

Gender and outcome of patients receiving immune checkpoint inhibitors in the treatment of advanced cancer

Antonino Grassadonia¹, Michele De Tursi¹, Isabella Sperduti², Clara Natoli¹, Maddalena Barba³, Nicola Tinari¹

¹Department of Medical, Oral and Biotechnological Sciences and CeSI-MeT, G. D'Annunzio University, Chieti, Italy; ²Department of Bio-Statistics, IRCCS Regina Elena National Cancer Institute, Rome, Italy; ³Division of Medical Oncology 2, IRCCS Regina Elena National Cancer Institute, Rome, Italy. Received 31 August 2019; accepted 16 January 2020.

Innate and adaptive immune responses are functionally influenced by gender. In fact, women exhibit a higher efficiency of antigen presenting cells (APCs) and macrophage activation, and higher levels of B cells, antibody production, CD4+ T cells, CD4/CD8 ratio, and T helper (Th) 2 cell response, while men have higher levels of CD8+ T cells, regulatory T (Treg) cells, and Th1 cell response.¹

Sexual immune dimorphism has been related to differences in the expression of chromosome X-linked immune-associated genes, such as TLR7, TLR8, IL-2, IL-4, IL-15, FOXP3,^{2,3} along with differences in the hormonal modulation of the immune response by estrogen, progesterone and testosterone^{4,5} and a different influence of gut microbiome on immune competency.^{6,7}

The increasing use of immune checkpoint inhibitors (ICIs) in the treatment of different types of cancer, including melanoma, non small-cell lung cancer (NSCLC), and renal cell carcinoma (RCC),⁸ has raised the question whether gender can affect the anti-tumor response to these agents.

In order to answer the question, we carried out a systematic review and meta-analysis of 21 phase III randomized controlled trials (RCTs) and analyzed the impact of gender on the outcome of patients with advanced cancer treated with ICIs.⁹ Patients were mostly affected by metastatic melanoma (5 studies) and NSCLC (10 studies). Other studies included 2 trials on RCC and single trials on small-cell lung carcinoma, head and neck cancer, urothelial carcinoma, and gastric cancer. Altogether, we collected overall survival (OS) data from 11,318 patients, and progression-free survival (PFS) data from 3,746 patients. All the selected studies compared ICIs (anti-CTLA-4, anti-PD1, or anti-PDL1) versus standard treatment or placebo, and reported the clinical outcomes of OS and/or PFS stratified by gender.⁹

By using the random effects model, our meta-analysis revealed that both males and females achieved a significant reduction in the risk of death when treated with ICIs, compared to control. HR was 0.73 for men (95% CI 0.66-0.80, $p < 0.001$, I² 66%) and 0.77 for women (95% CI 0.67-0.89, $p < 0.001$, I² 62%). Then, we separately analyzed the studies investigating anti-PD-1 or anti-PDL-1 agents and those investigating anti-

CTLA-4 agents. The use of anti-PD-1/PDL-1 resulted in a better outcome both in men and women (HR 0.69, 95% CI 0.62-0.78, $p < 0.001$ and HR 0.73, 95% CI 0.60-0.89, $p = 0.002$, respectively). In contrast, the anti-CTLA-4 treatment was effective in men (HR 0.77, 95% CI 0.63-0.94, $p = 0.012$), but did not reach significance in women (HR 0.89, 95% CI 0.76-1.05, $p = 0.162$). However, anti-CTLA-4 resulted in a similar benefit in men and women when the analysis was restricted to 4 studies on melanoma (HR 0.67, 95% CI 0.50-0.90, $p = 0.008$ and HR 0.80, 95% CI 0.68-0.94, $p = 0.006$, respectively).

Finally, we analyzed the effect of gender on PFS. All the 8 studies selected were trials conducted in patients affected by NSCLC. All but one investigated anti-PD-1/PDL-1 agents. We found a significant improvement in PFS in men (HR 0.67, 95% CI 0.55-0.80, $p < 0.001$, I² 73%), but not in women (HR 0.77, 95% CI 0.57-1.05, $p = 0.100$, I² 63%).

The results of this meta-analysis clearly indicate that both genders gained OS advantage from the treatment with ICIs compared to the standard treatment, with a tendency towards more favorable outcome in men, who achieved a lower HR. However, a significant improvement in PFS emerged exclusively in men.

A benefit in OS was observed in both genders when the analysis was focused on anti-PD-1/PDL-1. Interestingly, men – but not women – showed a significantly better OS when treated with anti-CTLA-4, with the exception of patients affected by melanoma, a disease in which women showed a benefit as well. To provide a biologic rationale to this finding, both gender-related differences in immune response¹ and the different mechanisms of action of anti-CTLA-4 and anti-PD-1/PDL-1 must be considered.

