Memory T-Cell Heterogeneity and Terminology

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Immunological memory and exhaustion are fundamental features of adaptive immunity. Recent advances reveal increasing heterogeneity and diversity among CD8 T-cell subsets, resulting in new subsets to annotate and understand. Here, we review our current knowledge of differentiation and maintenance of memory and exhausted CD8 T cells, including phenotypic classification, developmental paths, transcriptional and epigenetic features, and cell intrinsic and extrinsic factors. Additionally, we use this outline to discuss the nomenclature of effector, memory, and exhausted CD8 T cells. Finally, we discuss how new findings about these cell types may impact the therapeutic efficacy and development of immunotherapies targeting effector, memory, and/or exhausted CD8 T cells in chronic infections and cancer.

EFFECTOR AND MEMORY CD8 T-CELL DIFFERENTIATION

Following acutely resolved infection or vaccination, naive CD8 T cells are activated, undergo robust clonal expansion, and give rise to a diverse pool of effector CD8 T (Teff) cells. If activated by signals 1 (antigen), 2 (costimulation), and 3 (inflammation), most CD8 T cells differentiate into short-lived effector cells (SLECs). Although SLECs are heterogeneous, they often express KLRG1 and/or CX3CR1, produce cytokines and cytotoxic molecules, express activation markers, and acquire inflammatory homing properties. The majority of Teff cells, mostly SLECs, die in the weeks following the peak of expansion, usually (though not always) corresponding to clearance of antigen. However, a small subset of this activated, clonally expanded pool, known as memory precursors (Tmp cells), has the potential to differentiate into long-lived memory CD8 T (Tmem) cells. Some SLECs also persist, but these do not further differentiate into Tmem cells. These Tmem cells persist long term and acquire the ability to undergo antigen-independent self-renew via interleukin (IL)-7 and IL-15. This Tmem cell differentiation is accompanied by dynamic transcriptional, epigenetic, and metabolic reprogramming (Cui and Kaech 2010; Chang et al. 2014; Zehn and Wherry 2015; Jameson and Masopust 2018). Although this general outline describes the broad features of events following acute infection, there is considerable cellular and population heterogeneity at each stage of differentiation. Moreover, if antigen is not cleared, the development of exhausted CD8 T (Tex) cells occurs instead of Tmem cells, a topic discussed further below. Although parallels exist

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in concept for CD4 T-cell memory, there is clearly more complexity for CD4 T cells including the differentiation of Th1, Th2, Th17, and T follicular helper subsets. We will focus the following discussion on CD8 T cells. As a note, historically, the term effector and memory have been used in different contexts and meanings, such as in the context of time, function, and lineage, which are summarized in Table 1.

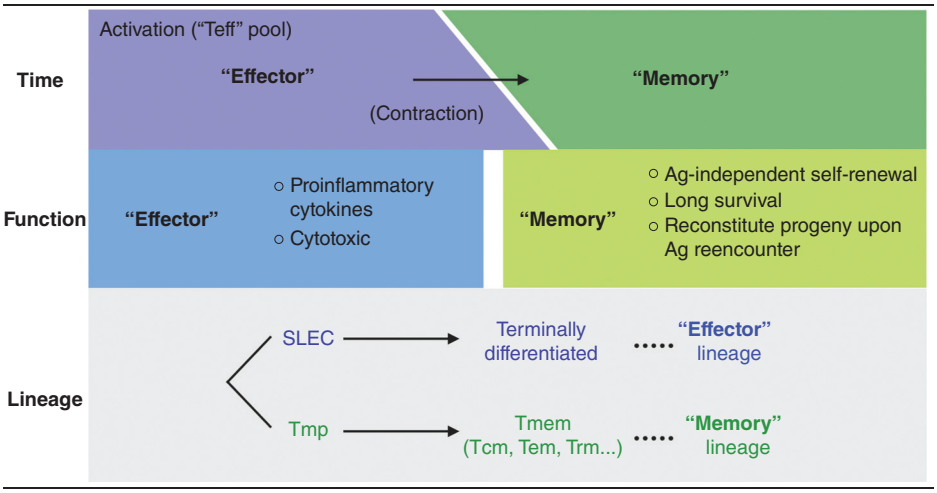
Teff AND Tmem CELL SUBSETS

Immunological memory is defined as the ability of the immune system to remember specific antigenic encounters and then mount enhanced responses upon reexposure. As such, Tmem cells are a key part of cellular immunological memory. Tmem cell subsets were originally defined based on homing and migration markers together with effector functions (Hamann et al. 1997; Sallusto et al. 1999). Cells expressing the lymph node (LN) homing receptors CCR7 and CD62L and possessing a high proliferative capacity but low cytotoxicity were termed central memory (Tcm) cells. Those lacking LN homing capacity and possessing inflammatory migration potential as well as effector function were termed effector memory (Tem) cells. In the subsequent two decades, concepts of Tem and Tcm cells have evolved to include additional subsets, such as effector memory rheumatoid arthritis (RA) (so called because they express CD45RA; Temra), transitional memory, memory stem (Tscm) cells, and tissue-resident memory (Trm) cells (Chang et al. 2014; Jameson and Masopust 2018). Our understanding of CD8 T-cell heterogeneity has advanced along with technology; increasingly large flow cytometry panels have generated new T-cell subsets. An early CyTOF study resolved ~200 different subpopulations of T cells based on high dimensional combinations of protein expression (Newell et al. 2012). It remains unclear whether these subpopulations represent distinct T-cell identities or simply different phenotypic states of a smaller number of T-cell fates. Nevertheless, it is clear that there are at least some major CD8 T-cell subtypes that can be defined by the combination of phenotype, behavior, and function. Some of these are outlined here.

Central Memory

Tcm cells are quiescent memory T cells that express high levels of molecules, such as CD62L and CCR7, that allow efficient homing to LNs, and low levels of cytotoxic proteins like granzymes and perforin. Functionally, Tcm cells possess low ex vivo cytotoxic activity and a high proliferative capacity (Wherry et al.

