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# UNIVERSITÀ DEGLI STUDI DI TORINO

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## Consensus nomenclature for CD8 T cell phenotypes in cancer

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## **Abstract**

Whereas preclinical investigations and clinical studies have established that CD8 T cells can profoundly affect cancer progression, the underlying mechanisms are still elusive. Challenging the prevalent view that the beneficial effect of CD8 T cells in cancer is solely attributable to their cytotoxic activity, several reports have indicated that the ability of CD8 T cells to promote tumor regression is dependent on their cytokine secretion profile and their ability to self-renew. Evidence has also shown that the tumor microenvironment can disarm CD8 T cell immunity, leading to the emergence of dysfunctional CD8 T cells. The existence of different types of CD8 T cells in cancer calls for a more precise definition of the CD8 T cell immune phenotypes in cancer and the abandonment of the generic terms “pro-tumor” and “anti-tumor”. Based on recent studies investigating the functions of CD8 T cells in cancer, we here propose some guidelines to precisely define the functional states of CD8 T cells in cancer.



## 1) Introduction: the relevance of CD8 T cells in cancer

CD8 T cells are essential for clearing viral, protozoan, and intracellular bacterial infections<sup>1</sup>. Multiple lines of evidence show that CD8 T cells are also a key component of anti-tumor immunity. Initial studies in preclinical cancer models showed that CD8 T cells have a role in the prevention of tumor growth. Uyttenhove et al showed that escape of P815 mastocytoma was due to loss of distinct CD8 T cell specificities<sup>2</sup> and Nakayama and Uenaka showed that antibodies against CD8 effectively blocked the spontaneous rejection of transplantable tumors<sup>3</sup>. Shankaran et al. and Smyth et al. later showed that adaptive immune responses were essential to prevent growth of mutagen-induced spontaneous tumors<sup>4,5</sup>. Interestingly, Shankaran et al. further reported that TAP1-transfected transplantable sarcomas were eliminated in wild-type mice in a CD8 T cell dependent-manner, suggesting that high expression of tumor antigens could drive activation of anticancer CD8 T cell responses<sup>4</sup>. Subsequent work from Koebel et al. showed that during the equilibrium phase of cancer growth, where cancer cells persist but are kept in check by the immune system<sup>6</sup>, depletion of CD8 T cells drives cancer cell growth, underscoring the importance of CD8 T cells in controlling cancer growth over long time periods<sup>7</sup>.

CD8 T cells have also been shown to be essential effector cells in the context of anticancer therapies. Depletion of CD8 T cells has been shown to abrogate the anticancer efficacy of oxaliplatin and doxorubicin against EL4 thymoma and MCA2 fibrosarcoma tumors, respectively<sup>8,9</sup>. Similarly, the therapeutic effect of local radiotherapy in melanoma, of interferon therapy in leukemia, and of bacille Calmette-Guerin therapy in bladder cancer is abrogated in the absence of CD8 T cells<sup>10-12</sup>. Altogether, these results establish that CD8 T cells can control spontaneous and carcinogen-induced tumor growth, invasiveness of transplantable cell lines as well as the therapeutic efficacy of some anticancer treatments.

In line with this substantial amount of preclinical work, it has been established in human cancers that CD8 T cell infiltrates can predict patients' survival. While in kidney cancer CD8 T cell infiltrates have been associated with worse outcome and with higher tumor grade<sup>13, 14</sup>, they are linked to a better clinical outcome in the vast majority of other cancer types. In ovarian cancer, the presence of CD3 T cell infiltrates have been shown to correlate with improved survival rates<sup>15</sup>. In colon cancer, tumors without signs of metastatic invasion exhibit increased numbers of effector- memory CD8 T cells, thereby indicating that the presence of effector-memory CD8 T cells in the tumor microenvironment correlates with a better prognosis<sup>16</sup>. These findings were subsequently confirmed in other cohorts and an

international consortium is currently evaluating the possible utilization of immune infiltrate data to predict patient survival in routine clinical settings<sup>17, 18</sup>. The favorable prognostic value of CD8 T cell infiltrates has also been documented in other cancer types, such as breast cancer and epithelial ovarian cancer<sup>19, 20</sup>. These findings suggest that, in humans, even in situations when tumors are detectable, CD8 T cells can control tumor progression. The clinical relevance of CD8 T cells in human cancer is further underscored by recent studies in breast cancer patients showing that the combination of high CD8 and low FOXP3 cell infiltrates after chemotherapy was significantly associated with favorable clinical responses<sup>21</sup>. These results were confirmed in two other studies, where CD8 tumor infiltrating T cells were found to be an independent predictive factor for pathological complete response after anthracycline or anthracycline-taxane-based chemotherapy<sup>9, 22</sup>. Collectively, these preclinical and clinical observations indicate that CD8 T cells should not only be contemplated as a putative therapeutic tool but also as a biomarker to monitor the efficacy of cytotoxic chemotherapy. However, recent data also indicate that intra-tumoral CD8 T cells often lose their effector functions and exhibit a dysfunctional state. Accordingly, the terms “anti-tumor” and “pro-tumor” have been used in the literature to describe CD8 T cells in cancer. Given the advances in our knowledge of CD8 T cell phenotypes in cancer, these terms are clearly an oversimplification. Here, we discuss the different functional states of CD8 T cells in cancer and propose some guidelines for more accurate designation of CD8 T cells that exhibit different functional phenotypes.

