CAMEL: the Clonally-Aware Measure of Effector Longevity

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January 26, 2023

1 Background

Previous work in both mice [1] and humans [3] has shown that a larger proportion of "progenitor exhausted/CD8_G" cells (marked by higher expression of TCF-1, lower expression of TIM-3) vs. "terminally exhausted/CD8_B" cells (marked by lower expression of TCF-1, higher of TIM-3) was predictive of response to immune checkpoint inhibition. It has been shown that these progenitor exhausted cells retain proliferative and tumor-killing capacity (as demonstrated in vitro and in vivo via transfer experiments), whereas the terminally exhausted cells do not [1]. Moreover, terminally exhausted cells were recently shown to themselves have suppressive capacity [5]. Therefore, it is tempting to assume that suppressing cells' differentiation to the terminally exhausted state will lead to sustained tumor killing and ultimate tumor clearance.

There are a few reasons to question this assumption. First, exhaustion is above all driven by persistent T cell receptor (TCR) stimulation, which is itself quite necessary for tumor-specific recognition and killing. Selection of genetic perturbations which are optimized for minimal terminal exhaustion are likely to select for genes which are necessary for T cell activation of TCR signal transduction (see Fig. 1). Secondly, genetic perturbations which do restore T cell function and tumor control have *not*, thus far, been found to suppress the differentiation of T cells to the terminally exhausted state. Most notably, genetic deletion of PD-1 has been shown to *promote* exhaustion [2]. Since disruption of PD-1 signaling can arguably described as the "gold standard" of cancer immunotherapy (with approved use for over 20 types of cancers), we should look for metrics that select perturbations that are known to drive tumor control and clearance.

One possible explanation for how PD-1 knockout (KO) drives tumor clearance without reversing or reducing T cell exhaustion is that PD-1 KO cells proliferate more ("quantity") and may also have greater effector potential ("quality") than PD-1 wild-type (WT) cells. Under this hypothesis, a set of PD-1 KO cells may develop such that they are more likely to be terminally exhausted, but because they proliferated more (quantity) and killed tumors more effectively (quality) in that time, they were more successful in aggregate. This idea is illustrated in Fig. 2.

As shown in Fig. 2, the "optimal" balance of T cell states changes over time. The data available are single cell "snapshots" of an overall curve whose "integral," roughly speaking, we wish to compute.

2 The Solution

To address this, we propose the "Clonally Aware Measure of Effector Longevity" (CAMEL). CAMEL functions by optimizing the overall behavior of clones (groups of cells descending from a single naive cell which is activated, trafficks to the tumor, and which differentiate into a number of states) rather than cells. That is, it seeks to capture the overall capacity of single progenitor cells, which we hypothesize is linked to tumor control.

T cell clonality must be assessed genetically—that is, there must be a genetic marker that is unique in the pre-activated T cells that will be propagated among all differentiated daughter cells. In the present dataset, this may be done using the combination of the guide RNA (gRNA) and the unique molecular identifier (UMI) contained in the hairpin region of the gRNA. Taking together, each gRNA-UMI combination represents one or few cells in the original transferred pool, and it may be assumed that all T cells in the tumor with a shared gRNA-UMI descend from a common ancestor. In experiments that utilize the endogenous T cell repertoire, sequencing of the TCR locus can also be used as a clonal indicator.

