

1 **The function and dysfunction of memory CD8⁺ T cells in tumor immunity**

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24 **Abstract:** The generation and maintenance of CD8⁺ T cell memory is crucial to long-
25 term host survival, yet the basic tenets of CD8⁺ T cell immunity are still being
26 established. Recent work has led to the discovery of tissue-resident memory cells and
27 refined our understanding of the transcriptional and epigenetic basis of CD8⁺ T cell
28 differentiation and dysregulation. In parallel, the unprecedented clinical success of
29 immunotherapy has galvanized an intense, global research effort to decipher and de-
30 repress the anti-tumor response. However, the progress of immunotherapy is at a
31 critical juncture, since the efficacy of immuno-oncology agents remains confined to a
32 fraction of patients and often fails to provide durable benefit. Unlocking the potential
33 of immunotherapy requires the design of strategies that both induce a potent effector
34 response and reliably forge stable, functional memory T cell pools capable of
35 protecting from recurrence or relapse. It is therefore essential that basic and emerging
36 concepts of memory T cell biology are rapidly and faithfully transposed to advance
37 therapeutic development in cancer immunotherapy. This review highlights seminal
38 and recent reports in CD8⁺ T cell memory and tumor immunology, and evaluates
39 recent data from solid cancer specimens in the context of the key paradigms from pre-
40 clinical models. We elucidate the potential significance of circulating effector cells
41 poised downstream of neoantigen recognition and upstream of T cell dysfunction and
42 propose that cells in this immunological ‘sweet spot’ may prolong survival and serve
43 as the substrate for checkpoint blockade.

44

45 **Naïve T cell activation**

46 CD8⁺ T cell responses are initiated in secondary lymphoid organs (SLOs) when naïve
47 CD8⁺ T cells (T_n) are activated by migratory dendritic cells (DC) presenting antigen-
48 derived peptides loaded on major histocompatibility complex (MHC) class I molecules.
49 T_n cells carrying epitope-specific T cell receptors (TCR) may undergo activation,
50 dysfunction, survival or deletion, contingent upon the following interdependent
51 variables: i) the cytokine/chemokine/metabolite milieu, ii) status of the dendritic cell
52 (DC) (e.g. activation, co-stimulatory/adhesion molecule profile, tissue of origin), iii)
53 TCR affinity for presented peptide, iv) epitope antigenicity (amino acid sequence, MHC
54 binding affinity, concentration) v) presence/quality of CD4⁺ T cell help and vi) duration
55 and frequency of contact at the immunological synapse¹⁻⁵. During acute viral infection,
56 T_n recognize antigenic peptides presented by migratory DC that have sensed
57 pathogen- or danger-associated molecular patterns (e.g. dsRNA via TLR3) and
58 subsequently expressed co-stimulatory molecules (e.g. CD80/CD86, CD40L, OX40L,
59 41BBL, CD70). After receiving sufficient signal 1 (TCR signaling), signal 2 (co-
60 stimulation e.g. CD80/CD86) and signal 3 (inflammatory cytokine e.g. IFN α/β , IFN γ ,
61 IL-2, IL-12, IL-21, IL-33, TNF α), CD8⁺ T cells clonally expand and give rise to vast
62 numbers of effector CD8⁺ T cells (T_{eff}). T_{eff} subsequently migrate to the infected
63 tissue through the bloodstream via chemokine receptor (e.g. CCR5) and adhesion
64 molecule (e.g. LFA-1) interactions where they recognize their cognate peptide:MHC-I
65 complex on target cells and exert cytolytic functions (secretion of perforin, GZMb,
66 TNF α , IL-2, IFN γ) to lyse infected cells. Following the effector phase, 90-95% of T_{eff}
67 cells undergo apoptosis whilst a pool of clonally expanded, antigen-experienced cells
68 persist to provide durable immunological memory⁶. Memory T cells are present at 10-
69 100 times their precursor frequency, and bear a distinctive migratory, molecular,

70 epigenetic, metabolic, phenotypic and functional profile relative to Tn and Teff cells ⁶⁻
71 ⁸. These properties enable memory T cells to traffic throughout the blood, SLOs and
72 tissues in a quiescent state yet hyper-proliferate and elicit augmented effector
73 responses during antigen re-encounter; thereby coordinating rapid pathogen
74 elimination.

75 **CD8⁺ memory T cell generation**

76 Several studies have suggested that T cells are programmed to become memory
77 during the early stages of the priming phase ⁹. In vaccinated humans, memory CD8⁺
78 T cells arise from a rapidly dividing effector pool formed in the first 14 days post
79 challenge, subsequent to re-engagement of naïve like chromatin landscapes ¹⁰.
80 Similarly, in the lymphocytic choriomeningitis virus (LCMV) model, long-lived memory
81 CD8⁺ T cells emerge from de-differentiation of fate-permissive Teff cells ¹¹. These
82 findings concur with single cell RNAseq (scRNAseq) analysis of early CD8⁺ T cell
83 specification during adoptive transfer in the LCMV model, in which Teff and memory
84 differentiation emerge from an early burst of transcriptional activity followed by
85 epigenetic refinement ¹². Work in the *Listeria monocytogenes* and LCMV models have
86 previously classified subsets of Teff cells based upon their ability to give rise to
87 memory CD8⁺ T cells. These precursor subsets are defined by differential expression
88 of the IL-7 receptor (CD127) and the killer cell lectin-like receptor G1 (KLRG1).
89 Memory precursor effector cells (MPEC; CD127^{hi}KLRG1^{neg}) are characterized by
90 BCL2 expression, a longer lifespan and proliferative potential in response to
91 homeostatic cytokines (IL-7/IL-15) or antigenic re-challenge, whilst short-lived effector
92 cells (SLEC; CD127^{lo}KLRG1^{hi}) have a shorter lifespan and reduced homeostatic
93 proliferative capacity ¹³⁻¹⁵. The recent finding that effector differentiation precedes
94 memory formation is complicit with this 'separate precursor' model, and the long-held

95 knowledge that memory potential is non-equivalent amongst Teff cells, since certain
96 effectors may preferentially re-engage naïve like programs that specify memory fate.
97 Although not necessarily contradictory, it is also noteworthy that production of memory
98 CD8⁺ T cells has also been reported to occur in the absence of an overt effector
99 response ¹⁶.

100 Data from several infection models have shown that SLEC differentiation is favored
101 by increased signal 1 (prolonged antigen exposure, affinity/avidity/concentration low
102 intracloal competition) and signal 3 (elevated inflammatory cytokine burden, IFN γ , IL-
103 12 directly or via CXCR3-mediated trafficking to the infected site), whilst brief TCR
104 stimulation, truncated infection periods (e.g. via administration of antibiotics), defects
105 in inflammatory cytokine signaling, enhanced anti-inflammatory cytokine availability
106 (e.g. TGF β , IL-10) or the presence of regulatory T cells promotes MPEC development
107 or derivation of less differentiated memory subsets ¹⁵. Costimulation via CD28-
108 CD80/CD86 is also required during priming to prevent anergy and adaptive tolerance,
109 whilst ligation of TNF super family receptors (TNFSRs) on CD8⁺ T cells (CD27, OX40,
110 41BB, CD30) promotes proliferation, survival and enhances the quality of the recall
111 response ¹⁷⁻²⁰. Similarly, ligation of HVEM receptor on CD8⁺ T cells by BTLA (on CD8 α
112 DC) is required for Teff cell survival and development of protective immune memory
113 in response to bacterial and viral infection, in part via promoting MPEC persistence ²¹.
114 Another key factor in the generation of memory CD8⁺ T cells is CD4⁺ T cell help. CD8⁺
115 T cells primed in the absence of CD4⁺ T cells have impaired long-term survival and
116 display defective ability to respond against secondary challenge ²². The mechanisms
117 behind the requirement of CD4⁺ T cells are not completely understood, however the
118 interaction between CD40 on CD8⁺ T cells with CD40L on CD4⁺ T cells and the
119 secretion of IL-15 from these cells have shown to be relevant in the generation Teff

120 cells with enhanced ability to become memory ^{23,24}. More recently, CTLA-4 on CD4⁺
121 T regulatory (Treg) cells has been shown to force memory T cell quiescence,
122 suggesting that helper and regulatory CD4⁺ T cell subsets may be required for optimal
123 memory CD8⁺ T cell generation and homeostasis, respectively ²⁵.

124 **Circulating memory CD8⁺ T cell subsets**

125 Memory CD8⁺ T cells are heterogeneous, and can be defined as one of four major
126 subsets according to their surface markers, effector potential, stemness and ability to
127 home lymphoid organs and non-lymphoid tissues (Figure 1). Circulating memory CD8⁺
128 T cells can be classified as stem central memory (Tscm), central memory (Tcm) and
129 effector memory (Tem), whereas memory CD8⁺ T cells that become established within
130 the infected/challenged tissue and do not re-circulate are termed tissue resident
131 memory (Trm). Tscm cells are present in mouse, human and non-human primates and
132 are endowed with the greatest stem potential of all memory subsets, allowing them to
133 give rise to Tcm and Tem cell populations upon antigen stimulation²⁶. Tscm cells have
134 a naïve-like phenotype with low expression of CD44 (mouse), high levels of CD62L
135 and co-express antigen-experienced CD8⁺ T cells molecules such as CD122, the
136 Stem Cell Antigen 1 (SCA-1), B cell lymphoma 2 (BCL-2), CXC-chemokine receptor 3
137 (CXCR3), and CD95 ²⁶. Tcm and Tem cells were originally described in mouse and
138 human based on the expression of CD44, CCR7 and CD62L, and CD45RO and CCR7
139 respectively ²⁷. Tcm cells display reduced effector function and have a stem-cell-like
140 phenotype given their ability to generate new Tem cells after antigen recognition ²⁸. In
141 mice, Tcm cells are CD44⁺CD62L⁺CCR7⁺ while in human these cells are
142 CD45RO⁺CCR7⁺ (and CD62L⁺). Expression of CCR7 and CD62L facilitate migration
143 through the high endothelial venules (HEV) into secondary lymphoid organs, where

144 Tcm cells preferentially accumulate²⁸. Tcm/Tscm cells show common transcriptomic,
145 epigenetic and proteomic features (e.g. high basal STAT5) that cluster them
146 separately from Tem cells²⁹. In comparison, Tem cells are more differentiated, display
147 a molecular fingerprint associated with Teff cell function (cytolytic Teff genes) and
148 exhibit immediate effector function upon antigen re-encounter³⁰. Mouse Tem cells
149 have a CD44⁺CD62L⁻ phenotype, whilst human Tem cells are defined by
150 CD45RO⁺CCR7⁻, with KLRG1 expression being common to Tem in both species³¹.
151 In humans, the markers CD27 and CD28 can be used to further define circulating
152 memory CD8⁺ T cells. Both markers are expressed by naïve, Tscm and Tcm cells,
153 whereas Tem cells can be divided into Tem 1 (CD28⁺CD27⁺), Tem 2 (CD28⁺CD27⁻ or
154 CD28⁻CD27⁺), or Tem 3 (CD28⁻CD27⁻) that exhibit progressively enhanced effector
155 potential *ex vivo*³². Terminal differentiation of human CD8⁺ T cells is demarcated by
156 re-expression of CD45RA within the Tem cell pool, giving rise to Temra cells
157 (Terminally differentiated effector memory cells re-expressing CD45RA; CCR7⁻CD28⁻
158 CD27⁻CD45RA⁺)³³. Temra cells exhibit potent effector function, poor proliferative
159 capacity, low IL-2 production and are enriched for phenotypic and functional (defective
160 telomerase activity) traits of senescence³³. One marker associated with Temra cells
161 is CD57, which correlates with a history of extensive cell division, short telomeres,
162 replicative senescence, ageing, cytomegalovirus (CMV) status, decreased *ex vivo*
163 IFN γ but enhanced cytotoxic function (i.e. GZMb and perforin expression)³³. Temra
164 cells may also (re)express KLRG1, which is enriched in populations specific for viruses
165 with latency periods³⁴. Interestingly, CD57⁺KLRG1⁻ and CD57⁺KLRG1⁺ CD8⁺ T cells
166 retain effector function but the latter subset fail to proliferate and have diminished
167 expression of CD27, CD28 and CD127, indicating more terminal differentiation³⁴.

168

169 Signals 1-3 form complex molecular circuitries, which enact key transcription factors
170 (T-bet, Eomes, Blimp-1, Bcl-6, Tcf7, Foxo1) to determine precursor fate and memory
171 CD8⁺ T cells subset differentiation. These findings have been expertly reviewed
172 elsewhere ⁶. An oversimplified consolidation of this data is that strong TCR signals,
173 IL-2 (inducing Tbet and BLIMP-1) and IL-12 (upregulating Tbet) favor Tem cell (and
174 SLEC) differentiation, whilst abrupt signal 1, IL-21, IL-10, TCF7, FOXO1, EOMES and
175 Bcl-6 support Tcm cell (and MPEC) specification, as summarized in ³⁵. Tcm cells
176 express higher levels of the latter two transcription factors, require Bcl-6 and sustain
177 Eomes expression via the Tcf-1-Wnt axis ³⁶. Together with augmented IL-7/IL-15-
178 driven Stat5 phosphorylation and induction of Bcl-2 this forms a module which confers
179 enhanced survival and self-renewal to Tcm/Tscm cells relative to Tem cells ³⁷.
180 Transcriptional networks downstream of increased inflammation and TCR signaling
181 (which favor Teff cell development during priming) in contrast drive Tem/Temra cell
182 differentiation ¹⁵. However, whether subset commitment depends on the malleability
183 of a single naïve CD8⁺ T cell population via alterations in TCR stimulation (signal
184 strength model) or successive rounds of antigen exposure (decreasing potential
185 model) has been contended ^{6,38,39}. It is noteworthy that a model in which repetitive
186 antigen exposure drives stepwise Tscm>Tcm>Tem>Temra cell differentiation is
187 supported by recent functional, transcriptomic and proteomic data and the
188 redistribution of these subsets following chronic immune stimulation ^{8,40-42}. In
189 accordance with this, CD8⁺ T cells in healthy human blood are predominantly of a
190 naïve phenotype (40%), Tem and Temra cells are present at approximately equal
191 proportions (20-25%) and a minority are of a Tcm cell phenotype ^{43,44}. However, this
192 is highly variable between donors and changes with age or antigen experience such
193 that Temra cells (but also to an extent Tem and Tcm cells) gradually increase at the

194 expense of naïve pools⁴⁴. This phenomenon of ‘immunosenescence’ is exemplified
195 in chronic infection (e.g HIV), auto-inflammatory disease (e.g. Rheumatoid arthritis)
196 and cancer, and can be tracked within antigen specific CD8⁺ T cells (e.g. HIV, CMV,
197 EBV), where progressive differentiation may result in clonal deletion³³. It should be
198 noted that, despite the discrete properties and phenotypes of memory CD8⁺ T cell
199 subsets observed in a variety of experimental and clinical settings, the concept of
200 linear differentiation remains a framework imposed upon a likely fluid spectrum of cell
201 fates; consequently, exceptions and regular revisions to this model are common and
202 necessary. An additional layer of complexity is that phenotypes used to describe
203 memory CD8⁺ T cell subsets derive largely from analysis of resting cells in the
204 circulation. Since activation *in vitro* and *in vivo* drastically affects expression of the
205 majority of markers used to define classical subsets, application of this nomenclature
206 in the context of an ongoing or experimental immune responses can be challenging
207⁴⁵.

208 **Tissue resident memory CD8⁺ T cells**

209 Tem cells within tissues were historically considered to be circulatory, however tissue-
210 resident memory CD8⁺ T (Trm) cells were formally described in 2009⁴⁶. Trm cells have
211 been shown to stably reside in the skin, lung, intestine, brain, female reproductive
212 tract, salivary glands and others, where they provide rapid and potent protective
213 immunity against re-infecting pathogens⁴⁶⁻⁵². Trm cells are long-lived, mediate
214 immediate protective immunity and are the most abundant T cell lineage in organisms
215 with natural infection experience^{53,54}. Phenotypically, Trm cells constitutively express
216 CD69, integrin $\alpha E(CD103)\beta 7$ (commonly referred to as CD103) and are devoid of
217 CD62L and CCR7⁵⁵. Given that CD103 is the ligand for E-cadherin, which is

218 expressed in epithelial cells, it has been proposed that CD103 is responsible for
219 residency in epithelial tissues⁵⁶. CD103 is also induced by TGF β (which is key to Trm
220 development) and competes for E-cadherin binding with KLRG1 creating a circuit in
221 which TGF β favors Trm cell abundance via induction of CD103 and interception of the
222 KLRG1-E-cadherin axis^{55,57}. CD69 upregulation abrogates tissue egress by
223 degrading sphingosine 1-phosphate (S1P) receptor 1 (S1P1R), disabling CD8⁺ T cells
224 to respond to S1P gradients, which is highly abundant in blood and lymph⁵⁸.
225 Interestingly, Trm cells from unrelated tissues share a core transcriptional program
226 that is different from Tem, Tcm and Tn cells, but may also diverge on the basis of
227 auxiliary, tissue-specific gene expression characteristics reflective of the site of origin
228^{55,59-61}.