Table 1. Terminology of "effector" and "memory"



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2003). These cells can produce interleukin (IL)-2 and, in some settings, interferon γ (IFN-γ) upon stimulation. Tcm cells also acquire the ability to self-renew in response to IL-7 and IL-15. This homeostatic proliferation is unique to memory T cells and, in the CD8 T-cell compartment, resides mainly in the Tcm subset. Tcm cells can rapidly proliferate and expand after reencountering antigen and can generate effector progeny upon antigen restimulation (Wherry et al. 2003). In humans, Tcm cells are often phenotypically characterized as $CD45RA^{-}CD45RO^{+}CCR7^{+}CD27^{+}CD28^{+}$ (Hamann et al. 1997; Mahnke et al. 2013), whereas in mice these cells are usually defined simply by high expression of CD62L and/or CCR7 with markers of antigen experience such as high CD44.

Effector Memory

Tem cells do not express LN homing receptors CD62L and CCR7, and thus cannot home to LNs. Rather, Tem cells often express molecules that allow homing to nonlymphoid tissues and/or inflammatory sites. The concept of "effector memory" has evolved considerably since the original definition, and today there is clearly both phenotypic and conceptual heterogeneity. In early studies, Tem cells were simply defined as a pool of memory cells that retained the ability to rapidly produce effector functions and were thus capable of providing a first line of defense against reinfection. For example, Tem cells were defined to produce mainly IFN-γ and tumor necrosis factor (TNF), but not IL-2, and were capable of rapid cytotoxicity ex vivo (Hamann et al. 1997; Sallusto et al. 1999). These cells were found in blood and possessed inflammatory homing molecules, and the model was proposed that Tem cells therefore surveyed peripheral sites for new reinfection. In the two decades since they were first described, it has become clear that the Tem cell compartment is more complex, with additional heterogeneity or subsets existing and the concept of tissue-resident memory (Trm) cells (Schenkel and Masopust 2014) that provide protection in peripheral tissues has emerged (see below). In the absence of antigen stimulation, a subset of Tem cells can convert to Tcm cells and, in so doing, acquire properties of self-renewal and long-term homeostatic maintenance (Wherry et al. 2003). This intersubset conversion suggests flexibility in Tcm and Tem cell lineages. However, not all Tem cells can convert to Tcm cells, suggesting the existence of other paths that give rise to Tcm cells. In humans, Tem cells are phenotypically characterized as $CD45RA^{-}CD45RO^{+}CCR7^{-}CD27^{-}$ (Hamann et al. 1997; Mahnke et al. 2013). In mice, Tem cells are often denoted by markers of antigen experience (i.e., $CD44^{Hi}$) and the absence of CD62L and/or CCR7 expression.

Effector Memory RA (Temra) Cells

Temra cells are a subset of human CD8 T cells that reexpress CD45RA in the absence of CD27 (i.e., $CD27^{-}CD45RA^{+}$; Hamann et al. 1997; Mahnke et al. 2013). Temra cells also do not express CCR7, CD62L, or CD28 ($CCR7^{-}CD62L^{-}CD28^{-}$) (Lugli et al. 2010). However, these cells express high levels of cytotoxic molecules and produce proinflammatory cytokines. They often accumulate with repetitive stimulation, as occurs during cytomegalovirus (CMV) infection in humans (Weekes et al. 1999; Patin et al. 2018). Temra cells are terminally differentiated or senescent and display the shortest telomeres among human T cells (Romero et al. 2007). These cells express markers of terminal differentiation/senescence such as KLRG-1 and/or CD57 (Brenchley et al. 2003; Henson and Akbar 2009). Terminally differentiated Teff cells in mice, marked by high KLRG1, are perhaps the closest counterpart to the Temra cells found in humans. However, $KLRG1^{Hi}$ Teff cells arise from a single infectious or stimulation event, not repetitive stimulation that gives rise to Temra cells in humans.

Memory Stem (Tscm) Cells

Tscm cells are a small subset of CD8 T cells, defined in humans as $CD45RA^{+}CCR7^{+}CD27^{+}CD28^{+}CD62L^{+}IL7R^{+}$, but distinguished from naive T cells by expression of CD95, CD122, CXCR3, and/or LFA-1 (Gattinoni et al. 2011).

Tscm cells are reportedly long-lived, have a stem cell–like ability to self-renew, and the multipotent capacity to differentiate into memory and effector CD8 T-cell subsets (Gattinoni et al. 2017). However, Tscm cells are rare and phenotypically appear less differentiated than Tcm cells. Although the precise ontogeny of Tscm cells remains to be defined, some evidence indicates that Tscm cells may arise prior to full differentiation into Teff or Tem cells, whereas many Tcm cells can arise from Tem cells.

Tissue-Resident Memory (Trm) Cells

Originally considered part of the Tem cell pool, Trm cells were first identified in the intestinal mucosa (Masopust et al. 2001), but now have been found in nearly all tissues in humans and mice (Schenkel and Masopust 2014; Thome et al. 2014). Trm cells primarily exist as residents of tissues without recirculating back to blood and function as a first line of defense against reinfection (Masopust et al. 2006; Schenkel and Masopust 2014; Mueller and Mackay 2016; Szabo et al. 2019). In many tissues, Trm cells express CD103 and CD69. They can be operationally defined as cells that are protected from labeling with intravenously injected antibody and do not equilibrate between hosts in parabiosis experiments.

Exhausted T (Tex) Cells

Tex cells were originally defined in chronic viral infections as antigen-specific CD8 T cells with decreased effector function (Gallimore et al. 1998; Zajac et al. 1998). Tex cells coexpress high levels of multiple inhibitory receptors (IRs), including PD-1, and have a unique transcriptional and epigenetic program (Barber et al. 2006; Wherry et al. 2007; Blackburn et al. 2009; Pauken et al. 2016; Sen et al. 2016). Tex cells are found in chronic infections and cancer in both humans and mice. In these settings of chronic disease, Tex cells likely provide some level of containment or delay in disease progression but are incapable of full control of infection or tumors (Wherry and Kurachi 2015; McLane et al. 2019). Based on canonical markers in humans, Tex cells often fall into the Tem or Tcm cell fraction of $CD45RA^{+}CD27^{+}$ cells but are distinguished by high expression of PD-1 and other IRs. In tissues, Tex cells may share features and/or have phenotypic overlap with Trm cells. Additional heterogeneity in the Tex cell compartment will be discussed in more detail below.

The overview of CD8 T-cell subsets and terminology is summarized in Figure 1 and Table 2. (Epigenetic lineage commitment will be further discussed in more detail below.)

