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Choosing the Best Chemotherapy Agent to Boost Immune Checkpoint Inhibition Activity

Marina Chiara Garassino[1], Valter Torri[2], Mario Paolo Colombo[3], and Antonio Sica[4,5]

Despite the fact that reactivation of specific antitumor immunity through inhibition of immune checkpoints represents a formidable therapeutic weapon against cancer, many patients are poorly reactive to this treatment. To overcome this limitation, efforts are being made to characterize the immunostimulatory properties of chemotherapeutic agents and how they can be best combined with immune checkpoint inhibitors. The work by Wanderley and colleagues indicates that the TLR4 agonist taxol can restore the anticancer activity of tumor-associated macrophages and improve the clinical efficacy of immune checkpoint inhibitors. *Cancer Res; 78(20); 5729–30. ©2018 AACR.*

See related article by Wanderley et al., p. 5891

In this issue of *Cancer Research*, the article by Wanderley and colleagues (1) provides new and interesting evidence that the TLR4 agonist taxol can restore antitumor activities *in vivo* by reprogramming tumor-associated macrophages (TAM) to an immunocompetent M1-profile, thus contributing to the antitumor effect of taxol in combination with immune-checkpoint inhibitors (ICI). In the last couple of years, ICIs have revolutionized the treatment of many cancers by increasing the number of long-term survivors. However, there are still many tumor types that do not benefit from these treatments and of the tumor types that potentially could benefit from a single ICI agent, only a fraction of patients have durable long-lasting responses and clinically meaningful increased survival. Despite an increasing number of phase I and II trials of drugs targeting new checkpoint agonists and antagonists, one of the most promising strategies for increasing the number of patients benefiting from ICIs is combination with "old" chemotherapies. Chemotherapy has several immunologic effects such as increasing immunogenic death, eliminating immunosuppressive cells [regulatory T cells, myeloid-derived suppressor cells (MDSC), and macrophages], and increasing the ability of T cells to recognize tumor antigens (2). Therefore, the addition of chemotherapy to ICIs could potentially overcome mechanisms of primary resistance by inducing certain nonimmunogenic tumors to become immunogenic and could potentially delay the appearance of secondary resistance. On the basis of this premise, several trials are now ongoing in multiple tumor types in which chemotherapy represents the cornerstone of treatment.

In non–small cell lung cancer (NSCLC), combination immunotherapy with chemotherapy is the only strategy that has shown a statistically significant increase in patient survival, independent of PD-L1 expression. Several chemotherapy regimens have been investigated in first-line NSCLC treatment. All regimens added an ICI to combination chemotherapy of platinum plus primarily taxanes or pemetrexed. However, there seems to be a difference between the different chemotherapy backbones in their ability to be synergistic with the ICIs. For example, if we compare the incremental effect of pembrolizumab added to platinum pemetrexed in Keynote 189 (3), the results seem superior [HR, 0.49, 95% confidence interval (CI), 0.38–0.64; $P < 0.00001$] to those of atezolizumab observed in IMPower 150 (4) with carboplatin and paclitaxel with or without bevacizumab (HR, 0.78; 95% CI, 0.64–0.96; $P = 0.0164$). An explanation for the observed difference could be differences in the immunogenic activities of the chemotherapy compounds. Interesting results presented earlier this year at the AACR Annual Meeting by Novosiadly and colleagues (5) suggest a different immunomodulatory effect of pemetrexed compared with paclitaxel. MC38 tumors treated for 2 weeks with pemetrexed, paclitaxel, and carboplatin were analyzed for more than 70 immune-related genes using a custom-made QuantiGene Plex panel. Results showed that pemetrexed induced upregulation of several genes involved in the IFNγ pathway and IFN type I response, as well as other genes involved in T-cell recruitment and activation. Of note, the immunomodulatory effect of paclitaxel was qualitatively different and mainly associated with modest upregulation of myeloid cell–related genes (Il6, Cxcl1, Ccl2, Ccl3, Ccl4, Timd4). Interestingly, whereas both pemetrexed and paclitaxel upregulated PD-L1, the latter has been shown to act as a TLR4 agonist, thus affecting the innate immune functions of infiltrating myeloid cells (6). Present among these macrophages are a major tumor-promoting myeloid population able to infiltrate solid tumors and elicit cytotoxic and antitumor activities (7). In addition to the presence of MDSCs, the number of macrophages correlates with poor prognosis in various human malignancies. This paradoxical relationship has been widely attributed to their "functional plasticity," defined as the capacity to express distinct functional programs in response to different environmental signals. This accepted paradigm indicates the M1 and M2 polarization states

[1]Thoracic Oncology Unit, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. [2]Methodology of Clinical Research Laboratory, Oncology Department, IRCCS Mario Negri Institute for Pharmacologic Research, Milan, Italy. [3]Department of Research, Molecular Immunology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. [4]Department of Pharmaceutical Sciences, University of Eastern Piedmont, A. Avogadro, Novara, Italy. [5]Department of Inflammation and Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy.

