PEM	1.07 (0.86 to 1.33)	NA	0.90 (0.63 to 1.29)	0, 97 (0, 74 to 1, 26)	1.15 (0.79 to 1.66)

*Numbers in parentheses in the PD-L1 status and histotype columns indicate the number of patients within each subgroup (ie, number of patients with squamous tumors and number of patients with nonsquaomus tumors in that trial). CBDCA = carboplatin; CDDP = cisplatin; CI = confidence interval; GEM = gemcitabine; HR = hazard ratio; ITT = intention to treat; nTxl = nab-pacltitaxel; OS = overall survival; PD-L1 = programmed death-ligand 1; PEM = pemetrexed; PFS = progression-free survival; TXL = paclitaxel.

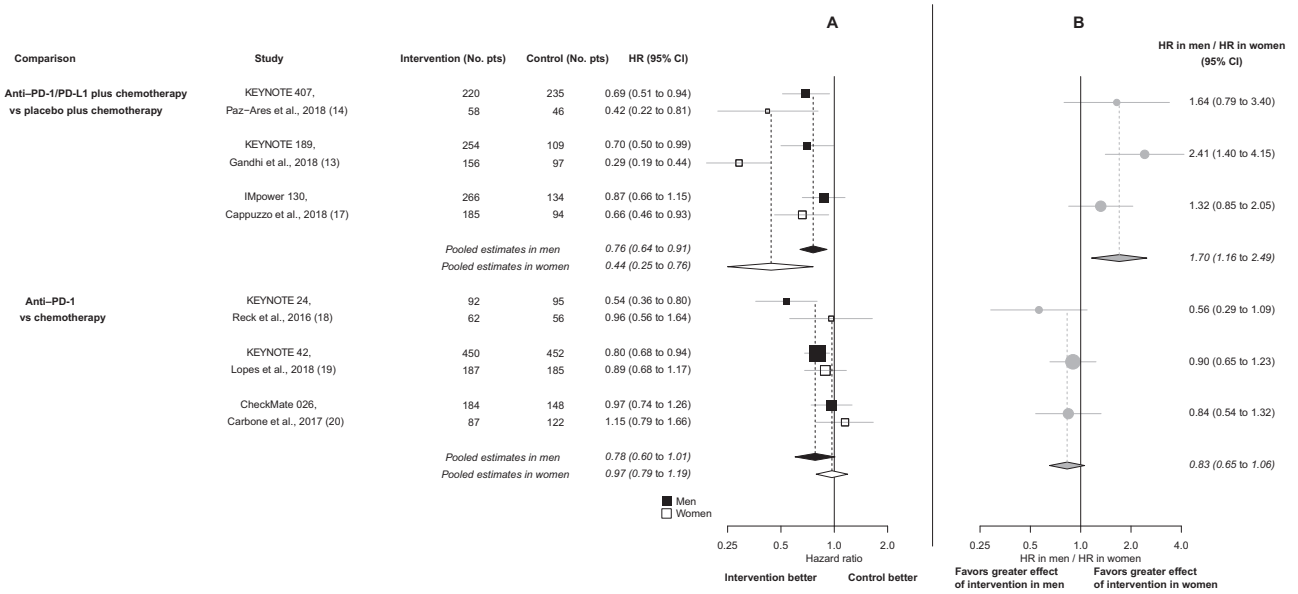

Figure 4. Hazard ratios of death according to sex and type of immunotherapeutic strategy. **A)** The hazard ratios (HRs) of death for patients assigned to intervention treatment compared with those assigned to control treatment, according to sex and type of immunotherapeutic strategy (ie, anti–programmed death-ligand 1 [anti–PD-1/PD-L1] given alone or combined with chemotherapy). **Squares** indicate study-specific hazard ratios. Values less than 1 indicate intervention is better than control. Size of the square is proportional to the precision of the estimate (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval (CI). **Diamonds** indicate the meta-analytic pooled hazard ratios, calculated separately in women and men within each treatment comparison, with their corresponding 95% confidence intervals. The **dashed vertical lines** indicate the sex-specific pooled hazard ratios, and the **solid vertical line** indicates a hazard ratio of 1, which is the null-hypothesis value (ie, no association between type of treatment and risk of death). **B)** The interaction between treatment efficacy and sex, according to the type of immunotherapeutic strategy (ie, anti–PD-1/PD-L1 given alone or combined with chemotherapy). Each **filled circle** indicates the study-specific ratio of hazard ratios, that is, the ratio of the reported hazard ratios of death in men and in women. Values greater than 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 (ie, the inverse of the variance). **Horizontal lines** indicate the 95% confidence interval. **Diamonds** indicate the meta-analytic pooled ratio of hazard ratios, calculated separately within each type of immunotherapeutic strategy, with its corresponding 95% confidence interval. The **dashed vertical lines** indicate the pooled ratios of hazard ratios, and the **solid vertical line** indicates a pooled hazard ratios ratio of 1, which is the null-hypothesis value (ie, no difference between men and women of the immune-therapy effect).