HETEROGENEITY IN THE Teff CELL POOLS

Developmental heterogeneity arises following the initial activation, differentiation, and clonal expansion of Teff cells (Cui and Kaech 2010; Chang et al. 2014; Herndler-Brandstetter et al. 2018). Heterogeneity may arise as early as the first cell division, with asymmetric partitioning of key signaling molecules reinforcing divergent developmental circuits (Chang et al. 2007; Reiner and Adams 2014). As the Teff cell pool continues to develop, two major populations, committing to distinct differentiation lineages, emerge. These two populations are defined as $KLRG1^{Hi}IL\text{-}7R^{Lo}$ SLECs and $KLRG1^{Lo}IL\text{-}7R^{Hi}$ memory precursor (Tmp) cells (Kaech et al. 2003; Joshi et al. 2007).

$KLRG1^{Hi}IL\text{-}7R^{Lo}$ SLECs comprise the majority of the Teff cell population. They have potent effector functions and produce cytokines, kill their targets, and disseminate widely. However, these SLECs become terminally differentiated and cannot form long-term Tcm cells. Many SLECs die during the contraction phase, but a subset may persist into the memory phase in the form of "terminal Tem" cells (Joshi et al. 2007). These terminal Tem cells, which are essentially resting SLECs, can persist for ~2–3 months or more in the absence of antigen in some settings. These cells remain $CD62L^{Lo}$ in the post-effector phase and, therefore, could fall into the category of Tem cells but will not undergo conversion to $CD62L^{Hi}$ Tcm cells. Rather, these SLEC-derived terminal Tem cells represent a pool of residual cells that may be retained for systemic surveillance and rapid protection from

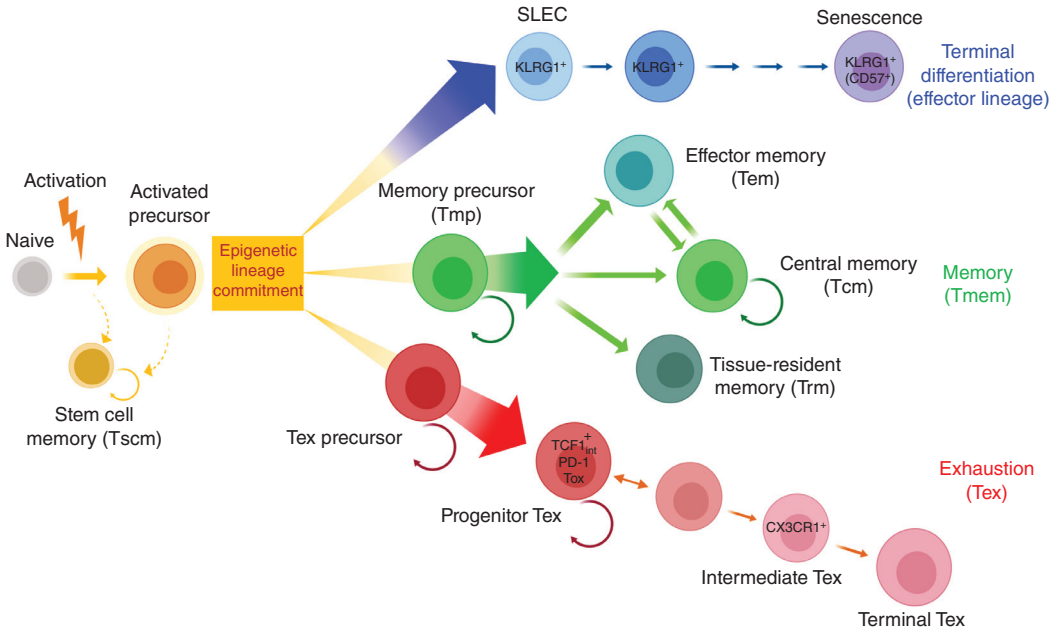

Figure 1. Schematic of CD8 T-cell differentiation and subsets. (Blue cells) terminally differentiated Teff cell lineage, (green cells) Tmem cell lineage, and (red cells) Tex cell lineage. The color scheme is used throughout this review.

reinfection. Such a scenario, however, suggests at least two distinct types of Tem cells. One type is Tem cells that can convert to Tcm cells and is derived from Tmp cells (see below), and another is derived from surviving KLRG1^{Hi} SLECs that eventually die off over time.

In contrast, the IL-7R^{Hi} Tmp cell subset preferentially forms Tmem cells, including CD62L^{Lo} Tem cells that are then capable of differentiating into CD62L^{Hi} Tcm cells. It is the latter population that responds to both IL-7 and IL-15 and undergoes antigen-independent self-renewal. In addition to giving rise to the systemic and circulating pools of Tcm and some Tem cells, Tmp cells can also give rise to Trm cells (Schenkel and Masopust 2014; Mueller and Mackay 2016). However, the potential to generate Trm cells peaks before the end of clonal expansion, typically around day 5 postinfection in mice (Masopust et al. 2001). Once in tissues, Tmp cells acquire a new residency program, upregulate CD103 and/or CD69, and durably occupy these tissues (Kaech et al. 2003; Joshi et al. 2007; Sarkar et al. 2008; Obar and Lefrançois 2010; Angelosanto et al. 2012; Mackay et al. 2013).

Although this Tmp/SLEC model has helped clarify Teff and Tmem cell differentiation, additional complexities in Teff and Tem (and likely Tcm) cell populations exist. For example, studies using reporter mice suggest that KLRG1^{+} Teff cells (i.e., SLECs) might possess developmental plasticity because a subset appeared to down-regulate KLRG1 and give rise to Tcm, Tem, and Trm cells (Herndler-Brandstetter et al. 2018). In other studies, CX3CR1 expression can further subdivide the Teff and early Tem cell populations into CX3CR1^{Lo}, CX3CR1^{Int}, and CX3CR1^{Hi} populations, each with different phenotypic and functional properties (Böttcher et al. 2015; Gerlach et al. 2016). Whereas the CX3CR1^{Lo} and CX3CR1^{Hi} populations were Teff-like (e.g., SLEC), the CX3CR1^{Int} population formed a transitional or Tmp-like pool that seeded the long-term Tmem cell pool. The CX3CR1^{Hi} subset is mainly a recirculating, vascular-patrolling population (Gerlach et al. 2016). These mouse studies indicate considerable complexity in Teff→

