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Blood 142 (2023) 244–246

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Advancing CAR-T Therapy in Acute Lymphoblastic Leukemia: Multi-Omic Analyses of CD19-Directed CAR-T Cells Enabled By an Ex Vivo Co-Culture Platform

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Background: Acute Lymphoblastic Leukemia (ALL) has witnessed remarkable progress in the treatment of patients with Chimeric Antigen Receptor T-cell (CAR-T) therapies, leading to high response rates and durable remissions. However, challenges persist for patients who do not respond to CAR-T treatment, experience relapse, or encounter adverse events such as cytokine release syndrome. Overcoming these challenges necessitates a deeper understanding of mechanisms and the development of advanced technologies and assays. This study focuses on harnessing the potential of an innovative microfluidic platform for high-throughput cell-based assays, enabling multi-omics and functional analysis using a minimal amount of primary patient cells. By utilizing this platform, we aim to gain insight into the dynamics of primary patient material by evaluating responses to aid in selection of promising CAR-T enhancements or combination therapies designed to overcome mechanisms of CAR-T failure or relapse to improve clinical outcomes.
Methods: For the cytotoxicity assay, we utilized autologous or allogeneic pairs of CD19-directed CAR-T cells and T-cell negative fractions from ALL patients' blood apheresis samples, containing CD19-expressing cancer cells. A negative control was established using untransduced T-cells. To ensure high cell viability and purity, the cells underwent careful processing and were labeled with long-lived dyes for tracking. Multiple cell suspensions with precise Effector to Target to Negative Fraction ratios were prepared. Only a few thousand cells from each coculture suspension were seeded in microfluidic culture chambers, which required no fluidic control system. Experiments were conducted for up to 96 hours with media replacement every 24 hours. At each endpoint, media was collected for cytokine quantification, and cells were stained for live/dead counting as well as quantification of intracellular or surface marker expression by imaging. After image acquisition, cells were collected for RNA extraction and gene expression analysis.
Results: Results demonstrated that the developed co-culture platform allows precise control over culture conditions, ensuring culture of accurate ratios of highly complex cell populations. The viability of primary cells in the device guaranteed functional relevance of the cytotoxicity assays for up to a 96-hour timepoint. Strong CAR-T-mediated cell killing was observed, with a dose-dependent effect depending on the co-culture's relative CAR-T content. Less than 50% cell survival was observed in cocultures with Effector to Target ratio 1:1, as compared to the untransduced T-cell control. Furthermore, strong CAR-T activation was observed with over 60% of CART expressing CD25 and CD69 surface markers, as measured by imaging. A multi-color staining panel allowed for precise monitoring of changes over time within the assay of immune cell populations, including cancer cells, monocytes/macrophages, and NK cells, among others. Cytokine analysis revealed an increased secretion of IL-2, TNFa, IFNg, and Granzyme B in coculture with CAR-T cells compared to untransduced T-cells, with a dose-dependent effect based on the CAR-T cell ratio. Finally, RNA isolated from remaining cells at the end of the assay was of high quality with yields sufficient to enable transcriptome analysis for each treatment condition.
Discussion: This study contributes to the development of innovative multiplexed high throughput strategies for in vitro functionality assessments in cell therapy, including combination studies and deeper mechanistic understandings. The platform's ability to generate fully integrated, multi-omics readings from complex allogeneic and autologous cocultures of CAR-T and patient cells offers a new frontier in early discovery and translational cancer research. This opens new possibilities for drug discovery, drug screening, and disease modeling, ultimately advancing our understanding of complex biological processes and accelerating therapeutic development. Future directions include exploring mechanisms of resistance and sensitivity, including donor-specific effector cell functionality, combination studies, impacts of immunosuppressive cell types, patient heterogene-

ity of target antigen expression or other co-stimulatory molecules, and evaluation of product potency or fitness of incoming apheresis material.

Disclosures Mattie: *Kite Pharma, a Gilead company:* Current Employment, Current equity holder in publicly-traded company. **Gillard:** *Lynx Biosciences:* Current Employment. **Calderon:** *Lynx Biosciences:* Current Employment. **Do:** *Lynx Biosciences:* Current Employment. **Salunkhe:** *Kite Pharma, a Gilead company:* Current Employment, Current equity holder in publicly-traded company. **Heeke:** *Kite Pharma, a Gilead company:* Current Employment, Current equity holder in publicly-traded company. **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. **Pak:** *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.