## **2) CD8 effector T cells in cancer**

Naïve CD8 T cells that undergo priming *in vivo* in the presence of helper factors produced by CD4 T cells differentiate into effector T cells that express high levels of perforin and granzymes<sup>23, 24</sup>. The coordinated delivery of these cytotoxic molecules to cancer cells can drive caspase activation and ultimately cell death<sup>23, 25-27</sup> (**Figure 1a**). Given the demonstrated potential of CD8 T cells to kill cancer cells, CD8 T cells are often referred to as cytotoxic T lymphocytes (CTLs). Several different methods can be employed to assess CD8 T cell cytotoxicity: direct measurement of target cell killing (for example by the chromium 51 release (<sup>51</sup>Cr) assay<sup>28</sup>), flow cytometry based or ELISPOT measurement of granzyme B, a component of lytic granules in CD8 T cells<sup>29, 30</sup>, and detection of the expression of CD107a, which is present on the cell surface of degranulating CD8 T cells. While the individual merits of these different methods have been debated, they have all been used to demonstrate CTL activity in cancer. Using quantification of CD107a, Rubio et al. showed that tumor-cytolytic T

cells could be elicited in patients after vaccination and that tumor cell killing is associated with the ability of CD8 T cells to recognize their targets<sup>31</sup>. Using a <sup>51</sup>Cr release assay, Takeshima et al. showed that in tumor-bearing mice local radiotherapy could elicit cytotoxic tumor-specific CD8 T cells that prevent tumor growth<sup>32</sup>. Importantly, they further demonstrated the importance of CD8 T cells in mediating tumor regression following radiotherapy *in vivo* by using a neutralizing CD8 antibody. This key experiment, which was replicated in other studies<sup>10</sup>, was essential because the detection of activated or even antigen-specific cytotoxic T cells in *ex vivo/in vitro* assays does not necessarily ensure that CD8 T cells drive tumor regression *in vivo*.

CD8 T cells can also kill tumors via the Fas/Fas ligand pathway. Indeed, it has been proposed that FasL-driven CD8 T cell killing could be essential for the elimination of large and/or disseminated tumors<sup>33-35</sup>. However, it should be noted that tumors can lose Fas expression or develop mutations in the cell death pathway engaged by FasL, thus developing resistance to FasL/Fas-mediated CD8 T cell cytotoxicity. Other mechanisms by which tumors can resist CD8 T cell cytotoxicity are increased expression of anti-apoptotic molecules such as Bcl-2, Bcl-xl, and Mcl-1 and changes in components of the cytoskeleton that impair the formation of stable immunological synapses between cytotoxic CD8 T cells and tumor cells<sup>36</sup>.

Strategies have also been developed to assess CTL activity *in vivo*. For this, the selective elimination of adoptively transferred carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled target cells bearing a specific CD8 T cell peptide has been examined in preclinical models<sup>38</sup>. While this technique has the major advantage of assessing CD8 T cell cytotoxicity *in vivo*, it does not inform as to the killing mechanism employed nor does it allow for visualization of the killing process. In regards to the latter, the development of intra-vital imaging represents a major advance in monitoring T cell anticancer functions *in vivo* in mice at the single-cell level. Using this technology, the group of Amigorena has found that activated cytotoxic CD8 T cells can infiltrate tumors and arrest in close contact to and kill tumor cells provided that the tumor cells express cognate antigen<sup>39</sup>. Using a similar methodology, Breart et al. found that in contrast to *in vitro* cytotoxic assays where tumor cell death occurs within minutes after incubation with cytotoxic T cells, the *in vivo* destruction of 1 tumor cell by a cytotoxic T lymphocyte in the tumor bed took on average six hours, possibly explaining the limited ability of CD8 T cells to eradicate established tumors<sup>40</sup>.

While the cytotoxicity of CD8 T cells against tumor cells has been a major focus, it is important to note that some studies suggest that direct tumor cell killing may not be the major

or only mechanism responsible for tumor regression. It has been shown that CD8 T cells can also recognize tumor antigens processed by the stroma<sup>41</sup> and studies using longitudinal confocal microscopy imaging have shown that vessel regression occurs immediately following CD8 T cell entry from the blood stream into the tumor<sup>42</sup>. Thus, cytotoxicity against tumor stroma may also be a major mechanism of tumor regression.

Although much attention has been given to the cytotoxic function of CD8 T cells, it is not the sole mechanism responsible for the anti-cancer activity of CD8 T cells. Activated CD8 T cells also secrete cytokines like TNF $\alpha$  and IFN $\gamma$ , which can induce cancer cell senescence and play essential roles in the control of anticancer immune responses and tumor growth<sup>43</sup> (**Figure 1a**). IFN $\gamma$  has indeed been shown to be critical for cancer immunosurveillance and its secretion by CD8 T cells can enhance antigen presentation, the anti-tumor functions of macrophages, and limit tumor angiogenesis<sup>44-46</sup>. CD8 T cell-derived IFN $\gamma$  was further shown to be critical for the anti-cancer efficacy of chemotherapeutic drugs such as doxorubicin and oxaliplatin<sup>8</sup>. Importantly, the ability of these drugs to prevent tumor outgrowth was not compromised in perforin-deficient mice, suggesting that in this system CD8 T cells do not prevent tumor growth through direct cytotoxic activity. Accordingly, immunization of mice with chemotherapy-treated dying tumor cells failed to elicit CD8 T cell cytotoxicity but instead induced their secretion of IFN $\gamma$ . Thus, in some contexts the ability of CD8 T cells to produce IFN $\gamma$  may be more critical than their cytolytic function for anti-tumor efficacy<sup>8</sup>. These observations are in line with previous studies that identified IFN $\gamma$ -dependent anti-angiogenesis as a general mechanism involved in tumor rejection by CD8 T cell effectors<sup>47</sup>.

