Transcriptomic analysis of the first in human phase 1 trial of intrathecal dendritic cells in patients with leptomeningeal disease from breast cancer reveals therapy induces immune response through DC-CD4-B cell axis

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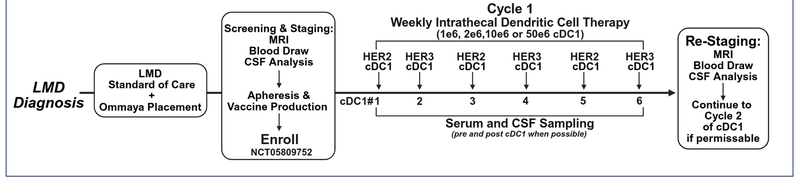
BACKGROUND

The CSF of patients with breast cancer leptomeningeal disease (BC LMD) have an innate, but not adaptive, immune cellular profile. Murine LMD models showed IT cDC1s were safe, induced a Th1 response (CD4+ > CD8+ T cell and B cell dependent), cured most HER2+ LMD, and prevented LMD recurrence. We conducted a phase I study of IT cDC1s and hypothesized the CSF would be remodeled to have a Th1 adaptive immunological profile.

METHODS

This is a phase I single-arm, dose escalation study to establish **1)** the safety and **2)** associations between clinical outcomes & translational CSF studies. Eligibility includes TNBC or HER2+ LMD pts, prior pCSpRT/WBRT, ECOG PS ≤2, and Ommaya reservoir. IT cDC1 IT were administered weekly x 12 at one of 4 dose levels (1×10^6 – 5×10^7) until PD/DLT. Endpoints were 1) safety, MTD RP2D, 2) association between clinical and immune profiles in the CSF. DLTs were defined as ≥ gr. 3 not due to LMD & didn't respond to intervention.

A pipeline diagram of phase I single-arm, dose escalation study



As of 08/01/25, the first 6 patients received 1×10^6 – 2×10^7 cDC1 therapy with noDLTs. Headaches were common and grade 3 in two (33%) patients. Two (33%) had PD of LMD within 13 weeks. One patient died from leukoencephalopathy. The average OS was 15.8 months and five (83%) are alive (**Table 1**)

Table 1: Patient Characteristics, treatment response, and life status of BC LMD patients in the Phase 1 trial.

Patient	Cohort #/ Dose	Age/ Gender	KPS on C1D1	Receptor Status	Cytol.	Completed Study Rx Y/N/ In-Progress	Grade ≥3 AE peak CSF WBCs	DLTs	Syst. Disease after Rx ie EOT imaging	LMD Response	Current KPS and Status (A or D)	Date of Follow-up**	Cause of Death	Survival from C1D1 (months)
#01	0 1×10^6	48/F	70	TNBC	+	Y	H/A Gr. 3 CSF WBC 330	N	PD	PD 3 wks s/p last cDC1 admin.	0 D	12/4/24	X-Ray induced leukoencep.	**14.6**
#02	0 1×10^6	33/F	90	HER2+, ER+, PR-	-	Y	None; CSF WBC 2250	N	PR	PD in LMD 1 wk s/p last cDC1 admin.	90 A	8/1/25		**21.7**
#03	0 1×10^6	41/F	80	HER2+, ER/PR-	-	N	None; CSF WBC 26	N	SD	SD	50 A	8/1/25		**19.9**
#05	1 2×10^6	48/F	90	TNBC	+	Y	None; CSF WBC 1705	N	NE	SD	90 A	8/1/25		**13.9**
#06	1 2×10^6	59/F	90	HER2+	-	Y	None; CSF WBC 183	N	NE	PD LMD 8 wks s/p last cDC1 admin.	80 A	8/1/25		**13.7**
#08	2 1×10^7	48/F	70	HER2+	-	N	H/A Gr. 3 i.c. venous thrombosis; CSF WBCs 275	N	SD	PD LMD 18 wks after 3 cDC1s	40 A	8/1/25		**11.2**
Abbreviations: + = positive cytology; - = negative cytology; A = alive; C = cycle; C1D1 = cycle 1 day 1; D = Day; Dose = IT dose of # of cDC1s; leukoencep. = leukoencepalopathy; N = no or none; NE = non-evaluable; PD = progressive disease; p.f = posterior fossa; Rx = treatment; s/p = status post; wk(s) = week(s); Y = yes; SD = Stable Disease														**Average Survival**
Patient #05 has a history of "+" cytology and the cytology was "-" at Moffitt before the trial														**15.8**

