



## Type 2 innate lymphoid cells: a novel actor in anti-melanoma immunity

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### ABSTRACT

Immunity to melanoma is thought to be mainly mediated by adaptive immune cells. To what extent innate immunity, particularly innate lymphoid cells, drive the immune response and impact melanoma prognosis and therapeutic responsiveness is not well understood. In a recent article published in *Nature Immunology*, we uncovered a critical role that ILC2 play in the control of melanoma. Using both complementary mouse models and human samples, we showed that ILC2-derived granulocyte macrophage-colony stimulating factor (GM-CSF) drives eosinophil tumor recruitment and activation. We found that ILC2 express PD-1 which inhibits ILC2 effector function and impairs anti-tumor responses. We further demonstrated that the combination of IL-33 and anti-PD-1 blocking antibodies improved anti-tumor responses through the expansion of splenic and tumor-infiltrating ILC2 and eosinophils. These findings have revealed an essential mechanism involving ILC2 and eosinophils necessary for anti-melanoma immunity and immunotherapy responses.

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The innate lymphoid cell (ILC) family encompass heterogeneous immune cells harboring pleiotropic function. They are grouped into five subsets, namely natural killer (NK) cells, ILC1, ILC2, ILC3 and lymphoid tissue-inducer cells (LTi).<sup>1</sup> While NK cells are found in the blood, other ILC subsets are mainly located in the tissues where they provide protection and support to maintain tissue homeostasis.<sup>1</sup> ILC are well equipped with receptors and ligands to sense their environment and to respond to an immune threat through cytokine and chemokine production.<sup>2</sup> The involvement of NK cells in anti-tumor immunity is well established; however, the role and function of other ILC subsets in cancer is only now emerging.<sup>3</sup> Considerable efforts, including our own, are currently being made to decipher the role and function of non-NK cells ILCs in various malignancies<sup>4-7</sup> including melanoma.<sup>8</sup>

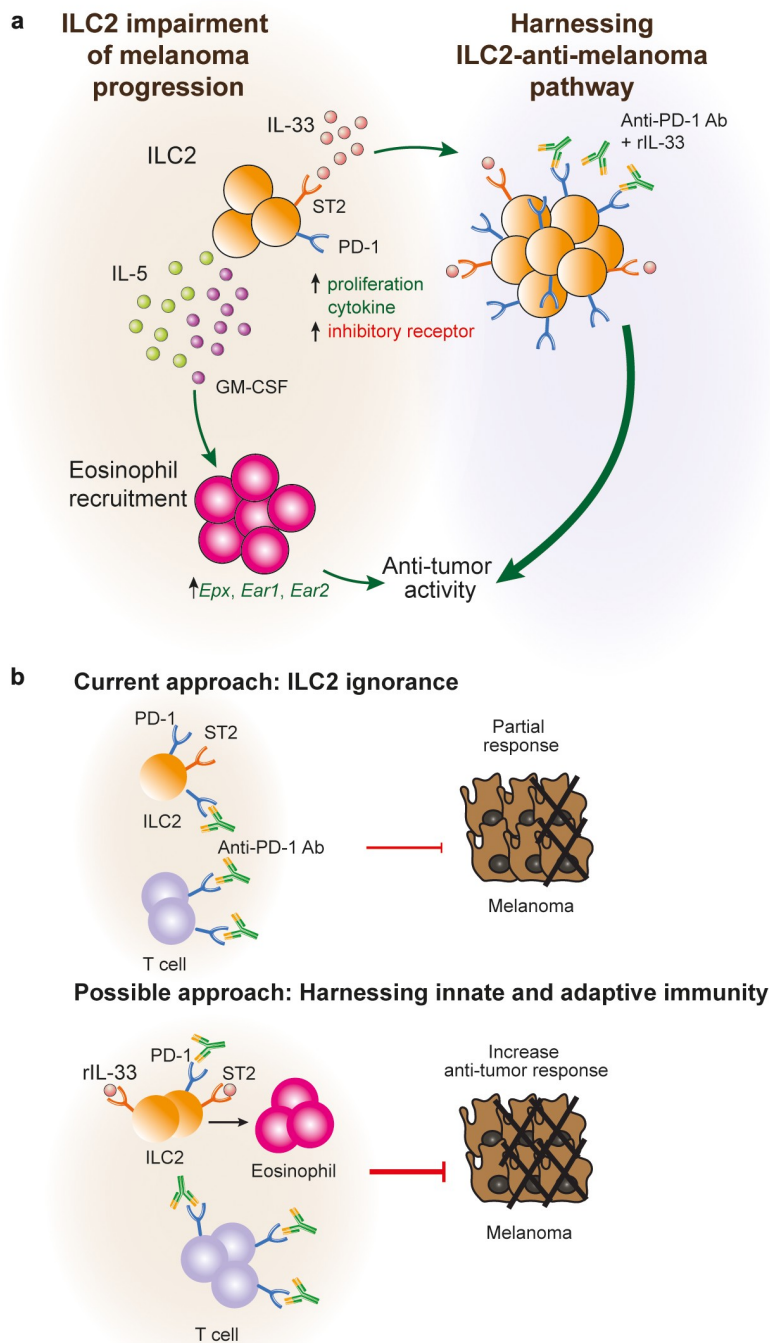
### ILCs infiltrate melanoma tumors, but only ILC2 protect against primary tumor development