Table 2. Characteristics of T-cell subsets

		Features associated with T-cell subtypes				
Subtype	Other names	Distinct epigenetic signature	Distinct transcriptional profile	Key transcription factors (TFs)[a]	Emblematic protein markers[b]	References
Activated precursor	(Subset of) Teff pool	?	?	BATF, TCF-1	?	Kurachi et al. 2014; Chen et al. 2019
Stem cell memory (Tscm)	Stem cell memory (Tscm)	?	Yes	TCF1 c-Myb	CD95, CD11a	Zhang et al. 2005; Gattinoni et al. 2017; Gautam et al. 2019
Memory precursor (Tmp)	Memory precursor effector cells (MPECs)	Yes	Yes	TCF-1, Foxo1	IL-7R (CD127)	Kaech et al. 2003; Joshi et al. 2007; Gray et al. 2017; Yu et al. 2017
Central memory (Tcm)	Central memory (Tcm)	Yes	Yes	TCF-1, Foxo1, Eomes	CD62L, CCR7	Wherry et al. 2003; Kim et al. 2013; Graef et al. 2014; Utzschneider et al. 2018
Effector memory (Tem)	Effector memory (Tem)	Yes	Yes	T-bet, Blimp-1, Zeb2, Id2	Lack of CD62L, CCR7	Hamann et al. 1997; Sallusto et al. 1999; Wherry et al. 2003
Tissue-resident memory (Trm)	Tissue-resident memory (Trm)	Yes	Yes	Runx3, Hobit, lack of KLF2	CD69, CD103 (depending on tissues)	Mackay et al. 2016; Milner et al. 2017
Exhaustion progenitor	Early exhaustion, progenitor exhausted T cell, stem-like, follicular-like, memory-like exhausted T cells	Yes	Yes	Tox, TCF-1	PD-1, CXCR5, TCF-1, Tox	Blackburn et al. 2008; Paley et al. 2012; He et al. 2016; Im et al. 2016; Utzschneider et al. 2016; Wu et al. 2016; Alfei et al. 2019; Chen et al. 2019; Hudson et al. 2019; Jadhav et al. 2019; Khan et al. 2019; Scott et al. 2019; Seo et al. 2019; Yao et al. 2019; Zander et al. 2019

Continued

Table 2. *Continued*

Subtype	Other names	Features associated with T-cell subtypes: Distinct epigenetic signature	Distinct transcriptional profile	Key transcription factors (TFs)[a]	Emblematic protein markers[b]	References
Terminal exhaustion	Terminally differentiated exhausted T cell	Yes	Yes	Tox, Eomes	PD-1, TIM-3, Tox, Eomes	Blackburn et al. 2008; Paley et al. 2012; He et al. 2016; Im et al. 2016; Utzschneider et al. 2016; Wu et al. 2016; Alfei et al. 2019; Chen et al. 2019; Hudson et al. 2019; Jadhav et al. 2019; Khan et al. 2019; Scott et al. 2019; Seo et al. 2019; Yao et al. 2019; Zander et al. 2019
Short-lived effector cells (SLECs)	SLECs	Yes	Yes	T-bet, Blimp-1, Zeb2, Id2	KLRG1	Kaech et al. 2003; Intlekofer et al. 2007; Joshi et al. 2007; Guan et al. 2018
Terminally differentiated cells		Yes	Yes	T-bet, Blimp-1, Zeb2, Id2	KLRG1	Kaech et al. 2003; Intlekofer et al. 2007; Joshi et al. 2007; Guan et al. 2018
Terminally differentiated effector cells	TEMRA, terminal effector (Tte) (human)	?	Yes	?	CD45RA, CD57, KLRG1, lack of CD27	Mahnke et al. 2013

[a]Examples of TFs for each T-cell type. Often, many other TFs not listed have been described for specific T-cell types.
[b]Examples of markers for each T-cell type. Often, many other markers not listed have been described for specific T-cell types.

Tem→Tmem subset biology. Although future studies are required to fully delineate the complexity and key lineage relationships underlying this heterogeneity, it is likely that the general concept of "effector memory" has value as an umbrella term under which there are likely multiple additional subsets.

REGULATION OF EFFECTOR VERSUS MEMORY CD8 T-CELL DEVELOPMENT

Defining the signals and mechanisms that regulate Teff and Tmem cell differentiation remains a major goal in the field of immunology. A considerable body of work has demonstrated that qualitative and quantitative differences in signals (antigen, cytokine, costimulation, CD4 T-cell help, metabolism, etc.) play an important role in memory versus effector CD8 T-cell differentiation (Chang et al. 2014).

Tmem cell differentiation is influenced by signals received during priming, including antigen levels, clonal competition, and/or the duration of stimulation (Sarkar et al. 2007). In particular, antigen recognition and TCR stimulation (e.g., signal 1) have been shown to differentially regulate Tmem cell development. Weaker TCR signaling upon initial antigen-recognition promotes a faster transition from Tem to a Tcm cell, and strong TCR signaling promotes terminal differentiation (Wherry et al. 2003; Teixeiro et al. 2009; Zehn et al. 2009; Smith-Garvin et al. 2010).

Costimulatory molecules (signal 2), such as CD28 and other members of the tumor necrosis factor receptor (TNFR) family, have also been shown to both play important roles in the primary response, and also regulate Tmem generation, function, and survival (Boise et al. 1995; Hendriks et al. 2000; Bertram et al. 2004; Borowski et al. 2007; Fuse et al. 2008; Garidou et al. 2009; Dong et al. 2012; Esensten et al. 2016; Schildberg et al. 2016).

CD4 T-cell help plays a key role in regulating Teff and Tmem cell differentiation, and early studies showed that development of Tmem and Tcm cells was compromised in the absence of CD4 T-cell help (Janssen et al. 2003; Shedlock and Shen 2003; Sun and Bevan 2003). These effects are associated with an increase in SLEC generation, dependent on the TF T-bet (Intlekofer et al. 2007). The mechanisms by which CD4 T cells influence Tmem cell differentiation remain unclear and may differ depending on the setting. Mechanisms may include licensing of dendritic cells (DCs) (Castellino et al. 2006) via CD40L (Bennett et al. 1998; Ridge et al. 1998; Schoenberger et al. 1998), production of IL-2 (Kalia et al. 2010; Makedonas et al. 2010; Pipkin et al. 2010), production of IL-21 (Cui et al. 2011), and supporting the production of effective antibodies (Bachmann et al. 2004).

