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### Commentary

The clinical benefits that have been achieved for a group of cancer patients with metastatic disease on checkpoint inhibitor therapy have kindled intense interest in understanding tumor-induced escape from T lymphocyte control. Other lymphoid cells also participate in tumor control; in particular, NK cells can limit hematogenous cancer metastasis spread and are also subject to negative regulation by developing cancers. In this issue of the *JCI*, Li and colleagues define an unanticipated role for the stress-induced protein CD155 in cancer metastasis. The presence of CD155 on the surface of cancer cells was shown to promote tumor invasiveness, while its upregulation in tumor environment-infiltrating myeloid cells restrained antitumor immunity by impairing antitumor T lymphocytes and NK cell function. Together, these results support further exploration of strategies for targeting CD155.

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# The expanding constellation of immune checkpoints: a DNAMic control by CD155

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The clinical benefits that have been achieved for a group of cancer patients with metastatic disease on checkpoint inhibitor therapy have kindled intense interest in understanding tumor-induced escape from T lymphocyte control. Other lymphoid cells also participate in tumor control; in particular, NK cells can limit hematogenous cancer metastasis spread and are also subject to negative regulation by developing cancers. In this issue of the *JCI*, Li and colleagues define an unanticipated role for the stress-induced protein CD155 in cancer metastasis. The presence of CD155 on the surface of cancer cells was shown to promote tumor invasiveness, while its upregulation in tumor environment-infiltrating myeloid cells restrained antitumor immunity by impairing antitumor T lymphocytes and NK cell function. Together, these results support further exploration of strategies for targeting CD155.

## NK cells and the nectin family of regulatory proteins

NK cells are members of the type 1 innate lymphoid cell family, which, like CD8<sup>+</sup> T lymphocytes, are capable of killing target cells and releasing IFN- $\gamma$ . Experimental evidence has implicated NK cells in immune surveillance; however, the bulk of preclinical and clinical data suggest a distinct and prevalent role of these lymphoid cells in the control of hematogenous metastases from solid tumors (1).

Different classes of activating and inhibitory receptors jointly operate to direct the activity of NK cells toward virus-infected, stressed, and cancerous cells. Among these receptors, nectin and nectin-like molecules can both positively and negatively influence NK cell activity. The DNAX accessory molecule (DNAM-1, also known as CD226) is likely among the most relevant NK cell receptors, as this adhesion molecule triggers both NK cell cytotoxicity and IFN- $\gamma$  production after encountering cancer cells. The ligands

for DNAM-1 are CD112 and CD155 (also known as poliovirus receptor [PVR]); however, these ligands can also bind, with different avidity, CD96 and T cell Ig and ITIM domain (TIGIT), both of which act as negative regulators of NK cell function (2). Clinical data depict an inverse correlation between the number of circulating and tumor-infiltrating NK cells and the presence of metastases at disease presentation in different patients (reviewed in ref. 1). More recently, a study in a small cohort of patients treated with anti-PD-1 showed that subsets of NK cells (but not T cells) detected at baseline using cytometry by time-of-flight (CyTOF) profiling correlated with the clinical response (3).

In this issue, Li and colleagues present evidence that expression of CD155 on both cancer and tumor-associated myeloid cells directly restrains antitumor immunity (Figure 1). These data thus identify CD155 as an additional checkpoint inhibitor, not only for NK cells but also for T lymphocytes (4).

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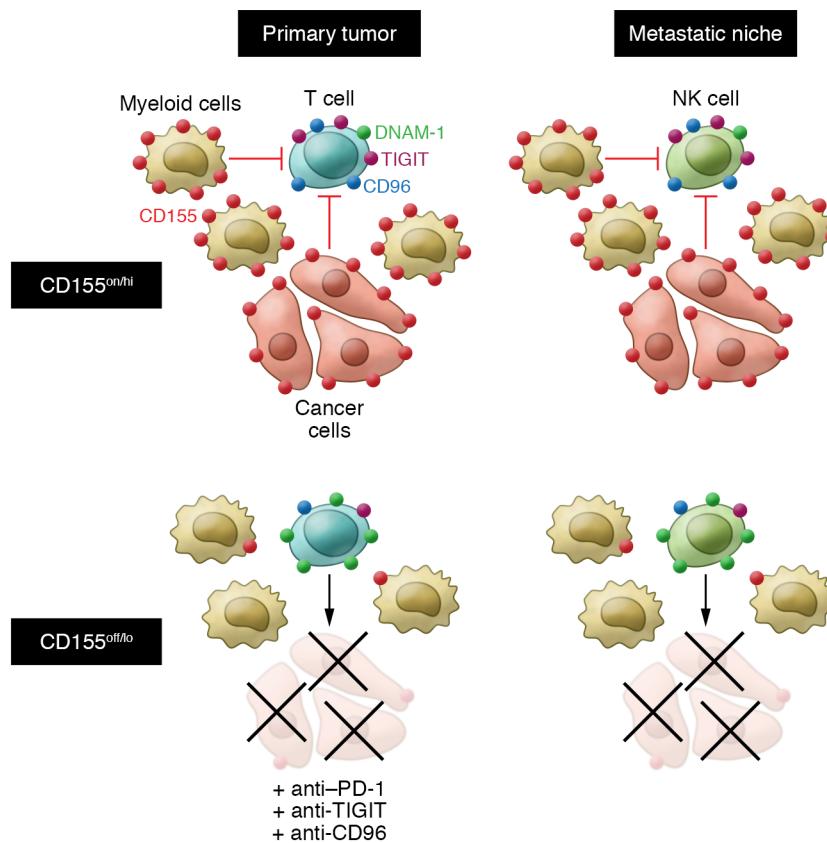
## Two-for-one: *CD155* as a checkpoint and protumor gene

