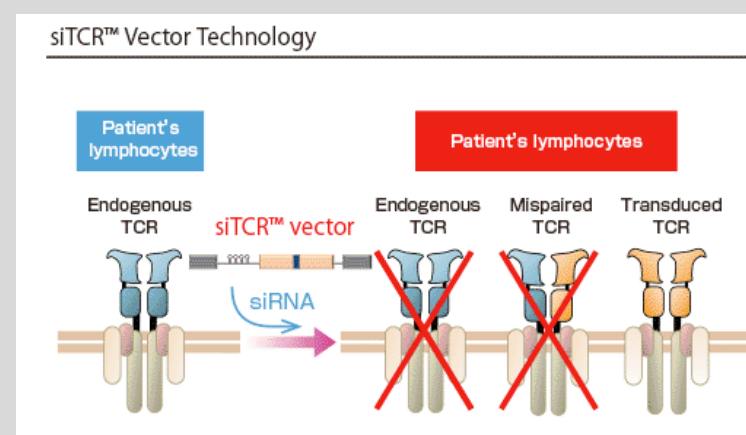


Background

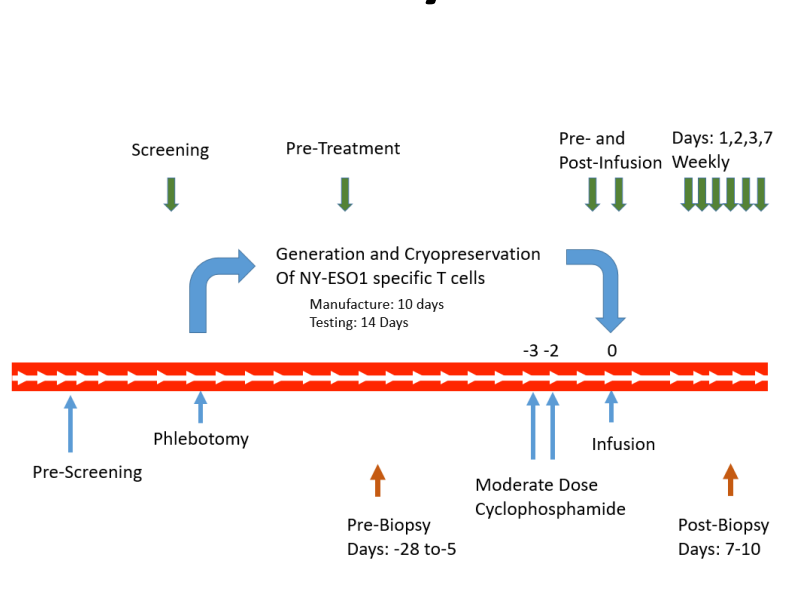
Adoptive transfer of T cell receptor gene-engineered T (TCR-T) cells can induce durable anti-cancer responses. TBI-1301 is a novel gene therapy produced by engineering autologous lymphocytes to express an NY-ESO-1-specific TCR using a retrovirus vector encoding siRNA to silence endogenous TCR. In this study, we characterize long-lived persisting transferred TBI-1301 TCR-T cells following adoptive transfer at the single-cell level.

Downregulation of endogenous TCR expression promotes efficient expression of the introduced TCR and reduces the risk of unknown side effects caused by the TCR mispairing. Using flow cytometry analysis we can track the persistence and phenotype of the infused cells for months after initial infusion.