CTLA-4 is expressed on T lymphocytes and, by binding B7 receptors on antigen-presenting cells (APCs), determines an inhibition of T-cell activation at the priming phase of the immune response, when a naïve T lymphocyte recognizes tumor antigens for the first time.¹⁰ Therefore, anti-CTLA-4 antibodies can re-activate suppressed T lymphocytes, stimulating their proliferation and triggering humoral and cytotoxic anti-tumor response. It is widely accepted that this early phase of immune system activation is stronger in women than in

men, since the former have more effective APCs and a higher count of CD4+ T cell.¹ It is conceivable that, in order to progress and overcome the host proficient immune response, a tumor growing in a human female organism must select cellular clones with low immunogenic potential, i.e., clones that do not display antigens able to elicit an anti-tumor response. Therefore, in this female-biased immune scenario, we may hypothesize that the lack of T cell activation, rather than the CTLA-4-mediated T cell inhibition, is responsible for the tumor escaping from immune surveillance. Alternatively, it is also possible that female T-cell suppression is driven by distinct cellular mechanisms which do not include CTLA-4. As a consequence, anti-CTLA-4 therapy fails to revert immune response in women. Moreover, it has been reported that CTLA-4 is expressed in Treg cells, a lymphocyte population with immunosuppressive effects, and that anti-CTLA-4 agents can restore immune competence partly by depleting Treg cells or abrogating their function.^{11,12} Since women have a lower Treg count than men, they may receive smaller benefits from an anti-CTLA-4 therapy.

Unexpectedly, this result was not duplicated in female patients affected by melanoma (4 trials). In this subgroup, women as well as men achieved a survival advantage with anti-CTLA-4 compared to control. We can hypothesize that tumor- and/or patient-specific factors other than gender may influence the response to anti-CTLA-4 agents in melanoma. In particular, melanoma is known to be a tumor with a very high mutational burden (0.5 to >100 mutations per megabase)¹³ and with a high propensity to generate neoantigens that are recognized by the immune system as nonself.¹⁴ The genetic bases for a clinical response to anti-CTLA-4 in melanoma have been recently clarified.¹⁴ We could speculate that melanoma cells are by themselves able to elicit a strong antitumor immune response, potentially able to destroy the tumor if the immunosuppressive effect of CTLA-4 expression on T cell does not occur. In this scenario, both men and women receive a benefit from CTLA-4 blockade in melanoma.

PD-1 is mostly expressed in CD8+ T lymphocytes, and inhibits their cytotoxic activity by binding PD-L1 and PD-L2 expressed in tumor cells.¹⁰ Thus, the inhibition of PD-1 interaction with its ligands, using anti-PD-1 or anti-PDL-1 antibodies, can re-activate effector CD8+ T cell to kill tumor cells. In light of our results, this peripheral immune mechanism seems to be resumed by anti-PD-1/PD-L1 in both genders. Men have a higher count of CD8+ T lymphocytes, but these cells are functionally more active in women.¹ Recent studies have shown that tumor cells of NSCLC express significant higher levels of PD-L1 in male compared to female patients.¹⁵ For this reason, anti-PD1/PDL-1 therapies may be more effective in men. Consistently, our study showed a better OS and

a significantly improved PFS in men compared to women, when treated with anti-PD1/PDL-1.

Prior to our study, 3 different meta-analyses by Botticelli et al,¹⁶ Wu et al¹⁷ and Conforti et al,¹⁸ have investigated the impact of gender-related differences on the efficacy of immune check-point inhibitors, and have been recently published. All included both phase II and phase III studies. This latter approach, i.e. the inclusion of phase II studies, may increase the degree of heterogeneity. On the contrary, we exclusively included phase III RCTs, not only to limit heterogeneity, but also because phase III studies are sufficiently powered to detect differences between two groups of treatment, and ensure a longer follow-up and a higher number of events compared to phase II studies.

Overall, the evidence observed supports more favorable outcomes in men than in women. In particular, the meta-analysis by Botticelli et al reported a statistically significant improvement in PFS, but not in OS, in men compared to women when treated with anti-PD-1, and a more favorable OS in males when treated with anti-CTLA4, although this was not statistically significant. The second study by Wu et al showed that PFS and OS were significantly improved by the treatment with ICIs in both genders, but men showed lower HR than women. Finally, Conforti et al showed that both men and women treated with ICIs obtained a reduced risk of death, but men had a significant lower HR.

Taken together, our data and the previous meta-analyses indicate that ICIs are better than the standard therapy in the overall population, but are more effective in men than in women. Importantly, only our study revealed that women do not gain benefits from an anti-CTLA-4 treatment compared to control (chemotherapy or placebo), with the exception of melanoma – as discussed above. Consistently, so far the anti-CTLA-4 therapy is used clinically only for patients affected by melanoma. Focusing on this topic, the studies by Botticelli and Wu showed a benefit for males vs females treated with anti-CTLA-4, but the difference did not reach a statistical significance, probably due to the small number of trials included in the analysis. The study by Conforti was statistically powered, but was aimed at comparing the pooled HR in men with the pooled HR in women, thus the authors emphasized the increased relative benefit in male compared to female patients treated with anti-CTLA-4. By looking back to their data, the anti-CTLA-4 therapy was not superior to control in female, consistently with our findings.

