Editorial

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Pharmacogenomics

Genetics and treatment of gastrointestinal stromal tumors with immune checkpoint inhibitors: what do we know?

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"tumor-infiltrating immune cells are present in GIST and seem to be associated with disease outcomes and also with increasing the activity of imatinib"

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Background

Gastrointestinal stromal tumors (GIST) are mesenchymal neoplasms, derived from the interstitial cells of Cajal and mainly characterized from driver mutation in tyrosine kinase receptor genes *KIT* and *PDGFRA*. During the last 20 years, the tyrosine kinase inhibitors (TKI) revolutionized the therapy of GIST and resulted in extreme improvement in the survival of patients with metastatic disease [1–3]. Unfortunately, responder patients invariably develop resistance over time to all TKI, imatinib as well as sunitinib and regorafenib. Recently, new TKI have demonstrated interesting results in the prolongation of survival in clinical studies [4,5]. However, the complexity of the molecular background of GISTs resistant to TKI is characterized by the acquisition of secondary mutations and additional genome alterations. The prolongation of patients' life expectancy and the complex biology involved in progressive disease led to a growing interest in developing new therapeutic approaches aimed at overcoming therapy resistance, including immunotherapy. Recently, immunotherapy in oncology has revolutionized the treatment of patients with cancer, improving survival outcomes [6–8].

In the last few years, several investigations on the GIST immune landscape have been conducted to characterize the immunological profile as a basis for immunotherapy in GIST.

According to this research, tumor-infiltrating immune cells are present in GIST and seem to be associated with disease outcomes and also with increasing the activity of imatinib. Tumor-associated macrophages (TAM) are described as being present in the disease microenvironment, in particular, M2 macrophages appear as the most enriched cells in metastatic and in imatinib-treated GIST cases [9–11]. Indeed, in a transgenic mouse model of GIST, imatinib therapy could polarize TAM to a M2-like macrophage phenotype [9]. Imatinib also showed the ability to promote the activation of natural killer (NK) cells and the production of IFN-γ, through the stimulation of a KIT-dependent cross-talk between host dendritic cells and NK cells [11]. Actually, in GIST, the NK infiltrate predicts progression free survival and patients treated with imatinib classified as immunologically active on the basis of NK-interferon levels show a better survival [12]. Tumor-infiltrating lymphocytes represent the second more enriched cell population in the GIST microenvironment [9,11,13,14]; however, only a few preclinical studies in mouse models of GIST were conducted on this immune population. In addition, $CD8^+$ T cells seem to synergize the antitumor effects of imatinib [15]. In fact, Balacharan *et al.* showed that imatinib leads to the activation of $CD8^+$ T cells and the inhibition of Treg through IDO inactivation. Finally, the therapeutic efficacy of imatinib may be increased by combination with immune checkpoint inhibitors as showed in preclinical GIST murine models [16].

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Recently, in our own research team, we investigated the GIST microenvironment and predictive signatures to immunotherapy, the expanded IFN-γ-induced immune signature (EIIS) and the T-cell-inflamed signature (TIS) [17]. The results confirmed the presence of immune infiltrate cells, with dominance of $CD4^+$ and $CD8^+$ T cells and M2 macrophages supporting a remarkable similarity with the microenvironment of melanoma. Moreover, additional interesting data were related to the presence of the gene expression signatures recently identified to be predictors of response to immune checkpoint inhibitors in multiple tumors. In particular, the EIIS is expressed in all GIST samples and, interestingly, positively correlates with *PD-L1* expression. This EIIS has been considered a predictor to immunotherapy in head and neck squamous cell carcinoma, melanoma and also gastric cancer [18,19]. As matter of fact, it is well known that *PD-L1* expression is directly induced in tumors, in the immune infiltrate and stromal cells by sustained IFN-γ signaling representing feedback inhibition of the immune responses. Currently, a number of studies have studied the PD-1/PD-L1 expression in GIST showing that PD-L1 tumor expression evaluated by immunohistochemistry was higher in GIST than in other sarcomas and *PD-L1* expression evaluated at the mRNA level was heterogeneous across tumors [14,20]. In our series, we found that the EIIS signature was positively correlated with *PD-L1* expression and *PD-L1* was positively correlated to both *CD8A* and *CD8B* gene expression.

This evidence supports the presence of an immune-active tumor microenvironment suggesting the involvement of adaptive immune-escape mechanisms, as described in other cancer settings.

In addition, in GIST, we found a high expression of the TIS signature and therefore high value of TIS score that is very close to other tumor types in which immunotherapy with PD1/PD-L1 inhibitors is clinically effective. The TIS score was introduced by Ayers *et al.* [18] and was initially derived using a set of melanoma samples and then improved using 220 patients with nine different cancer types. It considers genes related to IFN-γ response, antigen presentation, adaptive immune resistance, chemokine expression and cytotoxic activity and it correlates with clinical benefit from immune checkpoint inhibitor treatment.