229 Several transcription factors are involved in the generation and maintenance of Trm
230 cells. Downregulation of Eomes during Trm cell development is necessary for CD103
231 induction, whereas low levels of T-bet are necessary for the expression of IL-15
232 receptor (a key signal for the maintenance of these cells in the tissues)^{55,62}.
233 Furthermore, the Trm cell differentiation program is controlled by the expression of
234 Blimp-1 and the homolog of Blimp-1 in T cells (Hobit) transcription factors together
235 with downregulation of the transcription factor Krüppel-like factor 2 (Klf2), which
236 represses the expression of S1PR1 (receptor for S1P) thereby inhibiting tissue egress
237^{63,64}. RUNX3 was also recently described as a transcription factor required for the
238 establishment of Trm cells in different tissues and solid tumors, operating via induction
239 of tissue-residency genes and the suppression of loci related to tissue egress⁶⁵. Trm
240 cell commitment appears to be two stage process (Bcl-2 and CD69 induction followed
241 by CD103 expression), and Tem as well as Tcm cells can give rise Trm cells in
242 different tissues^{55,66,67}. It should be noted that, in a similar manner to their impact on

243 Tem cells, CD4⁺ T cell help has been shown to guide Trm cell formation ⁶⁸.

244 Functionally, the positioning of Trm cells at sites of previous antigen encounter
245 provides host organisms with a means of rapid response to reinfection and protection
246 from reactivated latent viruses ⁶⁹. Upon antigen recognition, Trm cells likely mediate
247 both immediate lytic activity via high constitutive production of GZMb and orchestrate
248 an alarm state at the local tissue site, recruiting and activating NK cells, DC and other
249 lymphocytes via secretion of IFN γ , IL-2 and TNF α ^{51,70,71}. Interestingly, Trm cells
250 recruit Tem cells into the tissues in an IFN γ -dependent manner, potentially inducing
251 bystander activation since recruited populations are GZMb⁺ ^{51,72}. Two recent studies
252 have extended these findings to show that Trm cell reactivation promotes their *in situ*
253 local proliferation and the recruitment of new Trm cells into the tissues without
254 displacement of pre-existing populations ^{72,73}. It is of relevance to tumor immunity and
255 vaccination strategies that Trm cell induction is, intuitively, site specific. For example,
256 cutaneous HSV infection establishes a virus-specific Trm cell pool at the challenge
257 site, but not the contralateral flank, providing protection upon challenge in the former
258 but not the latter ⁴⁶. Similarly heterosubtypic (cross strain) protection from influenza
259 virus can be achieved by influenza-specific lung Trm cells generated through
260 intranasal live attenuated influenza but not systemic administration of injectable
261 inactivated or live attenuated influenza ⁷⁴. Intriguingly, although Trm cells in various
262 barriers sites are maintained by IL-15, their turnover and persistence also appears to
263 be tissue and/or context-specific ⁵⁵. Trm cells in multiple target tissues have been
264 reported to exhibit extended life spans ^{59,75}. However, unlike the skin, lung Trm cells
265 undergo rapid turnover, with attrition after infection being partly counterbalanced by
266 ongoing recruitment from the circulation ⁶⁶.

267 In humans, pioneering work to produce a spatial map of T cells in tissues using brain
268 dead organ donors has illustrated that blood and lymph nodes have a diffuse
269 distribution of naïve (most abundant) > Temra/Tem > Tcm (least abundant) subsets,
270 whereas the spleen and lungs contain mainly Tem and Temra cells and in the
271 Jejunum, Ileum and Colon are predominantly of a Temra cell phenotype (approx. 80%)
272 ⁷⁶. Interestingly, CD103 expression was preferentially localized to the CD45RO⁺
273 fraction of CD8⁺ T cells (in the Jejunum, Ileum, colon and lung), whilst Temra cells
274 were largely, but not entirely CD103⁻ ⁷⁶. Only a small fraction of Trm cells produce
275 IFN γ or IL-2 following stimulation with PMA and Ionomycin (PMA/Io), thus the full
276 scope and magnitude of effector function in human Trm cells is likely under
277 appreciated ^{43,76}. Subsequent work by the same group demonstrated that a shift
278 towards more differentiated phenotypes (Tem cells > Temra cells, increased %CD57⁺
279 cells) occurred as a function of viral specificity, age and /or CMV status in both Trm
280 cell and circulatory compartments ^{76,77}. Of relevance, work in clinical samples unveiled
281 that lung-derived Trm cells but not skin or circulating CD8⁺ T cells elicit polyfunctional
282 responses to influenza challenge, confirming tissue-specific immunity of Trm cells
283 seen *in vivo* is common to humans ^{78,79}.

284 **Memory CD8⁺ T cells and immune homeostasis**

285 Genetic, pharmacological or pathogen-derived memory CD8⁺ T cell deficiency or
286 dysfunction renders the host susceptible to potentially fatal opportunistic infection and
287 tumor development, whilst de-restricted Teff cell responses precipitate lethal
288 autoimmunity, allergy or inflammatory tissue destruction. There is therefore strong
289 evolutionary pressure to develop tightly regulated, multilateral mechanisms of immune
290 homeostasis. In the memory CD8⁺ T cell pool, immune homeostasis is orchestrated

291 in several layers. The overall size of the CD8⁺ memory T cell pool is maintained by
292 balancing attrition with compensatory homeostatic proliferation driven by IL-7 and IL-
293 15^{80,81}. These cytokines reconstitute lymphopenic hosts by peripheral expansion
294 which simultaneously converts naïve and Tcm cells to a Tem-like 'memory phenotype'
295 with augmented effector potential^{82,83}. Memory CD8⁺ T cells are also restrained by a
296 myriad of T cell intrinsic and extrinsic regulators of effector function including Treg
297 cells, intracellular quiescence factors^{25,84-87}, cell surface proteins involved in ATP
298 hydrolysis (CD38, CD39, CD73)⁸⁸, antigen presenting cell-derived IFN γ -inducible
299 catabolic enzymes (i.e. IDO)⁸⁹, nitric oxide⁹⁰, arginase 1⁹¹, prostaglandin E2 and
300 anti-inflammatory cytokines (TGF β , IL-10, VEGF, IL-35)^{91,92} and T cell inhibitory
301 receptors (TCIR), many of which are currently targeted or under investigation in
302 immune oncology. The latter include well characterized receptors whose cognate
303 ligands are expressed on various cells in the tumor microenvironment (TME) and
304 lymph nodes, such as PD-1 (PD-L-1/PDL-2; antigen presenting cells (APC), tumor
305 cells or epithelial cells), CTLA-4 (CD80/86 on professional APC), Tim-3 (galectin-9 on
306 APC and tumor cells), and LAG-3 (MHC-II on APC)⁹³. The abundance of TCIRs is
307 restricted to activated CD8⁺ T cells, and terminally differentiated Tem/Temra cells,
308 whilst their ligands are found on activated APC or epithelial cells, illustrating spatial
309 and temporal restriction to balance immunity and tolerance.

310 **Memory CD8⁺ T cell dysregulation**

311 Perturbation of signals 1, 2 or 3 can dysregulate memory CD8⁺ T cell responses. This
312 includes the onset of self-tolerance and anergy; two differentiation programs that
313 share an overlapping molecular basis which manifests in hypo-responsiveness to self-
314 peptide⁹⁴. The deletion of autoreactive T cell clones during central tolerance is

315 incomplete. Therefore, peripheral self-tolerance is a necessary evolutionary strategy
316 that prevents autoimmunity via inhibition of effector responses to cognate antigen
317 following sub-optimal co-stimulation (i.e. in the absence of DAMP/PAMP signalling on
318 APC). Context and system-dependent differences (including cytokine environment
319 and TCR avidity) may bring about variable degrees of hyporesponsiveness, altering
320 the requirement for antigen persistence, as well as the magnitude or co-occurrence of
321 defects seen in cytokine production/proliferation, in some instances leading to T cell
322 deletion⁹⁴⁻⁹⁹. Self-tolerance may also result from induction of TCIRs, via suppression
323 from immune regulatory cell populations (e.g. Treg cells) or the action of anti-
324 inflammatory cytokines/cc (e.g. IL-10)^{94,98}. *In vivo*, tolerance can be rescued by IL-2,
325 IL-7 or lymphopenia, but this occurs transiently with resumption of tolerance occurring
326 in the absence of antigen, suggesting commitment to an epigenetically programmed
327 tolerogenic cell fate⁹⁸. Similar to self-tolerance, stimulation of T cell clones with
328 antigen or anti-CD3 in the absence of costimulation *in vitro* results in proliferative
329 inhibition via a process termed anergy¹⁰⁰, which is rescuable via addition of
330 exogenous cytokines^{101,102}. However, it has been suggested that anergy and
331 tolerance can be discriminated on the basis of functional and molecular
332 characteristics, despite overlapping features⁹⁴. Self-tolerance is engaged through a
333 CD8⁺ T cell intrinsic gene expression profile distinct to naïve or memory CD8⁺ T cells.
334 Relative to memory CD8⁺ T cells, tolerant CD8⁺ T cells exhibit enhanced expression
335 of TCIRs (e.g. LAG-3), transcriptional repressors (EGR1/2, DUSP2), loss of key
336 transcription factors (EOMES, T-BET, GATA-3), diminished expression of cytokine
337 receptors and chemokine receptors (e.g. IL12RB1, CXCR3, CCR5) and crucially, lack
338 of effector genes induction (e.g. IFN γ , PRF1)⁹⁴. *In vitro* anergy induces NFAT in the
339 absence of AP-1, leading to NFAT homodimers that induce Egr2, Ikaros, E2F

340 transcription factors and the E3 ubiquitin ligase family which inhibit IL-2, TNF α , IFN γ
341 and other effector genes ¹⁰³. Models of *in vivo* anergy are associated with defective
342 calcium signaling and nuclear translocation of NFAT2 in the absence of NFAT1
343 leading to anergy-associated gene expression ¹⁰⁴.

344 CD8⁺ T cells experience persistent antigen exposure in a range of pathologies and
345 microenvironments resulting in the onset of an unconventional cell fate often described
346 as T cell exhaustion ¹⁰⁵. During acute viral infection, host CD8⁺ T cell responses clear
347 pathogen during the effector phase, contract and form functional memory CD8⁺ T cells.
348 A failure to rapidly eliminate pathogen results in chronic infection, associated with
349 unremitting antigen load and high levels of inflammation that drives exhaustion.
350 Seminal studies using the LCMV clone 13 mouse model of chronic viral infection led
351 to the prototypic description of exhaustion as a state of T cell hyporesponsiveness ¹⁰⁶⁻
352 ¹⁰⁸. Despite common misconceptions, exhausted T (Tex) cells are not entirely devoid
353 of effector function, since they contribute to viral control ¹⁰⁹. Rather, Tex cells exhibit
354 a broad spectrum of dysfunctional states, characterised by stepwise loss of i) IL-2
355 production ii) *in vitro* cytotoxicity iii) IFN γ /TNF α production, iv) degranulation and in
356 some instances ultimately v) physical deletion ^{94,108,110,111}. Progression to a terminal
357 Tex cell fate coincides with altered metabolism and broad expression of TCIRs
358 including, PD-1, CTLA-4, LAG-3, CD160, BTLA and Tim-3 ¹¹²⁻¹¹⁴. The severity of
359 exhaustion has been further defined by altered transcription factor expression. In the
360 LCMV clone 13 infection model, a circulating progenitor pool of TNF α , and IFN γ -
361 producing EOMES^{lo}PD-1^{int} Tex cells gave rise to a tissue homing, poorly proliferative,
362 but cytotoxic EOMES^{hi}Tbet^{lo}PD-1^{hi} Tex cell progeny upon antigen restimulation ¹¹⁵.
363 Given that T-bet represses PD-1, LAG-3 and other TCIR in Teff cells, loss of this
364 transcription factor marks transition into severe exhaustion that facilitates increased

365 negative signaling ¹¹⁵. Conversely, NFAT signaling enhances the expression of PD-1
366 and Tim-3 ¹¹⁶; thus, a balance between T-bet and NFAT may be crucial determinants
367 of the TCIR profile of Tex cells. Interestingly BLIMP-1 and BATF also appear to display
368 a distinct-context-dependent role in Tex cells; the former is correlated with TCIR
369 expression but is necessary for GZMb expression, whilst the latter is induced by PD-
370 1 signaling to suppress effector function ^{117,118}. Thus, in chronic viral infections there
371 is a progenitor subset of Tex cells whose function is supported by T-bet which may
372 stall severe exhaustion, whilst in progressively exhausted CD8⁺ T cells BLIMP-1 and
373 EOMES provide residual cytotoxic function whilst BATF and NFAT limit effector
374 potential ¹¹⁹. It is also of note that the NFAT-EGR2 axis appears central in anergy, and
375 thus may be a master regulator of T cell hyporesponsiveness ¹¹⁶. Targeting TCIRs
376 with blocking antibodies has been suggested to reverse exhaustion in chronic infection
377 and tumors, however this appears to be stage and to an extent system-dependent. In
378 LCMV chronic infection, targeting Tim-3 and PD-1 synergistically restores effector
379 function of CD8⁺ T cells ¹²⁰. It has also been suggested that there is a differential
380 sensitivity amongst Tbet^{hi}PD-1^{int}EOMES^{lo} (reversible Tex cell phenotype) and
381 Tbet^{neg}PD-1^{hi}EOMES^{hi} (irreversible Tex cell phenotype) subsets to PD-L1 blockade in
382 LCMV chronic infection ^{115,121}. Similar to what has been proposed in tolerance, chronic
383 infection appears to impose epigenetic re-programming associated with T cell
384 exhaustion^{122,123}. In this module transcription factors, cytokine and TCR signaling loci
385 appear in closed chromatin conformations at later stages of infection coincident with
386 increased accessibility of the PD-1 locus ^{124,125}. It has been suggested that this
387 epigenetically fixed state of CD8⁺ T cell dysfunction is accountable for checkpoint
388 blockade activity¹²⁶. In agreement with this, two recent reports showed that i) PD-L1
389 blockade in the LCMV infection model only transiently engaged effector transcriptional

390 circuitry but did not alter the epigenetic landscape of Tex cells or induce functional
391 memory T cells and ii) determined a specific epigenetic basis of Tex cells in murine
392 and human chronic viral infections ^{122,123}. Indeed, Tex cells have been widely
393 described in chronic viral infection of higher primates, including humans with Hepatitis
394 C virus (HCV) infection, Hepatitis B virus (HBV) induced-hepatitis and both simian
395 immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) infection ¹²⁷.
396 CD8⁺ T cells in chronic SIV and HIV exhibit cardinal phenotypic (TCIR expression),
397 functional and molecular features of exhaustion described above. HIV-specific resting
398 and activated CD8⁺ T cells showed a Tbet^{int}EOMES^{hi} population marked with multiple
399 TCIRs, corresponding to the severely exhausted T cells found in LCMV chronic viral
400 infection models ¹²⁸, whilst CMV-specific CD8⁺ T cells showed balanced EOMES and
401 Tbet expression. HIV-specific Tbet^{int}EOMES^{hi} CD8⁺ T cells exhibited a Tem1 cell
402 phenotype with poor effector function, and persisted long after anti-retroviral therapy
403 initiation suggesting that exhaustion was not reversed and that these cells may remain
404 long after removal of high antigen load in humans ¹²⁸. Another report classified CD8⁺
405 T cells of HIV patients based on EOMES and CD57 expression showing that
406 EOMES^{int}CD57⁺(Tbet^{hi}GZMb⁺PRF⁺) cells retained functionality and correlated with
407 HIV control, whereas EOMES^{hi}CD57⁺(Tbet^{int}GZMb^{int}PRF^{lo}) cells were dysfunctional
408 ¹²⁹. A subsequent study showed that the frequency of activated/Tex cells (CD38⁺PD-
409 1⁺) correlated with viral load in plasma and rapid clinical progression in HIV infection
410 ¹³⁰. However, in line with findings in the LCMV model, it seems that Tex cells in chronic
411 SIV and HIV infection may exert residual cytolytic function to contribute to viral control,
412 since their depletion leads to virus rebound/disease progression in SIV ^{131,132}. CD8⁺ T
413 cell exhaustion therefore appears to result from the convergence of chronic antigen
414 stimulation and inflammation, leading to augmented TCIR signalling, *de novo* gene

415 expression, dysregulation of CD8⁺ T cell transcription factor networks and epigenetic
416 reprogramming.