Using complementary multiparametric flow and mass cytometric analyses, and multiplex immunochemistry, we monitored ILC infiltration in melanoma mouse models and human tumors.<sup>8</sup> We first investigated tumor ILC infiltration in four pre-clinical mouse models of melanoma, encompassing both intradermally injected tumor cell lines and chemically induced tumors. We found that all ILC subsets infiltrated melanoma tumors. Next, we were able to confirm these preclinical results by analyzing human samples using imaging and mass cytometry.<sup>8</sup> This revealed that ILC2 infiltrated primary melanoma tumors and all ILC subsets were detected in metastatic lesions (subcutaneous or invaded lymph nodes) of melanoma patients.

To understand the role played by these innate immune cells in melanoma immunity, we used genetically engineered tumor-bearing mice to specifically delete ILC populations and compared tumor growth and mouse survival to that of wild-type controls. Surprisingly, we did not observe any impact of deletion of NK cells, ILC1 and/or ILC3s on either tumor growth or mouse survival, indicating that these ILC subsets do not seem to be involved in the control of primary melanoma. In contrast, in two different mouse models deficient in ILC2, we saw increased melanoma tumor growth suggesting a potential role for this innate immune cell subset in anti-melanoma immunity.<sup>8</sup> When we reconstituted *Rag2<sup>-/-</sup>Il2rgc<sup>-/-</sup>* mice, a strain that is severely immunocompromised as it lack both innate and adaptive lymphocytes, with wild-type bone marrow-derived progenitors, we were able to differentially reestablish the innate and adaptive lymphoid compartments. In particular, we found that ILC2-replenished *Rag2<sup>-/-</sup>Il2rgc<sup>-/-</sup>* mice have reduced tumor size compared to non-reconstituted *Rag2<sup>-/-</sup>Il2rgc<sup>-/-</sup>* mice and achieved a level of anti-tumor protection similar to *Rag1<sup>-/-</sup>* mice (lacking adaptive lymphoid cells only).<sup>8</sup> Together, these results demonstrate the key role played by ILC2 in anti-tumor immunity in restricting the development of primary melanoma tumors.

### ILC2-derived GM-CSF production control eosinophil homeostasis and function

ILC2 are cytokine-producing cells and lack killing capacities.<sup>1</sup> Therefore, there is a need for them to partner with other cell subsets to exert tumoricidal activities against cancer cells. To identify these partners in crime and to understand the



**Figure 1.** Schematic representation showing a proposed model of ILC2-driven anti-tumor immunity in melanoma. (a) In tumors, ILC2-derived IL-5 and GM-CSF drive eosinophil recruitment and activation. Both ILC2 and eosinophils are essential to anti-melanoma immunity. However, ILC2 express PD-1 which inhibits ILC2 function. Combination of rIL-33 and anti-PD-1 antibodies enhanced anti-tumor immunity associated with increased ILC2 and eosinophils tumor recruitment. (b) Proposed approach to enhance treatment responses by targeting both innate and adaptive immunity, particularly ILC2, eosinophils and T cells.