interaction between patients' sex and the efficacy of different anticancer immunotherapies is to assess such interaction separately for each type of immunotherapeutic strategy (1,2). Indeed, the direction of the interaction could be the opposite for different types of treatments, as we showed for anti–PD-1/PD-L1 given alone or combined with chemotherapy. Analyzing together different immunotherapeutic approaches, with statistically significant interaction between their efficacy and patients' sex, but with the opposite direction of such interaction, would lead to diluting results and to misleading conclusions.

Recently, Wallis et al. published a meta-analysis that reported absence of statistically significant sex-based heterogeneity of efficacy of anticancer immunotherapies, apparently in contrast with the results of our previous meta-analysis, which reported a statistically significantly larger OS benefit in men treated with anti–PD-1 or anti–CTLA-4 drugs as compared with women (4,34). Wallis et al., however, added seven recently published RCTs to the 16 RCTs already included in our previous meta-analysis (34). Notably, four of the seven RCTs included in Wallis' work tested the combination of anti-PD1/PDL1 plus chemotherapy (ie, PACIFIC, IMpower 133, KEYNOTE 189, and 407 trials), and all four trials showed a large sex-based heterogeneity of efficacy in favor of women (11–14). To demonstrate that the inconsistent results between our and Wallis' meta-analyses were mainly due to the addition of these four trials, we updated our previous meta-analysis, including all RCTs with anti–PD-1, anti–PD-L1, or anti–CTLA-4 published in our meta-analysis (4), but excluding RCTs testing the combination of anti-PD1/PD-L1 plus chemotherapy. Results were strongly consistent with those that we previously reported, confirming a statistically significant interaction between patient sex and treatment efficacy, with men deriving a larger survival benefit than women from immunotherapies other than the combination of chemotherapy plus anti–PD-1/PD-L1.

A limitation shared both by Wallis' and our meta-analyses is that they rely 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 (1,2).

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 before being considered sufficient to support any change in clinical practice. However, data reported here and in our previous work (4) support the need for different therapeutic strategies to be tested in male and female populations to further improve the use of immunotherapy. For example, accrual and design of trials of immunotherapy might best be performed separately for men and women, with proper sample size planning for both. We recommend that future research with anticancer immunotherapy take into account sex-related heterogeneity of responsiveness to treatments.

Funding

There was no funding source for this study.

Notes

Affiliations of authors: Division of Medical Oncology for Melanoma, Sarcoma, and Rare Tumors (FC, LP, TDP, EC, PFF, AG), Division of Melanoma, Sarcoma, and Rare Tumors (EliP), Division of Thoracic Oncology (FDM), Department of Pathology (GV), and Scientific Directorate (AG), IEO, European Institute of Oncology IRCCS, Milan, Italy; Department of Statistics and Quantitative Methods, University of Milan-Bicocca, Milan, Italy (VB, EleP); State University of Milan, Milan, Italy (GV); Department of Data Sciences, Division of Biostatistics, Dana-Farber Cancer Institute, Harvard Medical School, Harvard T.H. Chan School of Public Health, and Frontier Science & Technology Research Foundation, Boston, MA (RDG); MultiMedica San Giuseppe Hospital, Milan, Italy (AG).

All authors contributed equally. The corresponding author (FC) had full access to all the data and the final responsibility for submission for publication.

RDG declares that his institution has received partial support for his salary from the following pharmaceutical companies during the past 3 years: Roche, Pfizer, Novartis, Merck, AstraZeneca, Ferring, Ipsen, Celgene, and GlaxoSmithKline. The other authors declare no conflicts of interest directly related to this work. We thank Shari Gelber for editorial assistance.