Clinical Trial

Cohort Cy



Cohort Cy + Flu

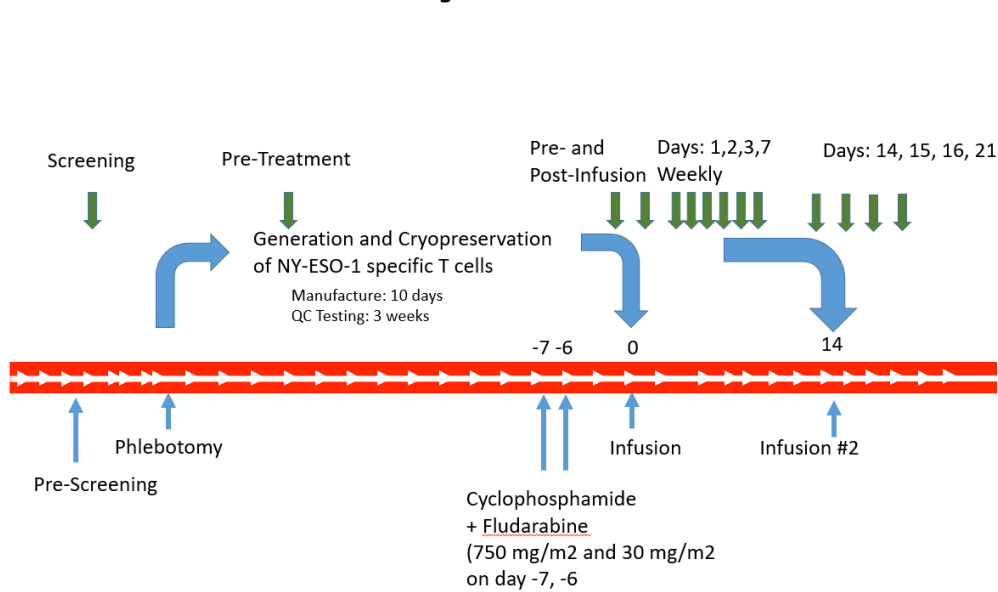


Figure 1. Schematic for TBI-1301 trial. HLA-A*02:01+ or HLA-A*02:06+ patients with NY-ESO-1 expressing tumor undergo phlebotomy at least 6 weeks prior to the planned infusion. Autologous lymphocytes are then transduced to express NY-ESO-1 specific TCR. The retroviral vector used to generate TBI-1301, MS3II-NY-ESO1-siTCR, encodes TCR α and β chains that specifically recognize an NY-ESO-1 derived peptide that is presented in the context of HLA-A*02:01 or HLA-A*02:06 molecules. The vector also encodes siRNA (small interfering RNA) that are homologous to the constant (C)-region sequence of endogenous, but not transduced, TCR α and β chain mRNAs (5-7). Patients undergo lymphodepleting chemotherapy with either 750mg/m²/d of Cyclophosphamide on Days -3 and -2 or harsher lymphodepletion consistent of Cyclophosphamide and Fludarabine before being infused with 5x10⁹ cells on Day 0. Radiographic imaging of sites of tumor was performed preinfusion and on Day 56 and every 3 months thereafter.

Methods

Patients eligible for the approved study (UHN REB 15-9534) included those with informed consent, HLA-A*02:01 or A*02:06 haplotype, and NY-ESO-1 expression by IHC. PBMCs were harvested and processed to generate engineered TBI-1301 TCR-T cells. Patients received an infusion of 5x10⁹ cells on day 0 after lymphodepletion with cyclophosphamide (CY) alone (750 mg/m² on day -7 and -6) or in combination with fludarabine (FLU) (30 mg/m² on day -7 and -6). Endpoints included safety, efficacy, and biological correlates for persistence of TCR-T cells post-infusion. The TBI-1301 infusion product and persisting TBI-1301 TCR-T cells were assessed by multi-parameter flow cytometry, single-cell RNA and TCR sequencing analysis.

Table 1. Characteristics of the Patients and Clinical Outcomes.

