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# POSTER

## 628. AGGRESSIVE LYMPHOMAS: CELLULAR THERAPIES

### Pre-manufacturing CD8+ central-memory type 2 T cells predict axicabtagene ciloleucel failure in large B-cell lymphoma

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**Abstract Introduction** Axicabtagene Ciloleucel (Axi-Cel), a CD28-based anti-CD19 CAR T-cell therapy, induces remission in approximately 50% of patients with large B-cell lymphoma (LBCL). As alternative immunotherapies emerge, developing individualized predictors for Axi-Cel response could guide treatment, identifying those most likely to achieve remission while directing others toward alternative strategies.

**Method** We analyzed clinical and biological data from 60 heavily pre-treated LBCL patients treated with Axi-Cel at Lille University Hospital, France. Eighty percent had received 2 prior therapies. Baseline blood (apheresis or D-7) was collected from 48 patients (18 discovery, 30 validation), with infusion product (IP) and D7 post-infusion samples collected in the discovery cohort. Samples were analyzed by single-cell RNA sequencing (scRNA-seq) and spectral flow cytometry (SFC).

**Results** Fifty-eight patients were evaluable for response via PET-CT at 1, 3, and 6 months. At infusion, 71.7% had progressive disease. Overall response rates (n=60) were 76.7%, 60.0%, and 56.7%, with complete response (CR) rates of 53.3%, 55.0%, and 57.6%. Multivariate analysis identified younger age (OR 0.94 per year, p=0.029), total metabolic tumor volume pre-lymphodepletion ≥60 cm³ (OR 13.10, p=0.0027), and primary refractory disease (OR 6.33, p=0.022) as adverse factors.

D7 scRNA-seq analysis showed that responders had higher levels of $CD4^+$ CAR T cells with a constrained activation signature (*TNFRSF1B*, *CD40LG*, *TNFRSF4*, *IL32*, and *IL7R*, and co-inhibitory receptors *LAG3*, *CTLA4*, *TNFRSF18* and *PDCD1*). Non-responders (NR) had more CAR $T_{regs}$ and proliferating $CD8^+$ CAR T cells with reduced effector function (high *MKI67*, low *KLRG1*, *GZMB*, *GZMH*, *PRF1*, *GNLY*, and *FASLG*). NR patients had lower absolute $CD8^+$ CAR T cell counts, suggesting poor expansion of other subsets rather than hyperproliferation. These differences were less evident in the IP, underscoring the importance of the in vivo environment.

Strikingly, cluster analysis of baseline samples revealed a robust signature of resistance to Axi-Cel, with a strong enrichment in naïve $CD8^+$ T cells (*TCF7*, *LEF1*, *CCR7*, *SELL*, and *IL7R*) and CM type 2 $CD8^+$ T cells (expressing *GATA3*, *CCR4*, and *CRTH2*, along with *IL7R*, *TCF7* and *LEF1*). Bulky disease correlated with the significant enrichment of these less differentiated $CD8^+$ T cells, confirmed by SFC. Validation in an independent cohort confirmed the biomarker profile, including enrichment in CM $CD8^+$ T cells and $CRTH2^+$ cells in NR patients, especially those with bulky disease. These findings suggest tumor burden may drive T cell quiescence in NR patients.

An unbiased SFC approach identified the top 5 markers descriminating CR from NR patients, defining a $CD8^+CD4^-CD28^+IL7R^+KLRG1^-$ population significantly enriched in NR patients. This manually gated population proved to be an independent predictor of treatment failure in our multivariate model (OR 1.21 per 1%-increase, p=0.0049), showing perfect predictive value in the discovery cohort (PPV and NPV 100%) and strong accuracy in the validation cohort (PPV 100%, NPV 84.2%), further improved when combined with primary refractory status (PPV 92%, NPV 100%).

**Conclusion**We identify a baseline CM $CD8^+$ T-cell signature ($CD8^+CD4^-CD28^+IL7R^+KLRG1^-$) as a robust predictor of resistance to 28ζ-based CAR T-cell therapy in LBCL, challenging the notion that memory T cells are universally beneficial for CAR T-cell efficacy. NR patients' pre-infusion $CD8^+$ T cells display type-2 polarization, likely reflecting tumor-driven adaptation that impair cytotoxicity. This contrasts with our previous findings on the BBζ-based CAR T-cell product Tisa-Cel in leukemias, where

memory function and type-2 CAR T cells are crucial for long-term efficacy and persistence (Fraietta *et al.*; Nat Med 2018, Bai *et al.*, Nature 2024), where memory function and type-2 polarization support durability. Axi-Cel, by contrast, relies on CD28 for rapid cytotoxic T cell expansion, rendering type-2 memory $CD8^+$ T cells suboptimal. Early identification of this phenotype at leukapheresis may enable better patient stratification and guide use of alternative products to maximize therapeutic success.

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