Altogether, these observations underscore that the “anti-tumor” activity of effector CD8 T cells in tumor tissue can be ascribed to both their direct cytolytic activity and their cytokine secretion. Indeed, poly-functional CD8 T cells that exhibit cytotoxicity along with production of TNF $\alpha$  and IFN $\gamma$  may be the most robust anti-tumor effectors. In this regard, it is also important to note that the efficacy of effector CD8 T cells in the tumor microenvironment may be limited as they undergo terminal differentiation and lose their ability to self-renew. The “anti-tumor” potential of CD8 T cells that retain self-renewing capacity is discussed below.

### **3) Cancer-driven CD8 T cell dysfunction**

Although effector CD8 T cells can be found in the tumor microenvironment, it is also well-established that tumors can drive CD8 T cell dysfunction. In the literature, the terms “anergic” and “exhausted” have both been used to describe dysfunctional CD8 T cells. Whether the CD8 T cells in cancer are anergic or exhausted has been a matter of debate. Here we will discuss the use of these terms to describe dysfunctional CD8 T cells in cancer.

Anergy typically refers to a general state of diminished function of a given immune response. In the 1980s, the term was applied to T cells induced into a state of non-responsiveness *in vitro* upon engagement of the T cell receptor (Signal 1) in the absence of a costimulatory signal (Signal 2). In a number of *in vivo* settings, such as tolerance induction by i.v. injection of antigens without adjuvants, it was hypothesized that T cell unresponsiveness was similarly induced by antigen recognition without appropriate co-stimulatory signals<sup>48</sup>. Anergic T cells fail to proliferate and produce effector cytokines in response to subsequent stimulation. T cell anergy is believed to be operative in cancer given that tumors often poorly express co-stimulatory molecules such as B7-1/B7-2, that dendritic cells present in tumor tissue express low MHC and low B7-1/B7-2 but high PD-L1 (B7-H1)<sup>49</sup>, and that myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAM) contribute to sub-optimal antigen presentation in the tumor environment<sup>50</sup>. Moreover, MDSCs and TAMs can produce arginase-1 and TGF- $\beta$  and drive oxidative stress, all of which drive suppression of CD8 T cell responses<sup>51</sup>.

The term “exhaustion” comes from the study of the CD8 T cell response to chronic viral infections in mouse models where antigen is not cleared despite ongoing stimulation. There is also evidence for virus-specific T cell “exhaustion” in humans in the setting of chronic HCV and HIV. Similar to anergic T cells, “exhausted” T cells exhibit defective responses to antigen stimulation; however, unlike anergy which develops as a result of a sub-optimal first encounter of T cells with cognate antigen, exhaustion develops progressively as a result of chronic stimulation of T cells in the face of high antigen burden<sup>52</sup>. Indeed, the T cells that develop an “exhausted” phenotype are those that undergo robust activation in the acute phase of the anti-viral response.

“Exhausted” CD8 T cells express high levels of co-inhibitory receptors such as PD-1, Lag-3, CD244, CD160, and Tim-3, and it has been shown that interfering with the signaling through one or more of these receptors can improve anti-virus CD8 T cell responses<sup>53, 54</sup>. CD8 T cells that express inhibitory molecules and exhibit severe functional deficits have also been described in cancer<sup>55-59</sup> (**Figure 1b**). These observations have led to the widespread use of the

term “exhaustion” to describe the dysfunctional CD8 T cells in cancer. However, whether the dysfunctional CD8 T cells observed in cancer are truly analogous to those that arise in chronic viral infection is an open question. Resolution of this issue awaits elucidation of the molecular programs specifically associated with dysfunctional T cells in cancer. These studies are currently at an early stage. An initial study of the dysfunctional CD8 T cells from the tumor-infiltrated lymph nodes of melanoma patients indicates that the gene profile of these cells is significantly enriched for genes identified in exhausted LCMV-specific murine CD8 T cells; however, these cells fail to up-regulate Batf, a key driver of T cell exhaustion in HIV infection<sup>57</sup>.

Moreover, it has recently been suggested that “exhaustion” is a misnomer as “exhausted” cells are not completely devoid of function as the term “exhaustion” implies. Rather these cells exhibit an attenuated response that is optimized for minimizing tissue damage while still preserving some level of response against abnormal cells (virally infected or cancerous)<sup>60</sup>. Indeed, a key function of co-inhibitory receptor expression on highly active T cells is to contract ongoing T cell responses in order to restore immune homeostasis and prevent immunopathology. Unfortunately, tumors have taken advantage of this mechanism to dampen anti-tumor T cell responses.

At this juncture, we recommend against ascribing the CD8 T cells in cancer as either “anergic” or “exhausted”. This terminology is not useful as these states have been defined and largely studied in other T cell types, such as CD4 T cells, or in disease conditions that differ significantly from cancer, namely chronic viral infection. We recommend that the term dysfunctional instead be used to describe the poorly functional CD8 T cells in cancer<sup>61</sup>. We further caution against ascribing cells as dysfunctional based on expression of co-inhibitory receptors alone as these molecules are also found on effector T cells that retain functional properties<sup>62</sup>. Indeed, expression of these inhibitory receptors could also reflect a state of previous activation of CD8 T cells indicating that expression of these receptors may identify anti-tumor specific T cells associated with a good prognosis<sup>63, 64</sup>. Dysfunctional CD8 T cells should be defined as cells that exhibit defects in proliferation, lack of inflammatory cytokine production and/or cytotoxic functions, together with expression of one or more co-inhibitory receptors (**Figure 1b**).