In conclusion, overall data support an improvement in the prognosis of metastatic cancer patients with the treatment with ICIs, although men achieve a greater benefit compared to women. The lack of benefits from anti-CTLA-4 in women is a relevant issue that has to be considered in prospective clinical trials, since conclu-

sions might be affected by the number of men and women enrolled in the study. To better understand the role of gender in the anti-tumor activity of ICIs, and in order to avoid any misinterpretation of the results, the patients selected for RCTs should be stratified by gender during the randomization process.

References

- Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16:626-38.
- Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol*. 2008;8:737-44.
- Kim CH. FOXP3 and its role in the immune system. *Adv Exp Med Biol*. 2009;665:17-29.
- Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol*. 2015;294:63-9.
- Mackern-Obertl JP, Jara EL, Riedel CA, Kalergis AM. Hormonal modulation of dendritic cells differentiation, maturation and function: implications for the initiation and progress of systemic autoimmunity. *Arch Immunol Ther Exp*. 2017;65:123-36.
- Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*. 2013;339:1084-8.
- Botticelli A, Zizzari I, Mazza F, Ascierio PA, Putignani L, Marchetti L, et al. Cross-talk between microbiota and immune fitness to steer and control response to anti PD-1/PDL-1 treatment. *Oncotarget*. 2017;8:8890-9.
- Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat Rev Clin Oncol*. 2016;13:273-90.
- Grassadonia A, Sperduti I, Vici P, Iezzi L, Brocco D, Gamucci T, et al. Effect of gender on the outcome of patients receiving immune checkpoint inhibitors for advanced cancer: a systematic review and meta-analysis of phase III randomized clinical trials. *J Clin Med*. 2018;7(12).pii:E542. doi: 10.3390/jcm7120542.
- Messerschmidt JL, Prendergast GC, Messerschmidt GL. How cancers escape immune destruction and mechanisms of action for the new significantly active immune therapies: helping nonimmunologists decipher recent advances. *Oncologist*. 2016;21:233-43.
- Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, et al. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. *J Immunol*. 2006;177:4376-83.
- Riella LV, Liu T, Yang J, Chock S, Shimizu T, Mfarrej B, et al. Deleterious effect of CTLA4-Ig on a Treg-dependent transplant model. *Am J Transpl*. 2012;12:846-55.
- Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415-21.
- Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371:2189-99.
- Petrelli F, Maltese M, Tomasello G, Conti B, Borgonovo K, Cabiddu M, et al. Clinical and molecular predictors of PD-L1 expression in non-small-cell lung cancer: systematic review and meta-analysis. *Clin Lung Cancer*. 2018;19:315-22.
- Botticelli A, Onesti CE, Zizzari I, Cerbelli B, Sciattella P, Occhipinti M, et al. The sexist behaviour of immune checkpoint inhibitors in cancer therapy? *Oncotarget*. 2017;8:99336-46.
- Wu Y, Ju Q, Jia K, Yu J, Shi H, Wu H, et al. Correlation between sex and efficacy of immune checkpoint inhibitors (PD-1 and CTLA-4 inhibitors). *Int J Cancer*. 2018;143:45-51.
- Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19:737-46.

Key messages

- In our systematic review and meta-analysis of phase III randomized clinical trials, we explored the impact of gender on survival in patients with advanced cancer treated with immune checkpoint inhibitors (ICIs).
- Both males and females showed reduced risk of death associated with ICIs use (HR 0.73, $p < 0.001$ and HR 0.77, $p < 0.001$, respectively).
- Subgroup analyses by specific ICI showed similar OS in both genders for anti-PD-1/PDL-1. Anti-CTLA-4 use was associated with longer OS in men only (HR 0.77, $p < 0.012$), with the exception of melanoma (in women, HR 0.80, $p = 0.006$). PFS was longer in men than in women (HR 0.67, $p < 0.001$ and HR 0.77, $p = 0.100$, respectively).
- The lack of benefits from anti-CTLA-4 in women is a relevant issue that has to be considered in prospective clinical trials, since conclusions might be affected by the number of men and women enrolled in the study.

Author contribution statement: CN and NT conceived the study and contributed to study design; AG wrote the manuscript and contributed to data interpretation; MDT critically reviewed the manuscript; IS and MD analysed the data and contributed to data interpretation. All authors have read and approved the manuscript.

Conflict of interest statement: the Authors declare no conflicts of interest.

Correspondence to:

Antonino Grassadonia

Department of Medical, Oral and Biotechnological Sciences and CeSI-MeT

G. D'Annunzio University

66100 Chieti, Italy

e-mail: antonino.grassadonia@unich.it