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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Unraveling the Dynamics of T Cell-Dependent Bispecific Antibodies in Multiple Myeloma: Multi-Omic Insights from Primary Patient Cells

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Introduction: Multiple myeloma (MM) is the second most prevalent hematologic malignancy. Despite significant advancements in immuno-oncology (IO) therapeutics, such as T-cell dependent bispecific (TDB) antibodies targeting MM antigens, MM remains incurable. This highlights the need for further research to develop personalized and effective treatments. The study of primary MM cells is pivotal for understanding therapeutic effects and patient variability but faces challenges due to cell scarcity and low *ex vivo* viability. Our microfluidic platform, requiring minimal cell input and no matrices or immobilization, maintains viability and offers versatile, integrated readouts, thereby deepening the breadth and depth of information from *ex vivo* experiments. In this study, we developed a multi-omic assay to gain insights into cytotoxicity, cytokine profiling, and gene expression changes to conduct a comprehensive analysis of TDBs targeting MM antigens in primary MM co-cultures.
Methods: Primary T cells and MM cells, isolated from cryopreserved patient samples, were cultured in the microfluidic platform, either autologously or allogeneically, at specific effector-to-target cell ratios. Cells were treated with TDBs with daily media replenishment for up to 72 hours. Following incubation, cells were stained with Hoechst, viability staining, CD25, and CD69 antibodies followed by high-content imaging. Supernatant from each condition was pooled for cytokine profiling, and nucleic acids were extracted from the cells post-imaging. Gene expression analysis was performed using NanoString's Pan-Cancer IO 360™ panel.
Results: Image analysis revealed that TDB treatment induced T cell-mediated cytotoxicity in four patients, with sub-optimal TDB-induced cytotoxicity observed in two patients, highlighting intrinsic resistance mechanisms to TDB treatment and the need for greater mechanistic understanding. Notably, all patients' effector cells exhibited upregulation of CD69, a T cell activation marker, after 24 hours post-TDB treatment which was sustained for 72 hours. TDB treatment also led to the upregulation of CD25, starting at 24 hours and peaking 72 hours post-treatment. Furthermore, enhanced secretion of interferon-gamma, tumor necrosis factor-alpha, and interleukin-2 (IL-2) was detected in samples that responded to TDB treatment, while samples that exhibited resistance to TDB treatment showed no IL-2 secretion. Gene expression analysis revealed an upregulation of genes linked to the interferon-gamma signaling pathway in TDB-treated samples. Additionally, when comparing the gene signatures between responders and non-responders at baseline, the non-responders' samples displayed increased expression of CD3D, CD3G, CD8a, and CD28. These markers, typically associated with T-cell signaling, hint at a more complex pre-existing T-cell activation landscape in non-responders, potentially influencing the outcome of T-cell engager therapies.
Discussion: We introduced a novel assay on our microfluidic platform to profile TDBs with primary MM and immune cells. This platform offers several key advantages. First, it enables robust dose-response evaluations of cytotoxicity, providing accurate assessments of the TDBs' effectiveness. Second, it allows for concurrent and longitudinal profiling of cytokine release and measurement of T cell activation markers, offering a comprehensive understanding of T cell responses. And third, the platform facilitates gene expression analysis using the same cells, enabling the generation of fully integrated readouts and deeper mechanistic understanding. This multi-omics approach not only deepens our understanding of IO therapies for MM but also offers potential for diverse hematological malignancies. Direct analysis of patient cells provides invaluable insights into individual responses, setting the stage for mechanistic discoveries and the development of more effective, personalized therapies. Ultimately, these insights underscore the transformative potential of integrative multi-omics in steering immune-centric therapies for hematologic malignancies.

Disclosures Wu: *Lynx Biosciences, Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Chen:** *Lynx Biosciences, Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Deangelis:** *Janssen Biotech:* Ended employment in the past 24 months. **Cortes:** *Janssen R&D:* Current Employment. **Morachis:** *Lynx Biosciences:* Current Employment, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees. **Titz:** *Lynx Biosciences:* Current Employment, Current holder of *stock options* in a privately-held company. **Cases:** *Lynx Biosciences, Inc.:* Consultancy. **Verona:** *Janssen:* Current Employment, Current equity holder in publicly-traded company, Patents & Royalties. **Pak:** *Lynx Biosciences, Inc.:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees.