417 As mentioned above, over-differentiation or immunosenescence of the CD8⁺ T cell
418 memory compartment is observed in ageing, chronic infection and cancer; resulting in
419 elevated apoptosis in addition to increased frequencies of terminally differentiated
420 memory CD8⁺ T cells (e.g. Temra cells) at the expense of progenitor pools (naïve
421 CD8⁺ T cells, Tscm cells and Tcm cells) that manifests in defective immune memory
422 ³³. This accelerated ageing of the immune system has been shown to accompany T
423 cell senescence, a triphasic process of cell cycle arrest that occurs following DNA
424 damage (either via insult such as irradiation or through exposure of DNA via telomere
425 erosion) (Phase 1) and involves a DNA damage response (Phase 2) and growth arrest
426 (Phase 3) ¹³³. In senescent CD8⁺ T (Ts) cells this process involves signaling via p53,
427 MAPK, p38, and CDK inhibitors, and is linked to progressive differentiation as marked
428 by CD57, KLRG1, loss of CD27 and CD28 expression and re-expression of CD45RA
429 ¹³³. However, distinguishing highly polyfunctional, pre-senescent CD8⁺ T cells
430 (including CD57⁺ cytotoxic CD8⁺ T cells and CD45RA re-expressing Temra cells)
431 appears challenging and relies on KLRG1 and CD57 dual expression as a minimum
432 ³⁴. Loss of telomerase, BCL-2 and phosphorylation of AKT^{Ser473} may also mark truly
433 senescent CD8⁺ T cell populations ¹³⁴. Senescent CD8⁺ T cells are sustained by IL-
434 15 to persist *in vivo* and home to inflamed tissues, through interaction with ICAM-1,
435 the extracellular matrix and Fractalkine (CCR7⁻,CD62L⁻,CD11a⁺CD18⁺, CD49e⁺,
436 CX3CR1⁺). Most crucially, when stimulated with appropriate APC/co-stimulatory
437 signals (41BBL) these cells down regulate CD45RA, become activated, proliferate and
438 mediate potent cytotoxic effector function including IFN γ , TNF α and a reduced amount
439 of IL-2 ¹³⁵.

440 Similar to Tex cells, Ts cells share a loss of proliferation and IL-2 production
441 accompanied by high TCIR expression but these programs differ in many features ¹³³.
442 Ts differentiation is associated with CD45RA re-expression, expression of CD57 and
443 KLRG1, the acquisition of enhanced IFN γ , TNF α , cytotoxicity, shortened telomeres
444 and reduced telomerase activity (many of which are also linked to pre-senescence)
445 ¹³³. In contrast, Tex cells have been described as CD57⁻, KLRG1⁻ exhibiting
446 progressive loss of effector function given by their low expression of IFN γ and TNF α .
447 Finally, the epigenetic status of Tex and Temra cells is divergent, with the IFN γ locus
448 being hyper and hypo-methylated, respectively^{33,105,133,136,137}.

449 **The inception and inhibition of anti-tumor immunity**

450 The unprecedented survival rates achieved with checkpoint blockade have fueled
451 renewed optimism in cancer immunotherapy. However, only a minority of patients are
452 sensitive to treatment and few experience durable clinical benefit ¹³⁸. Key correlates
453 of a therapeutic response to checkpoint inhibition include high tumor mutational
454 burden (TMB) and T cell infiltration; implying that mutation-encoded neoepitopes serve
455 as a substrate for tumor specific Teff cells and that these neoantigen reactive T
456 (NART) cells are actively suppressed by the targeted TCIRs ¹³⁹⁻¹⁴². However, the
457 increasingly appreciated transience of Teff cell reinvigoration and prevalence of
458 relapse collectively signify a defect in durable immune memory post checkpoint
459 blockade. As a field, we have therefore failed to design immunotherapeutic strategies
460 that reliably forge stable, functional memory T cell pools capable of protecting from
461 recurrence, indicating a lack of essential knowledge in the ontogeny and dysregulation
462 of anti-tumor T cell responses. Pioneering studies have shown that cross-presentation
463 by tumor resident DC and direct presentation on tumor cells can prime CD8⁺ T cell
464 cells at the tumor site, eliciting an efficient anti-tumor immune response in the absence

465 of lymph nodes ¹⁴³. More recently however, it has been proposed that tumor antigens
466 are most often presented in the tumor-draining lymph-nodes by migratory DC derived
467 from the tumor site ^{144,145}. Following priming, formation of functional immune memory
468 in the presence of chronic viral and tumor antigen is impaired, the basis of which
469 remains only partly understood¹⁴⁶. T cell extrinsic barriers of Teff and memory CD8⁺ T
470 cell function in anti-tumor immunity likely include i) inefficient priming (insufficient
471 antigen load via low mutation rate, a high sub-clonal neoantigen burden and/or poorly
472 expressed tumor antigens, similarity of tumor epitopes to self-peptides, inability of
473 epitopes to bind HLA, lack of danger signals low levels of co-stimulation or
474 inflammatory cytokines/chemokines, checkpoint ligand expression or tolerogenic
475 function(s) of APCs and lack of CD4⁺ T cell help) ii) local regulatory cell suppression
476 (Tregs, myeloid-derived suppressor cells, tumor associated macrophages, cancer
477 associated fibroblasts), iii) soluble inhibitory factors in the TME (e.g. TGF β , IL-10,
478 reactive oxygen species) iv) tumor-intrinsic resistance (expression of anti-apoptotic
479 molecules, mutations in tumor antigen processing/presentation machinery and IFN γ
480 signaling, down-regulation or loss of heterozygosity of MHC alleles), loss of
481 neoantigens, inhibitors of cytolytic compounds, expression of FASL and checkpoint
482 ligands) v) physical exclusion of T cells from the tumor vi) Metabolic and hypoxic
483 constitution of the TME ¹⁴⁷⁻¹⁵⁰. T cell intrinsic hurdles to generate a functional tumor
484 specific memory T cell pool include: i) the deletion of tumor-associated self-antigens
485 and potentially NART cells by central tolerance ii) low avidity TCR-peptide:MHC
486 interactions iii) increased sensitivity of T cells to apoptosis, iv) inability to migrate to
487 the tumor site, v) TCIR expression and vi) T cell dysfunction (anergy, tolerance,
488 exhaustion) ⁹⁴. The anti-tumor immune response therefore shares common features
489 with those discussed above for chronic viral infection. This includes prolonged antigen

490 stimulation, a predominance of T cell inhibitory networks, and regulatory cell
491 expansion⁹⁴. Some key differences in anti-tumor immunity include priming conditions,
492 where lower antigenicity of self- or modestly altered non-self peptides, the absence of
493 danger signals to activate APC and the initial lack of inflammation, collectively there
494 are reduced signals 1, 2 and 3 in cancers compared to viral infection. Thus, the context
495 of tumor-specific T cell priming in early disease is similar to conditions conducive to
496 tolerance, yet consequent antigen chronicity and increased inflammation thereafter
497 recapitulate cardinal aspects of exhaustion^{94,98,151,152}. It is therefore unsurprising that
498 T cells exhibiting TCIR expression, defective cytokine production, altered cytokine
499 production, modulated TCR signalling and epigenetic reprogramming have been
500 widely observed in experimental and clinical settings of cancer^{105,153}.

501 **Memory CD8⁺ T cells in tumor immunity: Pre-clinical models**

502 CD8⁺ T cells in pre-clinical cancer models exhibit profound TCIR expression and are
503 typically unable to reject even highly immunogenic tumors. However, experimental
504 interventions such as checkpoint blockade, adoptive cell therapy, vaccination or
505 induced lymphopenia can lead to tumor regression via inhibition of suppressive
506 signals, delivering agonistic co-stimulation or cytokine signals, depletion of Tregs or
507 provision of Teff cells devoid of inhibitory receptors^{154,155}. Given phenotypic, functional
508 and transcriptomic similarities, it has been proposed that an overlapping program of T
509 cell dysfunction occurs in tumors similar to T cell exhaustion seen in chronic viral
510 infection. For example, the co-expression of PD-1 and Tim-3 has been used to define
511 dysfunctional tumor infiltrating CD8⁺ T cells in colon and mammary mouse tumors,
512 where the blockade of these two molecules restores the functionality of CD8⁺ T cells
513¹⁵⁶. Another study has shown that co-blockade of TIGIT and PDL-1 can resurrect
514 functionality of intratumoral CD8⁺ T cells¹⁵⁷. However, targeting of different

515 checkpoints clearly elicits tumor regression via divergent mechanisms that may not
516 always reflect reversal of dysfunction. Indeed, whilst α -PD-1 treatment specifically
517 induces the expansion of PD-1^{hi}TIM-3⁺CD8⁺ T cells inside the tumor (which may point
518 to either transient rewiring of effector machinery or disengagement of CD8 T cell
519 dysfunction), α -CTLA-4 treatment induces the proliferation of peripheral ICOS⁺ Th1
520 CD4⁺ and CD8⁺ T cells and depletes Tregs, suggesting that these agents i) have vastly
521 different mechanisms of action beyond antagonism ii) de-repress CD8⁺ T cells via
522 disrupting CD8⁺ T cell intrinsic (PD-1) and/or CD8⁺ T cell extrinsic (Treg cell depletion)
523 regulation iii) mobilize independent memory or Teff cell subsets and thus iv) may
524 exhibit a differential impact on CD4⁺ and CD8⁺ T cell memory induction ¹⁵⁵. Loss of
525 effective CD8⁺ T cell responses in tumors also appears to involve transcription factor
526 dysregulation. Rescue of T-bet and EOMES phosphorylation was seen concomitant
527 with tumor clearance following α -PD-1 combined with α -CTLA-4 in a CT26 GVAX
528 tumor model ¹⁵⁸. Ablation of the key anergy gene *Ikaros* induced tumor rejection in a
529 melanoma model and loss of the transcription factor MAF augmented anti-tumor
530 responses in established melanoma ^{159,160}. More recently, the transcription factor Egr2
531 (implicated in T cell anergy) together with LAG-3 and 4-1BB expression was used to
532 define dysfunctional CD8⁺ T cells in the tumor microenvironment that can be
533 reactivated using blocking antibodies against these two molecules ¹²⁷. Furthermore,
534 these dysfunctional LAG-3⁺4-1BB⁺CD8⁺ T cells expressed a wide range of inhibitory
535 and stimulatory receptors including 2B4, TIGIT, CD160, CTLA-4, OX-40, GITR, NRP1
536 and ICOS and downregulated the IL-7 receptor which is essential for memory CD8⁺ T
537 cell survival ¹³. The ATPase CD39 has been recently used to define exhausted CD8⁺
538 T cells in a mouse model as well as in melanoma and breast cancer patients. Tumor-
539 infiltrating CD39^{hi}CD8⁺ T cells produce less TNF- α , IL-2 and express more PD-1, Tim-

540 3, LAG-3, TIGIT and 2B4 compared with the CD39^{int} and CD39^{neg} tumor infiltrating
541 CD8⁺ T cells ¹⁶¹. In addition to TCIRs, several immunosuppressive and pro-
542 tumorigenic factors, including adenosine, indoleamine 2,3 dioxygenase (IDO),
543 vascular endothelial growth factor (VEGF), type I interferons, glucose, Treg cells and
544 myeloid-derived suppressor cells (MDSC) have been implicated in CD8⁺ T cell
545 exhaustion or dysfunction ¹⁵³. However, direct evidence for the role of these factors in
546 mouse tumor models is scarce. VEGF has been shown to induce an exhausted
547 phenotype in tumor infiltrating CD8⁺ T cells characterized by the expression of several
548 inhibitory receptors including PD-1, CTLA-4 and TIM-3. Interestingly, VEGF blocking
549 antibodies synergize with α -PD-1 antibodies promoting CD8⁺ T cell reinvigoration and
550 slowing tumor growth ¹⁶². Treg cells induce a dysfunctional state of tumor-infiltrating
551 DC, promoting the induction of PD-1⁺TIM-3⁺ exhausted CD8⁺ T cells that produced
552 lower amounts of IFN- γ and TNF- α inside the tumor ¹⁶³. Thus, TCIR expression
553 appears to identify tumor reactive T cells that experience negative TCIR signaling,
554 transcription factor dysregulation, loss of cytokine-mediated homeostasis and extrinsic
555 regulation, with checkpoint inhibition (CPI) eliciting anti-tumor responses by inducing
556 heterogeneous effector T cell pools via interception of several pathways. However,
557 this evidence does not elucidate the inception of tumor specific T cell dysfunction.

558

559 Several recent studies have used inducible experimental neoantigen expression in
560 tissues to model the physiology of tumorigenesis. Elegant work demonstrated that
561 chronic neoantigen stimulation induced biphasic tumor-specific T cell dysfunction that
562 is initiated in early tumorigenesis. Using an inducible SV40 T antigen, it was shown that
563 neoepitope exposure resulted in first a plastic (Day 8) then irreversibly fixed (day 35)
564 state which could not be rescued *in vitro* via IL-2 or anti-PD-1 ¹⁵². More specifically, *in*

565 *vivo*, at day 35 post neoantigen induction, neoantigen-specific T cells showed
566 enhanced T-BET and Ki67 following anti-PD-1/PDL-1 but no reinvigoration of IFN γ or
567 TNF α production ¹⁵². Importantly, immunization of mice with epitopes for two TCR-
568 transgenic CD8⁺ T cell clones elicited comparable effector responses and migration to
569 the TME for corresponding adoptively co-transferred cells. However, cells specific for
570 the persistently (but not transiently) expressed neoantigen selectively developed
571 dysfunction; demonstrating that chronic neoantigen exposure rather than elements of
572 the TME were the key drivers of dysfunction ¹⁵². Molecular analysis of these cells
573 illustrated that an overlapping but not identical transcriptional profile existed for chronic
574 viral infection and tumor-specific dysfunctional cells. However, importantly, context-
575 specific differences were evident and tumor-specific CD8⁺ T cells also shared common
576 gene signatures with tolerised CD8⁺ T cells. The molecular basis of the aberrant
577 response showed that, relative to Teff cells, late dysfunctional cells exhibited
578 diminished key effector and memory transcription factors (*Eomes*, *Tbet*), with
579 progressive loss of genes involved in memory differentiation (*Tcf7*, *Foxo1*) and
580 attenuated expression of regulators of T_{RM} cell fate commitment (*Klf2*, *S1pr1*) whilst
581 at day 8 Teff cells up-regulated anergy or hypofunction related loci (*Egr2*, *E2f1*, *E2f2*)
582 ^{152,164}. At Day 34 memory CD8⁺ T cells upregulated multiple genes that were also
583 enriched in late stage patient melanoma samples. These included transcription factors
584 (e.g. *Blimp-1*, *Batf*, *Dusp1*), TCIR (*Ctla4*, *Lag3*, *CD137*) and down regulation of
585 memory and quiescence factors (*Tcf7*, *Foxo1*, *Bach2*) ¹⁵². Another difference to the
586 LCMV chronic infection model was the progressive loss of both *Tbet* and *Eomes*
587 expression in the tumor-specific dysfunction setting, which differed from the switch of
588 Tbet^{hi}PD-1^{int} into Tbet^{lo} EOMES^{hi}PD-1^{hi} discussed in previous sections of this review
589 ^{115,152}. Given that loss of EOMES and T-bet are necessary for CD103 and IL-15R

590 expression in Trm development (see **Tissue resident memory CD8⁺ T cells** section
591 above), this observation may reflect activity of the Trm program in solid tumors.

