

Stemming the Tide of Cancer

By Daniel E. Speiser and Werner Held

Tumors are relatively easy to treat if they stay put, but cancer cells become more deadly when they disseminate to distant parts of the body. Surgery and local irradiation are not well suited for treating cancers that have spread and formed metastases at multiple body locations, and most types of metastatic cancer become progressively resistant to treatment with chemotherapeutic drugs or small molecule inhibitors that aim to block tumor growth.

The development of immunotherapy, a treatment that does not act directly on the tumor but rather stimulates the immune system to more effectively defend the whole body, has improved prognoses for some types of metastatic cancer. About 50 years ago researchers found that one component of the immune system, CD8⁺ T cells, have the remarkable potential to detect and kill cancer cells. Groundbreaking research on melanoma, an aggressive type of skin cancer, and later on cancers of breast, prostate, ovaries, and colon **have** shown that patient survival was significantly extended when tumors harbored a high abundance of CD8⁺ T cells.[1] These studies provided compelling evidence that CD8⁺ T cells are in principle able to protect against cancer.

About 50 years ago, researchers established that CD8⁺ T cells isolated from tumors and cultured in vitro for several days to weeks could specifically detect and kill cancer cells. But a decade ago, we found that these cells targeted cancer relatively poorly when tested immediately after their isolation from tumors.[2] This indicated that, while tumor-reactive CD8⁺ T cells manage to get into tumors, the tumor environment somehow prevents the cells from efficiently killing tumor cells.

Then, an unusual feature of tumor-specific CD8⁺ T cells offered a clue to the reason for that waning effectiveness. The immune cells expressed receptors known as checkpoints that decreased, rather than improved, the capacity of CD8⁺ T cells to kill tumor cells. The importance of such cell-surface receptors in dampening the function of T cells was first recognized separately by Tasuku Honjo and James Allison in the 1990s. Their pioneering work, which was awarded the Nobel Prize in Physiology or Medicine in 2018, led to the development of antibodies that prevented inhibitory receptors, such as programmed death 1 (PD-1), from engaging their binding partners present on other cells. This tweak improved the function of CD8⁺ T cells in vitro.

Blocking inhibitory receptors has revolutionized the treatment of several cancers, including melanoma and certain types of lung, bladder, kidney, intestinal, and gynecological cancers. Metastatic melanoma, which formerly was untreatable, can now be cured in a significant fraction of patients thanks to these immunotherapies. When the inhibitory PD-1 receptor is blocked by treatment with antibodies, CD8⁺ T cells present in the tumor increase expression of the cytotoxic granzyme B protein, a hallmark of killer cells, and start to multiply; this is often associated with tumor shrinkage and therapy success.[3] However, PD-1 expressing CD8⁺ T cells present in tumors were thought to be “exhausted” and unable to divide. It was thus difficult for immunologists to understand the full cellular and molecular basis for the CD8⁺ T cell expansion in response to

checkpoint blockade. It was also unclear why the therapy was effective for some patients but not others.

Science often does not follow a linear path, and important new insights frequently derive from studying seemingly unrelated problems. For example, exhausted CD8⁺ T cells were first described in chronic viral infections more than 20 years ago. More recently, detailed analyses of virus-fighting T cells by us [4] and by Rafi Ahmed's group at Emory University [5] revealed that there are at least two distinct types of CD8⁺ T cells. A rare cell type does not engage directly with infected cells but rather sustains the CD8⁺ T cell response to infection by renewing itself and by dividing to form the more common type of CD8⁺ T cells that has the potential to kill virus-infected cells.

These findings raised the possibility that a similar division of labor among CD8⁺ T cells exists in tumors, and that this plays a role in the mechanisms of tumor immunotherapy.

Tumors harbor stem cell–like CD8⁺ T cells that express PD-1

Two recent papers, one by us [6] and one from Nick Haining's group at Harvard Medical School [7], used mouse models to look for different types of PD-1 expressing CD8⁺ T cells in tumors and found that there are indeed two main subsets of cells: one that produces granzyme B, a hallmark of cell-killing CD8⁺ T cells, and one that does not make granzyme B but instead expresses T cell factor 1 (TCF1), a transcription factor needed for CD8⁺ T cell memory formation, as we had shown 10 years earlier [8]. The granzyme B⁺ cells did not make TCF1; these subsets were not overlapping.

We wondered whether it is the non-killer, TCF1-expressing subset of cells that multiplies in response to PD-1 blockade. (See illustration.) In additional mouse experiments, we showed that the presence of PD-1⁺ TCF1⁺ CD8⁺ T cells was essential to increasing the abundance of T cells during checkpoint blockade immunotherapy and to controlling tumors [6].

As PD-1⁺ TCF1⁺ CD8⁺ T cells expanded, most of the offspring cells transitioned from one subtype to the other: TCF1 expression fell off, limiting the cells' capacity to divide, while granzyme B expression increased and the cells gained the potential to kill cancer cells. Some of the offspring maintained TCF1 expression, however, ensuring they maintained the potential for continued multiplication and killer cell production.