Lympho-depletion	Patient	Age/Sex - Diagnosis	Prior Treatment	NY-ESO-1 Expression by Tumour	# Infused Cells	Grade of CRS	Best Overall Response (RECIST)	Time to Progression (days)	OS (days)
Cy	060	40/F - Endometrial	Carbo/Tax; PI3K inh; Pembrolizumab; xrt	<5%	5.0	None	SD (3.6%)	136	1807+
Cy	159	49/M - Synovial	Doxo/Ifos; xrt	>75%	2.14	Grade 2 (tocilizumab)	SD (-2.7%)	165	811
Cy	208	38/M - Synovial	Doxo/Ifos	>75%	5.0	Grade 1	PR (-90.3%)	268	1171
Cy	003	30/F - Synovial	Doxo/Ifos; Treme/Durva	>75%	5.0	Grade 1	PR (-55.7%)	317	968
Cy	001-B	64/F - Melanoma	Nivo; Ipi; Dab/Tram; Carbo/Taxol	<5%	5.0	None	PD (+30%)	52	148
Cy	109	60/F - Melanoma	Encora/Binimetinib; Pembro/Carbo/Tax	>75%	5.0	None	SD (2.2%)	136	635
Cy	298	28/F - Synovial Sarcoma	Doxo/Ifos; xrt; gem/tax; pazopanib	>75%	5.0	Grade 1	SD (14.3%)	165	467
Cy	222	50/M - Melanoma	Encor/Bini; Ipi/Nivo; pemb/aICOS; durv/IMCp100	<5%	5.0	None	SD (1.3%)	145	291
Cy	166	79/F - Ovarian Carcinoma	carbo/tax; carbo/gem; doxil/aPDL1; wkly tax; phase 1; carbo	5-25%	5.0	Grade 2 (tocilizumab)	SD (8.5%)	141	277
Cy	391	17/M - Synovial Sarcoma	Doxo/Ifos; Pazopanib; Olaparib/Temodar	50-75%	5.0	Grade 2 (tocilizumab)	SD (-3.9%)	70	187
Cy + Flu	261	50/F - Synovial Sarcoma	Doxo/Ifos; Pazopanib; DTIC; TCR-T (MAGE-A4)	>75%	5.0	Grade 2 (tocilizumab)	SD (-29.1%)	308	483
Cy + Flu	368	61/M - Synovial Sarcoma	Epirub/Ifos; TCR-T (MAGE-A4)	>75%	5.0	None	SD (0%)	126	997
Cy + Flu	025	40/M - Melanoma	INF; DTIC; Ipi; Pembro; Carbo/Tax; TIL; Pembro rechallenge	>75%	5.0	None	PD (-2.2%)	26	237
Cy + Flu	457	40/M - Melanoma	Ipi/Nivo; Tram; DTIC	5-25%	5.0	Grade 2 (tocilizumab)	PD (30.7%)	43	195

