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## Revvng up dendritic cells while braking PD-L1 to refuel the Cancer-Immunity Cycle motor

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### Summary

Although successful for some, most melanoma patients are refractory to T cell checkpoint inhibition. In this issue of *Immunity*, Merad and colleagues (2016) describe a dendritic cell-based strategy to heighten the efficacy of therapeutic anti-PD-L1 and BRAF inhibitors in mouse melanoma models.

### Main text

To generate an effective and sustainable immune response against cancer, a number of biological events must be set into motion. These events start with release of cancer cell antigens, dendritic cell antigen capture and processing and T cell priming in the lymph node, followed by T cell trafficking to tumors where they recognize and kill cancer cells. In 2013, Chen & Mellman labeled these steps as the “Cancer-Immunity Cycle” (Chen and Mellman, 2013) (**Figure 1**). What we know from many experimental and clinical studies is that one or more steps in the Cancer-Immunity Cycle are often dysfunctional in tumor-bearing hosts, preventing durable anti-tumor immune responses. The malfunctions in the cycle run the gamut from insufficient antigen expression to dendritic cell paucity and immaturity to the lack of T cell infiltration into tumors to the inhibition of T cell effector functions. Enhancing the Cancer-Immunity Cycle and bypassing the tumor-induced roadblocks is one of the major clinical challenges in cancer research. In this issue of *Immunity*, Salmon *et al.* uncover several roadblocks stalling the Cancer-Immunity Cycle in melanoma mouse models and describe a multi-pronged approach to kick start the cycle again (Salmon *et al.*, 2016).

Blockade of inhibitory T cell checkpoint molecules, such as PD-1 and PD-L1, is a very successful strategy to amplify the cycle and circumvent some of the roadblocks imposed by tumors (Pardoll, 2012). For melanoma patients treated with PD-1 or PD-L1 antagonists, response rates range from 30-40% with long-term remissions, and many patients with other cancer types also greatly benefit from these drugs (Sharma and Allison, 2015). Unfortunately, these data also emphasize the less-than-optimal aspect of checkpoint immunotherapy, which is that the majority of patients are refractory to T cell checkpoint targeting. So how can the number of responsive patients be increased? Stimulating the initial steps of the Cancer-Immunity Cycle in combination with T cell checkpoint immunotherapy may be one answer.

Uptake of tumor-associated antigens by dendritic cells (and other antigen-presenting cells) is one of the first steps of the Cancer-Immunity Cycle (Chen and Mellman, 2013). Salmon *et al* found that human melanomas contain very few dendritic cells (Salmon et al., 2016). Similarly, tumors in two independent mouse models of melanoma – the transplantable B16 model and the tamoxifen-inducible *Braf*<sup>V600E</sup>;*Pten*<sup>F/F</sup>; *2 catenin*<sup>STA</sup>; *Tyr::CreERT2* model – show limited accumulation of dendritic cells. Not only are dendritic cells rare, they have to compete for antigen with the more abundant populations of macrophages and other myeloid cells. These data reveal the first roadblock in the cycle: insufficiency of dendritic cells in melanoma, which is a common and widespread feature of many experimental and human tumor types (Broz et al., 2014; Ruffell et al., 2014; Spranger et al., 2015).

One subset of melanoma-associated dendritic cells in mice is characterized by the positive expression of CD103, CD11c and BATF3 as well as negative expression of CD11b (Salmon et al., 2016). Salmon *et al* show that these CD103<sup>+</sup> dendritic cells are perfectly capable of taking up antigen, migrating to lymph nodes and activating cytotoxic T cells. In spite of their T cell-priming ability, B16 tumors transplanted into CD103<sup>+</sup> dendritic cell-deficient mice (*Batf3*<sup>-/-</sup> mice) grow as quickly as controls, indicating that dendritic cells are dysfunctional and dispensable in this model. An analogous population of dendritic cells with the same unique ability to stimulate T cells is found in other tumor models; these CD103<sup>+</sup> dendritic cells also fail to launch an immune response that is strong enough to control tumor growth (Broz et al., 2014; Ruffell et al., 2014; Sanchez-Paulete et al., 2016).

