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DIAGNOSTIC NUANCES OF CIRCULATING CAR-T CELL EVALUATION POST INFUSION – A TERTIARY CARE ONCOLOGY CENTER EXPERIENCE FROM NORTH INDIA

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INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as novel therapeutic agent for the treatment of relapsed/refractory diffuse large B-cell lymphoma (R/r DLBCL). The Landscape of dysfunctional immune mechanism in peripheral blood mononuclear cells (PMBC) post CAR-T remains largely unclear. Knowledge regarding kinetics of CAR-T cells post-infusion is limited and whether these features might have any predictive value in the long term has not been clearly elucidated.

AIM

Herein, we describe various diagnostic modalities to study CAR-T cell expansion in a series of 6 cases of R/r DLBCL post CAR-T infusion detected by morphological evaluation, complete blood counting and flowcytometric immunophenotyping (FCMI) and its impact on early treatment outcome.

METHOD

Study Setting: **We studied detailed features of the CAR-T cell product as well as CAR-T expansion and persistence after infusion using lymphocyte count, morphological evaluation and multiparametric flowcytometry (MFC) in a total of six R/r DLBCL patients receiving CAR-T at our center over a period of six months (July 2023 – January 2024).**

Intervention: **All patients received CAR-T therapy post Fludarabin – Cyclophosphomide conditioning as per protocol. Flowcytometric immunophenotyping was done at baseline, Day - 7, Day - 30 and Day – 60 post infusion.**

RESULTS

Lymphocytes showed a progressive increase from 0 - 2% at day 1 to a peak of 40-75 % on day 7-10. Peripheral smear (PS) examination in all cases revealed abnormal lymphoid cells from blastoid to large atypical forms by the end of first week. MFC showed median % of various lymphocyte subset on day 7 as follows: CD3 (90.88%; range: 69.00 – 99.81%), CD4 (35.39%; range: 9.09 – 69.85%), CD8 (46.51%; range: 17.09 – 76.61%), CD19 (0.00%; range: 0.00 – 0.32%) and NK cells (10.81%; range: 4.51 – 29.00%). At the time of peak expansion, there was predominance of CD8+ T cells with a reduction in the ratio of CD4/CD8 across all cases. All cases were associated with persistence of complete B-cell aplasia (BCA) on Day – 60 except one not achieving clinical remission.

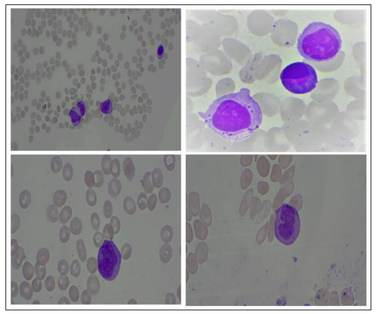


Figure 1 shows cytomorphology of atypical lymphoid cells which are small-to-medium-sized lymphocytes with abundant basophilic cytoplasm, thickened chromatin, and nuclear pleomorphism and indentation in nuclei with irregular outline & prominent granulations

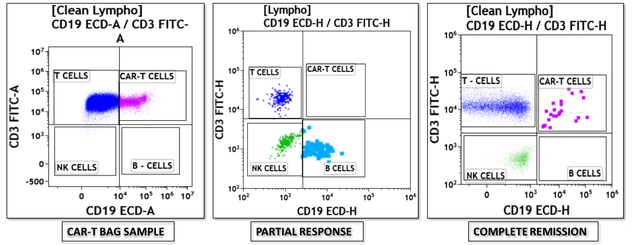


Figure 2 shows flowcytometry dot plots demonstrating CAR-T cells at various time points as assessed by Multiparametric Flowcytometry

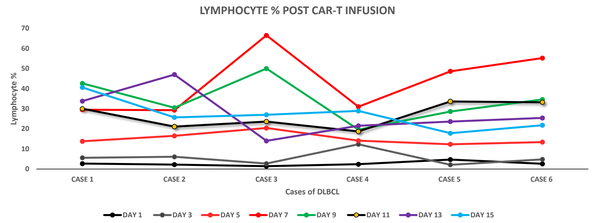


Figure 3 shows lymphocyte trend analysis on various days post-infusion

	%CD3+ Lymphocytes	%CD4+ Lymphocytes	%CD8+ Lymphocytes	CD4:CD8	%CD19+ B cells	%CD16+CD56+ NK Cells
CASE 1	--	---	--	--	--	--
CASE 2	97.89	69.85	27.19	2.57	0	13.35
CASE 3	99.81	35.39	57.52	0.62	0	4.51
CASE 4	88.19	66.84	17.08	3.91	0	10.81
CASE 5	90.88	10.56	76.61	0.14	0.07	7.75
CASE 6	69	9.09	46.51	0.2	0.32	29

Table 1 shows lymphocyte subset analysis by flowcytometry at the time of peak lymphocyte expansion

CONCLUSIONS

- A meticulous assessment of lymphocyte trend analysis, PS and bone marrow specimen may thus be utilised as a simple, cost-effective monitoring tool for proliferation and expansion of CAR-T cells.
- MFC gives supportive evidence of CAR-T cell expansion in the form of predominance of T-lymphocytes, reduced CD4/CD8 ratio and relative BCA.
- Furthermore, persistence of BCA can be explored as predictor of remission status in larger prospective study.

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