The presence of CD155 in human cancers has been documented in some studies (1, 4) and is an unfavorable prognostic marker in cervical, urothelial, and lung cancer (5). Li and colleagues characterized the cellular distribution of CD155 protein in primary cutaneous melanomas by IHC. CD155 expression was prevalent on HMB45<sup>+</sup> melanoma cells and various tumor-infiltrating myeloid cells, including CD14<sup>+</sup>CD11c<sup>−</sup> macrophages, CD14<sup>+</sup>CD11c<sup>+</sup> myeloid cells, and CD14<sup>−</sup>CD11c<sup>+</sup> DCs, with negligible expression on tumor-infiltrating lymphocytes (TILs).

While CD155 expression had a variable and incomplete impact on the growth of different subcutaneously implanted tumors, lack of CD155 in mice strongly reduced the metastatic diffusion of tumors to the lungs. Moreover, CD155 had variable effects in different arms of the immune system, as primary tumor growth was mostly controlled by CD8<sup>+</sup> T cells, but the absence of NK cells rendered both WT and *Cd155*<sup>−/−</sup> mice unable to restrain lung metastasis formation (4).

Elegant bone marrow chimera experiments unveiled a distinct contribution of CD155 expression within the hematopoietic compartment in restraining tumor development (4). Specifically, ablation of CD155 in bone marrow-derived cells, both systemically in tumor-free mice and within the tumor microenvironment, enhanced DNAM-1 surface expression in circulating T lymphocytes and NK cells, as well as in TILs. Administration of an anti-DNAM-1-blocking mAb to *Cd155*<sup>−/−</sup> mice abrogated NK cell-dependent control of metastatic spread, and, functionally, higher DNAM-1 expression was related to enhanced IFN- $\gamma$  cytokine production. These data suggest that CD155 acts *in trans* to inhibit DNAM-1 expression in different lymphoid cells, confirming and extending previously published data (6).

Li et al. also exploited the cancer cell-autonomous effect of CD155 expression



**Figure 1. CD155 and CD155-mediated pathways have potential as therapeutic targets for limiting tumor growth.** Within the cancer microenvironment of primary tumor lesions and the distant metastatic niche, the upregulation of CD155 ( $CD155^{on/hi}$ ) enforces a decrease in the expression of DNAM-1 in both T lymphocytes and NK cells, impairing the antitumor activity exerted by these two immune effectors at these different sites. CD155 can also bind TIGIT and/or CD96 to induce inhibitory signals on NK cells. Genetic ablation (and possibly targeted inhibition by small molecules or antibodies;  $CD155^{off/lo}$ ), combined with PD-1, TIGIT, and CD96 blockade, unleashes the full antitumor power at the primary tumor site. CD155 modulation as a single agent can have an effect on the control of metastases from NK cells.

and used the CRISPR-Cas9 system to delete *CD155* in different, transplantable tumor cell lines, which were then inoculated in mice either intravenously or orthotopically (4). Although not all the cell lines were compared in these two settings, the results of these experiments provided a clear indication that tumor cells lacking *CD155* have limited metastatic potential. *CD155*-deficient cancer grew more slowly *in vivo* and formed fewer metastases when inoculated in immunodeficient animals. *In vitro*, the number of cell colonies formed under limiting dilution and the ability of cells to migrate were both impaired by *CD155* deficiency, whereas the rate of apoptosis under hypoxic or nutrient limitation conditions increased. Overall, these findings reveal a global inability of cancer cells to sustain autonomous survival when lacking *CD155*.

Given these experiments, it is obvious to surmise that manipulation of *CD155* as a strategy to enhance immune-mediated rejection of tumors would probably require interference of both cancer and myeloid cell expression (Figure 1). Indeed, genetic ablation of *CD155* in both host and cancer cells resulted in the greatest control of

melanoma primary lesions and prevention of metastatic spread to the lungs (4). Moreover, anti-DNAM-1 mAb treatment ablated the beneficial anti-melanoma response achieved by *CD155* loss only in *Cd155*-deficient, but not WT, mice (4). These results indicate that, while DNAM-1 is the main *CD155*-activating signal, DNAM-1-independent mechanisms of the antitumor response operate in the lymphocytes of WT mice, and only the complete absence of *CD155* in both tumor and immune compartments can unleash a preeminent DNAM-1 activation.