References

1. Klein S, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16(10):626–638.
2. Markle JG, Fish EN. SeXX matters in immunity. *Trends Immunol*. 2014;35(3):97–104.
3. Özdemir BC, Csajka C, Dotto GP, et al. Sex differences in efficacy and toxicity of systemic treatments: an undervalued issue in the era of precision oncology. *J Clin Oncol*. 2018;36(26):2680–2683.
4. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19(6):737–746.
5. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary?. *Controlled Clin Trials*. 1996;17(1):1–12.
6. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177–188.
7. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539–1558.
8. Fisher DJ, Carpenter JR, Morris TP, Freeman SC, Tierney JF. Meta-analytical methods to identify who benefits most from treatments: daft, deluded, or deft approach? *BMJ*. 2017;356:j573.
9. Schenker N, Gentleman JF. On judging the significance of differences by examining the overlap between confidence intervals. *Am Statistic*. 2001;55(3):182–186.
10. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med*. 2018;378(24):2288–2301.
11. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med*. 2018;379(24):2342–2350.
12. Horn L, Mansfield AS, Szczęsna A, et al. First-line Atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N Engl J Med*. 2018;379(23):2220–2229.
13. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078–2092.
14. Paz-Ares LG, Luft A, Ali Tafresh A, et al. Phase 3 study of carboplatin-paclitaxel/nab-paclitaxel (chemo) with or without pembrolizumab (pembro) for patients (pts) with metastatic squamous (sq) non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2018;36(suppl 15):105.
15. Jotte RM, Cappuzzo F, Ihor Vynnychenko I, et al. IMpower131: primary PFS and safety analysis of a randomized phase III study of atezolizumab + carboplatin + paclitaxel or nab-paclitaxel vs carboplatin + nab-paclitaxel as 1L therapy in advanced squamous NSCLC. In: ASCO 2018. Abstract LBA9000.
16. Papadimitrakopoulou V, Cobo M, Bordoni R, et al. IMpower 132: PFS and safety results with 1L atezolizumab + carboplatin/cisplatin + pemetrexed in stage IV nonsquamous NSCLC. In: International Association for the Study of Lung Cancer 19th World Conference on Lung Cancer; 2018.
17. Cappuzzo F. Impower130: Progression-free survival (PFS) and safety analysis from a randomized phase 3 study of carboplatin + nab-paclitaxel (CnP) with or without atezolizumab (atezo) as first-line (1L) therapy in advanced non-squamous NSCLC. Paper presented at the ESMO 2018 Congress; October 19–23, 2018; Munich, Germany. Abstract LBA53
18. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823–1833.
19. Lopes G, Wu Y, Kudaba I, et al. Pembrolizumab (pembro) versus platinum-based chemotherapy (chemo) as first-line therapy for advanced/metastatic NSCLC with a PD-L1 tumor proportion score (TPS) $\geq$ 1%: open-label, phase 3 KEYNOTE-042 study. *J Clin Oncol*. 2018;36(suppl 18):LBA4.
20. Carbone DP, Reck M, Paz-Ares L, et al. First-line nivolumab in stage iv or recurrent non-small-cell lung cancer. *N Engl J Med* . 2017;376(25):2415–2426.
21. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. *Nat Rev Immunol*. 2010;10(8):594–604.
22. Kovats S, et al. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol*. 2015;294(2):63–69.
23. Polanczyk MJ, Hopke C, Vandenbark AA, et al. Treg suppressive activity involves estrogen-dependent expression of programmed death-1 (PD-1). *Int Immunol*. 2007;19(3):337–343.
24. Thorsson V, Gibbs DL, Brown SD, et al. The immune landscape of cancer. *Immunity*. 2018;48(4):812–830.
25. Loo K, Tsai K, Mahuron K, et al. Partially exhausted tumor-infiltrating lymphocytes predict response to combination immunotherapy. *JCI Insight*. 2017;2(14):e93433.
26. Conforti F, Pala L, Bagnardi V, et al. Sex-based differences of the tumor mutational burden and T-cell inflammation of the tumor microenvironment. *Ann Oncol*. 2019;30(4):653–655.
27. Salem ME, Xiu J, Lenz HJ, et al. Characterization of tumor mutation load (TML) in solid tumors. *J Clin Oncol*. 2017;35(suppl 15):11517.
28. Xiao D, Pan H, Li F, Wu K, Zhang X, He J. Analysis of ultra-deep targeted sequencing reveals mutation burden is associated with sex and clinical outcome in lung adenocarcinoma. *Oncotarget*. 2016;7(16):22857–22864.
29. Chalmers, Caitlin ZR, Connelly CF, Fabrizio D, et al. Analysis of 100.000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34.
30. Wanderley CW, Colón DF, Luiz JPM, et al. Paclitaxel reduces tumor growth by reprogramming tumor-associated macrophages to an M1 profile in a TLR4-dependent manner. *Cancer Res*. 2018;78(20):5891–5900.
31. Santa-Maria CA, Nanda R. Immune checkpoint inhibitor therapy in breast cancer. *J Natl Compr Canc Netw*. 2018;16(10):1259–1268.
32. Kok M, Winer E, Loi S. Passion for immune checkpoint blockade in triple negative breast cancer? Comment on the IMpassion130 study. *Ann Oncol*. 2019;30(1):13–16.
33. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med*. 2018;380(10):987–988.
34. Wallis CJD, Butaney M, Satkunasivam R, et al. Association of patient sex with efficacy of immune checkpoint inhibitors and overall survival in advanced cancers: a systematic review and meta-analysis [published online ahead of print January 3, 2019]. *JAMA Oncol*. doi:10.1001/jamaoncol.2018.5904.