Salmon *et al* then set out to understand why dendritic cells exhibit this inability to function properly. They discovered that tumor-associated CD103<sup>+</sup> dendritic cells express high levels of PD-L1, which may dampen the full activation of PD-1-expressing cytotoxic T cells. So, the authors treated mice bearing B16 tumors with anti-PD-L1 and showed that the moderate tumor growth delay is completely dependent on the presence of CD103<sup>+</sup> dendritic cells, while T cell infiltration and activation in tumors remained unchanged (Salmon et al., 2016). They performed a similar experiment using the BRAF-dependent, genetically engineered mouse (GEM) model, but in this case, anti-PD-L1 together with a BRAF inhibitor failed to significantly slow tumor progression. These cancer mouse models responded poorly to anti-PD-L1 therapy and reflect the anti-PD-L1 resistant patient populations, providing a platform to ask whether boosting dendritic cell function synergizes with checkpoint immunotherapy. To this end, Salmon *et al.* injected tumor-bearing mice with a cytokine that expands dendritic cells, FLT3L. Although this approach increased intratumoral dendritic cells and achieved moderate tumor control, the dendritic cells were largely immature. To assess whether greater benefit can be realized through dendritic cell maturation, the authors injected a known dendritic cell maturation compound – the Toll-like receptor 3 (TLR3) synthetic ligand, poly I:C – into tumors alone or in combination with systemic FLT3L. This combination therapy

led to static tumor growth, as well as priming and expansion of antigen-specific T cells in both the transplantable and GEM models. Reduced tumor growth was dependent on dendritic cells, TLR3 signaling, type I interferon signaling CD4 and CD8 T cells. Furthermore, adding anti-PD-L1 to the FLT3L:poly I:C combination resulted in the most optimal diminution of tumor burden.

Having established that stimulating dendritic cell expansion and maturation combined with T cell checkpoint immunotherapy augments the Cancer-Immunity Cycle (**Figure 1**), Salmon *et al* then explored the durability of this approach. Tumors in the GEM model were treated with the tri-therapy (anti-PD-L1, FLT3L and poly I:C) as before and a BRAF inhibitor. Afterwards, mice were repainted with tamoxifen on the opposite flank to induce new tumors. Strikingly, these mice developed much smaller secondary tumors due to the increased infiltration of activated CD8<sup>+</sup> T cells. These data not only indicate that restarting the Cancer-Immunity Cycle via expansion and activation of dendritic cells together with anti-PD-L1 establishes anti-tumor immunity against existing tumors, but it induces immunological memory to combat secondary tumors as well.

The findings of Salmon *et al* add to a growing number of studies that highlight the requirement and importance of dendritic cells in immunotherapy (Broz et al., 2014; Sanchez-Paulete et al., 2016; Spranger et al., 2015). These studies indicate that stimulating specific populations of tumor-associated dendritic cells may overcome non-responsiveness to PD-1/PD-L1 targeting and resistance to BRAF inhibitors (Salmon et al., 2016). Patients with tumors exhibiting low numbers of dendritic cells (Broz et al., 2014) or few tumor-infiltrating T cells (Tumeh et al., 2014) or scarce expression of PD-L1 (Herbst et al., 2014) would be prime candidates to test this strategy. Whether poly I:C is the best compound to induce dendritic cell maturation in patients and how to deliver it remains questionable. The current study and others inject poly I:C directly into tumors (Salmon et al., 2016; Sanchez-Paulete et al., 2016; Spranger et al., 2015), so tumors have to be easily accessible for delivery. Systemic administration is not likely a viable therapeutic option, as this molecule may induce autoimmunity, adversely affect other TLR3-expressing cells or induce other unwanted off-target effects.

Looking forward, it is critical to understand how the genetic makeup of individual tumors influences the behavior and maturation of dendritic cells. These data will produce new candidate molecules to potentiate the dendritic cell:checkpoint inhibitor combination. Work in this area was recently published and showed that the WNT:β-catenin signaling pathway suppresses dendritic cell recruitment into BRAF-dependent melanomas, resulting in tumor evasion (Spranger et al., 2015).

The next big challenge is to translate the observations of Salmon *et al.* to the metastasis setting. Metastasis, not primary tumor growth, is the major cause of cancer-

associated death. Although the current study demonstrated efficacy of dendritic cell stimulation and anti-PD-L1 against secondary *de novo* melanomas, it is unclear whether this strategy will also work against established metastases in visceral organs and whether the efficacy of these immunotherapy modalities is location dependent.