Finally, Li and colleagues focused on the therapeutic impact of *CD155* modulation in combination with checkpoint inhibitor therapy (4). Genetic ablation of *Cd155* in mice enhanced the response to anti-PD-1 mAb and to CTLA4/PD-1 blockade against various subcutaneously growing tumors. It is worth noting that triple blockade of TIGIT, CD96, and PD-1 had a higher therapeutic index in WT mice bearing a *CD155*-deficient melanoma than in animals that received control tumor cells. As both TIGIT and CD96 bind *CD155* and induce negative signals that restrain DNAM-1-dependent activation,

the inability of TIGIT and CD96 blockade to restrain the negative influence of *CD155* on the surface of cancer cells confirms the hypothesis that *CD155* can directly modulate DNAM-1, probably by reducing its expression on TILs.

## Open questions and future directions

The manuscript by Li and colleagues extends our knowledge of the intricate interplay between immune-activating molecules and their control circuits, an aspect that provides multiple avenues to ensure appropriate responses. DNAM-1 and other NK cell-activating receptors are indeed under the control of additional checkpoints that operate under steady-state conditions. For example, IL-1 receptor 8 (IL-1R8) is a negative regulator of NK cell maturation and effector function, as genetic *Il1r8* ablation leads to a cell-autonomous, IL-18-dependent increase in mature NK cell numbers and enhances IFN- $\gamma$  production upon *ex vivo* stimulation (7). Compared with WT animals, *Il1r8*<sup>-/-</sup> mice are more resistant to solid tumors in organs in which NK cells are more frequent, such as the liver and

lungs, are less prone to developing lung and liver metastases from sarcoma and colon carcinoma lines, respectively, and have a lower incidence of hepatocellular carcinoma after chemical-induced carcinogenesis (7). From a cancer immunology viewpoint, a lack of CD155 and IL-1R8 produces overlapping outcomes. However, it must be pointed out that IL-1R8 seems to be restricted to NK cells. The obvious question being, is there any benefit to targeting these different checkpoints at the same time?

Even though additional studies on different cancer types are necessary, the pattern of CD155 distribution within the tumor environment matches PD-L1 expression, with both cancer and myeloid cells primarily expressing the two proteins. This thought-provoking parallelism opens up a few issues that will require further analyses. The signals driving CD155 upregulation in either cancer cells or myeloid cells are not fully understood and might be dissimilar, with some evidence pointing to oncogenes, DNA damage, and the stress response as mediators of CD155 expression (8). In the case of PD-L1 regulation, a clear contribution of IFN- $\gamma$  released from activated immune cells has been documented (9), and host cells expressing PD-L1 might be more important than tumor cells in driving T lymphocyte exhaustion and the response to PD-1/PD-L1 blockade in experimental and human cancers, as was recently reported (10, 11). It remains to be determined whether IFN- $\gamma$  also controls CD155. Moreover, considering the evidence provided by Li et al., it will be important to correlate the presence of CD155 in human cancers with DNAM-1 downregulation in both circulating and tumor-infiltrating T lymphocytes and NK cells.

The cancer cell-intrinsic activity of CD155 also demands deeper exploration of the molecular pathways involved and the potential involvement of CD155 in the transformed phenotype. While a contribution of CD155 to tumor cell invasion and migration through the interplay with integrins and focal adhesion kinase is supported by *in vitro* experimental data (12, 13), the

molecular links to epithelial-to-mesenchymal transition, cell cycle, and cell survival need to be explored.

The clinical translation of these results and the exploitation of ideal immunotherapy combinations will rely on the necessary step of developing strategies to target and block CD155. Both small molecules and antibodies can be designed for this purpose. Indeed, Li and colleagues show that anti-CD155 mAb mimics genetic *Cd155* ablation and increases DNAM-1 in both T lymphocytes and NK cells of tumor-free mice (4). Additional data about the antitumor efficacy of this mAb, when administered alone or in combination with other checkpoint inhibitors, will be needed to pave the way for further development of this strategy. It must be stressed that any new CD155-targeting drugs will have to balance the DNAM-1/CD155 and TIGIT/CD96/CD155 axes to optimize the full activation of antitumor immune effectors. In theory, the best approach should maximize the blockade of CD155 interactions with TIGIT and CD96 and disrupt the negative influence of CD155 on DNAM-1 expression, but allow DNAM-1 to recognize CD155 and drive a full activation signal.

One final but important aspect of evaluating the effect of CD155 targeting for cancer therapy concerns the choice of tumor models used to assess combination therapy experiments, on the basis of the control of transplanted primary tumor growth. As exemplified by the findings of Li et al. about the split antitumor immune response, it is likely that these experiments address the key impact on the exhausted CD8 $^{+}$  T cell compartment. It will be essential to establish whether combination therapy also affects the NK cell-mediated control of metastatic disease, further improving the already remarkable effect of CD155 modulation.

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