Our results on the presence of the immune cells in the microenvironment in GIST and the presence of immune signatures known to be predictors of response to checkpoint inhibitors in oncology suggest that also in GIST a new approach with immunotherapy along with TKI may show a therapeutic benefit.

An evaluation of the GIST immune landscape was recently performed also by Vitiello *et al.* and comparatively analyzed the different molecular GIST subgroups. Through an integrated approach, based on bioinformatics, immunohistochemistry (IHC) and flow cytometry, they demonstrated that GIST with *PDGFRA* mutations are more immunogenic than GIST with other mutations. In particular, PDGFRA-mutant GIST showed a stronger gene expression-based immune signature together with a higher abundance of $CD45^+$ and $CD8^+$ cells in tumor microenvironment, confirming that this molecular class have higher cytolytic activity than GIST with similar clinic pathologic features but different oncogenic drivers (such as KIT-mutant). Despite no correlation between the immunological activity and neo-epitope burden nor number of high-affinity neo-epitopes being identified, this study showed that almost all GIST mutations (in *PDGFRA* or *KIT*) generated at least one high-affinity neo-epitope, which may be recognizable by the immune system. Interestingly, they showed that the *PDGFRA* D842V mutation produced six different high-affinity neo-epitopes able to bind several HLA types including the most common HLA in white individuals [21]. These findings are corroborated by the results obtained in our latest work on PDGFRA-mutant GIST (yet unpublished) in which we highlight that D842V-mutant GIST exhibits a remarkable enrichment of immune-signature, an increased TIS score and an abundance of $CD8^+$ infiltrating T cells with respect to non-D842V GIST.

Even though these recent findings seem to be promising for an immunotherapeutic approach in GIST, a clinical trial with dasatanib and ipilimumab as the KIT and CTLA-4 blockade combination did not report a synergistic activity, due to the limited clinical efficacy of this combination in GIST [22]. Regrettably, patients involved in this trial were extensively pretreated with TKI and most of them were considered unevaluable for tumor response since they developed a heavily TKI-resistant disease. As the authors also stated, this cohort is limited but generates data that, in the era of new effective checkpoint inhibitors, may be useful for future protocols with TKI or IDO combined with anti-PD-1 drugs or with other immunotherapeutic approaches.

Moreover, this trial was designated and conducted before the recent acquisition of data on the immunoprofiling of GIST and its correlation with the kinase genotype.

In fact, recently, new immunological data suggests that GIST patients could benefit from immunotherapy along with TKI. This hypothesis is extremely interesting since the new TKI can invariably lead to resistance development, even though they are extremely promising in clinical trials as potent inhibitors of many secondary-resistant mutations

located both in the ATP-binding pocket (mutations in the exons 13–14) and the activation loop (mutations in exons 17–18). The Phase III trial comparing ripretinib with placebo in ≥4 lines of therapy in metastatic GIST demonstrated a median progression free survival of 6.3 months for ripretinib, which was significantly improved compared with placebo (1.0 month) [5]. These clinical data suggest that in the advanced setting, the kinase genotype deregulation may not be the only driver of disease progression but a more complex biological background should be taken into account. Therefore, the strategy to overcome TKI inhibition as an exclusively therapeutic approach in GIST is still an unmet clinical need. New trials combining the checkpoint inhibitors to TKI are now planned firstly in third line of treatments and, if promising, in earlier settings.

Furthermore, other immunological approaches are now emerging, exploiting different immunomodulatory molecules to work together with TKI to be more effective. Of interest, the study proposed by Zhang *et al.*, in which the authors showed the ability of anti-CD40 antibody to revert the M2-like phenotype of TAM induced by KIT pathway inhibition, resulted in a notable improvement on the antitumor effects of imatinib therapy [23].

In conclusion, in the last few years, many preclinical translational studies investigating the immunological background of GIST have been conducted to explore the biological basis for the immunotherapy in this oncogene-driven tumor. GIST presents a rich immune cell-infiltration profile along with the expression of signatures predicting response to cancer immunotherapy, suggesting that GIST patients could benefit from a synergistic approach of immunotherapy along with TKI. On this basis, future clinical trials should be encouraged.