These capacities of a subset of tumor-resident PD-1⁺ CD8⁺ T cells—to multiply, differentiate, and self-renew—mirror those of memory CD8⁺ T cells and tissue-specific stem cells. The tumor environment therefore contains stem cell–like (in short, “stem-like”) CD8⁺ T cells whose presence is necessary for the expansion of tumor-specific CD8⁺ T cells in response to checkpoint blockade in cases where the immunotherapy successfully induces tumor control in a preclinical animal model [6]. Similar stem-like CD8⁺ T cells were found in protein-stained, microscopically analyzed sections of human melanoma and lung cancer biopsies, and through gene expression analysis of single CD8⁺ T cells from these tumors. [6] [7] [9]

Predicting and improving the efficacy of immunotherapy

The overall response rates of cancer patients treated with antibodies to PD-1 remain relatively low. Even in the case of metastatic melanoma, which has the highest response rate of any cancer type, a considerable fraction of patients does not benefit from this or other checkpoint blockade immunotherapies. Other patients show initial tumor shrinkage, but the effect is transient. It is vital to be able to predict which patients will respond, not only to avoid useless treatments and reduce side effects, but also to more rapidly offer patients alternative options. Unfortunately, current markers used to predict immunotherapy's success, including the number of somatic mutations in the tumor, the presence on the tumor of ligands that bind PD-1, and the abundance of T cells in the tumor, are rather poor predictors of clinical outcome in individual patients.

The importance of stem-like CD8⁺ T cells to controlling tumors in mice treated with checkpoint blockade therapy raises the question of whether the presence of these cells accurately predicts treatment response in humans. The jury is still out on this point. Stem-like TCF1⁺ PD-1⁺ CD8⁺ T cells have been found in the melanoma and lung cancer tissue of patients who subsequently responded to immunotherapy as well as of those who did not [7], indicating that the mere presence of stem-like CD8⁺ T cells does not guarantee treatment response. More patients will have to be analyzed to see whether the cells' abundance and/or function is different in responders and non-responders. Such predictive biomarkers would allow us to identify those patients who could benefit from the therapeutic potential of checkpoint blockade.

Such insights might also help boost the efficacy of other types of immunotherapy. Adoptive T cell therapy, for example, involves isolating lymphocytes, including CD8⁺ T cells, from resected tumors; culturing them in laboratory incubators to restore their function and expand them; and then reinfusing them into the patient. (See illustration on page tk.) It seems likely that the initial isolates used in adoptive T cell therapy contain variable numbers of stem-like PD-1⁺ CD8⁺ T cells, and that the long-term persistence and efficacy of the reinfused cells depends on the presence of CD8⁺ T cells with stem-like properties.

These are two predictions that can be tested now that scientists have identified the hallmarks of these stem-like cells. It is also essential to determine how culture conditions used in adoptive therapy affect stem-like cells. Indeed, the abundance and quality of stem-like CD8⁺ T cells obtained with current cell amplification procedures is unclear. Optimizations could include growth factors that favor the expansion of stem-like CD8⁺ T cells or limit the generation of killer CD8⁺ T cells, as the latter cells' multiplication capacity and persistence is limited. Improving the stem cell properties of the infused cells would likely improve the therapeutic efficacy and durability of adoptive cell therapy.

It is likely that current immunotherapy approaches do not fully unleash the functional potential of stem-like CD8⁺ T cells. A better understanding of these cells should improve immunotherapy for cancer patients. Going back to research the behavior of these immune cells in the context of infection may again help to gain critical insights. Besides infection and cancer, the discovery of stem-like T cells may be relevant in other situations where chronic T cell stimulation arises, such as autoimmune disease and transplantation.

Daniel E. Speiser and Werner Held are professors in the Department of Oncology at the University of Lausanne in Switzerland.

References

1. C.G. Clemente et al., "Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma," *Cancer*, 77:1303–10, 1996.
2. L. Baitsch et al., "Exhaustion of tumor-specific CD8⁺ T cells in metastases from melanoma patients," *J Clin Invest*, 121:2350–60, 2011.
3. P.C. Tumeh et al., "PD-1 blockade induces responses by inhibiting adaptive immune resistance," *Nature*, 515:568–71, 2014.
4. D.T. Utzschneider et al., "T Cell Factor 1-Expressing Memory-like CD8⁺ T Cells Sustain the Immune Response to Chronic Viral Infections," *Immunity*, 45:415–27, 2016.
5. S.J. Im et al., "Defining CD8⁺ T cells that provide the proliferative burst after PD-1 therapy." *Nature*, 537:417–21, 2016.
6. I. Siddiqui et al., "Intratumoral Tcf1⁺ PD-1⁺ CD8⁺ T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy," *Immunity*, 50:195–211.e10, 2019.
7. B.C. Miller et al., "Subsets of exhausted CD8⁺ T cells differentially mediate tumor control and respond to checkpoint blockade," *Nat Immunol*, 20:326–36, 2019.
8. G. Jeannet et al., "Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory," *PNAS*, 107:9777–82, 2010.
9. D.S. Thommen et al., "A transcriptionally and functionally distinct PD-1⁺ CD8⁺ T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade," *Nat Med*, 24:994–1004, 2018.

Key words:

Cancer, Immunotherapy, Checkpoint blockade, Cancer vaccine, T cells, Stem cell-like CD8⁺ T cells

CHECKPOINT INHIBITORS AND STEM-LIKE T CELLS

The body's defense system against infection also fights tumors, generating tumor resident stem-like T cells and killer T cells that express inhibitory receptors such as PD-1. When PD-1 binds to PD-L1 or PD-L2 on tumor cells or other cells, T cell functions are subdued. Checkpoint blockade treatments interrupt this interaction. This allows stem-like T cells to proliferate and to produce new killer T cells that can now kill tumor cells.