It is important to note that CD8 T cell dysfunction in the tumor microenvironment is believed to be reversible, at least to some extent. In pre-clinical cancer models, blockade of signaling through CTLA-4, PD-1, Tim-3, and Lag-3 have been shown to improve CD8 T cell responses (reviewed in <sup>65</sup>). Accordingly, the current success of strategies that interfere with

signaling through the PD-1 inhibitory receptor in the clinic is believed, at least in part, to be due to the ability of PD-1 blockade to re-invigorate CD8 T cell responses.

#### 4) CD8 T cell senescence in cancer

Senescent CD8 T cell phenotypes can also arise in the tumor microenvironment. Senescence refers to an irreversible state of growth arrest that develops in cells upon repeated cellular division, termed replicative senescence, or in response to DNA damage. General characteristics of senescent cells include: short telomeres, irreversible cell cycle-arrest, activation of DNA damage response (DDR) genes, robust secretion of factors that constitute the senescence-associated secretory phenotype (SASP), and accumulation of senescence associated heterochromatin foci (SAHF)<sup>66</sup>. Specific cell surface markers ascribed to senescent T cells are loss of CD28 and CD27 and high expression of CD57 and KLRG-1 (**Figure 1c**).

While senescence has historically been associated with aging, it is now recognized that replicative senescence also develops in the context of chronic antigen stimulation, such as that which occurs in cancer. Indeed, it has been shown that culture of tumor cells with normal healthy human T cells in low tumor to T cell ratios can induce a phenotype consistent with T cell senescence *in vitro*<sup>67</sup>. These cells exhibit decreased CD28 and CD27 expression along with concomitant up-regulation of  $\gamma$ H2AX (H2A histone family member X) and ATM (ataxia telangiectasia mutated), both of which are induced as part of the DDR to double strand DNA breaks (**Figure 1c**). A recent study further reported the presence of CD8 T cells that exhibit characteristics of senescence *in vivo* in human lung cancer tissue<sup>68</sup>. These cells are CD28<sup>-</sup>CD57<sup>+</sup> and exhibit accumulation of heterochromatin protein-1 gamma foci, a component of SAHF.

Although senescent T cells are irreversibly cell-cycle arrested, it is important to note that they are not completely devoid of function. The senescent CD8 T cells found in lung cancer tissue produce IL-6 and IL-8, two hallmark SASP factors<sup>68</sup>. These two features distinguish senescent CD8 T cells from dysfunctional CD8 T cells (**Figure 1b and c**) as the dysfunctional T cells are not irreversibly cell-cycle arrested they exhibit severely impaired production of pro-inflammatory cytokines and other effector molecules.

The two SASP factors that are reported to be expressed by senescent CD8 T cells, IL-6 and IL-8, are both pro-inflammatory cytokines. IL-6 can suppress regulatory T cell function (Treg)<sup>69</sup> and promote the differentiation of IL-17-producing Th17 cells<sup>70</sup>. The dampening of Treg function could benefit anti-tumor immunity by relieving an important mechanism of immune suppression in tumor tissue. However, the outcome of promotion of Th17 cells in

tumor tissue is less clear as both “pro-tumor” and “anti-tumor” properties for Th17 cells have been described<sup>71</sup>. Notwithstanding how IL-6 may shape anti-tumor T cell responses, IL-6 can promote tumorigenesis through its effects in driving cellular proliferation, promoting cell survival by delivering anti-apoptotic signals, and augmenting MDSC suppressive functions<sup>72, 73</sup>. Indeed, high levels of IL-6 have been associated with multiple cancers and are associated with poor prognosis<sup>74</sup>. IL-8 also exhibits pleiotropic tumor promoting effects. It can promote angiogenesis, cancer cell survival, proliferation, migration, and resistance to chemotherapy<sup>75</sup>. Thus, by virtue of their production of IL-6 and IL-8 senescent CD8 T cells could be considered “pro-tumor”.

It is also important to note that terminal effector CD8 T cells can also exhibit loss of CD28 and up-regulation of KLRG-1. Moreover, vaccine-induced CD8 T cells with optimal anti-tumor effector function have been noted to express high levels of KLRG-1<sup>76</sup>. Thus, senescent phenotype cannot be ascribed solely on the basis of loss of CD28 and expression of KLRG1. Senescent cells must further exhibit SAHF and activation of DDR genes (**Figure 1c**).

### **5) Stem-cell like memory CD8 T cells**

Terminal effector CD8 T cells limit tumor outgrowth. However, these cells can become dysfunctional or senescent in the tumor microenvironment. Recent studies that examine the efficacy of *ex vivo* generated CD8 T cells on tumor clearance after adoptive transfer into tumor-bearing hosts show that terminally differentiated effector CD8 T cells are ineffective at eliminating tumors *in vivo* compared to less differentiated T cells<sup>77, 78</sup> (**Figure 1a, Figure 2**). This occurs in spite of their higher secretion of IFN $\gamma$  and cytolytic activity. Instead, it has been suggested that CD8 T cells that share properties with naïve T cells such as CCR7 and CD62L expression and have the ability to self-renew are more potent for fighting tumors (**Figure 2**). Because of their ability to self-renew and persist for long periods of time, these CD8 T cells have been termed stem-cell like memory T cells. Unfortunately, in contrast to terminal effector CD8 T cells, stem-cell like memory CD8 T cells are predominantly found in lymphoid tissue and not in the tumor microenvironment.