Melanoma is a highly immunogenic cancer type, due in part to the high degree of UV light-induced non-synonymous mutations and subsequent production of (neo-) antigens – features that magnify the level of foreignness recognizable by the immune system. One particular issue with the models used in this study is that they are UV-independent, so they may be more representative of melanomas with low mutational load. In the GEM model, a BRAF inhibitor was used to induce cancer cell death and release antigens, suggesting that poorly immunogenic tumors may require chemotherapy, radiation or targeted therapy to overcome this roadblock in the cycle. However, it should be noted that the authors did not include controls without the BRAF inhibitor in the GEM model. For other cancer types with low immunogenicity, there will be additional antigen-independent roadblocks and speed bumps that are distinct from melanoma. For example, both paclitaxel and reprogramming of the immunosuppressive tumor microenvironment through depletion of interleukin 10 (IL-10)-expressing macrophages are necessary to reactivate dendritic cells and anti-tumor immunity in the *MMTV-PyMT* mammary tumor model (Ruffell et al., 2014). Thus, getting the Cancer-Immunity Cycle motor running in poorly immunogenic tumors may demand additional and/or alternative fuel – above and beyond the multi-pronged approach used by Salmon *et al.*

## References

- Broz, M.L., Binnewies, M., Boldajipour, B., Nelson, A.E., Pollack, J.L., Erle, D.J., Barczak, A., Rosenblum, M.D., Daud, A., Barber, D.L., *et al.* (2014). Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* 26, 638-652.
- Chen, D.S., and Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39, 1-10.
- Herbst, R.S., Soria, J.C., Kowanetz, M., Fine, G.D., Hamid, O., Gordon, M.S., Sosman, J.A., McDermott, D.F., Powderly, J.D., Gettinger, S.N., *et al.* (2014). Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 515, 563-567.
- Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 12, 252-264.
- Ruffell, B., Chang-Strachan, D., Chan, V., Rosenbusch, A., Ho, C.M., Pryer, N., Daniel, D., Hwang, E.S., Rugo, H.S., and Coussens, L.M. (2014). Macrophage IL-10 Blocks CD8(+) T Cell-Dependent Responses to Chemotherapy by Suppressing IL-12 Expression in Intratumoral Dendritic Cells. *Cancer Cell* 26, 623-637.

Salmon, H., Idoyaga, J., Rahman, A., Leboeuf, M., Remark, R., Jordan, S., Casanova-Acebes, M., Khudoynazarova, M., Agudo, J., Rivera, C., *et al.* (2016). CD103+ DC progenitor expansion and activation at the tumor site transform clinical response to PD-L1 and BRAF inhibition. *Immunity*.

Sanchez-Paulete, A.R., Cueto, F.J., Martinez-Lopez, M., Labiano, S., Morales-Kastresana, A., Rodriguez-Ruiz, M.E., Jure-Kunkel, M., Azpilikueta, A., Aznar, M.A., Quetglas, J.I., *et al.* (2016). Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti-PD-1 Monoclonal Antibodies Requires BATF3-Dependent Dendritic Cells. *Cancer Discov* 6, 71-79.

Sharma, P., and Allison, J.P. (2015). Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 161, 205-214.

Spranger, S., Bao, R., and Gajewski, T.F. (2015). Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* 523, 231-235.

Tumeh, P.C., Harview, C.L., Yearley, J.H., Shintaku, I.P., Taylor, E.J., Robert, L., Chmielowski, B., Spasic, M., Henry, G., Ciobanu, V., *et al.* (2014). PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 515, 568-571.

### Figure legend

#### **Figure 1. Dendritic cell expansion and maturation within the tumor microenvironment in combination with anti-PD-L1 and BRAF inhibition jump-starts the Cancer-Immunity Cycle.**

Cancer cells release antigens, which are taken up by dendritic cells and presented to T cells. Dendritic cells prime T cells in lymph nodes and these activated T cells migrate to tumors where they infiltrate and recognize tumor-associated antigens present on cancer cells. T cell killing of cancer cells then occurs followed by the release of additional antigens. This sequence of events is termed the Cancer-Immunity Cycle (Chen and Mellman, 2013); however, this cycle is often stalled in cancer. The cycle is restarted by BRAF inhibitor-induced release of antigens, dendritic cell expansion through FLT3L affects on the bone marrow, dendritic cell maturation via poly I:C-mediated TLR3 activation and the blockade of T cell checkpoint inhibitor, PD-L1 (Salmon *et al.*, 2016). Gray shading represents the melanoma microenvironment and blue shading represents peripheral tissue. (Adapted from (Chen and Mellman, 2013)).