mechanisms involved, we performed single cell RNA sequencing analyses of tumor-infiltrating leukocytes isolated from chemically induced mouse melanoma tumors.<sup>8</sup> In addition to IL-5 expression, we found that tumor-infiltrating ILC2, but not T cells or NK cells, were the major producers of the cytokine GM-CSF (Figure 1a). Of note, GM-CSF-deficient mice have increased tumor growth compared with wild-type control animals. Similar findings were observed when *Rag2*<sup>-/-</sup>*Il2rgc*<sup>-/-</sup>

mice were reconstituted with GM-CSF-deficient ILC2 suggesting that ILC2-derived GM-CSF, specifically, is essential to anti-melanoma responses.<sup>8</sup>

Next, driven by the fact that the cytokine GM-CSF is critical to myeloid cell homeostasis and function<sup>9</sup> and by our previous results demonstrating that anti-tumor immunity was reinstated in ILC2-reconstituted *Rag2*<sup>-/-</sup>*Il2rgc*<sup>-/-</sup> mice, we hypothesized that ILC2-derived GM-CSF production promoted myeloid cell-

dependent anti-tumor function. Consequently, we monitored the proportion of circulating and tumor-infiltrating myeloid cell subsets in *Rag2<sup>-/-</sup>Il2rgc<sup>-/-</sup>* mice reconstituted with distinct bone marrow progenitors.<sup>8</sup> We found that eosinophils, but not neutrophils, macrophages, or dendritic cells, were increased when ILC2 were restored in these mice. Furthermore, anti-tumor immunity was impaired in eosinophil-deficient mice and reduced eosinophils in circulation was observed in *Rag2<sup>-/-</sup>Il2rgc<sup>-/-</sup>* mice reconstituted with GM-CSF-deficient ILC2. To understand the impact of GM-CSF on eosinophil function, we purified wild type and GM-CSF-deficient ILC2 and cultured these cells with IL-33 to increase cytokine production. Two days later, we purified splenic eosinophils from wild-type mice and cultured them overnight with ILC2-conditioned media. After 18 h, eosinophils were collected, and qPCR analyses were performed. We uncovered that ILC2-derived GM-CSF production increased the expression of genes involved in eosinophil cytotoxic function (Figure 1a). In addition, the addition of recombinant GM-CSF in the culture media promoted eosinophil survival.<sup>8</sup> Collectively, these findings demonstrate that ILC2-derived GM-CSF production supports eosinophil survival and function which is necessary to promote efficient anti-tumor responses.

### Tumor-infiltrating ILC2 express PD-1, impairing ILC2-dependent anti-tumor immunity

We and others have found that ILC2 and their progenitors express the immune checkpoint molecule programmed cell-death 1 (PD-1)<sup>4,8,10–12</sup>, an inhibitory receptor well known for its involvement in the suppression of T cell function.<sup>13</sup> At steady-state, low levels of PD-1 were observed on the surface of ILC2 across different organs.<sup>8</sup> However, following *in vitro* activation with the alarmin IL-33 or *ex-vivo* examination of mouse and human melanoma tumors, we found that most of these innate cells express high levels of the inhibitory receptor PD-1<sup>8</sup> (Figure 1a) suggesting that it may play a role in the negative regulation of their effector functions. Supporting this hypothesis, we observed increased proliferation and cytokine production when ILC2 genetically lacked PD-1 confirming the deleterious role of this receptor on ILC2 activity.<sup>8</sup> In addition, PD-1 deletion in ILC2 was associated with increased anti-tumor responses highlighting that in the context of melanoma,<sup>8</sup> or similarly, in pancreatic tumors,<sup>4</sup> PD-1 acts as a general inhibitor of immune lymphoid cells.

### IL-33 combined with PD-1 blockade unleash anti-melanoma immunity

Building on these results and given the fact that ILC2 are associated with anti-tumor immunity, we aimed to increase ILC2 activity by using IL-33 with the ultimate goal of improving immune responses and disease outcomes. Moreover, we combined IL-33 treatment with anti-PD-1-blocking antibodies to overcome the negative impact of PD-1 expression on ILC2 activity. In this setting, we observed that mice treated with the combination therapy presented reduced tumor growth associated with increased ILC2 and eosinophil tumor infiltration<sup>8</sup> (Figure 1a). A moderate increase in tumor-infiltrating CD8<sup>+</sup> T cells was also noticed. These results suggest that harnessing

both the innate and adaptive immune compartments drive potent anti-tumor responses and further improve immunotherapy outcomes. These findings warrant further investigation particularly using human samples to confirm the relevance of ILC2 and eosinophils in the context of PD-1 blockade and highlight the possibility of taking advantage of targeting of these innate immune cells in our oncological armamentarium (Figure 1b).