It is well known that the omnipotency of naïve T cells is progressively lost with T cell differentiation to memory and effector T cells. Among antigen-experienced T cells, the stem-cell like memory T cells are the ones with the highest potency, producing progeny for both immediate immunity and its long-term maintenance, based on self-renewal. It is likely that tumor-antigen specific effector T cells depend on continuous differentiation from self-



renewing memory T cells. Therefore, it remains a major aim to develop methods inducing self-renewing T cells *in vitro* for adoptive transfer, or *in vivo* by active immunization. In this regard, the recent identification of IL-7 and IL-15 as molecular signals guiding human naive T lymphocytes to differentiate into stem-cell like memory CD8 T cells *in vitro* provides impetus to investigate their anticancer potential in clinical trials<sup>79, 80</sup>. Progress in basic research, bioengineering, and therapy development will likely further exploit the potential of T cell stemness, as a fundamental basis of robust and long-term T cell responses including the capability to home to tumors and exert effector functions therein.

## 6) CD8 T cells as regulatory cells in cancer?

The existence of several types of CD8 T cells with regulatory or suppressive properties in cancer has been proposed. These include: CD8<sup>+</sup> CD28<sup>-</sup>, CD8<sup>+</sup> CD25<sup>+</sup>, CD8<sup>+</sup> CD122<sup>+</sup>, and CD8<sup>+</sup> IL-10<sup>+</sup> T cells<sup>81, 82</sup>. At present, there seems to be no consensus in the field as to whether these are overlapping or dissimilar subsets and, moreover, whether these are truly distinct from other cell types that express some of the same surface markers. For these reasons, this potential class of CD8 T cells will not be further discussed here.

## 7) Conclusion

Our understanding of the CD8 phenotypes that arise in cancer necessitates that we move beyond the simplified nomenclature of “pro-tumor” vs “anti-tumor” T cells. We, and others, have now identified stem cell-like, terminal effector, dysfunctional, or senescent CD8 T cells that are functionally and in many cases molecularly distinct. Currently, there are no unique surface markers that allow for easy discrimination between these CD8 phenotypes. Thus, accurate identification requires a more in depth analysis that includes examination of cell surface phenotype, functional phenotype, and expression of intracellular markers. Here, we have summarized the current knowledge of CD8 phenotypes in cancer (**Box 1**). We recommend avoiding the use of broad terms like “pro-tumor” or “anti-tumor” CD8 T cells without providing information on their functional state. We further propose that the term CTL should only be employed when corresponding cytotoxic functions have been experimentally demonstrated and that the term “dysfunctional” rather than “anergic” or “exhausted” be used to describe CD8 T that exhibit functional deficits in cancer. Importantly, we caution against ascribing CD8 T cells as dysfunctional based on the expression of co-inhibitory receptors alone. Future studies incorporating T cell analyses should include appropriate markers and

functional assays to better define the phenotypes of T cells in peripheral blood, peripheral lymphoid tissues, and tumor biopsy samples.

### **Financial and competing interests disclosure**

MB is currently working as a scientific director of Institut Mérieux, a private company implementing *in vitro* diagnostics and immunotherapeutic approaches in oncology and infectious diseases. CM is Chief Scientific Officer of ISA Pharmaceuticals, a private biotech company developing synthetic therapeutic vaccines against cancer.

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### **Figure 1. CD8 T cell phenotypes in the tumor microenvironment**

**a)** Effector CD8 T cells that undergo terminal differentiation are characterized by low IL-2, strong IFN $\gamma$  and TNF $\alpha$  release as well as high expression levels of the transcription factors *Eomes* and *Id2*<sup>83-85</sup>. They do not express the surface markers CD62L, CCR7, CD27 but express killer cell lectin-like receptor G1 (KLRG-1) and PD-1<sup>63, 86-89</sup>. While terminal effector CD8 T cells exhibit strong cytolytic functions *in vitro*, their anticancer activity *in vivo* is limited because of their inability to self-renew compared to stem-cell like memory CD8 T cells<sup>78, 90, 91</sup>.

**b)** Dysfunctional CD8 T cells are characterized by cocomittant expression of two or more inhibitory receptors such as CTLA-4, PD-1, Lag-3, Tim-3 and BTLA<sup>65, 92, 93</sup>. These cells exhibit defects in cytotoxicity, proliferative capacity, and secretion of pro-inflammatory cytokines: IL-2, TNF $\alpha$  and IFN $\gamma$ <sup>55, 56, 94</sup>.

**c)** Senescent CD8 T cells express killer cell lectin-like receptor G1 (KLRG-1) and CD57 but not CD27 or CD28<sup>87, 95</sup>. They are characterized by short telomeres, poor proliferative capacity and activation of DNA damage response (DDR) genes<sup>66, 68, 95, 96</sup>. These cells were also shown to express PD-1 in chronic lymphocytic leukemia patients<sup>95</sup>. Senescent CD8 T cells lack cytotoxicity<sup>96</sup>, and were shown to express the proinflammatory mediators *Il6* and *Il8* in lung cancer tissue<sup>68</sup>.