In addition, inflammation (signal 3) modulates the balance between Teff/SLEC and Tmp/Tmem cell lineage commitment (Joshi et al. 2007; Harty and Badovinac 2008). For example, IL-12 (Joshi et al. 2007; Xiao et al. 2009), IFN-γ (Badovinac et al. 2000), and IL-27 (Yoshida and Hunter 2015) can enhance effector-like differentiation—particularly during SLEC generation—whereas IL-10 (Foulds et al. 2006; Laidlaw et al. 2015), IL-21 (Cui et al. 2011), and transforming growth factor β (TGF-β) (Zhang and Bevan 2013; Guan et al. 2018) can promote Tmem cell differentiation.

TFs and transcriptional circuits underlie these differentiation events, guiding Teff and Tmem cells. A series of TFs have been implicated in the formation of Tmem, Tmp, and/or Tcm cells, including Id3 (Yang et al. 2011), TCF-1 (Zhou et al. 2010; Utzschneider et al. 2016; Kratchmarov et al. 2018), Bcl6 (Ichii et al. 2002), STAT3 (Cui et al. 2011), Foxo1 (Utzschneider et al. 2018), Eomes (Banerjee et al. 2010), and Zeb1 (Guan et al. 2018; Scott and Omilusik 2019). On the other hand, the formation of Teff, SLECs, and/or Tem cells is promoted by T-bet (Intlekofer et al. 2007; Joshi et al. 2007), Id2 (Cannarile et al. 2006; Yang et al. 2011; Masson et al. 2013), Blimp-1 (Kallies et al. 2009; Rutishauser et al. 2009), STAT4 (Mollo et al. 2014), and Zeb2 (Guan et al. 2018; Scott and Omilusik 2019). Many of these TFs function in opposing pairs, such as T-bet and Eomes, Id2 and Id3, and Blimp1 and Bcl6. In these cases, one TF drives Teff-like differentiation and the other promotes Tmem-like differentiation. Many additional TFs are necessary for the initiation stages of T-cell activation, in-

cluding BATF and IRF4 (Kurachi et al. 2014), c-Myb (Chen et al. 2017), Runx3 (Wang et al. 2018), as well as other signal-dependent TFs directly downstream of TCR and/or costimulation including NFATs, Fos, Jun, and other AP-1 family members, NF-κB, and others (Daniels and Teixeiro 2015; Chen et al. 2018).

Most TFs function by binding to DNA in regions of epigenetically remodeled and/or open chromatin. Thus, the mechanisms of epigenetic regulation of Teff and Tmem cell differentiation are of interest (Araki et al. 2009; Zediak et al. 2011; Akondy et al. 2017; Abdelsamed et al. 2018; Carty et al. 2018; Tough et al. 2020). Histone modifications such as acetylation and methylation and associated alterations in chromatin accessibility have been implicated in differentiation of Tmem and Teff cells (Northrop et al. 2006; Araki et al. 2008; Zediak et al. 2011; Shin et al. 2013). Moreover, enzymes catalyzing epigenetic changes such as DNMT3a (Ladle et al. 2016) and TET2 (Carty et al. 2018), both of which are involved in regulating DNA methylation, are suggested to regulate early Teff cell differentiation and Teff versus Tmem cell fate decisions.

In addition to TFs and epigenetic changes, many other factors can influence Teff and Tmem cell differentiation, including metabolism (Pollizzi and Powell 2014; Buck et al. 2017), the microbiome (Bachem et al. 2019), aging (Eberlein et al. 2016), social stress (Weber et al. 2017), and neuroimmune interactions (Slota et al. 2015). Future studies are needed to determine whether some of these effects are linked through the TF pathways outlined above or whether their effects are mediated through distinct, potentially novel mechanisms.

Although much of the information discussed above has been derived from mouse models, it is becoming increasingly possible to interrogate these questions in humans. Studies using yellow fever vaccine (YFV) have led to seminal observations about human CD8 T-cell memory. The use of YFV in healthy subjects, combined with in vivo deuterium labeling, allowed detailed, longitudinal analysis of YFV-specific CD8 Teff and Tmem cells in humans in the absence of antigen reexposure (Akondy et al. 2017). These analyses showed that Tmem cells originated from extensively dividing CD8 T cells generated during the first 2 weeks after vaccination (i.e., derived from Teff cells). These Tmem cells were maintained and largely quiescent but divided once every year (doubling time of over 450 days). Epigenetic analysis revealed that the long-lived, YFV-specific Tmem cells retained epigenetic signatures of their Teff cell history. Moreover, these Teff cell epigenetic imprints were detected in Tmem cells decades after the initial vaccination (Akondy et al. 2017). Thus, these studies indicate one development pathway in which long-term Tmem cells develop from Teff cells can occur in humans.

TISSUE-RESIDENT MEMORY CELLS

Tissue-resident memory T (Trm) cells are a subset of Tmem cells that reside mainly in nonlymphoid tissues (although they can also be found in lymphoid tissues) and do not recirculate through blood and lymph. Trm cells function as a first line of defense against reinfection at barrier sites and can provide effective protective immunity (Masopust et al. 2006; Schenkel and Masopust 2014; Mueller and Mackay 2016; Szabo et al. 2019). Trm cells are often defined by expression of CD69 and CD103, each of which can contribute to tissue residency, but the expression of these molecules varies across tissues (Masopust et al. 2006; Schenkel and Masopust 2014; Mueller and Mackay 2016; Szabo et al. 2019). Compared to circulatory Tmem cells, Trm cells display distinct transcriptional (Wakim et al. 2012; Mackay et al. 2013, 2016; Milner and Goldrath 2018) and epigenetic programs (Milner et al. 2017), consistent with Trm cells representing a distinct subset of Tmem cells (Wakim et al. 2012; Mackay et al. 2013, 2016; Milner et al. 2017). Trm cells appear to arise from early Teff cells—likely Tmp cells that enter tissues and acquire additional environmental and transcriptional reprogramming—at least in some cases driven by TGF-β signaling (Zhang and Bevan 2013). Hobit, Blimp-1 (Mackay et al. 2016), and Runx3 (Milner et al. 2017) are key TFs expressed in Trm cells. Loss of TF KLF2 and subsequent down-regulation of the exit-control-

ling receptor S1P1 (Skon et al. 2013) is crucial for Trm cell identity. Thus, Trm cells are a distinct functional subset of Tmem cells endowed with tissue-specific residency, a unique role in barrier and tissue immunity, and a transcriptional program linked to their function.

