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Natural Killer Cells to the Attack: Combination Therapy against Neuroblastoma

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TGFβ in the tumor microenvironment diminishes natural killer (NK) cell–mediated anti-disialoganglioside (anti-GD2) mAb elimination of neuroblastoma cells. Consequently, blockade of TGFβ signaling with galunisertib in combination with the anti-GD2 mAb dinutuximab plus adoptively transferred NK cells is a promising tool for the treatment of neuroblastoma. *Clin Cancer Res; 23(3); 615–7. ©2016 AACR.*

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In this issue of *Clinical Cancer Research*, Tran and colleagues (1) report that galunisertib (LY2157299 monohydrate), an inhibitor of TGFβ type I receptor (TGFβRI) kinase, significantly increases the therapeutic effect mediated by adoptively transferred expanded and activated natural killer (aNK) cells in combination with the anti-disialoganglioside (anti-GD2) mAb dinutuximab (ch14.18) in a mouse model of neuroblastoma.

NK cells utilize an array of activating and inhibitory receptors to scrutinize for changes in the expression of their ligands that take place in tumor cells. Because NK cells are able to eliminate cancer cells without previous sensitization in a fast and strong manner, NK cell–based immunotherapy is becoming a reality in the clinic. In addition, NK cells have an important role in many mAb-mediated therapies, which is partly due to their ability to engage CD16 (the low-affinity receptor for IgG or FcγRIIIa) on NK cells and triggering of antibody-dependent cell-mediated cytotoxicity (ADCC). The final objective is to reverse the tumor-induced NK cell dysfunction by augmenting and sustaining NK cell effector functions (2, 3).

Neuroblastoma is the most common extracranial solid tumor during childhood. About half of patients have advanced stage neuroblastoma at diagnosis, and, in contrast to many other pediatric cancers, progress in its treatment has been relatively modest. Patients have poor long-term survival due to residual refractory disease, despite aggressive therapeutic approaches that include chemotherapy, surgery, radiotherapy, and autologous stem cell transplantation (4). Cancer progression depends not only on tumor biology but also on the host immune response. NK cells have been shown to eliminate neuroblastoma cells, and among others, the activating receptors DNAM-1, NKG2D, and NKp30 have been proposed to have a very important role in the killing of tumor cells (5, 6). New therapeutic options involve targeting of GD2, which is highly expressed on neuroblastoma cells (7). The chimeric anti-GD2 mAb dinutuximab in combination with granulocyte-macrophage colony–stimulating factor (GM-CSF), IL2, and 13-cis-retinoic acid (RA) was recently approved by the FDA for the treatment of high-risk neuroblastoma. The humanized 3F8 mAb is currently in clinical trials (8). Both anti-GD2 mAbs use NK cell–mediated ADCC as a mechanism to kill neuroblastoma cells, and their effect is modulated by the pattern of activating and inhibitory receptors expressed on NK cells and their corresponding ligands on tumor cells (5).

There is much evidence that the tumor microenvironment (TME) not only promotes tumor growth and survival but also induces immune tolerance (2). There are metabolites, soluble ligands of activating receptors, and cytokines that have been reported to suppress NK cell effector functions (2, 5, 6). Within the TME, due to its potent immunosuppressive functions, TGFβ has a very important role in tumor immune evasion, leading to tumor progression and metastasis. Active TGFβ binds to a tetrameric receptor that is composed of two TGFβRI chains and two TGFβRII chains. After binding of TGFβ, the type II receptors phosphorylate the type I receptors, which then propagate the signal by phosphorylating SMAD2 and SMAD3 transcription factors. TGFβ exerts its immunosuppressive activity partly by decreasing cell surface levels of activating receptors, and by inhibiting NK cell activation and proliferation, causing a significant diminution of the antitumor function of NK cells (9, 10). Therefore, hampering TGFβ-mediated signals to overcome the immunosuppressive TME, with the subsequent restoration and increase of NK cell killing abilities, is an appealing option in the treatment of cancer.

In this issue, Tran and colleagues have analyzed 249 neuroblastoma samples (1). Their data show that *TGFβR* (*TGFβRI* and *TGFβRII*) and *TGFβ* (*TGFβ1* and *TGFβ2*) genes are expressed at high levels, both in high-risk and low-risk neuroblastomas. Furthermore, using a reporter cell line system, they demonstrated that plasma from patients with neuroblastoma has significant amounts of TGFβ, as shown by the ability of galunisertib to inhibit SMAD activity mediated by patients' plasma. Galunisertib binds antagonistically to TGFβRI, preventing the intracellular phosphorylation of SMAD2 and SMAD3. These data suggest that

