Molecular Therapy
Commentary

Exploring innovative approaches in cancer immunotherapy by exploiting the role of translational readthrough-inducing drugs

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Cancer immunotherapy has dramatically reshaped the oncology therapeutic landscape by harnessing the immune system to recognize and eliminate tumor cells. Yet, a persistent challenge remains: the insufficient presentation of tumor-specific antigens that can trigger a robust, selective immune response. In this issue of *Molecular Therapy*, Lejeune and colleagues explored a novel approach to enhance tumor immunogenicity through translational readthrough of premature termination codons (PTCs).[1]

This commentary contextualizes their findings within the broader field of cancer immunotherapy and evaluates the experimental evidence while also discussing the conceptual and therapeutic implications of pharmacologically induced readthrough for neoantigen production and immune response activation. Indeed, a common hallmark of cancer cells is the accumulation of multiple genetic mutations,[2] but not all mutations contribute to the cancer phenotype. Driver mutations represent the most incisive mutations to trigger cancer progression; however, several passenger mutations occur during this process. Frameshift mutations are often the cause of PTC formation. Therefore, the induction of their translation could be used to produce neoantigens. Since neoantigen generation through mutations that alter peptide sequences lies at the core of an effective immune response activation,[3] this approach represents an innovative paradigm to enhance the efficacy of immunotherapy. In fact, while the introduction of PTCs (either by frameshift or point mutations) represents one of the mechanisms of tumor resistance to the immune response, the induction of readthrough of such PTCs could provide the most appropriate countermeasure. In this context, translational readthrough-inducing drugs (TRIDs) offer a variety of compounds, from aminoglycosides, such as geneticin G418, to small heterocycles, such as PTC124 and its analogs.[4] However, each TRID has its own mechanism of readthrough induction as well as different efficacy depending on the nature of the stop codon and, consequently, different side effects, often due to a lack of specificity.

Lejeune et al. explore an original strategy to enhance neoantigen diversity in tumors: pharmacological induction of translational readthrough using the purine analog 2,6-diaminopurine (DAP).[1] Building on prior work demonstrating DAP's capacity to restore full-length protein expression from PTCs in genetic diseases such as cystic fibrosis,[5] the authors extend this concept to oncology. They hypothesize that DAP-mediated readthrough of nonsense and frameshift-derived PTCs in cancer cells could generate novel C-terminal protein extensions, producing non-self-neoantigens recognizable by cytotoxic T lymphocytes (CTLs). Therefore, this work reveals a new intersection between molecular genetics and cancer immunotherapy, suggesting that therapeutic readthrough induction may potentiate immune recognition of tumors without genetic engineering.

Lejeune's group previously characterized DAP as a highly potent and specific readthrough-inducing compound acting preferentially at UGA stop codons.[6] In this new study, they combine molecular, proteomic, and immunological approaches to demonstrate DAP's ability to stimulate translational readthrough in cancer models while maintaining cellular and systemic safety. Using the CT26/BALB/c murine model of colon carcinoma, the authors show that tumor cells accumulated substantially more PTCs than normal tissues, confirming a prerequisite for selective neoantigen generation.

Significantly, chronic DAP administration did not alter the overall mutational burden or introduce new mutations, ruling out genotoxicity. Mass spectrometry revealed that less than 4% of the proteome was affected even at high DAP concentrations, indicating limited global perturbation of protein homeostasis. Blood composition and vascular integrity also remained unaltered after prolonged exposure, addressing potential systemic toxicities. These "safety features" are crucial when using TRIDs to selectively promote readthrough in tumor cells rather than in healthy cells.

To test whether DAP-induced readthrough can indeed generate immunogenic peptides, the authors developed a reporter construct encoding firefly luciferase interrupted by a premature UGA stop codon followed by the well-characterized SL8 epitope from ovalbumin. Upon DAP treatment, both luciferase activity and surface presentation of SL8-major histocompatibility complex (MHC) class I complexes increased significantly, surpassing the effects observed with the aminoglycoside G418. These results provide direct evidence that DAP-mediated readthrough can lead to the translation of C-terminal antigenic peptides, which are subsequently processed and presented by MHC class I molecules (Figure 1).