MEMORY STEM (Tscm) CELLS AND RELATIONSHIP TO OTHER Tmem SUBSETS

T memory stem (Tscm) cells are a rare subset of Tmem cells, endowed with the long-lived, stem cell-like ability to self-renew and the multipotent capacity to reconstitute a wide spectrum of Tmem and Teff cell subsets (Gattinoni et al. 2017). Tscm cells have a phenotype that overlaps with naive and Tcm cells in mice ($CD44^{Lo}$ $CD62L^{Hi}$ and high Sca-1, CD122, and Bcl-2) and humans ($CD45RA^{+}CD27^{+}$ and $CCR7^{+}$, $CD95^{+}$, $CXCR3^{+/-}$, $IL\text{-}2R\beta^{+}$, $CD58^{+}$ and $CD11a^{+}$) (Zhang et al. 2005; Gattinoni et al. 2011). These Tscm cells also express the TF TCF1 (Gautam et al. 2019). However, whereas studies have shown that at least some Tcm cells derive from Tem cells (Wherry et al. 2003) and cells that have gone through an effector phase (Akondy et al. 2017), studies on Tscm cells suggest that these cells may arise prior to full Teff cell differentiation (Gattinoni et al. 2009; Lugli et al. 2013). Future studies should help clarify the precise developmental origins of Tscm versus Tcm cells, test the comparative developmental potential to give rise to other subsets, and/or serially self-renew as well as define the epigenetic control mechanisms that might distinguish these cell types.

EXHAUSTION

During chronic infections or cancer, when antigen stimulation persists, activated T cells can leave the Teff and Tmem cell differentiation path and instead develop into exhausted CD8 T (Tex) cells (Wherry and Kurachi 2015). Tex cells are characterized by reduced (though not completely absent) effector function, high coexpression of IRs such as PD-1, TIM3, LAG3, CTLA4, and TIGIT, altered metabolism, and impaired proliferation when stimulated (Wherry and Kurachi 2015; McLane et al. 2019). These IRs restrain Tex cells and blockade of the PD-1 pathway reinvigorates Tex cells, thus enhancing disease control (Barber et al. 2006). Despite poor proliferative potential, Tex cells are maintained by constant in vivo cell division by a hierarchy of Tex cell subsets with progenitor or "stem-like" properties (Wherry et al. 2007; Blackburn et al. 2008; Paley et al. 2012). In addition, Tex cells have a distinct transcriptional and epigenetic landscape compared to Teff and Tmem cell subsets. Thus, Tex cells are a CD8 T-cell lineage distinct from Teff or SLEC and Tmem cell subsets (Wherry et al. 2007; Doering et al. 2012; Pauken et al. 2016; Scott-Browne et al. 2016; Sen et al. 2016; Philip et al. 2017; Chen et al. 2019). Several studies subsequently identified the high mobility group TF Tox as the key factor that initiates the epigenetic program and fate commitment of Tex cells by both promoting accessibility to Tex cell–specific open chromatin regions and suppressing the epigenetic program of the terminally differentiated Teff cell fate (Alfei et al. 2019; Khan et al. 2019; Scott et al. 2019; Seo et al. 2019; Yao et al. 2019). These data indicate that Tex cells are distinct from other mature CD8 T-cell types, an observation with implications for predicting how immunotherapies might target Teff versus Tex cells.

Tex cells are the major responding cell types following PD-1 pathway blockade in cancer and chronic infections (Im et al. 2016; Pauken et al. 2016; Utzschneider et al. 2016; Wu et al. 2016; Huang et al. 2017). Much of the clinical benefit of PD-1/PD-L1 blockade, and perhaps other checkpoint blockades, is likely mediated by reinvigoration of these cells (Topalian et al. 2015; Huang et al. 2017, 2019; LaFleur et al. 2018). Technological advances continually facilitate a deeper understanding of this cell type. Profiling open chromatin regions by ATAC-seq demonstrated that Tex cells differ from Teff and Tmem cells by approximately 6000 open chromatin regions (Pauken et al. 2016; Scott-Browne et al. 2016; Sen et al. 2016; Philip et al. 2017). The magnitude of this difference is similar to the ~5000–7000 open chromatin regions that differ between developing myeloid cells and B cells (Lara-Astiaso et al. 2014; Monticelli and Natoli

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2017). It was remarkable that this global open chromatin landscape did not change appreciably upon PD-1 pathway blockade, despite robust transcriptional reengagement of Teff cell–like genes (Pauken et al. 2016). These observations have two implications. First, the reinvigorated Tex cells did not convert to Teff cells, but instead retained their Tex cell identity while temporarily reacquiring Teff cell functions. Second, these observations highlight the notion that cells can exist in multiple transcriptional and/or phenotypic states despite harboring a single epigenetic state (Fig. 2). This concept is important for defining criteria that determine cell identities or subsets.

SUBSETS OF Tex CELLS

Tex cells in cancer and chronic infection are heterogeneous with multiple subsets (Wherry and Kurachi 2015; McLane et al. 2019). Tex cell subsets were first described based on different expression levels of PD-1 and CD44 and these subsets were linked to differential responsiveness to PD-1 pathway blockade (Blackburn et al. 2008). Subsequent studies demonstrated that Tex cell progenitor and terminal subsets were related in a proliferative hierarchy with the Tex cell progenitor population undergoing constant proliferation in response to persisting antigen to give rise to a more numerically abundant terminal Tex cell population (Paley et al. 2012). This progenitor or "stem-like" Tex cell subset is controlled by TCF1 (Im et al. 2016; Utzschneider et al. 2016; Wu et al. 2016) (see below). Notably, all Tex cell subsets express the Tex TF Tox (Alfei et al. 2019; Khan et al. 2019; Scott et al. 2019; Seo et al. 2019; Yao et al. 2019), but other TFs have sequentially important roles throughout the Tex proliferative hierarchy (Wherry and Kurachi 2015; McLane et al. 2019). Although Tex cell subset biology is consistent across studies, different nomenclature has been used. An attempt is made here to connect some of this nomenclature to the common underlying Tex cell subset biology.