592 Since CD8⁺ T cells in inducible neoantigen cancer models exhibit a truncated effector
593 phase, it remains possible that memory CD8⁺ T cell generation and CD8⁺ T cell
594 dysfunction occur in the absence of canonical fate commitment, and that memory cells
595 are formed without complete effector de-differentiation ¹¹. Results from these models
596 suggest that tumor-specific CD8⁺ T cell dysfunction represents a unique program of
597 differentiation, distinguishable from acute/chronic infection, or tolerance that is caused
598 by chronic neoantigen exposure in the TME. How this molecular program of
599 dysfunction is altered in models testing neoepitopes derived from mutated self-
600 proteins (that may have a broad range of affinities) remains to be seen. Work from
601 Schietinger's group has subsequently shown that the irreversible dysfunction in this
602 model is linked with epigenetic reprogramming and a fixed chromatin state ¹⁵¹. In this
603 report, changes in epigenetic landscape occur during the first 14 days (plastic state)
604 and not thereafter (fixed state), whilst PD-1 expression steadily increases. The fixed
605 state was consistent with inaccessible enhancer regions at the *Ifng* and *Tcf* family loci
606 whilst accessibility to the *Pdcd1* locus and predicted NFATC1- binding sites of anergy-
607 inducing (*Egr1/2*) or TCIR-encoding loci was increased ¹⁵¹. A crucial finding in this
608 report was that adoptively transferred memory CD8⁺ T cells also underwent rapid
609 dysfunction upon neoantigen exposure, implying that even the development of
610 functional memory may not overcome tumor-specific CD8⁺ T cell dysfunction. The
611 fixed chromatin state was also seen in human tumor infiltrating lymphocytes (TIL) from
612 melanoma and NSCLC (a common feature between species being altered *Tcf7*
613 accessibility) and correlated with the presence of surface markers, including co-
614 expression CD38 and CD101, which marked cells unable to respond to stimulation,

615 although a minor subset in these cultures were still able to produce cytokine (i.e. the
616 CD38-CD101- cells). Treatment of dysfunctional CD8⁺ T cells with IL-15 *in vitro* did
617 not reverse dysfunction, however it has been shown that IL-15 only epigenetically
618 altered specific loci (*Tcf7*) in CD8⁺ T cells that convert from a Tscm/Tcm to Tem cell
619 phenotype during homeostatic proliferation³⁰, and thus intuitively would be insufficient
620 to completely reverse dysfunction. In line with both of these findings, IL-15 has been
621 shown to sustain rather than reverse exhausted CD8⁺ T cell pools at the tumor site
622 ¹⁶⁵.

623 A combined inference of work in mouse models of cancer is therefore that chronic
624 antigen stimulation and negative co-inhibitory signaling appear to produce a positive
625 feedback loop reinforcing tumor specific CD8⁺ T cell dysfunction to a stabilized
626 epigenetic state of CPI non-responsiveness. Moreover, where effective, the
627 antagonism of single or multiple negative signaling cascades (e.g. the PD-1:BATF
628 module) may not re-shape Tex cells *per se* but offer transient reprieve from one of the
629 central orchestrators of the dysfunctional program, without altering remodeled
630 chromatin, as demonstrated in the mouse model of LCMV¹²². This theme may be
631 imperative to improving long-term memory T cell responses. It is perhaps of crucial
632 relevance that murine dysfunctional NART cells had gene expression profiles that
633 showed considerable overlap with MART-1 specific CD8⁺ T cells isolated from late
634 stage human cancers¹⁵². This speaks to a vast amount of data attesting that tumor
635 specific CD8⁺ T cell dysfunction is also a major feature and therapeutically critical facet
636 of T cells in human cancer.

637 **Memory T cell subsets in tumor immunity: Studies in clinical samples**

638 TILs isolated from colon, renal, lung, ovarian, bladder and melanoma tumors have
639 been phenotyped using various combinations of markers to define activation status
640 (e.g. HLA-DR, CD38, Ki67), cytotoxicity (PRF, GZMb), transcription factor profile
641 (EOMES, Tbet), tissue residency (CD69, CD103) and linear differentiation (CD45RA,
642 CCR7, CD27, CD28) ^{166,167}. The majority of tumor infiltrating CD8⁺ T cells exhibit
643 dysfunction-associated phenotypes, including broad and intense TCIR expression
644 (e.g. PD-1, LAG-3, TIM-3 and TIGIT) ¹⁰⁵. For example, in clear cell renal cell carcinoma
645 TILs exhibited increased markers of residency (CD69), activation (CD38) and TCIR
646 (ICOS, LAG-3, PD-1, TIM-3) relative to T cells in normal tissue ¹⁶⁷. In non-small cell
647 lung cancer (NSCLC) the frequency of GZMb⁺CD8⁺ T cells in early lung
648 adenocarcinoma was decreased relative to adjacent lung, whilst CD8⁺PD-1⁺ T cell
649 frequency was increased ¹⁶⁶. A separate study in early stage NSCLC revealed that
650 relative to adjacent tissue, tumor lesions contained increased activated (HLA-DR⁺),
651 Tem cells that co-expressed PD-1, Tim-3, CTLA-4, LAG-3 and TIGIT that were largely
652 KLRG1⁻CD127⁻, with PD-1⁺ cells specifically enriched for activation markers and
653 TCIRs (TGIT, TIM-3, CD137, CD38 and Ki67), but displaying lower EOMES
654 expression ¹⁶⁸. In this investigation, increased activation of CD8⁺ T cells was observed
655 relative to normal tissue, and to a greater extent in current or ex-smokers compared
656 to never smokers. Despite TCIR expression, CD8⁺ T cells appeared capable of
657 synthesizing IFN γ and IL-2 in response to synthetic stimuli (PMA/Io) and IL-2 in
658 response to autologous tumor antigens, suggesting that CD8⁺ T cells may be
659 preferentially activated in response to mutagens but that functional competence is
660 retained or can be recovered at the tumor site by at least a subset of cells ¹⁶⁸. In terms
661 of linear differentiation Kargl et al. showed that CD8⁺ TIL in NSCLC were
662 predominantly Tem, with a smaller population of Temra cells and that lung

663 adenocarcinoma (LUSC) had a higher Temra to Tem cell ratio compared to LUSC ¹⁶⁹.
664 CD8⁺ T cells from NSCLC in a second report were shown to be of a Tem or Temra cell
665 phenotype and able to produce IFN γ and IL-2 upon PMA/Ionomycin stimulation
666 following IL-2 pre-treatment ¹⁷⁰. In melanoma, two reports showed that CD8⁺ T cells
667 were largely CD45RO⁺CCR7⁻CD27⁺CD28⁺ (Tem1) though a smaller Temra cell
668 population were present ¹⁷¹. Moreover, in patients with advanced melanoma, NY-
669 ESO-1-specific memory CD8⁺ T cells displayed a dysfunctional phenotype
670 (CD45RO⁺CCR7⁻TIM-3⁺PD-1⁺) and lower *in vitro* production of IFN- γ , TNF- α and IL-2
671 compared to TIM-3⁻PD-1⁺ and TIM-3⁻PD-1⁻ CD8⁺ T cells ^{172,173}. In clinical specimens,
672 activation markers, TCIRs expression and a Tem cell phenotype therefore appear to
673 be associated with exposure to, or specificity for tumor antigens.

674 Intriguingly, a report by Baitsch et al, showed that virus-specific and vaccine-induced
675 CD8⁺ T cells specific to Melan-A/MART-1 melanoma antigens in the periphery
676 exhibited small but significant differences (higher expression of TIM3 and CTLA4 but
677 lower XCL1 in the latter) though both were noted to be late differentiated effector cells.
678 Tumor-specific CD8⁺ T cells in the tumor infiltrated lymph nodes however, showed
679 preferential overlap with LCMV-derived Tex cells, suggesting that tumour specific T
680 cell exhaustion or dysfunction is localised to the tissue site, but not a feature of cells
681 within the circulation ¹⁷⁴. Two articles from Rosenberg's lab identified that PD-1⁺ CD8⁺
682 T cells contained tumor-specific pools in melanoma. Firstly, it was discovered that
683 Melan-A/MART1 specific CD8⁺ T cells were predominantly (though not exclusively)
684 PD-1⁺. In this report, PD-1 expression tracked with signs of ongoing activation (HLA-
685 DR, CTLA-4, Ki67) and *ex vivo* dysfunction (lower IFN γ and IL-2 production) ¹⁷⁴. In the
686 second, report PD-1⁺CD45RO⁺CD8⁺ Tem cells in the blood were found to contain
687 circulating tumor-reactive CD8⁺ T cells that recognize neoantigens in the tumor ¹⁷⁵. In

688 agreement with this we have also identified NART cells in the tumor of NSCLC
689 patients, and found these cells to be largely PD-1⁺, with heterogenous expression of
690 LAG-3, GZMb and CTLA-4¹⁴⁷. These findings therefore confirm that PD-1 expression
691 coincides with tumor reactivity in humans and extends this to include NART cells.

692 In NSCLC it was shown that the level of co-expression of TCIRs on memory CD8⁺ T
693 cells associates with stage of disease and loss of functional competence, but that
694 CD8⁺ T cells expressing intermediate levels of TCIRs may retain function¹⁷⁶. Merad's
695 group showed that CD8⁺PD-1⁺ T cells correlated with TCR clonality, whilst Kargl et al
696 demonstrated that tumor-specific (private) clonal expansion was correlated with *in*
697 *vitro* reactivity to autologous tumor cells^{166,169}. Collectively, these findings imply that
698 activation and/or exhaustion correlates with clonal expansion to tumor antigens¹⁶⁹.
699 Recently, scRNAseq analysis was used to deconvolute the multicellular ecosystem of
700 the TME in melanoma in a report by Garraway's group. In this study, TCR expansion
701 was associated with enrichment of an exhaustion molecular signature, further
702 underlining that clonal expansion may predispose commitment to a dysfunctional state
703¹⁷⁷. An in-depth scRNAseq and TCRseq profile from PBMC, adjacent tissue and TILs
704 of six patients with hepatocellular carcinoma has supported this model where
705 expanded clonotypes enrich for exhaustion and suggested a cell fate trajectory from
706 naïve > Tem > Tex cells may occur in liver cancer¹⁷⁸. Interestingly, this report paid
707 attention to two subsets of CD8⁺ T cells that have been ill defined in human tumors;
708 mucosal associated invariant T (MAIT) cells and an intermediate subset of *GZMK*-
709 expressing cells positioned between the effector and Tex state¹⁷⁸. The study of MAIT
710 cells in tumors remains in its infancy, but has been recently reviewed elsewhere¹⁷⁹.
711 Clearly much work is required to reconcile these potential pathways of differentiation
712 with programs of gradual dysfunction observed in pre-clinical data. Two important

713 conclusions can be drawn from the data on memory CD8⁺ T cells in human samples;
714 i) Tumor reactivity is linked to a Tem cells expressing TCIRs (but not TEMRA cells,
715 which have lower TCIR expression) ii) Clonal expansion or disease progression
716 predicts T cell dysfunction. However, this does not explain the multitude of other
717 dynamic states that memory CD8⁺ T cells appear to adopt within human TILs,
718 especially those unveiled by recent scRNAseq studies. Indeed, the co-existence of
719 phenotypically diverse, antigen experienced CD8⁺ T cells is a common observation in
720 human TILs. Whilst a consistent finding is the presence of dysfunctional CD8⁺ T cells,
721 less attention has been paid to tumor-specific CD8⁺ T cells within the TME and blood
722 that retain *ex vivo* cytotoxicity consistent with functional Teff or Tem cells. Hallmarks
723 of these cells are i) an ability to circulate in the periphery ii) more primitive states linear
724 of differentiation (e.g. CD27⁺ or CD28⁺), ii) lower degrees of dysfunction, as shown by
725 decreased TCIRs expression, iii) the presence of activation markers and iv) *ex vivo*
726 cytolytic or Teff cell function ^{167,168,171,176,180}. These studies and others, therefore
727 suggest that, like viral infections, tumor-specific memory CD8⁺ T cells may be present
728 in solid cancers at multiple stages of differentiation and that an earlier stage of
729 differentiation may predict function. This paradigm is consistent with mouse models of
730 checkpoint blockade discussed above, where less differentiated (plastic) cells,
731 comprised the subset responsive to anti-PD-1 therapy compared to stably
732 dysfunctional cells ^{121,152}.

733 The enhanced activity of less antigen-experienced T cells can be extrapolated to the
734 setting of adoptive transfer. Results of pre-clinical experiments using Tcm and Tem
735 cell subsets (generated by IL-15 or IL-2 *in vitro*, respectively) showed that less
736 differentiated (lymph node-homing Tcm cells) had superior anti-tumor activity,
737 suggesting that expansion of a progenitor population is required to supply the anti-

738 tumor response (possibly by retaining non-exhausted pools) ¹⁸¹. Consistent with this,
739 infusions of human Teff cells bearing ectopic TCRs were inferior to Tcm cells of the
740 same specificity *in vivo*, with the latter giving rise to Teff and memory CD8⁺ T cells ¹⁸².
741 Furthermore, in a T cell competent patient-derived xenograft (PDX) mouse model,
742 adoptively transferred Tcm and Tem cells derived from breast cancer infiltrate and
743 rejected tumors ¹⁸³. *In vitro* generated Tscm cells transferred into lymphodepleted
744 mice have also showed enhanced capacity to mediate rejection of melanoma tumors
745 compared to Tcm and Tem cells ²⁶. In this report, the authors suggest that, given their
746 lower TCR signaling upon antigen recognition, Tscm cells survive better in
747 environments with persistent antigen stimulation such as tumors, potentially resisting
748 entry into a dysfunctional state. In the clinic, TIL therapy of metastatic melanoma
749 showed that infusions of polyclonal TIL with superior T cell persistence correlated with
750 better clinical outcome ¹⁸⁴, and that TIL retaining a 'young' (CD27⁺CD28⁺ expression,
751 longer telomeres) phenotype can mediate regression in melanoma ¹⁸⁵. On aggregate,
752 these data indicate that less differentiated, circulating memory CD8⁺ T cell subsets of
753 humans and mice exhibit favorable anti-tumor activity *in vivo*.

754 Remarkably, it has also been shown that peripheral activation of effectors may be
755 integral for the success of immunotherapy. Recent data from Nolan and Engleman's
756 laboratories demonstrated that sustained systemic immunity across different tissues
757 is required for tumor rejection in a range of immunotherapy models ¹⁸⁶. These pre-
758 clinical data, and the transient rewiring of Tex cells described by Pauken et al. may
759 explain the temporary clinical response observed in an anti-PD-1 treated NSCLC
760 patients, decline of which coincided with the contraction and dysfunction of NART cells
761 in the blood ¹⁴¹. Intratumoral expansion of Tem cells also was seen to associate with
762 response to anti-PD-1 therapy in clinical samples, however it is not evident whether

763 these cells were dysfunctional prior to therapy or emerged from increased migration
764 of newly primed cells into the tumor ¹⁸⁷. Of note, a high frequency of CD27⁺CD28⁺
765 Tem cells in the blood of late stage Ipilimumab-treated patients also correlated with
766 response rate and overall survival whilst Temra cells frequency negatively associated
767 with overall survival ¹⁸⁸. Furthermore, in clear cell RCC CD8⁺ T cells with lower levels
768 of activation markers and TCIR (termed immune silent or activated) in the tumor were
769 linked to disease-free survival, whilst cells exhibiting co-expression of multiple TCIR
770 (immune regulated) were associated with worse outcome ¹⁶⁷. Work from Wherry's
771 group additionally suggested that activation or reinvigoration of circulating cells is
772 associated with clinical response to anti-PD-1 ¹⁶⁷. Several correlative *in silico* studies
773 further support that intratumoral Tem cells or activated Teff cells may offer protection
774 in primary disease as well as following CPI treatment. Charaentong et al. have made
775 *in silico* predictions that suggest activated CD8⁺ T cells could be major substrates for
776 immune checkpoint inhibition (CPI) in solid tumors, whilst Tem cells could be important
777 for control of primary disease ¹⁸⁹. This is in accord with previous work highlighting a
778 correlation of Tbet expression and Tem cell signatures with clinical outcome in solid
779 tumors ^{190,191}. These data therefore suggest that i) Tscm and Tcm cells capable of
780 differentiating into Teff cells are the most potent memory T cell subsets for tumor
781 rejection in adoptive cell therapy (due to their enhanced persistence, expansion,
782 lymph-node homing and resistance to dysfunction). ii) T cell subsets associated with
783 survival in primary disease and CPI are Teff, Tem and activated CD8⁺ T cells. iii)
784 Dysfunctional CD8⁺ T cells and Temra cells may appear later in disease or negatively
785 associate with outcome.

