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HIGH-CONTENT SCREENING TO MAP CAR-T FUNCTION ACROSS HYPOXIA-NORMOXIA GRADIENTS IN A 2D TUMOR-STROMA MODEL

JPZ Murarolli, GBC Moreto, ML Arrojo, AM Martins, SR Caruso, MD Orellana, RA Panepucci

Regional Blood Center of Ribeirão Preto, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil

Introduction: The tumor microenvironment (TME) imposes structural and metabolic barriers that impair CAR-T cell function. Hypoxia is a central factor in this resistance, yet conventional in vitro cytotoxicity assays lack the spatial resolution to quantify CAR-T performance across oxygen gradients. **Aim:** To develop an advanced heterotypic 2D co-culture model of $CD19^+$ lymphomas that mimics radial oxygen and metabolic gradients using the "coverslip hypoxia" approach, enabling automated, spatially resolved quantification of CAR-T activity via automated quantitative fluorescence microscopy - high-content screening (HCS). This configuration allows simultaneous assessment of hypoxic and normoxic niches within the same well, supporting high-throughput screening of pharmacological or genetic strategies in 96-well plates. **Material and methods:** HS-5 stromal cells (10^4/well) were seeded and, the next day, co-cultured with $CD19^+$ Raji or $CD19^-$ K562 tumor cells (10^4/well, eFluor 670-labeled) and either anti-CD19 CAR-T or non-transduced T cells (2×10^4/well, CellTrace Violet-labeled) under four conditions, in triplicate. Hypoxia was induced with 5 mm glass coverslips (CS), with wells without coverslips (No-CS) as normoxic controls. Whole-well images (5×5 fields, $10\times$ Obj.) were acquired on an ImageXpress MICRO XLS HCS system, stitched, and processed in CellProfiler to define four spatially distinct regions: CS_InnerCore (severe hypoxia at CS center), CS_OuterCore (moderate hypoxia), CS_Periphery (transition at CS edge), Outside (normoxia beyond CS). Cy5 and DAPI channels enabled segmentation of tumor and T cells, respectively, assigning each to its oxygen-defined compartment. Mean SYTOX Green intensity (FITC channel) identified dead cells, while eccentricity (ranging from 0, round, to 1, elongated) served as a T cell activation metric. **Results:** In Raji co-cultures, CAR-T cells induced marked tumor death after 24h, highest in Outside (~40%) and No-CS (~60%) regions, but minimal in hypoxic regions (< 10%). Non-transduced T cells showed negligible cytotoxicity, with only modest increases (~20%) in Outside and No-CS regions. In $CD19^-$ K562 co-cultures, tumor death was negligible across all regions (< 5–10% in normoxia), confirming antigen specificity. Effector death was greater in normoxia (~25–40% in Outside/No-CS) versus hypoxia (~5–10%), suggesting low oxygen protects both targets and effectors. Morphometric analysis showed CAR-T with Raji targets had increased eccentricity (up to ~0.6 at 24h), with a hypoxia-to-normoxia gradient, indicating greater motility under oxygenated conditions. CAR-T with K562 showed no significant eccentricity changes, confirming antigen-specific activation. Non-transduced T cells maintained lower eccentricity (~0.45–0.5) in all conditions. **Discussion and conclusion:** These findings show that hypoxia gradients selectively impair CAR-T cytotoxicity, survival, and motility, while preserving antigen specificity. The assay integrates physiologically relevant oxygen gradients with automated, multiparametric image analysis, enabling real-time, region-specific quantification of CAR-T function across parallel conditions. Unlike bulk readouts, this platform captures microenvironment-driven functional heterogeneity and directly compares matched hypoxic vs. normoxic zones, making it a powerful preclinical screening tool to identify strategies restoring CAR-T efficacy in the hostile stromal–hypoxic TME. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process Number 2022/12856-6.

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HIV INFECTION IS INDEPENDENTLY ASSOCIATED WITH DELAYED PLATELET AND LEUKOCYTE ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

AR Belisário, LT Mendonça, MC Martins, KL Prata

Centro de Tecidos Biológicos de Minas Gerais, Fundação Hemominas, Belo Horizonte, MG, Brazil

Background: HIV infection affects over 40 million people globally. Antiretroviral therapy has transformed HIV into a chronic, manageable condition, with life expectancy now approaching that of the general population. As people living with HIV (PLHIV) age, hematological and non-hematological comorbidities requiring autologous stem cell transplantation (ASCT) are increasingly common. While ASCT is feasible in selected PLHIV, the impact of HIV on engraftment kinetics remains poorly defined, limiting evidence-based optimization of transplant strategies. **Aim:** This study evaluated the association of HIV infection with infusion-related adverse events and outcomes after ASCT. **Material and methodos:** In this nested case-control study, 114 patients underwent ASCT at six Brazilian centers (2014–2025), with cryopreservation at a single facility. Nineteen (16.7%) were HIV-positive; all had positive serology at clinical/social screening and HPC collection, and four also had positive NAT results. Each case was matched by age, sex, diagnosis, and center to five HIV-negative controls. Data abstraction captured mobilization parameters, cell yield/viability, engraftment times, and infusion-related adverse events. Engraftment was defined as the time from infusion (Day 0) to neutrophil $> 0.5 \times 10^9$/L for three days, leukocyte $> 1 \times 10^9$/L for three days, or platelet $>20 \times 10^9$/L for seven days without transfusion. **Results:** Baseline characteristics were balanced between groups. Infusion-related adverse events were infrequent, with nausea, vomiting, flushing, and fever occurring in < 10% of participants, and rates were similar between HIV-positive (11.8%) and HIV-negative (16.3%) groups. Mobilization parameters, collection yield, and cell viability did not differ by HIV status. In