Progenitor Tex Cells

Tex cell subsets were first defined by PD-1 and CD44 expression, with PD-1^{Int} CD44^{Hi} Tex cells readily distinguished from PD-1^{Hi} CD44^{Int} Tex cells in chronic lymphocytic choriomeningitis virus (LCMV) infection (Blackburn et al. 2008). When purified and tested separately, PD-1^{Int} CD44^{Hi} cells retained proliferative potential and were the only subset of Tex cells capable of responding to PD-1 pathway blockade (Blackburn et al. 2008). This PD-1^{Int} pool was then shown to be maintained over time, retain

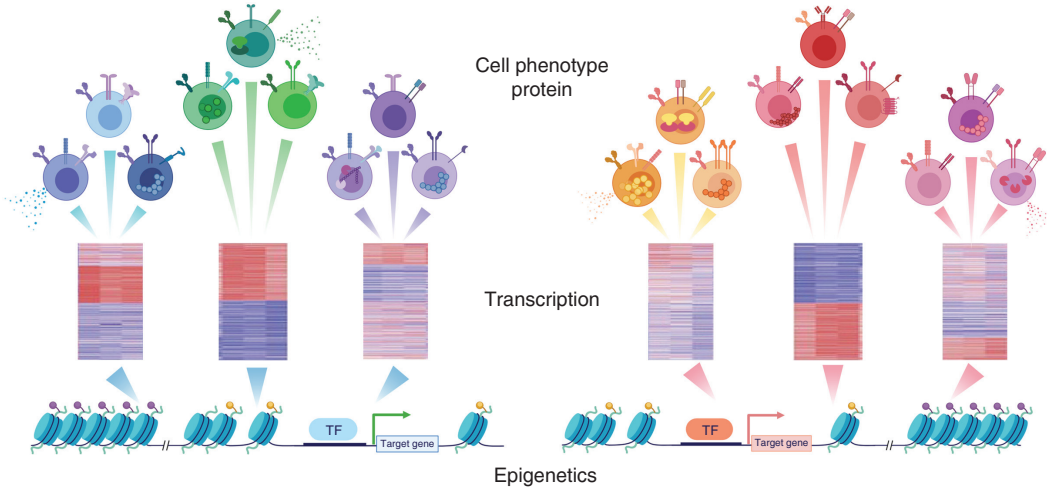

Figure 2. From open chromatin landscape to diverse cell phenotype. Observations highlight the notion that cells can exist in multiple transcriptional and/or phenotypic states despite harboring a single epigenetic state. (TF) Transcription factor.

some self-renewal capacity, and directly give rise to the more terminal PD-1^{Hi} population through cell division in vivo (Paley et al. 2012). Thus, the PD-1^{Int} subset of Tex cells possessed "progenitor" activity within the Tex cell population, whereas the PD-1^{Hi} subset was terminal (Paley et al. 2012). These studies also defined a role for the TFs T-bet and Eomes in this process. Loss of T-bet and increase in Eomes was associated with a transition from the PD-1^{Int} progenitor pool to the more terminal PD-1^{Hi} Tex cell subset (Paley et al. 2012; Odorizzi et al. 2015). Network analysis also suggested a potential role for Wnt signaling and/or TCF1 associated with the PD-1^{Int} Tex cell subset (Doering et al. 2012). Indeed, a series of subsequent studies refined the definition of Tex cell progenitors using CXCR5 and/or TCF1 (and Ly108 as a surrogate) to identify smaller populations that possessed progenitor activity and the ability to respond to PD-1 blockade (Im et al. 2016; Utzschneider et al. 2016; Wu et al. 2016). It was clear from these studies that TCF1 is a critical TF for this Tex cell progenitor subset. Some of these studies used the term "stem-like" Tex cells for the population that expressed TCF1 and possessed the ability to respond to PD-1 blockade. Likely, this stem-like Tex cell subset is a subpopulation of the original Tex cell progenitor subset. Additional heterogeneity in this TCF1^{+} progenitor population has also been identified; one subpopulation of the TCF1^{+} Tex cell progenitor ("Tex^{prog1}") is quiescent and confined to lymphoid tissue, whereas a second subpopulation of TCF1^{+} Tex progenitors ("Tex^{prog2}") displays more blood accessibility, has robustly up-regulated cell cycle genes, and is actively dividing (Beltra et al. 2020). At the Tex^{prog2} stage, TCF1 expression begins to decrease and T-bet expression begins to increase (Beltra et al. 2020). These Tex cell progenitors have been identified in blood (Huang et al. 2017, 2019; Bengsch et al. 2018) and tumors (Sade-Feldman et al. 2018; Li et al. 2019; Miller et al. 2019) and have direct implications for antitumor immunity in patients because of their responsiveness to PD-1 pathway blockade. However, in human tumors, the identification of tumor-specific Tex cell progenitors remains elusive.

Intermediate Tex Cells

This Tex cell subset expresses T-bet, but not TCF1, and has evidence of recent proliferation (Hudson et al. 2019; Zander et al. 2019; Beltra et al. 2020). These cells also express CX3CR1 and possess robust circulatory or homing capacity. There is evidence that this T-bet^{+} Tex cell intermediate subset gives rise to the more terminal Tex cell population (Hudson et al. 2019; Beltra et al. 2020), although some recent data suggests that perhaps in some settings this Tex cell intermediate subset might not generate terminal Tex cells (Zander et al. 2019). Discrepancies in the relationships between Tex cell subsets could relate to antigen load, inflammation, and/or CD4 T-cell help and should be clarified by future studies including lineage tracing.

Terminal Tex Cells

Early studies of Tex cell subsets defined terminal Tex cells as PD-1^{Hi}CD44^{Int} cells that lacked proliferative potential and were unresponsiveness to antigen stimulation or PD-1 pathway blockade (Blackburn et al. 2008). These cells also had a relatively short half-life in vivo and were characterized by high Eomes (Paley et al. 2012). Terminal Tex cells also expressed higher amounts of many IRs and were less polyfunctional by cytokine production (Blackburn et al. 2009; Wherry and Kurachi 2015; McLane et al. 2019). This terminal subset has also been identified as TCF1^{-}, CXCR5^{-}, and/or TIM3^{+} (He et al. 2016; Im et al. 2016), although some of these phenotyping approaches capture only partially overlapping populations (Beltra et al. 2020). However, this Tex cell terminal subset retains cytotoxic killing activity (Blackburn et al. 2009; Wherry and Kurachi 2015; McLane et al. 2019) and, because it is numerically abundant in most tissues, may play a role in at least temporary or partial disease control. Moreover, terminal Tex cells are also present in and accumulate to different degrees in nonlymphoid tissues, in some cases with phenotypic and operational (i.e., lack of blood accessibility) similarity to Trm cells (Schenkel and Masopust 2014; He et al. 2016;

Im et al. 2016; Mueller and Mackay 2016; Jansen et al. 2019; Szabo et al. 2019).

