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

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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

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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

unadjusted analyses, platelet engraftment occurred later in HIV-positive participants. Multivariable analysis confirmed HIV-positive status as an independent predictor of delayed platelet engraftment ($\beta = 1.85$ days; 95% CI: 0.63–3.07; p = 0.003) and leukocyte engraftment ($\beta = 1.002$ days; 95% CI: 0.13–1.87; p = 0.025). Neutrophil engraftment was not significantly associated with HIV status (p = 0.075). Higher infused $CD34^+$ doses were consistently associated with faster recovery across all cell lineages, whereas the use of 10% DMSO as the cryopreservation solution was associated with longer engraftment times. **Discussion and conclusion:** In conclusion, this study provides novel evidence that HIV infection independently delays platelet and leukocyte engraftment after ASCT, even when controlling for cell dose and other key transplant variables. The magnitude of the effect on platelet recovery is clinically meaningful, with potential to increase bleeding risk, prolong transfusion dependence, and delay full hematologic recovery. These findings underscore the need for tailored supportive care, optimized $CD34^+$ collection, and proactive post-transplant monitoring in PLHIV. By addressing modifiable factors and integrating HIV-specific considerations into transplant planning, clinicians may mitigate the adverse impact of HIV on hematopoietic recovery. Future multicenter prospective studies with comprehensive immunologic profiling are warranted to elucidate underlying mechanisms and inform evidence-based guidelines. Overall, HIV infection should be recognized as an independent predictor of delayed engraftment after ASCT, reinforcing the importance of individualized transplant strategies for this population.

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IMMUNE EFFECTOR CELL-ASSOCIATED HLH IN HIGH TUMOR BURDEN PATIENTS TREATED WITH CAR-T: LESSONS FROM BRAZILIAN CASES

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Introduction: CAR-T cell therapy is based on the modification of T lymphocytes to express chimeric receptors. Although effective in the treatment of B-cell malignancies, these therapies are associated with inflammatory toxicities such as Cytokine Release Syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), and, more rarely, Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis (IEC-HLH). We report two cases of IEC-HLH in patients who received anti-CD19 CAR-T infusion as part of a Brazilian academic study. **Case report:** Case 1: Male, 70 years old, with refractory CLL (TP53 mutated, 11 prior lines, including allo-HSCT). Pre-CAR-T bone marrow showed 83% clonal lymphocytes. Developed grade I CRS on D+1, treated with tocilizumab 8 mg/kg. On D+15, presented with fever, cytopenias, hyperferritinemia (7,417 ng/mL), hypofibrinogenemia, hypertriglyceridemia, elevated transaminases and LDH, with an H-Score of 200. Treated with a cumulative dose of 329 mg dexamethasone and anakinra (10–5 mg/kg/day) for 7 days, achieving complete resolution. Case 2: Male, 9 years old, with ALL and bone marrow showing 95% blasts, received prophylactic tocilizumab. Developed grade III CRS (D+1) and grade IV ICANS (D+5), both treated with immunosuppressants. On D+6, presented with findings suggestive of IEC-HLH: ferritin 43,044 ng/mL, cytopenias, hypofibrinogenemia, elevated transaminases, and triglycerides, with an H-Score of 184. Received anakinra (10–7 mg/kg/day for 7 days) with complete clinical and laboratory response. On D+30 bone marrow reassessment, he achieved MRD-negative complete remission, maintained to date (D+367). **Conclusions:** Both patients had high tumor burden and developed IEC-HLH later than CRS onset. The CLL patient showed late CAR-T cell expansion (0.45% to 38.1% between D+14 and D+21) coinciding with a decline in CD19+ cells (82.2% to 1.2%), suggesting a correlation between cell expansion and IEC-HLH. Both cases were treated with anakinra, in line with prior experience in secondary HLH. Therapeutic response was satisfactory. Both patients remain in complete remission (>1 year post-infusion). These cases highlight the importance of recognizing IEC-HLH in patients with high tumor burden. The favorable outcomes with anakinra suggest its potential as a standardized therapeutic strategy. Specific protocols are essential for the diagnosis and management of this rare but potentially severe complication.

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IMPACT OF SHORT-TERM TRANSFER FROM NITROGEN TO -80°C FREEZER ON THE VIABILITY OF CRYOPRESERVED CELLULAR THERAPY PRODUCTS: AN IN VITRO STUDY

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Introduction: Liquid or vapor nitrogen is considered the gold standard for the storage of cellular therapy products, ensuring long-term viability and stability. However, contingency planning remains a challenge, as each cryopreserved unit requires an available slot in another tank for redundancy in case of failure. Additionally, most transplant centers in Brazil lack liquid nitrogen storage tanks, and cryopreserved cellular products should be delivered before conditioning begins. **Aim:** This study aimed to evaluate the impact of short-term