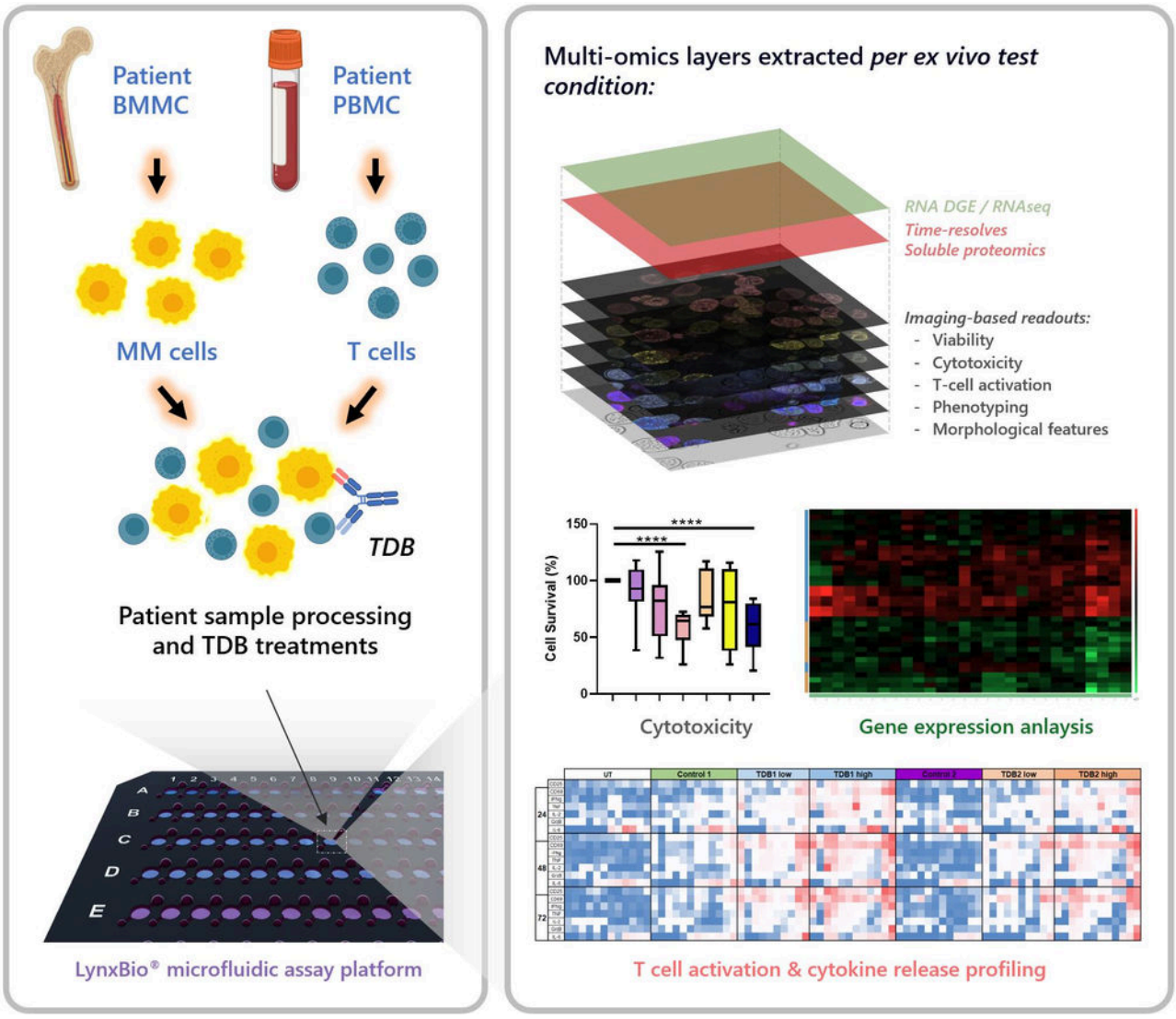

Figure 1

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