### **Figure 2. Features of stem-cell like CD8 T cells.**

Stem-cell like memory CD8 T cells share many phenotypic features with naïve T cells (reviewed in <sup>97</sup>). They typically express the CD45RA phosphatase, the lymph node homing molecules CCR7 and CD62L as well as the costimulatory receptors CD27 and CD28<sup>77, 98</sup>. These cells express the transcription factors *Id3*<sup>77</sup> and *Tcf7*<sup>91</sup>, secrete IL-2 and low levels of TNF $\alpha$  or IFN $\gamma$ . These cells also have the ability to self-renew and exhibit potent anticancer responses *in vivo*<sup>78, 90</sup>.

## References

1. Harty JT, Tvinnereim AR, White DW. CD8+ T cell effector mechanisms in resistance to infection. *Annual review of immunology* 2000; 18:275-308.
2. Uyttenhove C, Maryanski J, Boon T. Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression. *J Exp Med* 1983; 157:1040-52.
3. Nakayama E, Uenaka A. Effect of in vivo administration of Lyt antibodies. Lyt phenotype of T cells in lymphoid tissues and blocking of tumor rejection. *J Exp Med* 1985; 161:345-55.
4. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFN $\gamma$  and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001; 410:1107-11.
5. Smyth MJ, Thia KY, Street SE, MacGregor D, Godfrey DI, Trapani JA. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. *J Exp Med* 2000; 192:755-60.
6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002; 3:991-8.
7. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* 2007; 450:903-7.
8. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1 $\beta$ -dependent adaptive immunity against tumors. *Nature medicine* 2009; 15:1170-8.
9. Mattarollo SR, Loi S, Duret H, Ma Y, Zitvogel L, Smyth MJ. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res* 2011; 71:4809-20.
10. Lee Y, Auh SL, Wang Y, Burnette B, Wang Y, Meng Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. *Blood* 2009; 114:589-95.
11. Ratliff TL, Ritchey JK, Yuan JJ, Andriole GL, Catalona WJ. T-cell subsets required for intravesical BCG immunotherapy for bladder cancer. *J Urol* 1993; 150:1018-23.
12. Gresser I, Maury C, Carnaud C, De Maeyer E, Maunoury MT, Belardelli F. Anti-tumor effects of interferon in mice injected with interferon-sensitive and interferon-resistant Friend erythroleukemia cells. VIII. Role of the immune system in the inhibition of visceral metastases. *Int J Cancer* 1990; 46:468-74.
13. Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean MC, Riquet M, et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2013; 19:4079-91.
14. Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, et al. Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. *Cancer research* 2001; 61:5132-6.
15. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003; 348:203-13.
16. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. *The New England journal of medicine* 2005; 353:2654-66.
17. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. *The Journal of pathology* 2014; 232:199-209.
18. Koelzer VH, Lugli A, Dawson H, Hadrich M, Berger MD, Borner M, et al. CD8/CD45RO T-cell infiltration in endoscopic biopsies of colorectal cancer predicts nodal metastasis and survival. *J Transl Med* 2014; 12:81.

19. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011; 29:1949-55.
20. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America* 2005; 102:18538-43.
21. Ladoire S, Mignot G, Dabakuyo S, Arnould L, Apetoh L, Rebe C, et al. In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival. *J Pathol* 2011; 224:389-400.
22. Seo AN, Lee HJ, Kim EJ, Kim HJ, Jang MH, Lee HE, et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. *British journal of cancer* 2013; 109:2705-13.
23. Barry M, Bleackley RC. Cytotoxic T lymphocytes: all roads lead to death. *Nature reviews Immunology* 2002; 2:401-9.
24. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 1998; 393:480-3.
25. Masson D, Tschopp J. A family of serine esterases in lytic granules of cytolytic T lymphocytes. *Cell* 1987; 49:679-85.
26. Pasternack MS, Verret CR, Liu MA, Eisen HN. Serine esterase in cytolytic T lymphocytes. *Nature* 1986; 322:740-3.
27. Podack ER, Konigsberg PJ. Cytolytic T cell granules. Isolation, structural, biochemical, and functional characterization. *The Journal of experimental medicine* 1984; 160:695-710.
28. Brunner KT, Mauel J, Cerottini JC, Chapuis B. Quantitative assay of the lytic action of immune lymphoid cells on 51-Cr-labelled allogeneic target cells in vitro; inhibition by isoantibody and by drugs. *Immunology* 1968; 14:181-96.
29. Griffiths GM, Isaacs S. Granzymes A and B are targeted to the lytic granules of lymphocytes by the mannose-6-phosphate receptor. *The Journal of cell biology* 1993; 120:885-96.
30. Wever PC, Van Der Vliet HJ, Spaeny LH, Wolbink AM, Van Diepen FN, Froelich CJ, et al. The CD8+ granzyme B+ T-cell subset in peripheral blood from healthy individuals contains activated and apoptosis-prone cells. *Immunology* 1998; 93:383-9.
31. Rubio V, Stuge TB, Singh N, Betts MR, Weber JS, Roederer M, et al. Ex vivo identification, isolation and analysis of tumor-cytolytic T cells. *Nature medicine* 2003; 9:1377-82.
32. Takeshima T, Chamoto K, Wakita D, Ohkuri T, Togashi Y, Shirato H, et al. Local radiation therapy inhibits tumor growth through the generation of tumor-specific CTL: its potentiation by combination with Th1 cell therapy. *Cancer research* 2010; 70:2697-706.
33. Caldwell SA, Ryan MH, McDuffie E, Abrams SI. The Fas/Fas ligand pathway is important for optimal tumor regression in a mouse model of CTL adoptive immunotherapy of experimental CMS4 lung metastases. *Journal of immunology* 2003; 171:2402-12.
34. Seki N, Brooks AD, Carter CR, Back TC, Parsonneault EM, Smyth MJ, et al. Tumor-specific CTL kill murine renal cancer cells using both perforin and Fas ligand-mediated lysis in vitro, but cause tumor regression in vivo in the absence of perforin. *Journal of immunology* 2002; 168:3484-92.
35. Afshar-Sterle S, Zotos D, Bernard NJ, Scherger AK, Rodling L, Alsop AE, et al. Fas ligand-mediated immune surveillance by T cells is essential for the control of spontaneous B cell lymphomas. *Nat Med* 2014; 20:283-90.
36. Jazirehi AR, Economou JS. Proteasome inhibition blocks NF-kappaB and ERK1/2 pathways, restores antigen expression, and sensitizes resistant human melanoma to TCR-engineered CTLs. *Molecular cancer therapeutics* 2012; 11:1332-41.
37. Abouzahr S, Bismuth G, Gaudin C, Carroll O, Van Endert P, Jalil A, et al. Identification of target actin content and polymerization status as a mechanism of tumor resistance after cytolytic T lymphocyte pressure. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103:1428-33.