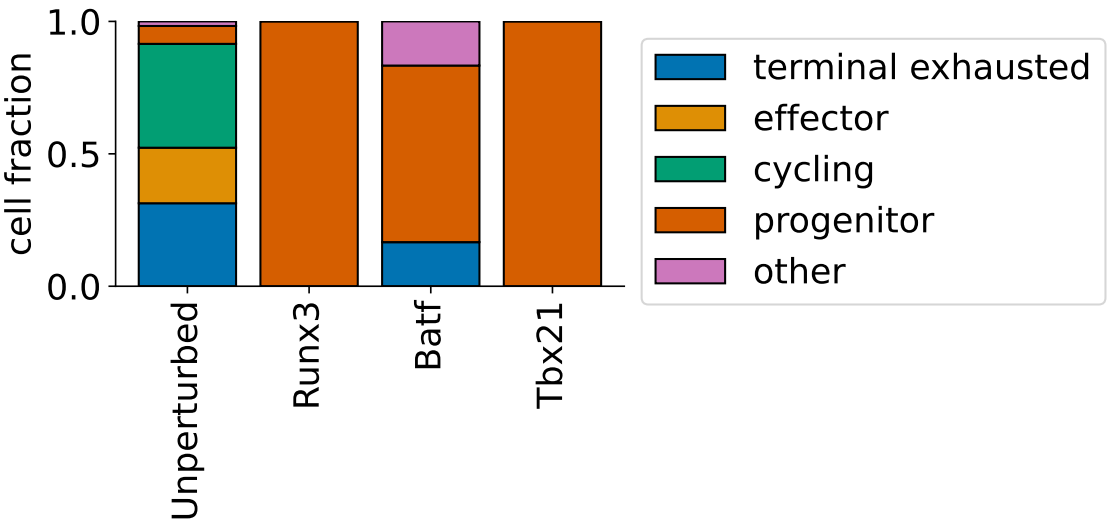

Figure 1: KO of Runx3, Batf, and Tbx21 all drive a significant progenitor fraction, despite the fact that deletion of Runx3 [6] and Tbx21 [8] have been shown to inhibit T cell function, and overexpression of Batf in CAR-T cells has been shown to increase T cell function [4] (though, it should be noted, Batf depletion has also been linked to tumor control [7], so further research is warranted).

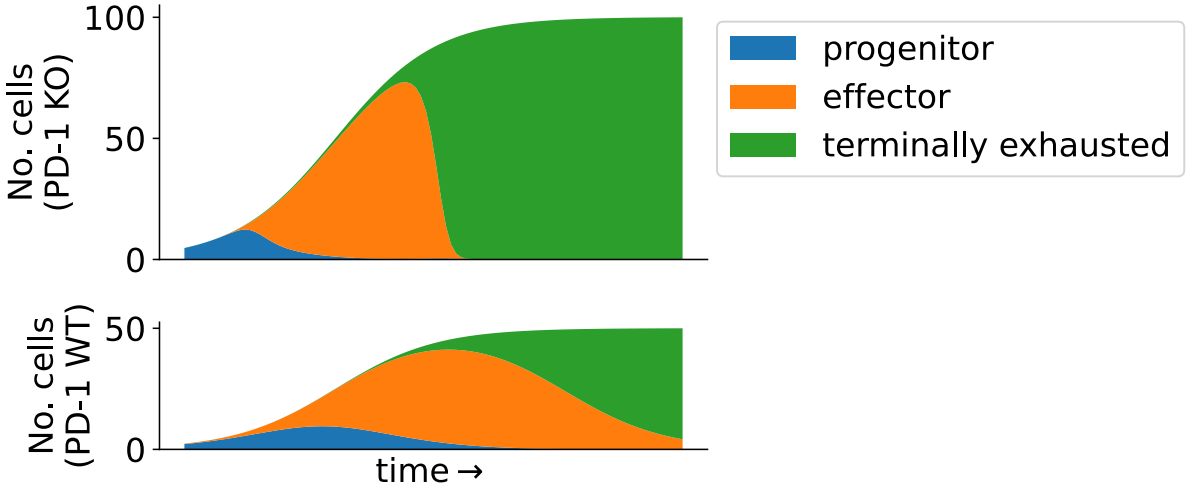

Figure 2: Exaggerated schematic illustrating how PD-1 KO cells could be more functional (i.e., drive more tumor killing) without, on a cell-intrinsic level, being less prone to terminal exhaustion. Here, we hypothesize that PD-1 KO drives more proliferation on a clone-by-clone basis, and, while onset of terminal exhaustion occurs more rapidly, it occurs after T cells have gone through the tumor-killing effector stage. This does not contradict the finding that, in the aggregate, a group of cells with a larger progenitor fraction is more capable of tumor killing than one with a higher terminally exhausted fraction.