RESULTS

CSF cytokines Th1 cytokines included IFN-γ, IL-6, IL-12, TNF-α; Th2-related IL-4 was not elevated (**Fig. 1**). Transcriptomics showed a marked increase in CD4+ T, CD8+ T and B cells with a reduction of tumor cells (**Fig. 2**). Antibodies to HER2 or HER3 developed in four of five (80%) patients (**Fig. 3**). IT cDC1 therapy increases T cell receptor (TCR) clonality (**Fig. 4**) and B cell receptor (BCR) repertoire sharing similar CDRH3 motifs similar to known HER2 and HER3 therapeutic antibodies (**Fig. 5**).

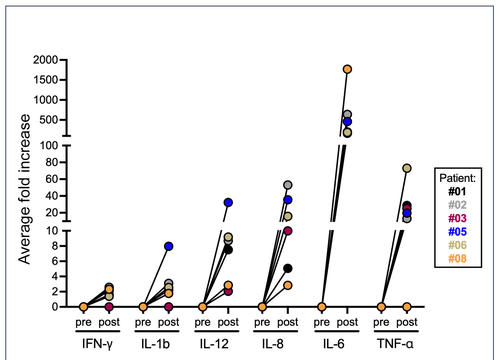


Fig 1: IT cDC1 induces cytokine spikes in the CSF in phase I trial BC LMD patients.

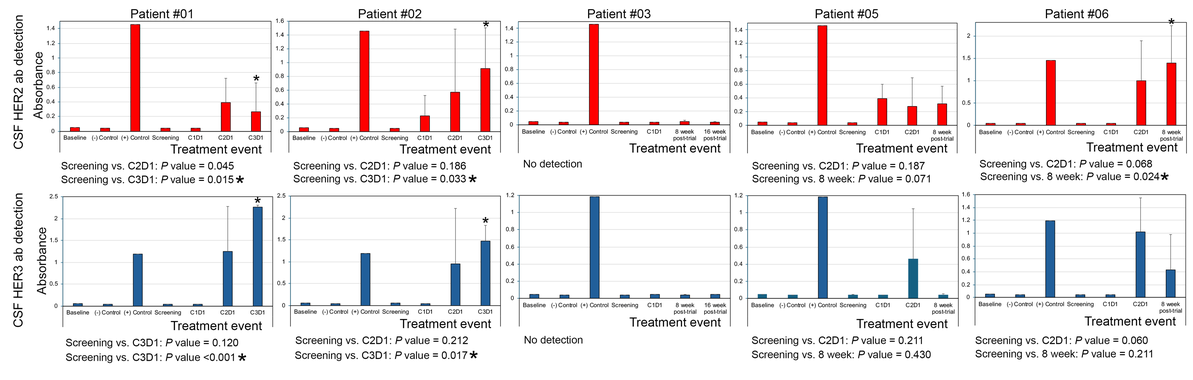


Fig 3: Detection of HER2 and HER3 IgG in patient CSF. The presence of HER2- and HER3-specific IgG in the CSF was measured in LMD patients at various time points during IT cDC1 therapy. Baseline sample was the CSF before treatment; negative control was the assay background (without IgG). Purified human recombinant anti-HER2 and anti-HER3 antibodies were used as positive controls.