786

787 The current body of T cell profiling data from solid cancer specimens raises several
788 central questions, especially when considered in the context of basic T cell
789 immunology and murine tumor models. Firstly, what are the cellular, molecular, clinical
790 and tumor-associated factors which determine T cell differentiation in the anti-tumor
791 response in humans? The current data suggest that clinical stage, clonal T cell
792 expansion, metastasis, stromal architecture (e.g. presence of tertiary lymphoid
793 structures) histological subtype or mutagen exposure may influence the level of
794 dysfunction or activation, but beyond that there is little evidence ^{166,169,171,176}. A second
795 question is whether T cell differentiation links with immune editing? Clonal expansion
796 in melanoma and liver cancer TIL was linked to an 'exhausted' molecular profile by
797 scRNAseq or high PD-1 expression, whilst loss of heterozygosity at HLA alleles in non
798 small cell lung cancer (NSCLC) associated with an increased cytolytic score,
799 suggesting that tumor antigen recognition exerts selection pressure to alter the tumor
800 genomic landscape and synchronously shapes co-evolution of memory CD8⁺ T cell
801 differentiation ^{149,177,178}. Moreover, therapeutic NART cell infusion clearly causes loss
802 of neoantigen presentation by tumors, demonstrating that selection pressure can drive
803 evolutionary tumor escape ¹⁴⁹. Thus, existing evidence supports that clonal expansion
804 and immune editing likely co-evolve, associated with increased PD-1 expression.
805 Related to this it is worth considering that CD8⁺ T cells specific for edited or lost
806 neoantigens may persist in the TME. Indeed, although Tex cells in viral infections are
807 maintained after antigen withdrawal, the turnover of tumor reactive cells in humans is
808 uncertain, and tissue resident populations such as those in the lung in fact experience
809 rapid attrition ⁶⁶. This becomes particularly cogent when considering the impact of
810 surgery on immune memory and in the context of clinical decisions to offer adjuvant
811 or neoadjuvant CPI, i.e. will removal of the main source of antigen impede formation

812 of memory following treatment and/or will Tex cells recover? Longitudinal studies will
813 likely determine this conundrum. Thirdly does the nature of antigen shape the T cell
814 response? T cells in cancer may recognise tumor-associated, tumor-specific or viral-
815 derived and mutation-encoded neoantigens. However, whether T cells recognising
816 these antigens adopt phenotypes consistent with divergence of tolerance induction
817 (i.e. due to degrees of self-similarity, or resemblance to viral epitopes) or
818 chronicity/level/dosage of exposure (i.e. ubiquitous truncal neoantigens present in
819 every tumor cells and thus appearing early in tumor development compared to branch,
820 sub clonal antigens that may appear later in tumor evolution) remains to be seen
821 ^{147,150,192}. In this regard it is interesting that the burden of clonal neoantigens and high
822 affinity frameshift insertion and deletion encoded neoantigens associate with response
823 to checkpoint blockade, yet how the pool of cells fostered by these favourable genomic
824 landscapes differs from low mutational burden patients is largely unknown ^{147,193}.
825 Fourthly, what is the differentiation program, ontogeny and fate of tumor reactive
826 memory CD8⁺ T cells in humans? The limited data on phenotypes of NART cells and
827 MART-1/Melan-A in humans suggest high TCIR expression but also heterogeneity,
828 provoking the idea that specific tumor reactive clonotypes may differentiate from
829 functional and dysfunctional states ^{147,174}. In acute viral infection and vaccination, we
830 have discussed that memory CD8⁺ T cells emerge from effector de-differentiation ¹¹.
831 *In vivo*, NART cells appear to become activated then rapidly and progressively adopt
832 dysfunctional states ¹⁵². In human TILs we find Tem, Temra and Teff cells and
833 phenotypically dysfunctional cells some of which may be connected by clonotype.
834 Thus, there appears to be a cell fate trajectory in tumor specific cells that is vastly
835 different cells differentiating in optimal conditions of immune memory (acute viral
836 infection) that leads to a spectrum of differentiation whereby less antigen experienced

837 cells (e.g. those recently migrated to the TME) are functional and those with prolonged
838 antigen exposure gradually acquire high TCIRs expression. The multitude of
839 phenotypes emerging from high dimensional flow cytometry and scRNAseq analysis
840 may also arise from different priming environments (tumor or APC in situ vs lymph
841 node) have different specificities (for tumor versus common pathogens) and/or be
842 interconvertible. Regardless of the pathway of differentiation, the ultimate cell fate of
843 tumor specific T cells in humans requires better definition. Tumor driven T cell
844 dysfunction appears to be distinguishable from classical T cell exhaustion in viral
845 infections, consequently the level of assumed dysfunction in these cells requires full
846 clarification. Fifth, connected to this is whether there such thing as a tumor-reactive T
847 cell phenotype? There is certainly a predisposition of pathogens to evoke responses
848 dominated by specific T cell subsets. For example, in humans, the majority of
849 respiratory syncytial virus (RSV), influenza (Flu) and Epstein bar virus (EBV) specific
850 CD8⁺ T cells show a Tem1 (CD45RA⁻CCR7⁻CD28⁺CD27⁺) profile, whilst HIV-specific
851 CD8⁺ T cells tend to be Tem 2-3 and CMV-specific CD8⁺ T cells split between Tem1
852 or Temra ¹⁹⁴. Furthermore, clonal dominance may influence this hierarchy, since CMV-
853 specific CD8⁺ T cells show a different phenotype in healthy vs HIV infected individuals
854 (increased Temra cells in the latter) ⁴². For tumor specific T cells this is less clear,
855 multimer technologies are an immensely powerful tool, being implemented widely and
856 expertly in the study of NART cells and other tumor specific T cell populations, but the
857 current data does not provide a consensus on a tumor reactive phenotype ¹⁹⁵. Despite
858 PD-1 and TCIR co-expression proving useful to enrich for tumor specificity, these
859 markers are also expressed on activated cells ¹⁷⁵. Co-expression of multiple TCIR, the
860 presence of CD38 and CD101 or high levels of PD-1 expression perhaps may be the
861 most accurate predictors of tumor reactivity, since these are a feature of chronic

862 stimulation, not likely to be shared by bystander cells ¹⁹⁶. However, these phenotypes
863 likely only enrich for a subset of tumor reactive cells which are dysfunctional. Recently
864 primed or functional anti-tumor Teff cells and circulating Tem cells present in the TME,
865 blood, adjacent tissue or LN may be more challenging to distinguish given that such
866 phenotypes are common to viral specific T cell populations. Indeed, although
867 pathways of differentiation may be gleaned from coupled TCRseq and scRNAseq
868 analysis of CD8⁺ T cells in human TILs, an integral missing component to these data
869 is antigen specificity. Finally, which T cell subset elicits therapeutic responses to CPI?
870 This will clearly depend on the TCIR targeted and the context. For anti-PD-1/PD-L1
871 evidence suggests that intratumoral T cells with high TCIR expression expand in the
872 TME ¹⁸⁷. On the other hand the most exhausted PD-1^{hi} cells in tumor and chronic
873 infection settings appear to be refractory to rescue and responses in the clinic and *in*
874 *vivo* rather associate with peripheral effector cell expansion ^{140,185,121,151,152}. It remains
875 possible therefore that several subsets are mobilised in response to anti-PD-1, but
876 that de-repression of a key non-exhausted effector pool facilitates durable clinical
877 benefit.

878 Whilst functionally relevant TCRs may be recovered from cells expressing high TCIR
879 levels, the intrinsic dysfunction of such populations may limit their utility in adoptive
880 cell transfer. This limitation would be evident both when preventing *in vitro* expansion
881 and by an inability to induce a response *in vivo* via compromised persistence or
882 inability to recirculate to the LN to serve as progeny. Efforts to reverse exhaustion may
883 assist in generation of functional TIL products from such populations, and may include
884 cytokines (e.g. IL-21), agonistic antibodies or epigenetic modifiers ^{126,197,198}. Although
885 potentially not as efficient, reversal of exhaustion *in vitro* may be possible with current
886 methods of rapid expansion, given the ability of several groups to detect neo-antigen

887 reactivity in expanded products, though it is uncertain if these cells were Tex or
888 functional *ex vivo* ¹⁹⁹. Engineering of exhaustion-resistant TILs (i.e. use of CRISPR
889 technology to remove TCIRs) for adoptive cell therapy, or combining adoptive cell
890 therapy with checkpoint blockade may avoid recrudescence of T cell dysfunction and
891 improve the efficacy of cellular cancer therapeutics ²⁰⁰. Whilst these findings underline
892 the crucial contribution of circulating CD8⁺ T cell in anti-tumor immunity, emerging
893 evidence also points towards a key role for Trm cells in some cancers.

894 **Tissue resident memory cells in anti-tumor immunity**

895 The role of Trm cells in tumor protection is yet to be fully discerned. Two reports in
896 mouse models of melanoma indicate these cells may have anti-tumor activity. Trm
897 cells driven by autoimmune vitiligo were shown to protect from melanoma in a CD103-
898 dependent manner ²⁰¹ and OVA-encoding vaccinia virus was shown to generate Trm
899 cells that delayed growth of OVA-expressing melanoma ⁶⁷. The prevalence, if not
900 relevance of Trm cells in clinical specimens however, is clear. Tumor samples from
901 patients with ovarian, endometrial, breast and lung cancers exhibit infiltration of
902 CD8⁺CD103⁺ TIL, the abundance of which correlates with prolonged survival and
903 better prognosis ²⁰²⁻²⁰⁶. Counterintuitively, in ovarian and lung tumors, the
904 CD103⁺CD8⁺ TIL subset express the highest levels of inhibitory immune checkpoints
905 such as PD-1, TIM-3, CTLA-4 and LAG-3, indicating that Trm cells may preferentially
906 adopt a dysfunctional phenotype, likely due to chronic antigen stimulation ²⁰⁷.
907 However, it is not certain whether a subset of Trm cells in tumors retain functionality
908 ^{204,206}. Although a CD103⁺CD8⁺ T cell signature associated with prolonged survival in
909 NSCLC, and total CD8⁺ T cells from CD103^{hi} TIL produced increased GZMb, no
910 difference was observed between CD103⁺ and CD103⁻ CD8⁺ T cells in the production
911 of GZMb, IFN γ or CD107, and PFN expression was lower in CD103⁺ cells ²⁰⁶. This

912 implies that CD103⁺CD8⁺ T cell accumulation may, like intense PD-1 expression,
913 reflect a history of cells with previous effector function that have converted to Trm/ Tex
914 cells, or that Trm cells confer a survival advantage through indirect mechanisms.
915 Interestingly, a major function of Trm cells is recruitment of cells from the circulation
916 ^{51,72,73}. Given the significant role of circulating Teff or Tem cells in anti-tumor immunity,
917 it remains possible that Trm cells confer protection via recruitment of bystander
918 circulating, tumor-specific T cells. Indeed, this mechanism may contribute to
919 heterosubtypic immunity in influenza models and may facilitate the immigration of
920 recently primed effectors from the tumor draining lymph nodes ⁷⁴.

921 In melanoma tumors, nearly 60% of all CD8⁺ T cells have a CD45RO⁺CD69⁺CCR7⁻
922 phenotype with nearly 50% being CD103⁺ ²⁰⁷. However, the presence of Trm cells in
923 melanoma tumors has not been correlated with enhanced survival or better prognosis,
924 suggesting an unknown mechanism by which a Trm cell phenotype is associated with
925 good prognosis and survival in some types of tumors while not in others ²⁰⁷. This could
926 be accounted for by a difference in subsets of Trm cells and their relative ability for
927 cytotoxicity, dysfunction retention/ turnover and recruitment at different sites. Both
928 mouse Trm cells in the lung and Trm cells of NSCLC samples were shown to have
929 increased sensitivity to apoptosis, a feature of lung Trm cell biology linked to
930 maintenance of antigen diversity and prevention of autoinflammatory tissue damage
931 at this sensitive host site ^{66,208}. Direct, *ex vivo* analysis of lung Trm cells has shown
932 that IL-2 can selectively induce cytotoxic features in CD103⁺ Trm cells, and that
933 blocking CD103 reduced *in vitro* lysis of autologous targets in the context of PD-1/PD-
934 L-1 blockade, suggesting that in the appropriate cytokine environment or following
935 CPI, Trm cells become potent anti-tumor effectors ²⁰⁸.

936 A recent report examining the TCR diversity between metastatic lesions of melanoma
937 patients suggests that Trm in tumors are less competent (e.g. lower IFN γ , IL-2) than
938 circulating populations, and that TCR diversity in Trm cells among lesions exceeds
939 that expected by changes in genomic landscape (although this prediction may be
940 challenging)²⁰⁷. It was suggested by the authors of the article that the interlesional
941 diversity in TCR sequences may explain differential responses to checkpoint inhibitors
942 and therefore that Trm cells are a major target of these therapies. Whether Trm cells
943 contribute directly or indirectly to tumor destruction during checkpoint blockade in the
944 clinic is currently unknown. The data above propounds that Trm cells may indirectly or
945 directly promote anti-tumor immunity yet may be selectively prone to TCIRs linked
946 dysfunction, and that the relative contributions of these features may be context and
947 possibly tissue specific.

948 A potential disadvantage of a stable pool of Trm cells in the TME is the retention of
949 cells with a reduced capacity for anti-tumor function, competing for trophic factors with
950 *de novo* primed or functional circulating Tem/Teff cells pools. This may occur due to
951 either cumulative antigen-driven dysfunction, or through maintenance of CD8⁺ T cells
952 with specificity for epitopes that have been edited, down-regulated or lost. A recent
953 report in a breast cancer model suggests that recent arrivals in the tumor exhibit
954 functionality, but that Trm cells established previously are dysfunctional¹⁶⁵. Tumor-
955 reactive Trm cells in this model persisted at the tumor site independent of antigen and
956 were sustained by TAM-derived IL-15, where Trm cells act as a 'sink' for cytokine.
957 This is in keeping with the persistence of Tex cells in the absence of antigen in viral
958 infection *in vivo* and in the clinic. Furthermore, in line with this it is likely that induction
959 of a Trm cell profile in the tumor may both allow recruitment of functional circulating
960 cells, and facilitate direct anti-tumor responses but as these cells become

961 dysfunctional or relevant epitopes are eliminated in later phases, Trm cells may exert
962 a negative impact by occupying the niche and preventing accommodation of more
963 functional or relevant cells (Figure 2). Indeed, it is possible that enforcing the Trm
964 program, which incurs loss of T-bet and EOMES transcription factors and ostensibly
965 reduced Teff potential may be a mechanism of tumor immune evasion^{47,62,76,77}. Two
966 reports this year have shown that existing Trm cells proliferate and give rise to
967 secondary Trm cells upon re-challenge, and that initial seeding populations are not
968 replaced upon recruitment of antigen-specific and bystander populations- implying
969 once more that irrelevant cell specificities may be accrued in the tumor, harnessing
970 cytokine resources at the expense of functional recent arrivals^{72,73}. Furthermore, the
971 second generation of Trm cells in the TME would presumably inherit the inhibitory
972 chromatin landscape of their progenitors, further expanding the dysfunctional pool.
973 Interestingly, a major molecular mechanism of anti-PD-1 is the increase of cell motility,
974 it is thus a possibility that mobilization of Trm cells may favor enhanced intratumoral
975 responses by permitting entry to the niche²⁰⁹. However, the turnover and attrition of
976 Trm cells is site-specific and this may have crucial implications of local anti-tumor
977 responses in different malignancies. Evidence from pulse-chase experiments in the
978 influenza model shows that Trm cells in the lung have a short half-life^{66,210}, and that
979 the gradually waning numbers after infection reflect the net effect of this loss partly
980 counterbalanced by continual reseeding from circulating memory CD8⁺ T cells pools
981⁶¹. Whether this is also true in lung cancer is unknown.

982 Similar to circulating tumor specific-cells, the origin of tumor reactive Trm cells is
983 undefined, though this subset is likely to emerge from the Tem or Tcm cell pool.
984 Whether Trm cells acquire dysfunctional characteristics or whether tumor reactive
985 cells acquire dysfunction and a Trm cell gene expression signature synchronously is

986 also not clear. It is possible that the high concentration of TGF β inside the tumor
987 induces CD103 on cells that do not bear transcriptional hallmarks of Trm cells, or
988 equally that TGF β driven *bona fide* Trm cell formation is directly accountable for
989 increased frequency of Trm cells in solid tumors relative to adjacent tissue ^{55,206,208,211}.
990 Further research to converge the nascent fields of Trm cell biology and T cell
991 dysfunction is required to better define this process and the role of Trm cells in tumor
992 immunity. In keeping with the 'streetlight hypothesis', our current attention may be
993 guided to analysis of effector functions common to circulating CD8⁺ T cells, whilst thus
994 far under-appreciated facets of Trm cell biology may be more significant in the anti-
995 tumor response.

996

997 **Conclusion**

998 The generation and maintenance of CD8⁺ T cell memory subsets is crucial to host
999 survival. Dysregulation of the central orchestrators in these networks leads to
1000 defective immune memory and host pathology. Recent work has made evident the
1001 complexity of memory T cell ontogeny, epigenetic reprogramming and the
1002 fundamental role that Trm cells play in immediate protection at portals of pathogen
1003 entry. Transposing our evolving knowledge of anti-tumor immunity onto this framework
1004 is a demanding but essential challenge, given the promise of immunotherapy and
1005 clinical need to broaden and optimize its application. The recent pre-clinical data
1006 suggest that following immune checkpoint inhibitors in the clinic i) the inability to revert
1007 the dysfunctional state and ii) the onset of fixed dysfunction in existing or *de novo*
1008 memory cells may both contribute to a lack of durable immune memory ^{122,152}.
1009 However, TIL therapy can lead to durable and complete responses and a minority of

1010 patients receiving immune checkpoint inhibitors experience long-term clinical benefit,
1011 suggesting either or both of these limitations may be overcome. It will be imperative to
1012 monitor memory T cell function in this sub group of patients to decipher the
1013 requirements for generation of functional tumor specific memory.

