



# A new tool in the cancer immunotherapy toolbox?

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Programmed cell death-1 (PD-1), a member of the CD28 superfamily, is primarily expressed on the surface of T cells. The normal function of PD-1 is to dampen the immune activity and promote self-tolerance of activated CD8<sup>+</sup> cytotoxic T cells by suppressing T cell inflammatory reactions. Binding of PD-1 with its ligand PD-L1 (or B7-H1) or PD-L2 (or B7-DC) excessively leads to the exhaustion of CD8<sup>+</sup> cytotoxic T cells (1). In most of patients with advanced cancers, CD8<sup>+</sup> cytotoxic T cells are exhausted owing to the engagement of PD-1 by the aberrantly expressed PD-L1 on tumor cells and/or nurse cells in the tumor microenvironment. Cancer immunotherapy aims to rejuvenate the exhausted CD8<sup>+</sup> cytotoxic T cells by targeting the PD-1 immune checkpoint through blocking of PD-1/PD-L1 binding with antibodies specific to either PD-1 (Nivolumab or Pembrolizumab) or PD-L1 (Atezolizumab, Avelumab, or Durvalumab) (1). Such antibody mediated-blocking overcomes the exhaustion of these CD8<sup>+</sup> cytotoxic T cells. Recently, Rudd's group identified that glycogen synthase kinase 3 (GSK3) is an upstream kinase that regulates the expression of PD-1 in CD8<sup>+</sup> cytotoxic T cells. By use of siRNA and small molecule inhibitors they demonstrated that inactivation of GSK3 could down-regulate PD-1 transcription resulting in enhanced CD8<sup>+</sup> cytolytic T cell response measured by viral clearance activity (2). This study suggests the tantalizing possibility that inactivation of GSK3 may constitute a new way of reversing the exhausted CD8<sup>+</sup> cytotoxic T cells to become functional cancer killing cytotoxic T cells.

In a recently published paper in *Cancer Research* (3),

the same group tested this very idea and demonstrated the potential utility of GSK3 inhibitors in cancer immunotherapy. Using different mouse model systems, they showed that SB415286, a small molecule inhibitor to GSK3, provided impressive tumor suppression benefits similar to that of anti-PD-1 blocking antibody. Mechanistically, GSK3 inhibition resulted in induced expression of T-bet, a repressor of PD-1 gene transcription, which leads to the reduction of PD-1 expression in CD8<sup>+</sup> cytotoxic T cells. Specifically, the authors first demonstrated that SB415286 significantly reduced the number of tumor metastasis nodules in lungs harvested from C57/B6 mice injected with mouse B16 tumor cells IV 14 days prior. This anti-tumor effect of SB415286 was comparable to that of using the anti-PD-1 blocking antibody. Furthermore, the combination of the anti-PD-1 blocking antibody and SB415286 did not show much additional effect suggesting the two agents are pharmacologically similar. Using conditional knockout mouse models in which GSK3 genes were exclusively deleted from T cells, they further showed that mice with GSK3 deficient T cells had a significant reduction in tumor growth (or engraftment), highlighting the direct role of GSK3 in modulating the antitumor function of cytotoxic T cells. Finally, the authors addressed the antigen specificity aspect of the tumor suppression using an Ova specific OT-1 transgenic mouse model engrafted with EL4 lymphoma tumor. They observed that Ova-antigen-bearing tumor xenografts grow slower than non-Ova-antigen-bearing ones; however, dramatic tumor clearance occurred only when the mice were treated with SB415286. This provides further

evidence that the effect of GSK3 is to modulate antigen-specific tumor killing by CD8<sup>+</sup> T cells. These results are consistent with an earlier report that in graft-versus-host disease hyperactive and hyperproliferative T cells from the transplant possess much lower GSK3 activity (4).

Aside from its role in the function of CD8<sup>+</sup> cytotoxic T cells, GSK3 activity is also important to the function of NK cells. Parameswaran and colleagues (5) reported that NK cells in acute myelogenous leukemia patients have heightened expression of GSK3; inhibition of GSK3 activity significantly improved NK cell-mediated anti-tumor activity through the increased production of TNF- $\alpha$  and increased NK-tumor cell conjugation. Finally, Cichocki and coauthors (6) found in addition to enhanced tumor-killing activity, GSK3 inhibition could also drive the maturation of NK cells both *in vitro* and in mouse xenograft models.

GSK3 includes functionally related GSK3 $\alpha$  and GSK3 $\beta$  each encoded by two distinct genes. Both GSK3 $\alpha$  and GSK3 $\beta$  are multifunctional serine/threonine protein kinases involved in many key signaling pathways. Aside from its roles in CD8<sup>+</sup> cytotoxic T cells and NK cells, GSK3 has long been suspected to play direct roles in the biology of cancer. However, Taylor *et al.* showed GSK3 inhibitor SB415286 did not have any direct effect on B16 or EL4 derived tumors engrafted in T cell deficient RAG2 knockout mice (3). The status of the expression and function of GSK3 proteins in these two cell lines used in their study is not known.

The latest work by Taylor *et al.* nicely validated their earlier prediction that GSK3 inhibition may be a viable alternative to PD-1/PD-L1 checkpoint blocking antibodies in cancer immunotherapy. However, this was done only in mouse systems; whether it also holds true in human patients remains to be demonstrated by clinical trials. Given that GSK3 is one of the earliest discovered protein kinases, it is not surprising that there have been over 20 different GSK3 inhibitors with varying specificity, potency and adverse effects developed to date. However, none are approved for cancer therapy. Only lithium is FDA approved and on available for the treatment of bipolar disorder. Lithium compound is unlikely to be useful in cancer given that it is neither a specific nor potent GSK3 inhibitor. Many of the other agents are primarily useful as tool compounds and are not undergoing clinical development. However, it is likely, given these reports, that pharmaceutical companies will be developing new GSK inhibitors. It is important that these new GSK inhibitors be evaluated *in vivo* since their primary anti-tumor effects may be mediated through

immune stimulatory activity rather than a direct effect on the tumor cells. These trials will need to carefully evaluate not only tumor response but also effects on T cells and NK cells as described above. Indeed, it will be fascinating to see if GSK3 inhibitors alone, and in combination with immune checkpoint inhibitors, can induce similar PD-1/PD-L1 checkpoint blocking function in human CD8<sup>+</sup> T cells, as well as NK cell mediated anti-tumor activities in patients. If so, GSK3 inhibitors could become a new tool in the ever-expanding immunotherapy armamentarium available to the oncologist.

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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