Integrated DNA methylation and flow cytometric immune profiling identifies signatures associated with recurrence after CAR-T therapy: a pilot study

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Background

CAR-T cell therapy has improved outcomes in relapsed and refractory hematologic cancers. However, predicting patient trajectories and durable remissions has been elusive especially in patients with high risk factors such as high tumor burden and early progression. Many mechanisms of resistance have been proposed including impaired tumor infiltration, CAR-T cell exhaustion, antigen loss or down regulation, and immunosuppressive interactions. Lymphodepletion, prior to CAR-T infusion, alters immune cell populations, reshapes cytokine networks and modulates co-stimulatory signaling thus affecting response and toxicity.

Methods

We conducted a pilot study (n = 10 patients) to characterize peripheral immune alterations in patients receiving lymphodepleting chemotherapy followed by CAR-T therapy. Serial peripheral blood samples were collected at predefined time points: Day -5 (before the start of lymphodepletion therapy), Day 0 (on the day of CAR-T cell infusion), and Day 28 (28 days post-infusion). Flow cytometry was used to quantify immune cell subsets, evaluate immune checkpoint expression and correlate to DNA methylation data.

Conclusions

LAG-3 and VISTA emerged as candidates for recurrence-associated immune checkpoint proteins, with increased expression observed in patients who experienced recurrence. Methylation-derived lineage proportions correlated strongly with matched flow-cytometry populations, indicating methylation deconvolution is a valid surrogate for the assay. Future studies should evaluate whether LAG-3 expression identifies patients who may benefit from LAG-3 blockade, checkpoint-combination therapy, or selective LAG-3–directed immune-modulating platforms. Longitudinal studies are needed to validate these findings

Results

Figure 1: LAG-3 and VISTA Flow Cytometric Checkpoint Signatures Distinguish Recurrence

Figure 1A

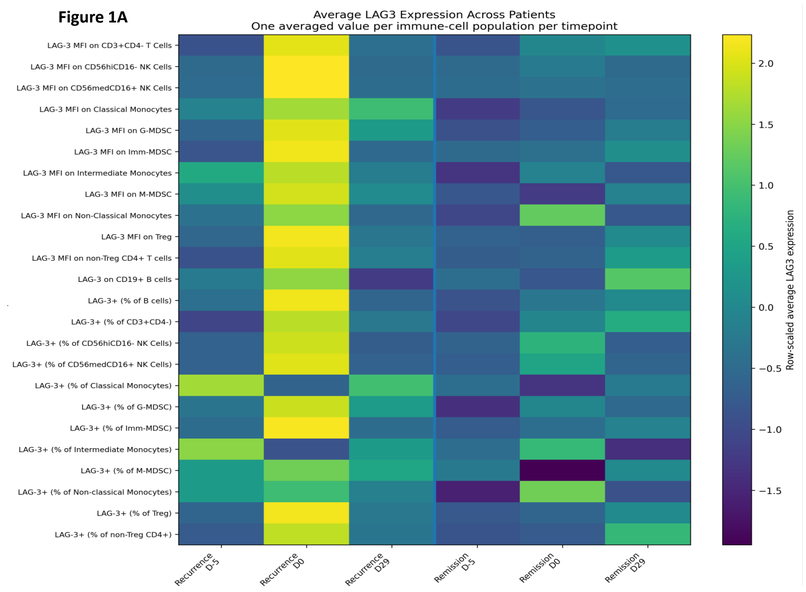


Figure 1B LAG-3 MFI on Classical Monocytes

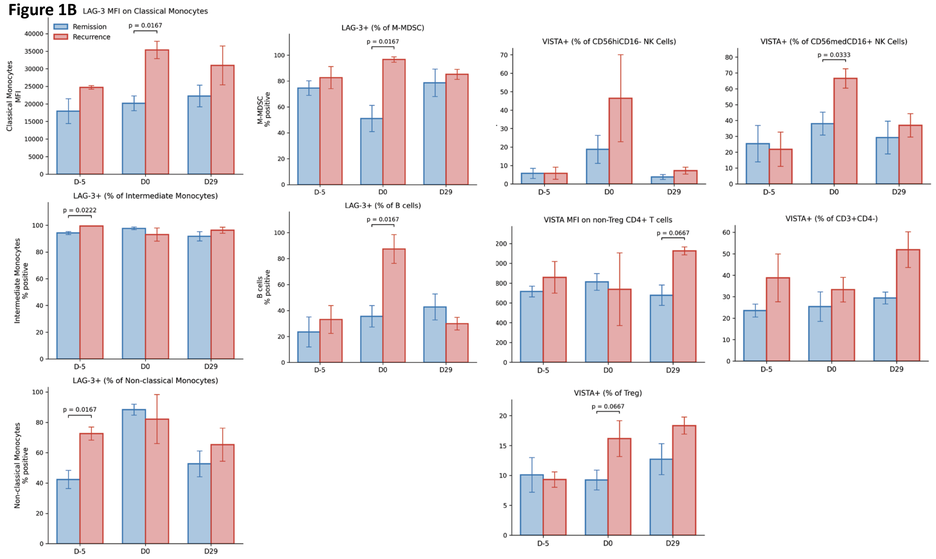


Figure 2: Methylation-Derived Immune Composition Recapitulates Flow Cytometry and CBC Data

Figure 2A

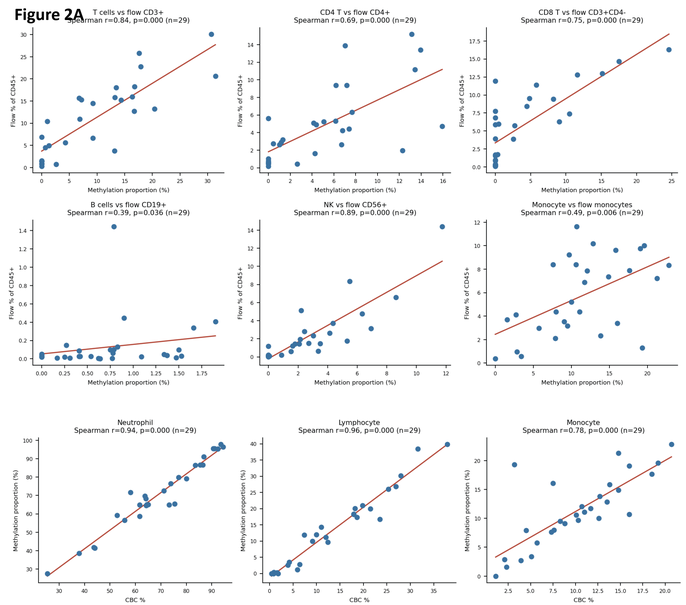


Figure 2B