A key strength of the study lies in its comprehensive *in vivo* validation. Daily

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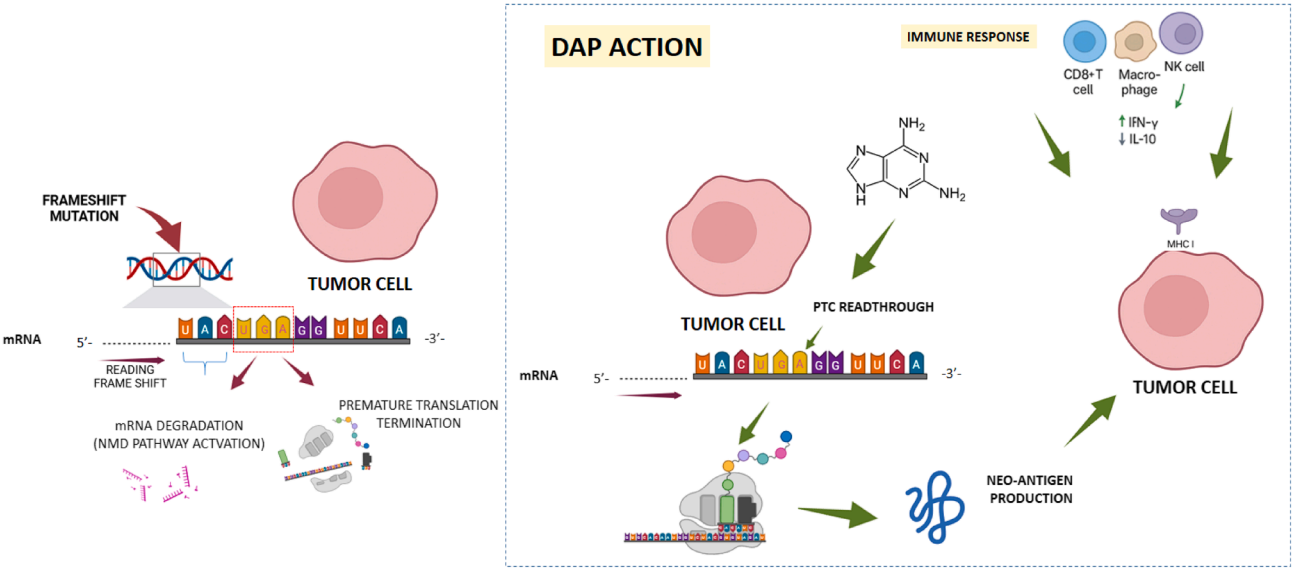

Figure 1. 2,6-diaminopurine promotes translational readthrough of premature termination codons in tumor cells, generating C-terminal neoantigens that are presented by MHC class I molecules and recognized by cytotoxic T lymphocytes, leading to an antitumor immune response

oral DAP administration significantly delayed tumor growth in immunocompetent mice but not in immunodeficient mice, demonstrating that its antitumor effect requires a functional immune system. Histological analyses revealed marked infiltration of $CD4^+$ and $CD8^+$ T lymphocytes, natural killer (NK) cells, and macrophages within DAP-treated tumors, accompanied by elevated interferon (IFN)-γ and reduced interleukin (IL)-10 expression, all hallmarks of an activated cytotoxic immune response (Figure 1).

The authors further strengthen the causal link between readthrough-induced antigenicity and immune activation through an elegant two-step injection experiment. Mice first injected with DAP-pretreated tumor cells exhibited immune infiltration and IFN-γ induction not only in those tumors but also in subsequently implanted, untreated tumors, suggesting that the immune system, once primed by DAP-induced neoantigens, can mount systemic antitumor immunity, akin to a vaccination effect.

Despite the originality of the work and the innovative potential of its application, several aspects need to be addressed to warrant future development. Further research is therefore encouraged, since the direct molecular identification of neoantigens generated by DAP-induced readthrough remains incomplete, and the endogenous repertoire of DAP-induced peptides in real tumors has not yet been characterized. Additionally, proteogenomic profiling of MHC-bound peptides would be essential to confirm the hypothesized diversity of novel epitopes. Furthermore, comparison with other UGA-specific TRIDs, such as ataluren or its analogs, to verify a similar immune response triggered by UGA readthrough could provide further validation of the proposed mechanism. Since DAP selectively induces readthrough at UGA codons, which represent only a subset of nonsense mutations, expanding this strategy toward UAA and UAG stop codons would require exploration of TRIDs with a broader spectrum of readthrough. While the murine CT26 model provides a proof of concept, it is microsatellite stable and only moderately immunogenic. Therefore, future work in microsatellite-instability-high tumor models could more robustly demonstrate the synergy between high mutational load, readthrough activity, and immune responsiveness.

In conclusion, Lejeune and colleagues present compelling preclinical evidence that the readthrough-inducing molecule DAP can reshape tumor immunogenicity and elicit an adaptive antitumor response. Their findings bridge two previously distinct research domains: the pharmacology of translational termination and the immunobiology of cancer. By converting translational noise into an immunological signal, DAP exemplifies how manipulating the fidelity of the genetic code can have profound therapeutic consequences. The future challenge will be to refine this approach to achieve maximal selectivity, minimal toxicity, and synergistic integration into the expanding arsenal of cancer immunotherapies.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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