38. Durward M, Harms J, Splitter G. Antigen specific killing assay using CFSE labeled target cells. *Journal of visualized experiments : JoVE* 2010.
39. Boissonnas A, Fetler L, Zeelenberg IS, Hugues S, Amigorena S. In vivo imaging of cytotoxic T cell infiltration and elimination of a solid tumor. *The Journal of experimental medicine* 2007; 204:345-56.
40. Breart B, Lemaitre F, Celli S, Bousso P. Two-photon imaging of intratumoral CD8+ T cell cytotoxic activity during adoptive T cell therapy in mice. *The Journal of clinical investigation* 2008; 118:1390-7.
41. Spiotto MT, Rowley DA, Schreiber H. Bystander elimination of antigen loss variants in established tumors. *Nat Med* 2004; 10:294-8.
42. Schietinger A, Arina A, Liu RB, Wells S, Huang J, Engels B, et al. Longitudinal confocal microscopy imaging of solid tumor destruction following adoptive T cell transfer. *Oncoimmunology* 2013; 2:e26677.
43. Braumuller H, Wieder T, Brenner E, Assmann S, Hahn M, Alkhaled M, et al. T-helper-1-cell cytokines drive cancer into senescence. *Nature* 2013; 494:361-5.
44. Street SE, Cretney E, Smyth MJ. Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. *Blood* 2001; 97:192-7.
45. Street SE, Trapani JA, MacGregor D, Smyth MJ. Suppression of lymphoma and epithelial malignancies effected by interferon gamma. *The Journal of experimental medicine* 2002; 196:129-34.
46. Sato N, Nariuchi H, Tsuruoka N, Nishihara T, Beitz JG, Calabresi P, et al. Actions of TNF and IFN-gamma on angiogenesis in vitro. *The Journal of investigative dermatology* 1990; 95:85S-9S.
47. Qin Z, Schwartzkopff J, Pradera F, Kammertoens T, Seliger B, Pircher H, et al. A critical requirement of interferon gamma-mediated angiostasis for tumor rejection by CD8+ T cells. *Cancer research* 2003; 63:4095-100.
48. Schwartz RH. T cell anergy. *Annual review of immunology* 2003; 21:305-34.
49. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med* 2003; 9:562-7.
50. Melief CJ. Cancer immunotherapy by dendritic cells. *Immunity* 2008; 29:372-83.
51. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; 9:162-74.
52. Wherry EJ. T cell exhaustion. *Nature immunology* 2011; 12:492-9.
53. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006; 439:682-7.
54. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nature immunology* 2009; 10:29-37.
55. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010; 207:2187-94.
56. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. *J Exp Med* 2010; 207:2175-86.
57. Baitsch L, Baumgaertner P, Devereux E, Raghav SK, Legat A, Barba L, et al. Exhaustion of tumor-specific CD8(+) T cells in metastases from melanoma patients. *J Clin Invest* 2011; 121:2350-60.
58. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood* 2011; 117:4501-10.
59. Yang ZZ, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, et al. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. *The Journal of clinical investigation* 2012; 122:1271-82.