https://doi.org/10.1182/blood-2023-180395

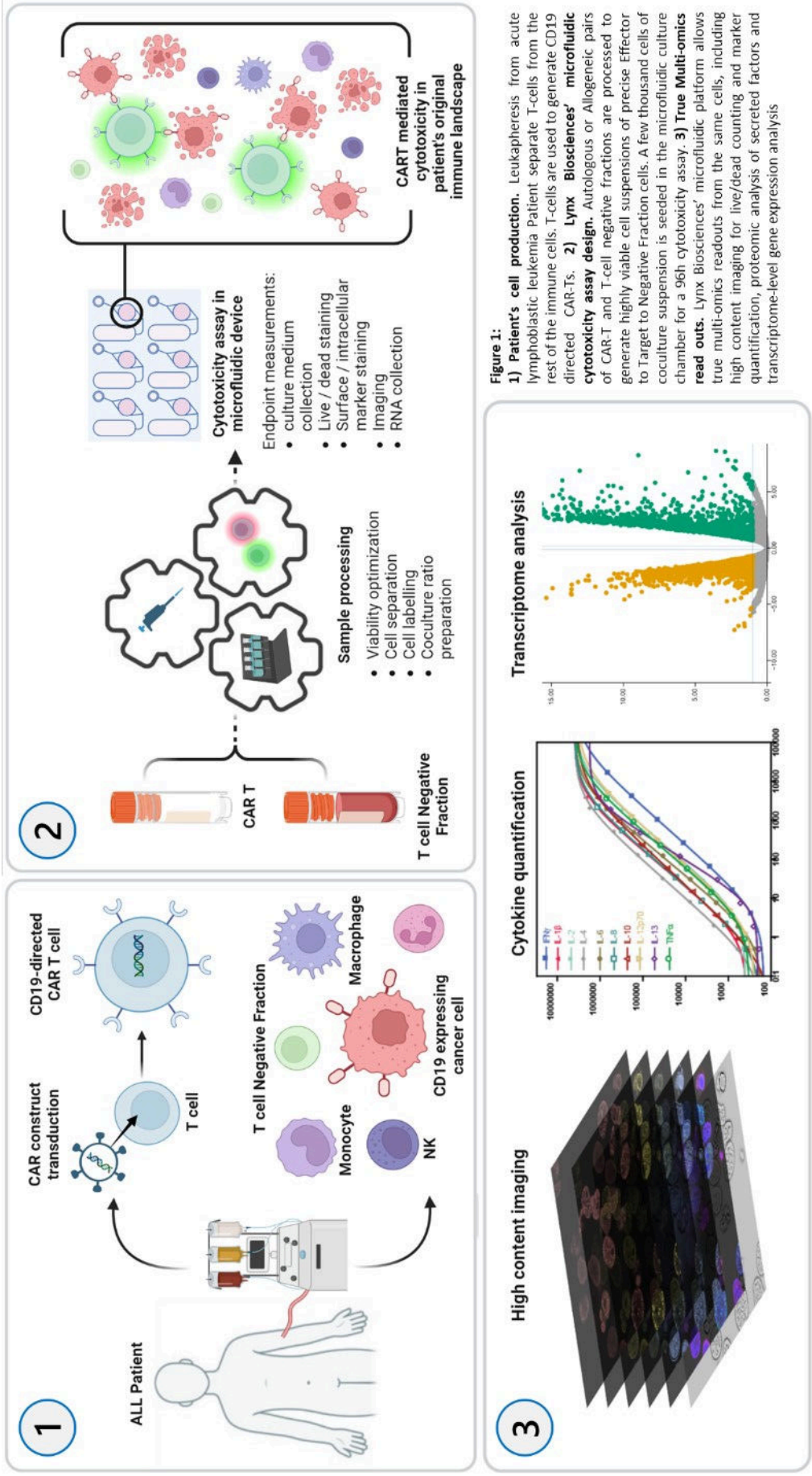

Figure 1:
1) Patient's cell production. Leukapheresis from acute lymphoblastic leukemia Patient separate T-cells from the rest of the immune cells. T-cells are used to generate CD19 directed CAR-Ts. **2) Lynx Biosciences' microfluidic cytotoxicity assay design.** Autologous or Allogeneic pairs of CAR-T and T-cell negative fractions are processed to generate highly viable cell suspensions of precise Effector to Target to Negative Fraction cells. A few thousand cells of coculture suspension is seeded in the microfluidic culture chamber for a 96h cytotoxicity assay. **3) True Multi-omics read outs.** Lynx Biosciences' microfluidic platform allows true multi-omics readouts from the same cells, including high content imaging for live/dead counting and marker quantification, proteomic analysis of secreted factors and transcriptome-level gene expression analysis

Figure 1