1014 The reversal of T cell dysfunction and the availability of neoepitopes represent two
1015 recently defined hurdles for tumor reactive memory CD8⁺ T cell maintenance and
1016 generation, respectively. Together with an inhibitory TME and lack of infiltration we
1017 now have four major hurdles to overcome for the development of effective
1018 immunotherapy in solid cancers. Therefore, combinatorial treatments providing i) a
1019 stimulatory priming environment (e.g. TLR agonists) ii) source of antigen (personalised
1020 neoepitope or tumor specific/associated antigen vaccines) or antigen-specific T cells
1021 (e.g. targeting neoepitopes) iii) enhanced infiltration (e.g. anti-TGFb, or anti-VEGF)
1022 and iv) a means to prevent exhaustion and/or regulation (e.g. CPI) may ultimately be
1023 fruitful if proven economically and clinically feasible²¹²⁻²¹⁴. Correspondingly, a high
1024 frequency of endogenous NART effector cells in an immunological 'sweet-spot' that
1025 are effectively primed, but non-exhausted may provide these benefits to prolong
1026 survival during primary disease or enhance responses to CPI. This is consistent with
1027 the mounting evidence which supports a major contribution of systemic activation and
1028 circulating Tem cells in effective anti-tumor responses. Unveiling the mechanisms
1029 which can form and maintain functional, tumor specific effector and memory cells
1030 remains key to the success of next generation immunotherapeutic strategies in solid
1031 cancers.

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1038 **References**

- 1039 1. Henry CJ, Ornelles DA, Mitchell LM, Brzoza-Lewis KL, Hiltbold EM. IL-12
1040 produced by dendritic cells augments CD8+ T cell activation through the
1041 production of the chemokines CCL1 and CCL17. *J Immunol.* 2008;181(12):8576-8584.
1042
- 1043 2. Tian S, Maile R, Collins EJ, Frelinger JA. CD8+ T cell activation is governed by
1044 TCR-peptide/MHC affinity, not dissociation rate. *J Immunol.* 2007;179(5):2952-
1045 2960.
- 1046 3. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help
1047 for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature.*
1048 1998;393(6684):480-483.
- 1049 4. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR. Help
1050 for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature.*
1051 1998;393(6684):478-480.
- 1052 5. Desch AN, Gibbings SL, Clambey ET, et al. Dendritic cell subsets require cis-
1053 activation for cytotoxic CD8 T-cell induction. *Nat Commun.* 2014;5:4674.
- 1054 6. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell
1055 differentiation. *Nat Rev Immunol.* 2012;12(11):749-761.
- 1056 7. Crompton JG, Narayanan M, Cuddapah S, et al. Lineage relationship of CD8(+)
1057 T cell subsets is revealed by progressive changes in the epigenetic landscape.
1058 *Cell Mol Immunol.* 2016;13(4):502-513.
- 1059 8. van Aalderen MC, van den Biggelaar M, Remmerswaal EBM, et al. Label-free
1060 Analysis of CD8(+) T Cell Subset Proteomes Supports a Progressive
1061 Differentiation Model of Human-Virus-Specific T Cells. *Cell Rep.*
1062 2017;19(5):1068-1079.
- 1063 9. Mercado R, Vijh S, Allen SE, Kerksiek K, Pilip IM, Pamer EG. Early
1064 programming of T cell populations responding to bacterial infection. *J Immunol.*
1065 2000;165(12):6833-6839.
- 1066 10. Akondy RS, Fitch M, Edupuganti S, et al. Origin and differentiation of human
1067 memory CD8 T cells after vaccination. *Nature.* 2017;552(7685):362-367.
- 1068 11. Youngblood B, Hale JS, Kissick HT, et al. Effector CD8 T cells dedifferentiate
1069 into long-lived memory cells. *Nature.* 2017;552(7685):404-409.
- 1070 12. Kakaradov B, Arsenio J, Widjaja CE, et al. Early transcriptional and epigenetic
1071 regulation of CD8(+) T cell differentiation revealed by single-cell RNA
1072 sequencing. *Nat Immunol.* 2017;18(4):422-432.
- 1073 13. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective
1074 expression of the interleukin 7 receptor identifies effector CD8 T cells that give
1075 rise to long-lived memory cells. *Nat Immunol.* 2003;4(12):1191-1198.
- 1076 14. Dunkle A, Dzhagalov I, Gordy C, He YW. Transfer of CD8+ T cell memory using
1077 Bcl-2 as a marker. *J Immunol.* 2013;190(3):940-947.
- 1078 15. Joshi NS, Cui W, Chandele A, et al. Inflammation directs memory precursor
1079 and short-lived effector CD8(+) T cell fates via the graded expression of T-bet
1080 transcription factor. *Immunity.* 2007;27(2):281-295.
- 1081 16. Lauvau G, Vijh S, Kong P, et al. Priming of memory but not effector CD8 T cells
1082 by a killed bacterial vaccine. *Science.* 2001;294(5547):1735-1739.
- 1083 17. Maxwell JR, Weinberg A, Prell RA, Vella AT. Danger and OX40 receptor
1084 signaling synergize to enhance memory T cell survival by inhibiting peripheral
1085 deletion. *J Immunol.* 2000;164(1):107-112.

- 1086 18. Sanchez PJ, McWilliams JA, Haluszczak C, Yagita H, Kedl RM. Combined
1087 TLR/CD40 stimulation mediates potent cellular immunity by regulating dendritic
1088 cell expression of CD70 in vivo. *J Immunol.* 2007;178(3):1564-1572.
- 1089 19. Tan JT, Whitmire JK, Ahmed R, Pearson TC, Larsen CP. 4-1BB ligand, a
1090 member of the TNF family, is important for the generation of antiviral CD8 T cell
1091 responses. *J Immunol.* 1999;163(9):4859-4868.
- 1092 20. Ellis TM, Simms PE, Slivnick DJ, Jack HM, Fisher RI. CD30 is a signal-
1093 transducing molecule that defines a subset of human activated CD45RO+ T
1094 cells. *J Immunol.* 1993;151(5):2380-2389.
- 1095 21. Steinberg MW, Huang Y, Wang-Zhu Y, Ware CF, Cheroutre H, Kronenberg M.
1096 BTLA interaction with HVEM expressed on CD8(+) T cells promotes survival
1097 and memory generation in response to a bacterial infection. *PLoS One.*
1098 2013;8(10):e77992.
- 1099 22. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG,
1100 Schoenberger SP. CD4+ T cells are required for secondary expansion and
1101 memory in CD8+ T lymphocytes. *Nature.* 2003;421(6925):852-856.
- 1102 23. Bourgeois C, Rocha B, Tanchot C. A role for CD40 expression on CD8+ T cells
1103 in the generation of CD8+ T cell memory. *Science.* 2002;297(5589):2060-2063.
- 1104 24. Oh S, Perera LP, Terabe M, Ni L, Waldmann TA, Berzofsky JA. IL-15 as a
1105 mediator of CD4+ help for CD8+ T cell longevity and avoidance of TRAIL-
1106 mediated apoptosis. *Proc Natl Acad Sci U S A.* 2008;105(13):5201-5206.
- 1107 25. Kalia V, Penny LA, Yuzefpolskiy Y, Baumann FM, Sarkar S. Quiescence of
1108 Memory CD8(+) T Cells Is Mediated by Regulatory T Cells through Inhibitory
1109 Receptor CTLA-4. *Immunity.* 2015;42(6):1116-1129.
- 1110 26. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-
1111 like properties. *Nat Med.* 2011;17(10):1290-1297.
- 1112 27. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory
1113 T cell subsets: function, generation, and maintenance. *Annu Rev Immunol.*
1114 2004;22:745-763.
- 1115 28. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of
1116 memory T lymphocytes with distinct homing potentials and effector functions.
1117 *Nature.* 1999;401(6754):708-712.
- 1118 29. Willinger T, Freeman T, Hasegawa H, McMichael AJ, Callan MF. Molecular
1119 signatures distinguish human central memory from effector memory CD8 T cell
1120 subsets. *J Immunol.* 2005;175(9):5895-5903.
- 1121 30. Abdelsamed HA, Moustaki A, Fan Y, et al. Human memory CD8 T cell effector
1122 potential is epigenetically preserved during in vivo homeostasis. *J Exp Med.*
1123 2017;214(6):1593-1606.
- 1124 31. Olson JA, McDonald-Hyman C, Jameson SC, Hamilton SE. Effector-like
1125 CD8(+) T cells in the memory population mediate potent protective immunity.
1126 *Immunity.* 2013;38(6):1250-1260.
- 1127 32. Appay V, Dunbar PR, Callan M, et al. Memory CD8+ T cells vary in
1128 differentiation phenotype in different persistent virus infections. *Nat Med.*
1129 2002;8(4):379-385.
- 1130 33. Henson SM, Riddell NE, Akbar AN. Properties of end-stage human T cells
1131 defined by CD45RA re-expression. *Curr Opin Immunol.* 2012;24(4):476-481.
- 1132 34. Ibegbu CC, Xu YX, Harris W, Maggio D, Miller JD, Kourtis AP. Expression of
1133 killer cell lectin-like receptor G1 on antigen-specific human CD8+ T
1134 lymphocytes during active, latent, and resolved infection and its relation with
1135 CD57. *J Immunol.* 2005;174(10):6088-6094.

- 1136 35. Reiser J, Banerjee A. Effector, Memory, and Dysfunctional CD8(+) T Cell Fates in the Antitumor Immune Response. *J Immunol Res.* 2016;2016:8941260.
- 1137
- 1138 36. Zhou X, Yu S, Zhao DM, Harty JT, Badovinac VP, Xue HH. Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1. *Immunity.* 2010;33(2):229-240.
- 1139
- 1140
- 1141 37. Tripathi P, Kurtulus S, Wojciechowski S, et al. STAT5 is critical to maintain effector CD8+ T cell responses. *J Immunol.* 2010;185(4):2116-2124.
- 1142
- 1143 38. Kaech SM, Ahmed R. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol.* 2001;2(5):415-422.
- 1144
- 1145
- 1146 39. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity.* 2007;27(3):393-405.
- 1147
- 1148
- 1149 40. Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J Exp Med.* 2008;205(3):625-640.
- 1150
- 1151
- 1152 41. Wirth TC, Xue HH, Rai D, et al. Repetitive antigen stimulation induces stepwise transcriptome diversification but preserves a core signature of memory CD8(+) T cell differentiation. *Immunity.* 2010;33(1):128-140.
- 1153
- 1154
- 1155 42. van Aalderen MC, Remmerswaal EB, Versteegen NJ, et al. Infection history determines the differentiation state of human CD8+ T cells. *J Virol.* 2015;89(9):5110-5123.
- 1156
- 1157
- 1158 43. Sathaliyawala T, Kubota M, Yudanin N, et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity.* 2013;38(1):187-197.
- 1159
- 1160
- 1161 44. Saule P, Trauet J, Dutriez V, Lekeux V, Dessaint JP, Labalette M. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. *Mech Ageing Dev.* 2006;127(3):274-281.
- 1162
- 1163
- 1164
- 1165 45. van Lier RA, ten Berge IJ, Gamadia LE. Human CD8(+) T-cell differentiation in response to viruses. *Nat Rev Immunol.* 2003;3(12):931-939.
- 1166
- 1167 46. Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol.* 2009;10(5):524-530.
- 1168
- 1169
- 1170 47. Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defence. *Nat Rev Immunol.* 2016;16(2):79-89.
- 1171
- 1172 48. Wu T, Hu Y, Lee YT, et al. Lung-resident memory CD8 T cells (TRM) are indispensable for optimal cross-protection against pulmonary virus infection. *J Leukoc Biol.* 2014;95(2):215-224.
- 1173
- 1174
- 1175 49. Masopust D, Vezys V, Wherry EJ, Barber DL, Ahmed R. Cutting edge: gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J Immunol.* 2006;176(4):2079-2083.
- 1176
- 1177
- 1178 50. Wakim LM, Woodward-Davis A, Bevan MJ. Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. *Proc Natl Acad Sci U S A.* 2010;107(42):17872-17879.
- 1179
- 1180
- 1181 51. Schenkel JM, Fraser KA, Vezys V, Masopust D. Sensing and alarm function of resident memory CD8(+) T cells. *Nat Immunol.* 2013;14(5):509-513.
- 1182
- 1183 52. Thom JT, Weber TC, Walton SM, Torti N, Oxenius A. The Salivary Gland Acts as a Sink for Tissue-Resident Memory CD8(+) T Cells, Facilitating Protection from Local Cytomegalovirus Infection. *Cell Rep.* 2015;13(6):1125-1136.
- 1184
- 1185

- 1186 53. Steinert EM, Schenkel JM, Fraser KA, et al. Quantifying Memory CD8 T Cells
1187 Reveals Regionalization of Immunosurveillance. *Cell*. 2015;161(4):737-749.
- 1188 54. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection
1189 generates non-migratory memory CD8+ T(RM) cells providing global skin
1190 immunity. *Nature*. 2012;483(7388):227-231.
- 1191 55. Mackay LK, Rahimpour A, Ma JZ, et al. The developmental pathway for
1192 CD103(+)/CD8+ tissue-resident memory T cells of skin. *Nat Immunol*.
1193 2013;14(12):1294-1301.
- 1194 56. Cepek KL, Shaw SK, Parker CM, et al. Adhesion between epithelial cells and
1195 T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. *Nature*.
1196 1994;372(6502):190-193.
- 1197 57. Grundemann C, Bauer M, Schweier O, et al. Cutting edge: identification of E-
1198 cadherin as a ligand for the murine killer cell lectin-like receptor G1. *J Immunol*.
1199 2006;176(3):1311-1315.
- 1200 58. Mackay LK, Braun A, Macleod BL, et al. Cutting edge: CD69 interference with
1201 sphingosine-1-phosphate receptor function regulates peripheral T cell
1202 retention. *J Immunol*. 2015;194(5):2059-2063.
- 1203 59. Wakim LM, Woodward-Davis A, Liu R, et al. The molecular signature of tissue
1204 resident memory CD8 T cells isolated from the brain. *J Immunol*.
1205 2012;189(7):3462-3471.
- 1206 60. Kumar BV, Ma W, Miron M, et al. Human Tissue-Resident Memory T Cells Are
1207 Defined by Core Transcriptional and Functional Signatures in Lymphoid and
1208 Mucosal Sites. *Cell Rep*. 2017;20(12):2921-2934.
- 1209 61. Hombrink P, Helbig C, Backer RA, et al. Programs for the persistence, vigilance
1210 and control of human CD8(+) lung-resident memory T cells. *Nat Immunol*.
1211 2016;17(12):1467-1478.
- 1212 62. Mackay LK, Wynne-Jones E, Freestone D, et al. T-box Transcription Factors
1213 Combine with the Cytokines TGF-beta and IL-15 to Control Tissue-Resident
1214 Memory T Cell Fate. *Immunity*. 2015;43(6):1101-1111.
- 1215 63. Mackay LK, Minnich M, Kragten NA, et al. Hobit and Blimp1 instruct a universal
1216 transcriptional program of tissue residency in lymphocytes. *Science*.
1217 2016;352(6284):459-463.
- 1218 64. Skon CN, Lee JY, Anderson KG, Masopust D, Hogquist KA, Jameson SC.
1219 Transcriptional downregulation of S1pr1 is required for the establishment of
1220 resident memory CD8+ T cells. *Nat Immunol*. 2013;14(12):1285-1293.
- 1221 65. Milner JJ, Toma C, Yu B, et al. Runx3 programs CD8(+) T cell residency in non-
1222 lymphoid tissues and tumours. *Nature*. 2017;552(7684):253-257.
- 1223 66. Slutter B, Van Braeckel-Budimir N, Abboud G, Varga SM, Salek-Ardakani S,
1224 Harty JT. Dynamics of influenza-induced lung-resident memory T cells underlie
1225 waning heterosubtypic immunity. *Sci Immunol*. 2017;2(7).
- 1226 67. Enamorado M, Iborra S, Priego E, et al. Enhanced anti-tumour immunity
1227 requires the interplay between resident and circulating memory CD8(+) T cells.
1228 *Nat Commun*. 2017;8:16073.
- 1229 68. Laidlaw BJ, Zhang N, Marshall HD, et al. CD4+ T cell help guides formation of
1230 CD103+ lung-resident memory CD8+ T cells during influenza viral infection.
1231 *Immunity*. 2014;41(4):633-645.
- 1232 69. Rosato PC, Beura LK, Masopust D. Tissue resident memory T cells and viral
1233 immunity. *Curr Opin Virol*. 2017;22:44-50.