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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### References

1. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie ANJ, Mebius RE, et al. Innate lymphoid cells: 10 years on. *Cell*. 2018 Aug 23;174(5):1054–1066. doi:10.1016/j.cell.2018.07.017.
2. Seillet C, Jacquelot N. Sensing of physiological regulators by innate lymphoid cells. *Cell Mol Immunol*. 2019 May;16(5):442–451. doi:10.1038/s41423-019-0217-1.
3. Demaria O, Cornen S, Daéron M, Morel Y, Medzhitov R, Vivier E. Harnessing innate immunity in cancer therapy. *Nature*. 2019 Oct;574(7776):45–56. doi:10.1038/s41586-019-1593-5.
4. Moral JA, Leung J, Rojas LA, Ruan J, Zhao J, Sethna Z, Ramnarain A, Gasmi B, Gururajan M, Redmond D, et al. ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity. *Nature*. 2020 Mar;579(7797):130–135. doi:10.1038/s41586-020-2015-4.
5. Dadi S, Chhangawala S, Whitlock BM, Franklin RA, Luo CT, Oh SA, Toure A, Pritykin Y, Huse M, Leslie C, et al. Cancer immunosurveillance by tissue-resident innate lymphoid cells and innate-like T cells. *Cell*. 2016 Jan 28;164(3):365–377. doi:10.1016/j.cell.2016.01.002.
6. Huang Q, Jacquelot N, Preaudet A, Hediye-Zadeh S, Souza-Fonseca-Guimaraes F, McKenzie ANJ, Hansbro PM, Davis MJ, Mielke LA, Putoczki TL, et al. Type 2 innate lymphoid cells protect against colorectal cancer progression and predict improved patient survival. *Cancers*. 2021 Feb 1;13(3):559. doi:10.3390/cancers13030559.
7. Carrega P, Loiacono F, Di Carlo E, Scaramuccia A, Mora M, Conte R, Benelli R, Spaggiari GM, Cantoni C, Campana S, et al. NCR(+)ILC3 concentrate in human lung cancer and associate with intratumoral lymphoid structures. *Nat Commun*. 2015 Sep 23;6:8280. doi:10.1038/ncomms9280.
8. Jacquelot N, Seillet C, Wang M, Pizzolla A, Liao Y, Hediye-zadeh S, Grisar-Tal S, Louis C, Huang Q, Schreuder J, et al. Blockade of the co-inhibitory molecule PD-1 unleashes ILC2-

- dependent antitumor immunity in melanoma. *Nat Immunol.* 2021 Jun 7;1–14.
9. Dougan M, Dranoff G, Dougan SK. GM-CSF, IL-3, and IL-5 family of cytokines: regulators of inflammation. *Immunity.* 2019 Apr 16;50(4):796–811. doi:10.1016/j.immuni.2019.03.022.
  10. Seillet C, Mielke LA, Amann-Zalcenstein DB, Su S, Gao J, Almeida FF, Shi W, Ritchie M, Naik S, Huntington N, et al. Deciphering the innate lymphoid cell transcriptional program. *Cell Rep.* 2016 Oct 4;17(2):436–447. doi:10.1016/j.celrep.2016.09.025.
  11. Yu Y, Tsang JCH, Wang C, Clare S, Wang J, Chen X, Brandt C, Kane L, Campos LS, Lu L, et al. Single-cell RNA-seq identifies a PD-1hi ILC progenitor and defines its development pathway. *Nature.* 2016 Nov 3;539(7627):102–106. doi:10.1038/nature20105.
  12. Taylor S, Huang Y, Mallett G, Stathopoulou C, Felizardo TC, Sun M-A, Martin EL, Zhu N, Woodward EL, Elias MS, et al. PD-1 regulates KLRG1+ group 2 innate lymphoid cells. *J Exp Med.* 2017 Jun 5;214(6):1663–1678. doi:10.1084/jem.20161653.
  13. Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. *N Engl J Med.* 2016 Nov 3;375(18):1767–1778. doi:10.1056/NEJMra1514296.