Corresponding Author: Marina Chiara Garassino, Thoracic Oncology Unit, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan 20133, Italy. Phone: 3902-2390-3813; Fax: 39-0223902630; E-mail: marina.garassino@istitutotumori.mi.it

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as the extremes of a continuum of different functional states, which define cytotoxic/inflammatory (M1) and immunosuppressive/proangiogenic (M2) macrophages, induced by inflammatory signals, such as TLR4 agonists (e.g., LPS), and IL4, respectively (7). In response to microenvironmental signals, TAMs preferentially acquire an M2-like and tumor-promoting phenotype and are consequently considered a promising target for anticancer interventions. Strategies targeting TAMs include the inhibition of pathways promoting their recruitment, their pharmacologic depletion, and interventions promoting their M2 to M1 functional switch aimed at restoring antitumor functions (7). Within this scenario, the work by Wanderley and colleagues (1) provides a rationale for new combination regimens comprising paclitaxel and immunotherapies as an anticancer treatment. However, based on Novosiadly and colleagues (5), to optimize immunotherapeutic regimens, it is fundamental to clarify the mechanisms that make a chemotherapeutic less or more immunostimulatory than others. Of note, long exposure to TLR4 agonists (e.g., LPS) has been shown to promote a tolerant "M2-like" macrophage phenotype (8), associated with immunosuppressive properties. It was indeed reported that LPS-driven activation of macrophages induces a dynamic reprogramming of macrophage functions, encompassing an early and transient M1 polarization state, characterized by high levels of expression of prototypical M1 genes (e.g., IL1β, TNFα, IL6, IL12), followed by an M2-like polarization state, characterized by decreased expression of M1 genes and elevation of immunosuppressive M2 genes (e.g., IL10, TGFβ, arginase I; ref. 8). This reprogramming satisfies the primary and early (M1 polarization) requirement to eliminate danger signals (e.g., microbial agents) with the late (M2 polarization) need to restore tissue homeostasis. Hence, the weaker antitumor effects of taxol, as compared with pemetrexed, observed in combination with anticheckpoint inhibitors, may potentially rely on a long-lasting pharmacologic stimulation of TLR4. On the basis of this hypothesis, it would be imperative to further explore the proper dose and time of TLR4 stimulation provided by taxol treatment *in vivo* to refine a better treatment regimen to maximize its synergy with immunotherapy. An additional mechanism by which combination of TLR4 agonists with mAbs (e.g., pembrolizumab, nivolumab) might be dampened is provided by the observation that macrophages exposed to combination of immune complexes and TLR4 agonists (e.g., LPS) are characterized by an $IL10^{high}$ and $IL12^{low}$ phenotype and promote type II responses, with immunoregulatory functions (7). Finally, an additional complication may stem from the observation that high levels of TLR4 in cancer cells (ovarian cancer) correlate with acquired resistance to taxol (9). This evidence should be considered because reactivation of specific immunity and elevated levels of IFNγ may enhance TLR4 expression (10).

Hence, even though the combination of chemotherapy and immunotherapy provides new therapeutic opportunities, there is a compelling need to better understand the immunomodulatory properties of different chemotherapies to optimize their clinical use in combination with ICIs. It is likely that in the future, an understanding of the immunopharmacology of chemotherapeutic agents will represent a large chapter of translational research with broad clinical implications.

In conclusion, there could be a new life for the old chemotherapy for its immunomodulant properties. This will open a new area of research with several unsolved questions. First, we must identify either the best chemotherapy agent or the best combination to be added to immunotherapy. Furthermore, historically, chemotherapy was developed on the basis of dose-limiting toxicity and its cytotoxic properties. When combined with immunotherapy, the final goal of chemotherapy is to promote more immunogenic death and consequent T-cell activation, by decreasing suppressor myeloid cell components (MSDCs, TAMs). However, it is possible that conventional dosages of chemotherapy by inducing necrosis and release of endogenous TLR4 ligands (e.g., HGMB1) could potentially induce earlier tolerance and M2 transition compared with new dosages specifically studied not only for their cytotoxic properties. Similarly, it is possible that continuous administration compared with intermittent treatments may again shift the immunologic status versus a less activated status. The work by Wanderley and colleagues is one of the first studies to begin unraveling the intricate canvas of the new life of chemotherapy.

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