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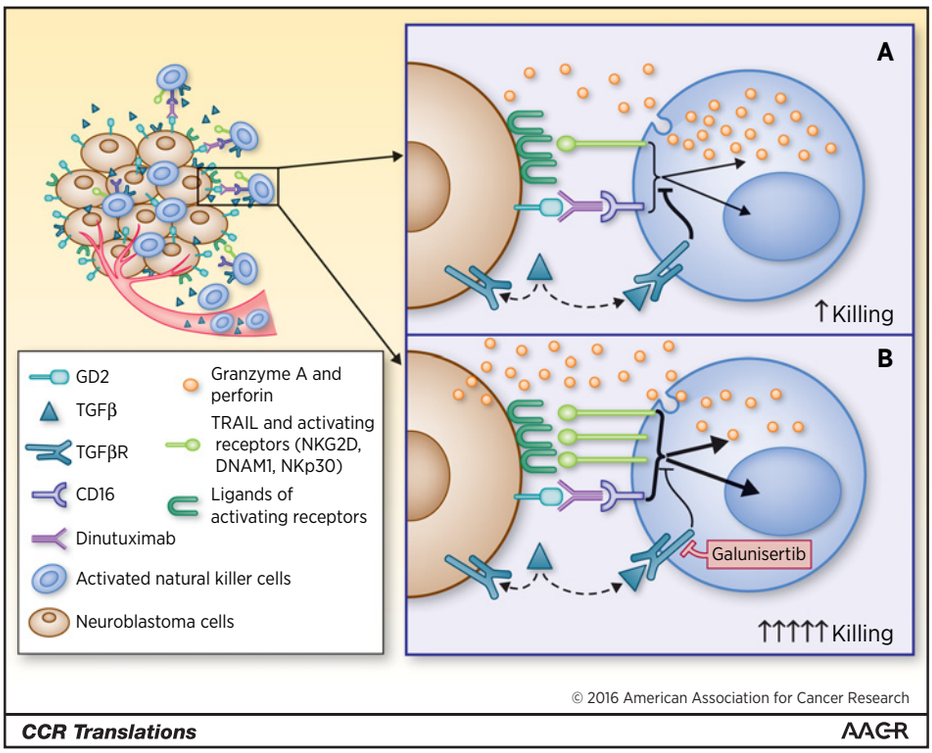

Figure 1.
Galunisertib-mediated TGFβ signaling inhibition increases the therapeutic effect of dinutuximab plus adoptively transferred aNK cells against neuroblastoma. **A,** TGFβ that is present in the plasma and in the TME downregulates the cell surface levels of activating receptors on aNK cells and the release of cytolytic granules after interaction with target cells, resulting in a decreased natural cytotoxicity and dinutuximab-mediated ADCC. **B,** Inhibition of TGFβRI kinase with the small-molecule inhibitor galunisertib restores the cell surface levels of NK cell–activating receptors and the release of perforin and granzyme A, leading to an increase in the elimination of neuroblastoma cells. The TGFβ receptor and TGFβ are drawn in a simplified manner. The TGFβ receptor is a tetramer: two TGFβRI chains and two TGFβRII chains. TGFβ binds as a dimer to the tetrameric receptor.

tumor-derived TGFβ could have an important role in inducing an immunosuppressive state in neuroblastoma patients and, consequently, in diminishing the effectiveness of immune-based therapies, such as dinutuximab and adoptive NK cell therapy. To test this hypothesis, Tran and colleagues performed a series of experiments. First, they studied the role of galunisertib in reverting TGFβ1-mediated suppressive effects on NK cells. TGFβ1-induced downregulation of TRAIL and the activating receptors DNAM-1, NKG2D, and NKp30 was reversed by galunisertib. However, neither TGFβ1 nor galunisertib had any effect on the expression of the ligands of these receptors and on GD2 levels on neuroblastoma cell lines, although galunisertib decreased SMAD2 phosphorylation on neuroblastoma xenografts in mice. This indicates that TGFβ1 has other effects on tumor cells that may or may not be relevant to their recognition by NK cells. TGFβ1 also reduced the release of perforin and granzyme A by aNK cells cultured with neuroblastoma cells, but, interestingly, the intracellular levels of these two cytolytic mediators were not affected, which is in agreement with previous reports showing that TGFβ1 abrogates perforin polarization to the immune synapse (11). These TGFβ1-mediated outcomes on NK cells lead to the inhibition of their natural cytotoxic activity against neuroblastoma cells. On the other hand, although CD16 levels were not modified by the treatment of aNK cells with TGFβ1, dinutuximab-mediated ADCC was diminished, in part probably due to a decrease in the release of perforin and granzyme A. Very importantly, galunisertib was able to inhibit all TGFβ1-induced effects on aNK cells and restore their ability to kill *in vitro* neuroblastoma cells (Fig. 1).

Finally, the authors tested on one hand the therapeutic capability of galunisertib alone, and on the other in combination with adoptively transferred aNK cells plus dinutuximab in mice injected with two laboratory-established neuroblastoma cell lines and with patient-derived xenograft neuroblastoma cells. Several points should be stated: First, galunisertib alone had no significant effect, indicating that blocking only TGFβ-mediated signaling had no influence in this model. Second, dinutuximab plus aNK cells showed a modest effect on tumor growth and extended survival that was only statistically significant when mice were injected with patient-derived xenograft neuroblastoma cells. Third, and more important, there was an increased therapeutic effect when galunisertib was combined with aNK cells plus dinutuximab, as shown by a significant increase in the survival of tumor-injected mice and significantly reduced tumor growth.

The data from Tran and colleagues indicate that to design new therapeutic approaches for solid tumors, inhibiting TGFβ signaling in the TME should be taken into account (1). Galunisertib has already been tested in the clinic and showed an acceptable tolerability and safety profile for clinical investigation in patients with solid tumors (12). But also, it is very important to highlight the need of combination therapy to improve the survival of patients with solid tumors. Targeting several players that participate in tumor growth and evasion will have a better effect on our success in treating cancer. In this context, infusion of activated and expanded NK cells is a safe therapeutic option, and we should think about the possibility of using them in the clinic in combination with dinutuximab and galunisertib. The results from Tran and colleagues represent a new step in combining immunotherapy with small-molecule inhibitors for the treatment of refractory neuroblastoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: F. Borrego
Writing, review, and/or revision of the manuscript: O. Zenarruzabeitia, J. Vitallé, I. Astigarraga, F. Borrego
Study supervision: F. Borrego
Other (figure design): O. Zenarruzabeitia, J. Vitallé

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