60. Utzschneider DT, Legat A, Fuertes Marraco SA, Carrie L, Luescher I, Speiser DE, et al. T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. *Nat Immunol* 2013; 14:603-10.
61. Hailemichael Y, Dai Z, Jaffarad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. *Nat Med* 2013; 19:465-72.
62. Legat A, Speiser DE, Pircher H, Zehn D, Fuertes Marraco SA. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than "Exhaustion" of Human CD8 T Cells. *Front Immunol* 2013; 4:455.
63. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *The Journal of clinical investigation* 2014; 124:2246-59.
64. Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. *Cancer research* 2013; 73:128-38.
65. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews Cancer* 2012; 12:252-64.
66. Rodier F, Campisi J. Four faces of cellular senescence. *The Journal of cell biology* 2011; 192:547-56.
67. Montes CL, Chapoval AI, Nelson J, Orhue V, Zhang X, Schulze DH, et al. Tumor-induced senescent T cells with suppressor function: a potential form of tumor immune evasion. *Cancer research* 2008; 68:870-9.
68. Mondal AM, Horikawa I, Pine SR, Fujita K, Morgan KM, Vera E, et al. p53 isoforms regulate aging- and tumor-associated replicative senescence in T lymphocytes. *The Journal of clinical investigation* 2013; 123:5247-57.
69. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* 2003; 299:1033-6.
70. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; 441:235-8.
71. Martin F, Apetoh L, Ghiringhelli F. Controversies on the role of Th17 in cancer: a TGF-beta-dependent immunosuppressive activity? *Trends in molecular medicine* 2012; 18:742-9.
72. Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. *Trends in molecular medicine* 2008; 14:109-19.
73. Apetoh L, Vegran F, Ladoire S, Ghiringhelli F. Restoration of antitumor immunity through selective inhibition of myeloid derived suppressor cells by anticancer therapies. *Curr Mol Med* 2011; 11:365-72.
74. Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. *The lancet oncology* 2013; 14:e218-28.
75. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2008; 14:6735-41.
76. van Duikeren S, Fransen MF, Redeker A, Wieles B, Platenburg G, Krebber WJ, et al. Vaccine-induced effector-memory CD8+ T cell responses predict therapeutic efficacy against tumors. *J Immunol* 2012; 189:3397-403.
77. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stemness: building the ultimate antitumour T cell. *Nature reviews Cancer* 2012; 12:671-84.
78. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, et al. A human memory T cell subset with stem cell-like properties. *Nature medicine* 2011; 17:1290-7.
79. Cieri N, Camisa B, Cocchiarella F, Forcato M, Oliveira G, Provasi E, et al. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. *Blood* 2013; 121:573-84.
80. Gattinoni L, Restifo NP. Moving T memory stem cells to the clinic. *Blood* 2013; 121:567-8.

81. Wang RF. CD8+ regulatory T cells, their suppressive mechanisms, and regulation in cancer. *Human immunology* 2008; 69:811-4.
82. Wei S, Kryczek I, Zou L, Daniel B, Cheng P, Mottram P, et al. Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma. *Cancer Res* 2005; 65:5020-6.
83. Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. *Science* 2003; 302:1041-3.
84. Ji Y, Pos Z, Rao M, Klebanoff CA, Yu Z, Sukumar M, et al. Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits the formation of memory CD8+ T cells. *Nat Immunol* 2011; 12:1230-7.
85. Yang CY, Best JA, Knell J, Yang E, Sheridan AD, Jesionek AK, et al. The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets. *Nat Immunol* 2011; 12:1221-9.
86. Henson SM, Franzese O, Macaulay R, Libri V, Azevedo RI, Kiani-Alikhan S, et al. KLRG1 signaling induces defective Akt (ser473) phosphorylation and proliferative dysfunction of highly differentiated CD8+ T cells. *Blood* 2009; 113:6619-28.
87. Voehringer D, Koschella M, Pircher H. Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1). *Blood* 2002; 100:3698-702.
88. Gothert JR, Eisele L, Klein-Hitpass L, Weber S, Zesewitz ML, Sellmann L, et al. Expanded CD8+ T cells of murine and human CLL are driven into a senescent KLRG1+ effector memory phenotype. *Cancer Immunol Immunother* 2013; 62:1697-709.
89. Fourcade J, Kudela P, Sun Z, Shen H, Land SR, Lenzner D, et al. PD-1 is a regulator of NY-ESO-1-specific CD8+ T cell expansion in melanoma patients. *J Immunol* 2009; 182:5240-9.
90. Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. *J Clin Invest* 2005; 115:1616-26.
91. Lugli E, Dominguez MH, Gattinoni L, Chattopadhyay PK, Bolton DL, Song K, et al. Superior T memory stem cell persistence supports long-lived T cell memory. *J Clin Invest* 2013; 123:594-9.
92. Fourcade J, Sun Z, Pagliano O, Guillaume P, Luescher IF, Sander C, et al. CD8(+) T cells specific for tumor antigens can be rendered dysfunctional by the tumor microenvironment through upregulation of the inhibitory receptors BTLA and PD-1. *Cancer Res* 2012; 72:887-96.
93. Mittal R, Wagener M, Breed ER, Liang Z, Yoseph BP, Burd EM, et al. Phenotypic T cell exhaustion in a murine model of bacterial infection in the setting of pre-existing malignancy. *PLoS One* 2014; 9:e93523.
94. Fourcade J, Sun Z, Pagliano O, Chauvin JM, Sander C, Janjic B, et al. PD-1 and Tim-3 regulate the expansion of tumor antigen-specific CD8(+) T cells induced by melanoma vaccines. *Cancer Res* 2014; 74:1045-55.
95. Nunes C, Wong R, Mason M, Fegan C, Man S, Pepper C. Expansion of a CD8(+)PD-1(+) replicative senescence phenotype in early stage CLL patients is associated with inverted CD4:CD8 ratios and disease progression. *Clin Cancer Res* 2012; 18:678-87.
96. Beatty GL, Smith JS, Reshef R, Patel KP, Colligon TA, Vance BA, et al. Functional unresponsiveness and replicative senescence of myeloid leukemia antigen-specific CD8+ T cells after allogeneic stem cell transplantation. *Clin Cancer Res* 2009; 15:4944-53.
97. Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: human memory T-cell subsets. *Eur J Immunol* 2013; 43:2797-809.
98. De Rosa SC, Herzenberg LA, Roederer M. 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nat Med* 2001; 7:245-8.