- 1234 70. Ariotti S, Hogenbirk MA, Dijkgraaf FE, et al. T cell memory. Skin-resident
1235 memory CD8(+) T cells trigger a state of tissue-wide pathogen alert. *Science*.
1236 2014;346(6205):101-105.
- 1237 71. Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. T cell
1238 memory. Resident memory CD8 T cells trigger protective innate and adaptive
1239 immune responses. *Science*. 2014;346(6205):98-101.
- 1240 72. Park SL, Zaid A, Hor JL, et al. Local proliferation maintains a stable pool of
1241 tissue-resident memory T cells after antiviral recall responses. *Nat Immunol*.
1242 2018;19(2):183-191.
- 1243 73. Beura LK, Mitchell JS, Thompson EA, et al. Intravital mucosal imaging of
1244 CD8(+) resident memory T cells shows tissue-autonomous recall responses
1245 that amplify secondary memory. *Nat Immunol*. 2018;19(2):173-182.
- 1246 74. Zens KD, Chen JK, Farber DL. Vaccine-generated lung tissue-resident memory
1247 T cells provide heterosubtypic protection to influenza infection. *JCI Insight*.
1248 2016;1(10).
- 1249 75. Mackay LK, Stock AT, Ma JZ, et al. Long-lived epithelial immunity by tissue-
1250 resident memory T (TRM) cells in the absence of persisting local antigen
1251 presentation. *Proc Natl Acad Sci U S A*. 2012;109(18):7037-7042.
- 1252 76. Thome JJ, Yudanin N, Ohmura Y, et al. Spatial map of human T cell
1253 compartmentalization and maintenance over decades of life. *Cell*.
1254 2014;159(4):814-828.
- 1255 77. Gordon CL, Miron M, Thome JJ, et al. Tissue reservoirs of antiviral T cell
1256 immunity in persistent human CMV infection. *J Exp Med*. 2017;214(3):651-667.
- 1257 78. Purwar R, Campbell J, Murphy G, Richards WG, Clark RA, Kupper TS.
1258 Resident memory T cells (T(RM)) are abundant in human lung: diversity,
1259 function, and antigen specificity. *PLoS One*. 2011;6(1):e16245.
- 1260 79. Jozwik A, Habibi MS, Paras A, et al. RSV-specific airway resident memory
1261 CD8+ T cells and differential disease severity after experimental human
1262 infection. *Nat Commun*. 2015;6:10224.
- 1263 80. Kennedy MK, Glaccum M, Brown SN, et al. Reversible defects in natural killer
1264 and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med*.
1265 2000;191(5):771-780.
- 1266 81. Kieper WC, Tan JT, Bondi-Boyd B, et al. Overexpression of interleukin (IL)-7
1267 leads to IL-15-independent generation of memory phenotype CD8+ T cells. *J*
1268 *Exp Med*. 2002;195(12):1533-1539.
- 1269 82. Goldrath AW, Sivakumar PV, Glaccum M, et al. Cytokine requirements for
1270 acute and Basal homeostatic proliferation of naive and memory CD8+ T cells.
1271 *J Exp Med*. 2002;195(12):1515-1522.
- 1272 83. Surh CD, Sprent J. Homeostasis of naive and memory T cells. *Immunity*.
1273 2008;29(6):848-862.
- 1274 84. Tsukumo S, Unno M, Muto A, et al. Bach2 maintains T cells in a naive state by
1275 suppressing effector memory-related genes. *Proc Natl Acad Sci U S A*.
1276 2013;110(26):10735-10740.
- 1277 85. Bista P, Mele DA, Baez DV, Huber BT. Lymphocyte quiescence factor Dpp2 is
1278 transcriptionally activated by KLF2 and TOB1. *Mol Immunol*. 2008;45(13):3618-
1279 3623.
- 1280 86. Yang K, Neale G, Green DR, He W, Chi H. The tumor suppressor Tsc1
1281 enforces quiescence of naive T cells to promote immune homeostasis and
1282 function. *Nat Immunol*. 2011;12(9):888-897.

- 1283 87. Kuo CT, Veselits ML, Leiden JM. LKLF: A transcriptional regulator of single-
1284 positive T cell quiescence and survival. *Science*. 1997;277(5334):1986-1990.
- 1285 88. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive
1286 adenosine in cancer. *Nat Rev Cancer*. 2017;17(12):709-724.
- 1287 89. Liu Z, Dai H, Wan N, et al. Suppression of memory CD8 T cell generation and
1288 function by tryptophan catabolism. *J Immunol*. 2007;178(7):4260-4266.
- 1289 90. Sato K, Ozaki K, Oh I, et al. Nitric oxide plays a critical role in suppression of
1290 T-cell proliferation by mesenchymal stem cells. *Blood*. 2007;109(1):228-234.
- 1291 91. Kung JT, Brooks SB, Jakway JP, Leonard LL, Talmage DW. Suppression of in
1292 vitro cytotoxic response by macrophages due to induced arginase. *J Exp Med*.
1293 1977;146(3):665-672.
- 1294 92. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. *Cell*
1295 *Death Dis*. 2015;6:e1792.
- 1296 93. Sledzinska A, Menger L, Bergerhoff K, Peggs KS, Quezada SA. Negative
1297 immune checkpoints on T lymphocytes and their relevance to cancer
1298 immunotherapy. *Mol Oncol*. 2015;9(10):1936-1965.
- 1299 94. Schietinger A, Greenberg PD. Tolerance and exhaustion: defining mechanisms
1300 of T cell dysfunction. *Trends Immunol*. 2014;35(2):51-60.
- 1301 95. Ohashi PS, Oehen S, Buerki K, et al. Ablation of "tolerance" and induction of
1302 diabetes by virus infection in viral antigen transgenic mice. *Cell*.
1303 1991;65(2):305-317.
- 1304 96. Ramsdell F, Fowlkes BJ. Maintenance of in vivo tolerance by persistence of
1305 antigen. *Science*. 1992;257(5073):1130-1134.
- 1306 97. Ohlen C, Kalos M, Cheng LE, et al. CD8(+) T cell tolerance to a tumor-
1307 associated antigen is maintained at the level of expansion rather than effector
1308 function. *J Exp Med*. 2002;195(11):1407-1418.
- 1309 98. Schietinger A, Delrow JJ, Basom RS, Blattman JN, Greenberg PD. Rescued
1310 tolerant CD8 T cells are preprogrammed to reestablish the tolerant state.
1311 *Science*. 2012;335(6069):723-727.
- 1312 99. Curtsinger JM, Lins DC, Mescher MF. Signal 3 determines tolerance versus full
1313 activation of naive CD8 T cells: dissociating proliferation and development of
1314 effector function. *J Exp Med*. 2003;197(9):1141-1151.
- 1315 100. Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated
1316 signalling co-stimulates murine T cells and prevents induction of anergy in T-
1317 cell clones. *Nature*. 1992;356(6370):607-609.
- 1318 101. DeSilva DR, Urdahl KB, Jenkins MK. Clonal anergy is induced in vitro by T cell
1319 receptor occupancy in the absence of proliferation. *J Immunol*.
1320 1991;147(10):3261-3267.
- 1321 102. Brown IE, Blank C, Kline J, Kacha AK, Gajewski TF. Homeostatic proliferation
1322 as an isolated variable reverses CD8+ T cell anergy and promotes tumor
1323 rejection. *J Immunol*. 2006;177(7):4521-4529.
- 1324 103. Baine I, Abe BT, Macian F. Regulation of T-cell tolerance by calcium/NFAT
1325 signaling. *Immunol Rev*. 2009;231(1):225-240.
- 1326 104. Srinivasan M, Frauwirth KA. Reciprocal NFAT1 and NFAT2 nuclear localization
1327 in CD8+ anergic T cells is regulated by suboptimal calcium signaling. *J*
1328 *Immunol*. 2007;179(6):3734-3741.
- 1329 105. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion.
1330 *Nat Rev Immunol*. 2015;15(8):486-499.

- 1331 106. Zajac AJ, Blattman JN, Murali-Krishna K, et al. Viral immune evasion due to
1332 persistence of activated T cells without effector function. *J Exp Med.*
1333 1998;188(12):2205-2213.
- 1334 107. Gallimore A, Glithero A, Godkin A, et al. Induction and exhaustion of
1335 lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized
1336 using soluble tetrameric major histocompatibility complex class I-peptide
1337 complexes. *J Exp Med.* 1998;187(9):1383-1393.
- 1338 108. Wherry EJ. T cell exhaustion. *Nat Immunol.* 2011;12(6):492-499.
- 1339 109. Blattman JN, Wherry EJ, Ha SJ, van der Most RG, Ahmed R. Impact of epitope
1340 escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection.
1341 *J Virol.* 2009;83(9):4386-4394.
- 1342 110. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral
1343 persistence alters CD8 T-cell immunodominance and tissue distribution and
1344 results in distinct stages of functional impairment. *J Virol.* 2003;77(8):4911-
1345 4927.
- 1346 111. Fuller MJ, Zajac AJ. Ablation of CD8 and CD4 T cell responses by high viral
1347 loads. *J Immunol.* 2003;170(1):477-486.
- 1348 112. Wherry EJ, Ha SJ, Kaech SM, et al. Molecular signature of CD8+ T cell
1349 exhaustion during chronic viral infection. *Immunity.* 2007;27(4):670-684.
- 1350 113. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8
1351 T cells during chronic viral infection. *Nature.* 2006;439(7077):682-687.
- 1352 114. Bengsch B, Johnson AL, Kurachi M, et al. Bioenergetic Insufficiencies Due to
1353 Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early
1354 Driver of CD8(+) T Cell Exhaustion. *Immunity.* 2016;45(2):358-373.
- 1355 115. Paley MA, Kroy DC, Odorizzi PM, et al. Progenitor and terminal subsets of
1356 CD8+ T cells cooperate to contain chronic viral infection. *Science.*
1357 2012;338(6111):1220-1225.
- 1358 116. Martinez GJ, Pereira RM, Aijo T, et al. The transcription factor NFAT promotes
1359 exhaustion of activated CD8(+) T cells. *Immunity.* 2015;42(2):265-278.
- 1360 117. Doering TA, Crawford A, Angelosanto JM, Paley MA, Ziegler CG, Wherry EJ.
1361 Network analysis reveals centrally connected genes and pathways involved in
1362 CD8+ T cell exhaustion versus memory. *Immunity.* 2012;37(6):1130-1144.
- 1363 118. Quigley M, Pereyra F, Nilsson B, et al. Transcriptional analysis of HIV-specific
1364 CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF.
1365 *Nat Med.* 2010;16(10):1147-1151.
- 1366 119. Man K, Gabriel SS, Liao Y, et al. Transcription Factor IRF4 Promotes CD8(+)
1367 T Cell Exhaustion and Limits the Development of Memory-like T Cells during
1368 Chronic Infection. *Immunity.* 2017;47(6):1129-1141 e1125.
- 1369 120. Jin HT, Anderson AC, Tan WG, et al. Cooperation of Tim-3 and PD-1 in CD8
1370 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A.*
1371 2010;107(33):14733-14738.
- 1372 121. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a
1373 subset of exhausted CD8 T cells by alphaPD-L1 blockade. *Proc Natl Acad Sci*
1374 *U S A.* 2008;105(39):15016-15021.
- 1375 122. Pauken KE, Sammons MA, Odorizzi PM, et al. Epigenetic stability of exhausted
1376 T cells limits durability of reinvigoration by PD-1 blockade. *Science.*
1377 2016;354(6316):1160-1165.
- 1378 123. Sen DR, Kaminski J, Barnitz RA, et al. The epigenetic landscape of T cell
1379 exhaustion. *Science.* 2016;354(6316):1165-1169.

- 1380 124. Youngblood B, Oestreich KJ, Ha SJ, et al. Chronic virus infection enforces
1381 demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T
1382 cells. *Immunity*. 2011;35(3):400-412.
- 1383 125. Youngblood B, Noto A, Porichis F, et al. Cutting edge: Prolonged exposure to
1384 HIV reinforces a poised epigenetic program for PD-1 expression in virus-
1385 specific CD8 T cells. *J Immunol*. 2013;191(2):540-544.
- 1386 126. Ghoneim HE, Zamora AE, Thomas PG, Youngblood BA. Cell-Intrinsic Barriers
1387 of T Cell-Based Immunotherapy. *Trends Mol Med*. 2016;22(12):1000-1011.
- 1388 127. Williams JB, Horton BL, Zheng Y, Duan Y, Powell JD, Gajewski TF. The EGR2
1389 targets LAG-3 and 4-1BB describe and regulate dysfunctional antigen-specific
1390 CD8+ T cells in the tumor microenvironment. *J Exp Med*. 2017;214(2):381-400.
- 1391 128. Buggert M, Tauriainen J, Yamamoto T, et al. T-bet and Eomes are differentially
1392 linked to the exhausted phenotype of CD8+ T cells in HIV infection. *PLoS*
1393 *Pathog*. 2014;10(7):e1004251.
- 1394 129. Simonetta F, Hua S, Lecuroux C, et al. High eomesodermin expression among
1395 CD57+ CD8+ T cells identifies a CD8+ T cell subset associated with viral
1396 control during chronic human immunodeficiency virus infection. *J Virol*.
1397 2014;88(20):11861-11871.
- 1398 130. Hoffmann M, Pantazis N, Martin GE, et al. Exhaustion of Activated CD8 T Cells
1399 Predicts Disease Progression in Primary HIV-1 Infection. *PLoS Pathog*.
1400 2016;12(7):e1005661.
- 1401 131. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after
1402 CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques.
1403 *J Exp Med*. 1999;189(6):991-998.
- 1404 132. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian
1405 immunodeficiency virus infection by CD8+ lymphocytes. *Science*.
1406 1999;283(5403):857-860.
- 1407 133. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or
1408 unrelated processes that compromise immunity? *Nat Rev Immunol*.
1409 2011;11(4):289-295.
- 1410 134. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit
1411 T-cell activation by distinct mechanisms. *Mol Cell Biol*. 2005;25(21):9543-9553.
- 1412 135. Waller EC, McKinney N, Hicks R, Carmichael AJ, Sissons JG, Wills MR.
1413 Differential costimulation through CD137 (4-1BB) restores proliferation of
1414 human virus-specific "effector memory" (CD28(-) CD45RA(HI)) CD8(+) T cells.
1415 *Blood*. 2007;110(13):4360-4366.
- 1416 136. Akbar AN, Henson SM, Lanna A. Senescence of T Lymphocytes: Implications
1417 for Enhancing Human Immunity. *Trends Immunol*. 2016;37(12):866-876.
- 1418 137. Tserel L, Kolde R, Limbach M, et al. Age-related profiling of DNA methylation
1419 in CD8+ T cells reveals changes in immune response and transcriptional
1420 regulator genes. *Sci Rep*. 2015;5:13107.
- 1421 138. Sharma P. Immune Checkpoint Therapy and the Search for Predictive
1422 Biomarkers. *Cancer J*. 2016;22(2):68-72.
- 1423 139. Gubin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer
1424 immunotherapy targets tumour-specific mutant antigens. *Nature*.
1425 2014;515(7528):577-581.
- 1426 140. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to
1427 CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371(23):2189-2199.

