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MOLECULAR EVALUATION OF ROCK2 AND IGFBP6 DYNAMICS FOLLOWING CAR-T THERAPY: POTENTIAL BIOMARKERS OF INFLAMMATION

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Introduction: CAR-T therapy has revolutionized the treatment of relapsed/refractory hematologic malignancies by enabling genetically modified T lymphocytes to target and eliminate tumor cells. However, it is often associated with severe toxicities, including cytokine release syndrome (CRS) and neurotoxicity (ICANS). Molecules such as ROCK2 (Rho-associated protein kinase 2) and IGFBP6 (Insulin-like Growth Factor Binding Protein 6), previously linked to inflammation in rheumatologic diseases and myelofibrosis, may influence these toxicities. Additionally, monitoring CAR-T expansion in peripheral blood may help predict clinical outcomes and toxicity risk.

Aim: This study aimed to validate digital droplet PCR (ddPCR) for monitoring CAR-T expansion in 29 patients with aggressive B-cell lymphomas and to assess ROCK2 and IGFBP6 expression in relation to toxicity.

Methods: Peripheral blood samples were collected at +24 h, +72 h, +7 d, +10 d and +14 days post-infusion. CAR-T cells were quantified by ddPCR using primers for the VH region of the anti-CD19 scFv domain (clone FMC63), with albumin as reference. A multiplex ddPCR assay was developed to measure ROCK2, IGFBP6 and GAPDH (housekeeping gene) expression. Droplets were generated and analyzed using the Bio-Rad QX200 system to quantify CAR-T copies and gene expression ratios.

Results: All patients showed peak CAR-T expansion at day 7. Tisa-cel–treated patients had lower overall expansion than those receiving Axi-cel or Brexu-cel, but showed higher CAR-T copies at 24 h ($p=0.03$). Based on CAR-T score, patients were grouped into 'low' and 'high' CRS. At 72 h, the high CRS group had fewer CAR-T copies (485 vs 2160/µg gDNA; $p=0.07$). To assess CRS prediction, ROCK2 and IGFBP6 mRNA levels were measured. IGFBP6 levels remained consistently low. ROCK2 showed a biphasic trend with a peak at 24 h. An inverse correlation with CRP was found at 24 h and 72 h ($p=0.034$; $p=0.048$). Notably, lower ROCK2 levels were associated with a rise in Tregs, supporting its potential immunomodulatory role.

Conclusions: ddPCR is a reliable tool for CAR-T cell monitoring. Our multiplex assay also enabled simultaneous evaluation of inflammatory markers. ROCK2 expression dynamics may reflect immune regulation and help identify patients at risk of CRS. In contrast, IGFBP6 appears less informative. Larger cohort studies are necessary to confirm these results.
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