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References

1. Demetri GD, von Mehren M, Blanke CD *et al.* Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 347(7), 472–480 (2002).
2. Demetri GD, van Oosterom AT, Garrett CR *et al.* Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumor after failure of imatinib: a randomised controlled trial. *Lancet* 368(9544), 1329–1338 (2006).
3. Demetri GD, Reichardt P, Kang Y-K *et al.* Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumors after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, Phase III trial. *Lancet* 381(9863), 295–302 (2013).
4. Heinrich M, von Mehren M, Jones RL *et al.* Avapritinib is highly active and well-tolerated in patients (pts) with advanced GIST driven by diverse variety of oncogenic mutations in KIT and PDGFRA. Presented at: *2018 CTOS Annual Meeting*. Rome, Italy, 14–17 November 2018.
5. von Mehren M, Serrano C, Bauer S *et al.* LBA87INVICTUS: A Phase III, interventional, double-blind, placebo-controlled study to assess the safety and efficacy of ripretinib as ≥4th-line therapy in patients with advanced gastrointestinal stromal tumors (GIST) who have received treatment with prior anticancer therapies (NCT03353753). *Ann. Oncol.* 30(Suppl.5), v925–v926 (2019).
6. Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).
7. 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.* 375(19), 1823–1833 (2016).
8. Motzer RJ, Escudier B, McDermott DF *et al.* Nivolumab versus everolimus in advanced renal-cell carcinoma. *N. Engl. J. Med.* 373(19), 1803–1813 (2015).
9. van Dongen M, Savage NDL, Jordanova ES *et al.* Anti-inflammatory M2 type macrophages characterize metastasized and tyrosine kinase inhibitor-treated gastrointestinal stromal tumors. *Int. J. Cancer* 127(4), 899–909 (2010).
10. Cavnar MJ, Zeng S, Kim TS *et al.* KIT oncogene inhibition drives intratumoral macrophage M2 polarization. *J. Exp. Med.* 210(13), 2873–2886 (2013).
11. Tan Y, Trent JC, Wilky BA, Kerr DA, Rosenberg AE. Current status of immunotherapy for gastrointestinal stromal tumor. *Cancer Gene Ther.* 24(3), 130–133 (2017).
12. Rusakiewicz S, Perier A, Semeraro M *et al.* NKp30 isoforms and NKp30 ligands are predictive biomarkers of response to imatinib mesylate in metastatic GIST patients. *Oncoimmunology* 6(1), e1137418 (2017).
13. Rusakiewicz S, Semeraro M, Sarabi M *et al.* Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res.* 73(12), 3499–3510 (2013).

14. D'Angelo SP, Shoushtari AN, Agaram NP *et al.* Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. *Hum. Pathol.* 46(3), 357–365 (2015).
15. Balachandran VP, Cavnar MJ, Zeng S *et al.* Imatinib potentiates antitumor T-cell responses in gastrointestinal stromal tumor through the inhibition of Ido. *Nat. Med.* 17(9), 1094–1100 (2011).
16. Seifert AM, Zeng S, Zhang JQ *et al.* PD-1/PD-L1 blockade enhances T-cell activity and antitumor efficacy of imatinib in gastrointestinal stromal tumors. *Clin. Cancer Res.* 23(2), 454–465 (2017).
17. Pantaleo MA, Tarantino G, Agostinelli C *et al.* Immune microenvironment profiling of gastrointestinal stromal tumors (GIST) shows gene expression patterns associated to immune checkpoint inhibitors response. *OncoImmunology* 8(9), e1617588 (2019).
18. Ayers M, Lunceford J, Nebozhyn M *et al.* IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J. Clin. Invest.* 127(8), 2930–2940 (2017).
19. Danaher P, Warren S, Lu R *et al.* Pan-cancer adaptive immune resistance as defined by the tumor inflammation signature (TIS): results from The Cancer Genome Atlas (TCGA). *J. Immunother. Cancer* 6(1), 63 (2018).
20. Bertucci F, Finetti P, Mamessier E *et al.* PDL1 expression is an independent prognostic factor in localized GIST. *Oncoimmunology* 4(5), e1002729 (2015).
21. Vitiello GA, Bowler TG, Liu M *et al.* Differential immune profiles distinguish the mutational subtypes of gastrointestinal stromal tumor. *J. Clin. Invest.* 129(5), 1863–1877 (2019).
22. D'Angelo SP, Shoushtari AN, Keohan ML *et al.* Combined KIT and CTLA-4 blockade in patients with refractory GIST and other advanced sarcomas: a Phase Ib study of dasatinib plus ipilimumab. *Clin. Cancer Res.* 23(12), 2972–2980 (2017).
23. Zhang JQ, Zeng S, Vitiello GA *et al.* Macrophages and CD8+ T-cells mediate the antitumor efficacy of combined CD40 ligation and imatinib therapy in gastrointestinal stromal tumors. *Cancer Immunol. Res.* 6(4), 434–447 (2018).