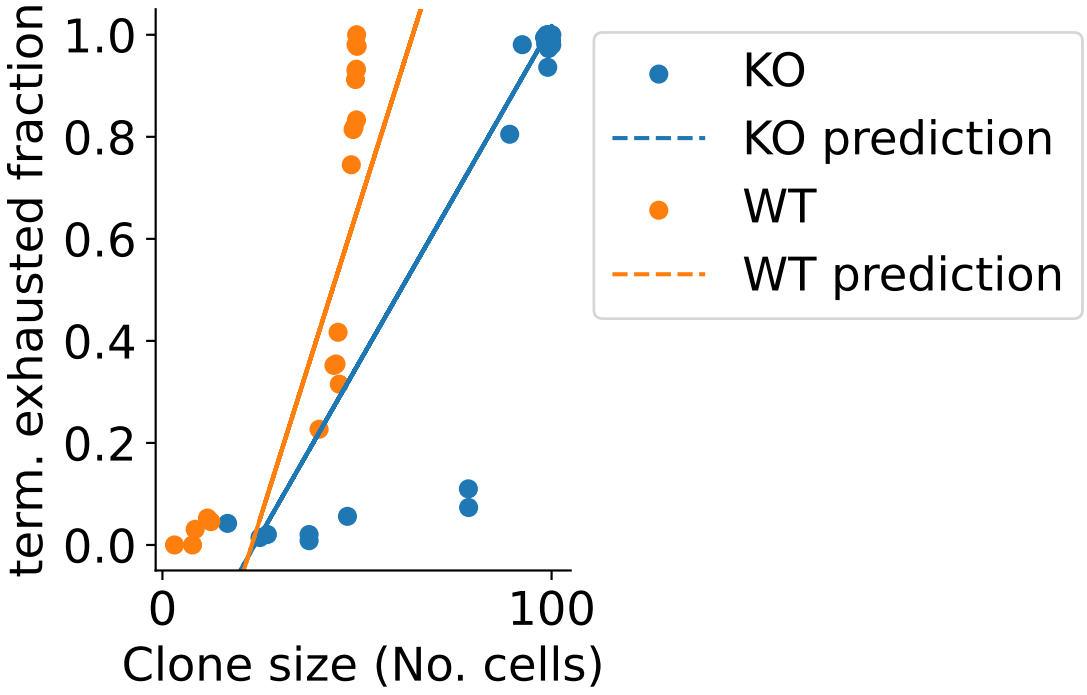

Figure 3: Exaggerated schematic illustrating how PD-1 KO cells could be more functional (i.e., drive more tumor killing) without, on a cell-intrinsic level, being less prone to terminal exhaustion. Here, we hypothesize that PD-1 KO drives more proliferation on a clone-by-clone basis, and, while onset of terminal exhaustion occurs more rapidly, it occurs after T cells have gone through the tumor-killing effector stage. This does not contradict the finding that, in the aggregate, a group of cells with a larger progenitor fraction is more capable of tumor killing than one with a higher terminally exhausted fraction.

Assuming that T cell clonality may be measured, we hypothesize that the most relevant measure of clonal capacity is not the terminally exhausted fraction as a function of *time*, but as a function of *clone size*.

In Fig. 3, we present simulated data for the terminally exhausted fraction (based on the composition curves in 2) as a function of clone size. Here, while the KO clones demonstrate a larger terminally exhausted fraction at every point in time, this fraction is lesser in "clonal expansion time." Therefore, the problem reduces to one of summarizing the relationship between "clonal expansion time" and "terminally exhausted fraction."

In practice, we recommend a very simple, robust means of regression between these variables, because, while a sample may contain many cells, it will contain many fewer clones, and therefore we expect that in most cases, a linear regression is most suitable. In particular, we recommend the Theil-Sen estimator, which infers the slope and intercept from data by taking the median thereof between all possible pairs of the independent and dependent variable. For the simulated data in Fig. 3, these are shown.

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