1023 THE SIGNAL STRENGTH OF SIGNAL 2 AND 3 DURING T CELL PRIMING AFFECT THE FUNCTIONAL FATE OF AN ANTI-TUMOR T CELL RESPONSE

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Background Fate decision in $CD8^+$ T cells refers to the process of differentiation that occurs when a naïve T cell enters into effector, memory, exhausted, or other states. Multiple models of fate decision have been proposed, but the exact determinants of T cell fate are unknown. Evidence in LCMV and cancer models support the notion that signals during T cell priming are especially important in deciding T cell fate and that fate decisions are made early in the priming process. Priming interactions are highly complex, requiring the integration of multiple signals. Each of these signals can vary in strength and it is the sum of these signals that decides the T cell response. Thus, it is unsurprising that we currently do not know the combinatorial specificities and molecular underpinnings of priming interactions. Understanding the factors that drive these different fates could be therapeutically harnessed to reduce exhaustion phenotypes and increase effector functions and memory potential of $CD8^+$ T cells in cancer.

Methods We used therapeutic interventions as a tool to alter the signals received by a T cell during priming and characterized the impact on T cell fate. Using a $BRAF^{V600E}PTEN^{-/-}$ melanoma cell line that expresses the SIYRYYGL (SIY) model antigen, we implanted tumors and provided therapy during priming. We surveyed the effects of checkpoint blockade therapy (anti-PD1 and anti-CTLA4), costimulatory agonists (anti-CD40 and anti-41BB), and Type-I Interferon (IFNa and IFNb) on tumor-reactive $CD8^+$ T cell phenotypes at early timepoints during priming via flow cytometry and single-cell RNA sequencing.

Results All treatments resulted in an increase of effector-like ($TCF1^-TIM3^+$) tumor-reactive T cells. Interestingly however, each intervention induced differential expression of a specific set of transcription factors. For example, expression of Eomes is significantly increased upon IFNb treatment at both protein and RNA level, while it is decreased in T cells exposed to agonistic anti-CD40. Analysis of single-cell RNA sequencing of tumor-reactive $CD8^+$ T cells revealed unsupervised clustering of cells based on the therapeutic intervention administered during priming. Tumor outgrowth and phenotypic studies at later timepoints indicated that $CD8^+$ T cells primed with therapeutic intervention adopt different cell fates.

Conclusions Priming signals are crucial determinants of T cell fate. Changing the signals received by a $CD8^+$ T cell during priming results in an altered phenotypic and transcriptional profile early during priming. These early transcriptional changes appear to generate different $CD8^+$ T cell fates, providing an opportunity to improve anti-tumor immunity in a neoadjuvant setting.

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