- 1428 141. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational
1429 landscape determines sensitivity to PD-1 blockade in non-small cell lung
1430 cancer. *Science*. 2015;348(6230):124-128.
- 1431 142. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to
1432 CTLA-4 blockade in metastatic melanoma. *Science*. 2015;350(6257):207-211.
- 1433 143. Thompson ED, Enriquez HL, Fu YX, Engelhard VH. Tumor masses support
1434 naive T cell infiltration, activation, and differentiation into effectors. *J Exp Med*.
1435 2010;207(8):1791-1804.
- 1436 144. Salmon H, Idoyaga J, Rahman A, et al. Expansion and Activation of CD103(+)
1437 Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to
1438 Therapeutic PD-L1 and BRAF Inhibition. *Immunity*. 2016;44(4):924-938.
- 1439 145. Roberts EW, Broz ML, Binnewies M, et al. Critical Role for CD103(+)/CD141(+)
1440 Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T
1441 Cell Immunity in Melanoma. *Cancer Cell*. 2016;30(2):324-336.
- 1442 146. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer.
1443 *Trends Immunol*. 2015;36(4):265-276.
- 1444 147. McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell
1445 immunoreactivity and sensitivity to immune checkpoint blockade. *Science*.
1446 2016;351(6280):1463-1469.
- 1447 148. Speiser DE, Ho PC, Verdeil G. Regulatory circuits of T cell function in cancer.
1448 *Nat Rev Immunol*. 2016;16(10):599-611.
- 1449 149. McGranahan N, Rosenthal R, Hiley CT, et al. Allele-Specific HLA Loss and
1450 Immune Escape in Lung Cancer Evolution. *Cell*. 2017;171(6):1259-1271
1451 e1211.
- 1452 150. Luksza M, Riaz N, Makarov V, et al. A neoantigen fitness model predicts tumour
1453 response to checkpoint blockade immunotherapy. *Nature*.
1454 2017;551(7681):517-520.
- 1455 151. Philip M, Fairchild L, Sun L, et al. Chromatin states define tumour-specific T cell
1456 dysfunction and reprogramming. *Nature*. 2017;545(7655):452-456.
- 1457 152. Schietinger A, Philip M, Krishawan VE, et al. Tumor-Specific T Cell Dysfunction
1458 Is a Dynamic Antigen-Driven Differentiation Program Initiated Early during
1459 Tumorigenesis. *Immunity*. 2016;45(2):389-401.
- 1460 153. Zarour HM. Reversing T-cell Dysfunction and Exhaustion in Cancer. *Clin*
1461 *Cancer Res*. 2016;22(8):1856-1864.
- 1462 154. Disis ML. Mechanism of action of immunotherapy. *Semin Oncol*. 2014;41 Suppl
1463 5:S3-13.
- 1464 155. Wei SC, Levine JH, Cogdill AP, et al. Distinct Cellular Mechanisms Underlie
1465 Anti-CTLA-4 and Anti-PD-1 Checkpoint Blockade. *Cell*. 2017;170(6):1120-
1466 1133 e1117.
- 1467 156. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC.
1468 Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore
1469 anti-tumor immunity. *J Exp Med*. 2010;207(10):2187-2194.
- 1470 157. Johnston RJ, Comps-Agrar L, Hackney J, et al. The immunoreceptor TIGIT
1471 regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell*.
1472 2014;26(6):923-937.
- 1473 158. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G. Dual blockade of PD-1
1474 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection
1475 function in tumors. *Cancer Res*. 2013;73(12):3591-3603.

- 1476 159. O'Brien S, Thomas RM, Wertheim GB, Zhang F, Shen H, Wells AD. Ikaros imposes a barrier to CD8+ T cell differentiation by restricting autocrine IL-2
1477 production. *J Immunol.* 2014;192(11):5118-5129.
1478
- 1479 160. Giordano M, Henin C, Maurizio J, et al. Molecular profiling of CD8 T cells in
1480 autochthonous melanoma identifies Maf as driver of exhaustion. *EMBO J.*
1481 2015;34(15):2042-2058.
- 1482 161. Canale FP, Ramello MC, Nunez N, et al. CD39 Expression Defines Cell
1483 Exhaustion in Tumor-Infiltrating CD8(+) T Cells. *Cancer Res.* 2018;78(1):115-
1484 128.
- 1485 162. Voron T, Colussi O, Marcheteau E, et al. VEGF-A modulates expression of
1486 inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med.* 2015;212(2):139-
1487 148.
- 1488 163. Bauer CA, Kim EY, Marangoni F, Carrizosa E, Claudio NM, Mempel TR.
1489 Dynamic Treg interactions with intratumoral APCs promote local CTL
1490 dysfunction. *J Clin Invest.* 2014;124(6):2425-2440.
- 1491 164. Waugh KA, Leach SM, Moore BL, Bruno TC, Buhrman JD, Slansky JE.
1492 Molecular Profile of Tumor-Specific CD8+ T Cell Hypofunction in a
1493 Transplantable Murine Cancer Model. *J Immunol.* 2016;197(4):1477-1488.
- 1494 165. Boldajipour B, Nelson A, Krummel MF. Tumor-infiltrating lymphocytes are
1495 dynamically desensitized to antigen but are maintained by homeostatic
1496 cytokine. *JCI Insight.* 2016;1(20):e89289.
- 1497 166. Lavin Y, Kobayashi S, Leader A, et al. Innate Immune Landscape in Early Lung
1498 Adenocarcinoma by Paired Single-Cell Analyses. *Cell.* 2017;169(4):750-765
1499 e717.
- 1500 167. Giraldo NA, Becht E, Vano Y, et al. Tumor-Infiltrating and Peripheral Blood T-
1501 cell Immunophenotypes Predict Early Relapse in Localized Clear Cell Renal
1502 Cell Carcinoma. *Clin Cancer Res.* 2017;23(15):4416-4428.
- 1503 168. Tassi E, Grazia G, Vegetti C, et al. Early Effector T Lymphocytes Coexpress
1504 Multiple Inhibitory Receptors in Primary Non-Small Cell Lung Cancer. *Cancer*
1505 *Res.* 2017;77(4):851-861.
- 1506 169. Kargl J, Busch SE, Yang GH, et al. Neutrophils dominate the immune cell
1507 composition in non-small cell lung cancer. *Nat Commun.* 2017;8:14381.
- 1508 170. Sheng SY, Gu Y, Lu CG, et al. The Characteristics of Naive-like T Cells in
1509 Tumor-infiltrating Lymphocytes From Human Lung Cancer. *J Immunother.*
1510 2017;40(1):1-10.
- 1511 171. Mortarini R, Piris A, Maurichi A, et al. Lack of terminally differentiated tumor-
1512 specific CD8+ T cells at tumor site in spite of antitumor immunity to self-antigens
1513 in human metastatic melanoma. *Cancer Res.* 2003;63(10):2535-2545.
- 1514 172. Fourcade J, Sun Z, Benallaoua M, et al. Upregulation of Tim-3 and PD-1
1515 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in
1516 melanoma patients. *J Exp Med.* 2010;207(10):2175-2186.
- 1517 173. Chauvin JM, Pagliano O, Fourcade J, et al. TIGIT and PD-1 impair tumor
1518 antigen-specific CD8(+) T cells in melanoma patients. *J Clin Invest.*
1519 2015;125(5):2046-2058.
- 1520 174. Baitsch L, Baumgaertner P, Devevre E, et al. Exhaustion of tumor-specific
1521 CD8(+) T cells in metastases from melanoma patients. *J Clin Invest.*
1522 2011;121(6):2350-2360.
- 1523 175. Gros A, Parkhurst MR, Tran E, et al. Prospective identification of neoantigen-
1524 specific lymphocytes in the peripheral blood of melanoma patients. *Nat Med.*
1525 2016;22(4):433-438.

- 1526 176. Thommen DS, Schreiner J, Muller P, et al. Progression of Lung Cancer Is
1527 Associated with Increased Dysfunction of T Cells Defined by Coexpression of
1528 Multiple Inhibitory Receptors. *Cancer Immunol Res.* 2015;3(12):1344-1355.
- 1529 177. Tirosh I, Izar B, Prakadan SM, et al. Dissecting the multicellular ecosystem of
1530 metastatic melanoma by single-cell RNA-seq. *Science.* 2016;352(6282):189-
1531 196.
- 1532 178. Zheng C, Zheng L, Yoo JK, et al. Landscape of Infiltrating T Cells in Liver
1533 Cancer Revealed by Single-Cell Sequencing. *Cell.* 2017;169(7):1342-1356
1534 e1316.
- 1535 179. Haeryfar SMM, Shaler CR, Rudak PT. Mucosa-associated invariant T cells in
1536 malignancies: a faithful friend or formidable foe? *Cancer Immunol Immunother.*
1537 2018.
- 1538 180. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen-specific CD8
1539 T cells infiltrating the tumor express high levels of PD-1 and are functionally
1540 impaired. *Blood.* 2009;114(8):1537-1544.
- 1541 181. Klebanoff CA, Gattinoni L, Torabi-Parizi P, et al. Central memory self/tumor-
1542 reactive CD8+ T cells confer superior antitumor immunity compared with
1543 effector memory T cells. *Proc Natl Acad Sci U S A.* 2005;102(27):9571-9576.
- 1544 182. Wu F, Zhang W, Shao H, et al. Human effector T cells derived from central
1545 memory cells rather than CD8(+)T cells modified by tumor-specific TCR gene
1546 transfer possess superior traits for adoptive immunotherapy. *Cancer Lett.*
1547 2013;339(2):195-207.
- 1548 183. Beckhove P, Feuerer M, Dolenc M, et al. Specifically activated memory T cell
1549 subsets from cancer patients recognize and reject xenotransplanted
1550 autologous tumors. *J Clin Invest.* 2004;114(1):67-76.
- 1551 184. Robbins PF, Dudley ME, Wunderlich J, et al. Cutting edge: persistence of
1552 transferred lymphocyte clonotypes correlates with cancer regression in patients
1553 receiving cell transfer therapy. *J Immunol.* 2004;173(12):7125-7130.
- 1554 185. Tran KQ, Zhou J, Durlinger KH, et al. Minimally cultured tumor-infiltrating
1555 lymphocytes display optimal characteristics for adoptive cell therapy. *J*
1556 *Immunother.* 2008;31(8):742-751.
- 1557 186. Spitzer MH, Carmi Y, Reticker-Flynn NE, et al. Systemic Immunity Is Required
1558 for Effective Cancer Immunotherapy. *Cell.* 2017;168(3):487-502 e415.
- 1559 187. Ribas A, Shin DS, Zaretsky J, et al. PD-1 Blockade Expands Intratumoral
1560 Memory T Cells. *Cancer Immunol Res.* 2016;4(3):194-203.
- 1561 188. Wistuba-Hamprecht K, Martens A, Heubach F, et al. Peripheral CD8 effector-
1562 memory type 1 T-cells correlate with outcome in ipilimumab-treated stage IV
1563 melanoma patients. *Eur J Cancer.* 2017;73:61-70.
- 1564 189. Charoentong P, Finotello F, Angelova M, et al. Pan-cancer Immunogenomic
1565 Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of
1566 Response to Checkpoint Blockade. *Cell Rep.* 2017;18(1):248-262.
- 1567 190. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of
1568 immune cells within human colorectal tumors predict clinical outcome. *Science.*
1569 2006;313(5795):1960-1964.
- 1570 191. Hackl H, Charoentong P, Finotello F, Trajanoski Z. Computational genomics
1571 tools for dissecting tumour-immune cell interactions. *Nat Rev Genet.*
1572 2016;17(8):441-458.
- 1573 192. Ghorani E, Rosenthal R, McGranahan N, et al. Differential binding affinity of
1574 mutated peptides for MHC class I is a predictor of survival in advanced lung
1575 cancer and melanoma. *Ann Oncol.* 2018;29(1):271-279.

- 1576 193. Turajlic S, Litchfield K, Xu H, et al. Insertion-and-deletion-derived tumour-
1577 specific neoantigens and the immunogenic phenotype: a pan-cancer analysis.
1578 *Lancet Oncol.* 2017;18(8):1009-1021.
- 1579 194. Remmerswaal EB, Klarenbeek PL, Alves NL, et al. Clonal evolution of CD8+ T
1580 cell responses against latent viruses: relationship among phenotype,
1581 localization, and function. *J Virol.* 2015;89(1):568-580.
- 1582 195. Bentzen AK, Hadrup SR. Evolution of MHC-based technologies used for
1583 detection of antigen-responsive T cells. *Cancer Immunol Immunother.*
1584 2017;66(5):657-666.
- 1585 196. Philip M, Schietinger A. Beyond Genomics: Multidimensional Analysis of
1586 Cancer Therapy Resistance. *Trends Immunol.* 2015;36(11):665-667.
- 1587 197. Santegoets SJ, Turksma AW, Suhoski MM, et al. IL-21 promotes the expansion
1588 of CD27+ CD28+ tumor infiltrating lymphocytes with high cytotoxic potential
1589 and low collateral expansion of regulatory T cells. *J Transl Med.* 2013;11:37.
- 1590 198. Chacon JA, Wu RC, Sukhumalchandra P, et al. Co-stimulation through 4-
1591 1BB/CD137 improves the expansion and function of CD8(+) melanoma tumor-
1592 infiltrating lymphocytes for adoptive T-cell therapy. *PLoS One.*
1593 2013;8(4):e60031.
- 1594 199. Saini SK, Rekers N, Hadrup SR. Novel tools to assist neoepitope targeting in
1595 personalized cancer immunotherapy. *Ann Oncol.* 2017;28(suppl_12):xii3-xii10.
- 1596 200. Su S, Hu B, Shao J, et al. CRISPR-Cas9 mediated efficient PD-1 disruption on
1597 human primary T cells from cancer patients. *Sci Rep.* 2016;6:20070.
- 1598 201. Malik BT, Byrne KT, Vella JL, et al. Resident memory T cells in the skin mediate
1599 durable immunity to melanoma. *Sci Immunol.* 2017;2(10).
- 1600 202. Webb JR, Milne K, Watson P, Deleeuw RJ, Nelson BH. Tumor-infiltrating
1601 lymphocytes expressing the tissue resident memory marker CD103 are
1602 associated with increased survival in high-grade serous ovarian cancer. *Clin*
1603 *Cancer Res.* 2014;20(2):434-444.
- 1604 203. Webb JR, Wick DA, Nielsen JS, et al. Profound elevation of CD8+ T cells
1605 expressing the intraepithelial lymphocyte marker CD103 (alphaE/beta7
1606 Integrin) in high-grade serous ovarian cancer. *Gynecol Oncol.*
1607 2010;118(3):228-236.
- 1608 204. Webb JR, Milne K, Nelson BH. PD-1 and CD103 Are Widely Coexpressed on
1609 Prognostically Favorable Intraepithelial CD8 T Cells in Human Ovarian Cancer.
1610 *Cancer Immunol Res.* 2015;3(8):926-935.
- 1611 205. Wang ZQ, Milne K, Derocher H, Webb JR, Nelson BH, Watson PH. CD103 and
1612 Intratumoral Immune Response in Breast Cancer. *Clin Cancer Res.*
1613 2016;22(24):6290-6297.
- 1614 206. Ganesan AP, Clarke J, Wood O, et al. Tissue-resident memory features are
1615 linked to the magnitude of cytotoxic T cell responses in human lung cancer. *Nat*
1616 *Immunol.* 2017;18(8):940-950.
- 1617 207. Boddupalli CS, Bar N, Kadaveru K, et al. Interlesional diversity of T cell
1618 receptors in melanoma with immune checkpoints enriched in tissue-resident
1619 memory T cells. *JCI Insight.* 2016;1(21):e88955.
- 1620 208. Djenidi F, Adam J, Goubar A, et al. CD8+CD103+ tumor-infiltrating
1621 lymphocytes are tumor-specific tissue-resident memory T cells and a
1622 prognostic factor for survival in lung cancer patients. *J Immunol.*
1623 2015;194(7):3475-3486.

- 1624 209. Zinselmeyer BH, Heydari S, Sacristan C, et al. PD-1 promotes immune
1625 exhaustion by inducing antiviral T cell motility paralysis. *J Exp Med.*
1626 2013;210(4):757-774.
- 1627 210. Ely KH, Cookenham T, Roberts AD, Woodland DL. Memory T cell populations
1628 in the lung airways are maintained by continual recruitment. *J Immunol.*
1629 2006;176(1):537-543.
- 1630 211. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression
1631 and cancer progression. *Nat Genet.* 2001;29(2):117-129.
- 1632 212. Lanitis E, Dangaj D, Irving M, Coukos G. Mechanisms regulating T-cell
1633 infiltration and activity in solid tumors. *Ann Oncol.* 2017;28(suppl_12):xii18-
1634 xii32.
- 1635 213. Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome
1636 vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature.*
1637 2017;547(7662):222-226.
- 1638 214. Pickup M, Novitskiy S, Moses HL. The roles of TGFbeta in the tumour
1639 microenvironment. *Nat Rev Cancer.* 2013;13(11):788-799.

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1643 **Figure legends**

1644 **Figure 1. Linear differentiation refreshed.**

1645 A composite of seminal work that has defined the lineage relationships of human CD8
1646 memory T cells. The conversion of naïve cells to Teff and consequent de-
1647 differentiation gives rise to diverse memory cells subsets with specific migratory
1648 potential. Re-stimulation of T subsets gives rise to progeny later in the scheme. See
1649 main text for a detailed description.

1650 **Figure 2. A putative model of co-evolution of T cell dysfunction and tumor**

1651 **genomics within the TME:** Phase I, Circulating tumor-specific Teff or Tem migrate to
1652 the tumor alongside bystander cells via chemotactic and inflammatory signaling.
1653 Teff/Tem convert to Trm and elicit cytotoxic effector function whilst experiencing
1654 chronic antigen stimulation. Selection pressure from T cell responses drives tumor
1655 evolution, including loss of class I presentation. Phase II, Tumor-specific cells undergo
1656 clonal expansion, consume IL-15 and experience progressive dysfunction. IL-15
1657 resources for incoming circulating Tem/Teff are depleted as dysfunctional T cells with
1658 specificity to lost antigen dominate the niche, facilitating tumor escape and disease
1659 progression.

1660 **Acknowledgements and conflicts of interest**

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