Tex Cell Precursor

A major question has been: from what precursor population do Tex cells develop? One possibility was that Tex cells arise from overstimulated Teff cells (SLECs). However, lineage-tracing experiments demonstrated that Tex cells did not arise from SLECs, but rather were only generated from the pool of CD127^{+} Tmp cells present in the effector phase (Angelosanto et al. 2012). In recent studies, scRNA-seq of cells isolated during the early phase of chronic LCMV (clone 13) infection identified a branched trajectory at an early stage CD8 T-cell differentiation where Tex versus Teff cell development diverged (Chen et al. 2019). Together with lineage tracing experiments, a TCF-1^{+}Ly108^{+}PD-1^{+} CD8 T-cell population present in the effector phase was identified as the Tex cell precursor that seeds development of mature Tex cells (Chen et al. 2019). These studies also distinguished this Tex cell precursor from the Tmp cell population (Chen et al. 2019). These early Tex cell precursors are not yet developmentally fixed, however, and can give rise to Tmem cells if removed from chronic antigen stimulation (Angelosanto et al. 2012), likely because the full epigenetic program of mature Tex cells is not established for 2–3 weeks following infection (Pauken et al. 2016; Sen et al. 2016; Philip et al. 2017; Khan et al. 2019). Tex cell precursors require Tox and TCF1 (Chen et al. 2019), with Tox directly controlling TCF1 expression. Tox is dispensable for TCF1 expression in the Tmem cell lineage (Khan et al. 2019; Scott et al. 2019). Tox also directly represses the SLEC Teff cells to help enable Tex cell precursor development and formation of the Tex cell lineage (Alfei et al. 2019; Khan et al. 2019; Scott et al. 2019; Seo et al. 2019; Yao et al. 2019). Moreover, PD-1 is required to preserve this initial Tex cell precursor population and allows formation of the initial pool of Tex cell progenitors (Chen et al. 2019), perhaps explaining why the Tex cell population is not sustained long term in the genetic absence of PD-1 (Odorizzi et al. 2015).

Thus, Tex cell subsets represent a multistage developmental hierarchy with Tex cell progenitor subsets (and likely biologically distinct Tex^{prog1} and Tex^{prog2} populations) giving rise to a Tex cell intermediate and then finally a terminal Tex cell subset. Additional heterogeneity may exist. Future profiling of Tex cell subset heterogeneity in humans, linked to tumor specificity, will help improve immunotherapies targeting Tex cells such as immune checkpoint blockade.

FROM EPIGENETIC SIGNATURES TO DIVERSE T-CELL PHENOTYPE

Given the critical roles of epigenetics and chromatin remodeling in lineage commitment at the early stage of T-cell differentiation, we propose the following model for Tmem and Tex cell differentiation (Fig. 1; Table 2). After naive T cells are activated and primed by antigen stimulation, they develop into a common precursor population with the potential to commit to multiple CD8 T-cell lineages. Transcriptional and initial epigenetic rewiring then establishes gene expression programs biased toward the development of each lineage, generating the lineage-committed progenitors (Tmem, Teff/SLEC, and Tex cells). These different initial lineage-biased precursors initially retain fate flexibility, but then become progressively more committed as the epigenetic regulation becomes more fixed over time. These epigenetic changes induce transcriptional circuits and establish the full lineage program. However, the open chromatin landscape of these major lineages determines the potential for gene expression, and each major lineage may access different components of this epigenetic and gene expression potential depending on environmental cues. Thus, CD8 T cells of a single lineage fate could display distinct protein or mRNA phenotypes depending on recent signals received and yet have the same underlying open chromatin landscape (Fig. 2). Such is the case, for example, for Tex cells following PD-1 blockade. Upon PD-1 blockade, Tex cells change transcriptionally and functionally but retain largely the same open chromatin profile (Pauken et al. 2016).

THERAPEUTIC IMPLICATIONS

The biology of subsets of Teff, Tmem, and Tex cells has direct clinical relevance for protection from infectious disease, vaccination, autoimmunity, and cancer immunotherapy. Immune memory, a hallmark of effective vaccination, is maintained in the T-cell compartment by subsets of Tmem cells that can give rise to protective activities, including direct effector function or generation of downstream progeny including Teff cells. Tex cells may be critical to establishing a host–pathogen stalemate and providing limited pathogen (or tumor) control while avoiding immunopathology. Moreover, understanding the diverse biological features of these cell populations should help improve efficacy of vaccines and cellular and biological therapies for infectious disease and cancer.

SUMMARY AND OPEN QUESTIONS

Despite tremendous advances in our understanding of the diversity of Teff, Tmem, and Tex cells in the past decade, a number of questions and challenges for the field remain. For example, how many subsets of Teff, Tmem, and Tex cells exist? What defines a discreet subset? How can we as a field harmonize the information about Teff, Tmem, and Tex cell biology even if nuances and distinctions (sometimes important ones) exist between studies?

CD8 T-cell differentiation may operate like fractal geometry, filling the existing biological "space" yet having distinct features, the deeper one examines the cells. Perhaps a key future goal will be to attempt to delineate a set of biologically relevant, measurable, and stable features of CD8 T-cell subsets and link these to nomenclature, knowing that under any subset designation there will be additional phenotypic, functional, transcriptional, and epigenetic diversity. This is not simply about naming cell types. These immune cell types are central to a wide variety of human diseases and our nomenclature implies relationships that may be important for vaccines and therapeutic approaches. In addition, because CD8 T cells can behave extraordinarily differently, determining which subset is desirable (or undesirable) to target could be the difference between clinical success and failure. Thus, establishing a comprehensive understanding of CD8 T-cell heterogeneity and development will significantly impact a wide variety of human disease and treatment, including vaccine development for infectious diseases, immunosuppressants for autoimmunity, and checkpoint blockade for cancer.

COMPETING INTEREST STATEMENT

E.J.W. has consulting agreements with and/or is on the scientific advisory board for Merck, Roche, Pieris, Elstar, and Surface Oncology. E.J.W. has a patent licensing agreement on the PD-1 pathway with Roche/Genentech. E.J.W. is a founder of Surface Oncology and Arsenal Biosciences. Y.M. has no COI to disclose.

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