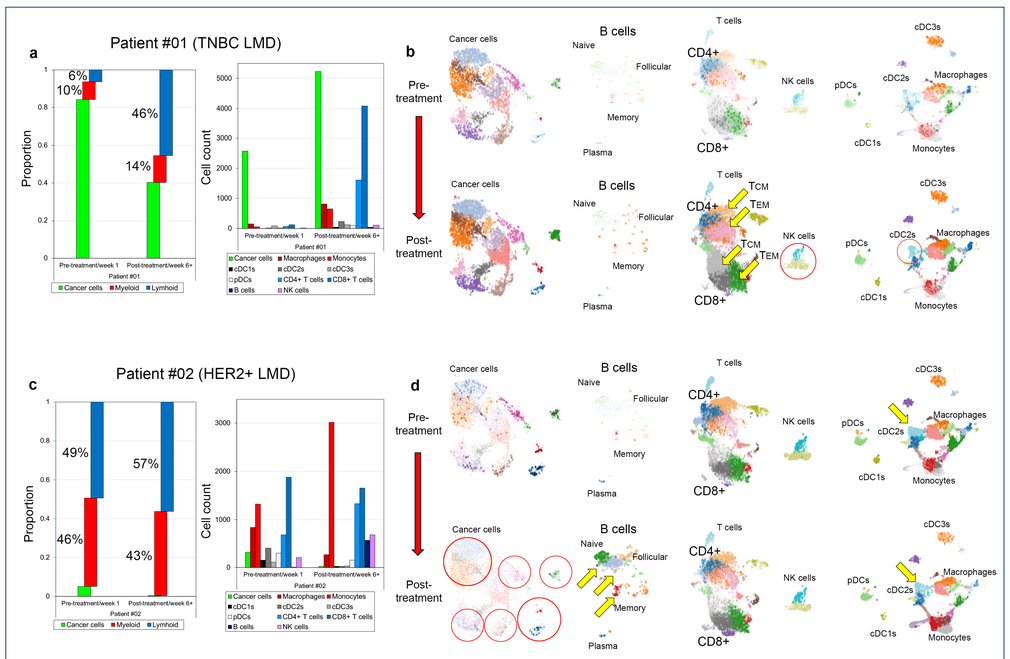
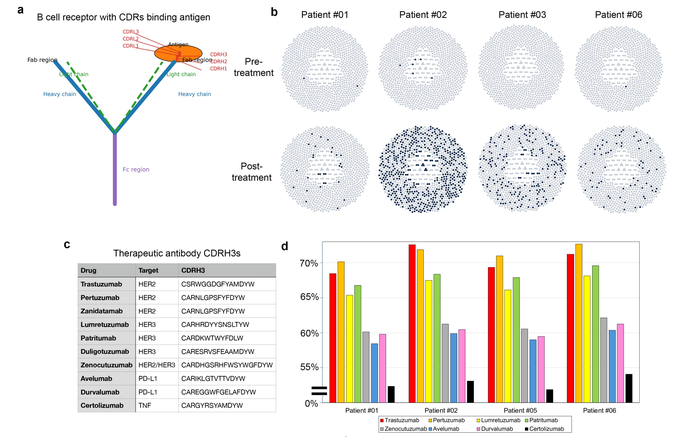


Fig. 2: Transcriptomic analysis of the CSF in patients in Phase 1 cDC1 therapy. a, Cellular compositions in the CSF of patient #01 (primary TNBC) before treatment (pre) and after six doses of IT cDC1s (post). **b,** UMAP plots of different immune cell types pre- and post-IT cDC1 treatments. **c,** Cellular compositions in the CSF of patient #02 (primary HER2+ BC) before treatment (pre) and after six doses of IT cDC1s (post). **d,** UMAP plots of different immune cell types pre- and post-IT cDC1 treatments.



Fig 4: IT cDC1 therapy induces polyclonality in TCR repertoire in patients.



Drug	Target	CDRH3
Trastuzumab	HER2	CSRWGGDGFYAMDYW
Pertuzumab	HER2	CARNLGPSFYFDYW
Zanidatamab	HER2	CARNLGPSFYFDYW
Lumretuzumab	HER3	CARHRDYYSNSLTYW
Patritumab	HER3	CARDKWTWYFDLW
Duligotuzumab	HER3	CARESRVSFEAAMDYW
Zenocutuzumab	HER2/HER3	CARDHGSRHFWSYWGFDYW
Avelumab	PD-L1	CARIKLGTVTTVDYW
Durvalumab	PD-L1	CAREGGWFGELAFDYW
Certolizumab	TNF	CARGYRSYAMDYW

Fig 5: IT cDC1 therapy induces repertoire remodeling toward therapeutic antibody-like BCR motfis. a, An illustration diagram of an antibody. b, Comparison of BCR clonality between untreated and IT cDC1 treated CSF in patients. Each shaded dot is a unique BCR sequence clone. **c,** A list of CDRH3 sequences from HER2, HER3, PD-L1 and TNF therapeutic antibodies. **d,** Strict alignment analysis revealed patients produce BCRs with structurally similar motifs – especially towards HER—directed (Trastuzumab, Pertuzumab), and secondarily toward HER3-directed antibodies in selected cases.

CONCLUSION

Our results suggest this approach activates immune responses through CD4-B cell axis that drives adaptive antitumor activity which is otherwise lacking in LMD (NCT05809752).

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