TUMOUR IMMUNOLOGY

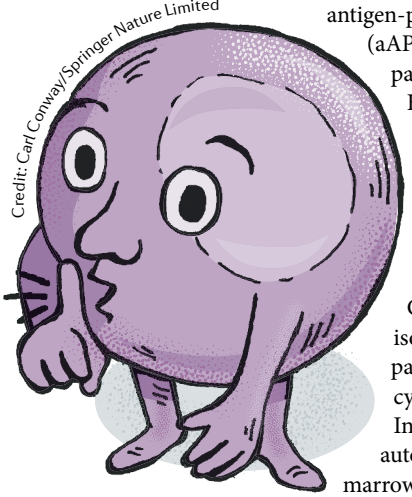
# Challenging (immunogenically) silent assumptions

> despite their low TMB, robust antitumour T cell responses occur in paediatric ALLs

As only ~2% of predicted neoepitopes mount detectable antitumour immune responses, a prevailing assumption — supported by poor immunotherapy response rates — has been that cancers with low tumour mutational burdens (TMBs), such as paediatric acute lymphoblastic leukaemia (ALL), are immunogenically silent. A new study now challenges this notion, reporting that paediatric ALLs are, in fact, antigenically visible and elicit robust, albeit ineffective, antitumour immune responses.

Using diagnostic biopsy samples and matched germline tissues from paediatric patients with ALL, Zamora et al. employed high-throughput genomic sequencing and HLA haplotyping to screen ALL-specific somatic alterations for their predicted ability to generate neoepitopes. Despite the low TMB, 5–28 predicted neoepitopes were identified per patient.

Functional assays were then used to determine if predicted neoepitopes elicit T cell responses. Upon co-culture with artificial antigen-presenting cells (aAPCs) expressing patient-specific HLAs and treated with synthetic neoantigenic peptides (corresponding to the predicted neoepitopes), $CD8^+$ T cells isolated from three patients generated cytokine responses. In a similar assay, autologous bone marrow cells (comprising tumour cells, APCs and $CD8^+$ T cells) derived from six patients elicited cytokine responses to at least one neoantigenic peptide, with 86% of tested peptides found to be immunogenic. These ex vivo findings probably mirror endogenous responses, as additional assays demonstrated antitumour $CD8^+$ T cell responses during co-culture with autologous $CD19^+$ tumour cells or aAPCs transfected to express mutant neoepitopes. These findings (and those of other experiments) imply that, despite the low TMB, paediatric-ALL-associated neoantigens are processed, presented and elicit immune responses.



Credit: Carl Conway/Springer Nature Limited

Next, neoantigen-specific $CD8^+$ T cell responses were mapped to specific neoepitopes using patient-specific tetramers (HLA–neoepitope complexes) corresponding to predicted neoepitopes across multiple HLA types. In bone marrow specimens from all six tested patients, at least one neoepitope-specific $CD8^+$ T cell population — inferred using a tetramer–T cell binding assay — was identified. Importantly, 68% of tested tetramers bound to T cells, suggesting that the majority of predicted neoepitopes elicit endogenous responses. Moreover, neoantigen-specific $CD8^+$ T cell responses were identified against the common *ETV6–RUNX1* gene fusion, which has a prevalence of ~20–25% in paediatric ALL and is associated with favourable prognosis.

Notably, tetramer assays also revealed immunodominance hierarchies, with most tetramer-bound T cell populations restricted to 1–2 neoepitopes. In the context of a low TMB, which might push the immune response to target most of the potential neoantigens, such immunodominance effects could explain the strong immune responses to an antigen subset.

Finally, single-cell transcriptomic profiling of tetramer-binding ALL-specific $CD8^+$ T cells with an effector phenotype ($CCR7^-CD45RO^+$) revealed three hierarchical clusters with distinct gene expression signatures, associated with functional effector, dysfunctional or exhausted $CD8^+$ T cells. Moreover, analysis of the methylation status of several key promoters associated with T cell function suggested that neoepitope-specific $CD8^+$ T cell pools contain various differentiation states, including functional effectors.

Overall, the findings indicate that, despite their low TMB, robust antitumour T cell responses occur in paediatric ALLs and are shaped at the cellular level through immunodominance. "While these immune responses are not controlling the tumour in these patients, they demonstrate that the tumour is antigenically visible to the immune response," concludes author Paul Thomas. "We are currently exploring the functional regulation of these responses to understand why they fail to control the tumour and whether there are therapeutic opportunities to enhance their activity and promote tumour clearance."

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**ORIGINAL ARTICLE** Zamora, A. E. et al. Pediatric patients with acute lymphoblastic leukemia generate abundant and functional neoantigen-specific $CD8^+$ T cell responses. *Sci. Transl Med.* https://doi.org/10.1126/scitranslmed.aat8549 (2019)