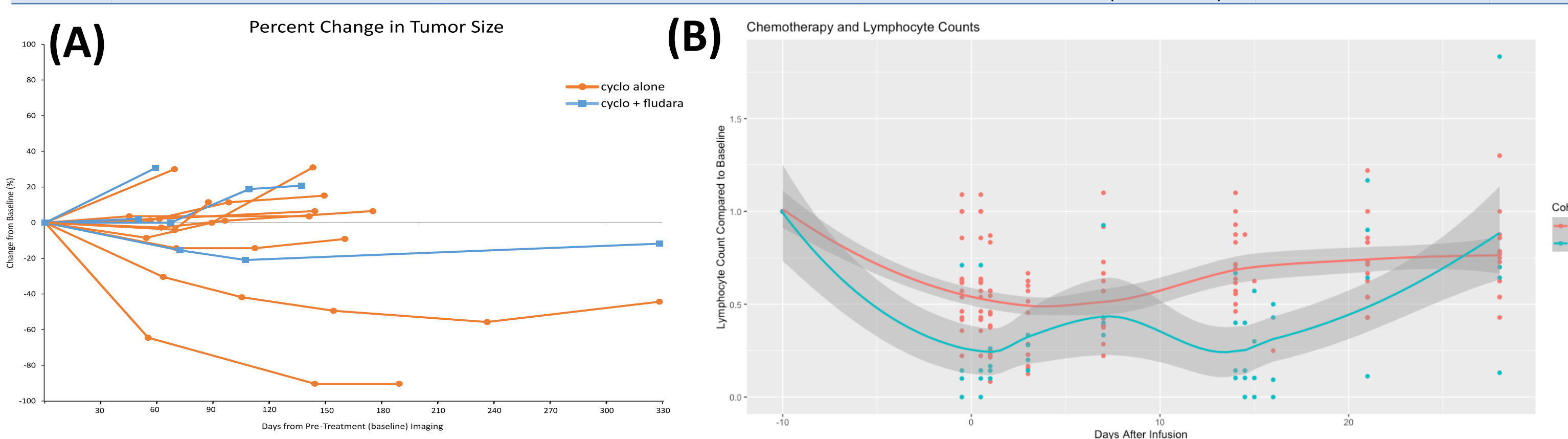


Figure 1 Patients underwent lymphodepleting chemotherapy with either cyclophosphamide alone or a combination of cyclophosphamide and fludarabine, followed by infusion of NY-ESO-1-specific T cells. A spider plot depicts the percentage change in tumor size post-infusion (A). Lymphocyte counts indicate that the combination of cyclophosphamide and fludarabine (Cy+Flu) resulted in more effective lymphodepletion (B).

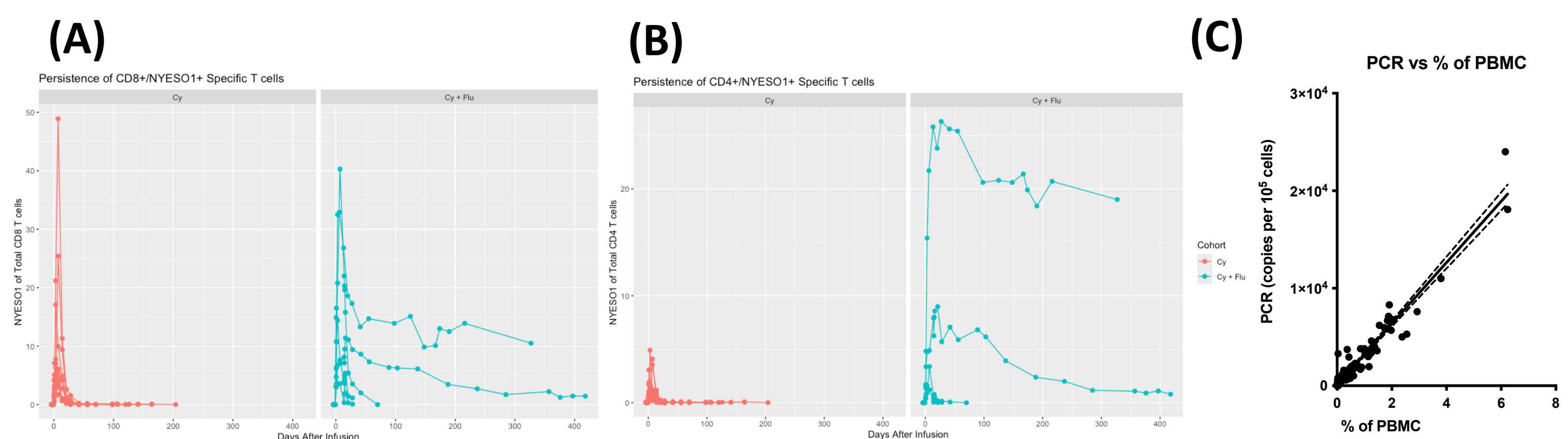


Figure 2. Peripheral blood mononuclear cells (PBMCs) were isolated via ficoll density gradient centrifugation and stained with a flow cytometry panel, including a NY-ESO-1 tetramer for specific detection of the transferred T cells (A, B). The flow cytometry results were compared to quantitative PCR analysis of DNA extracted from the samples, revealing a strong correlation between the two detection methods (C).

Results

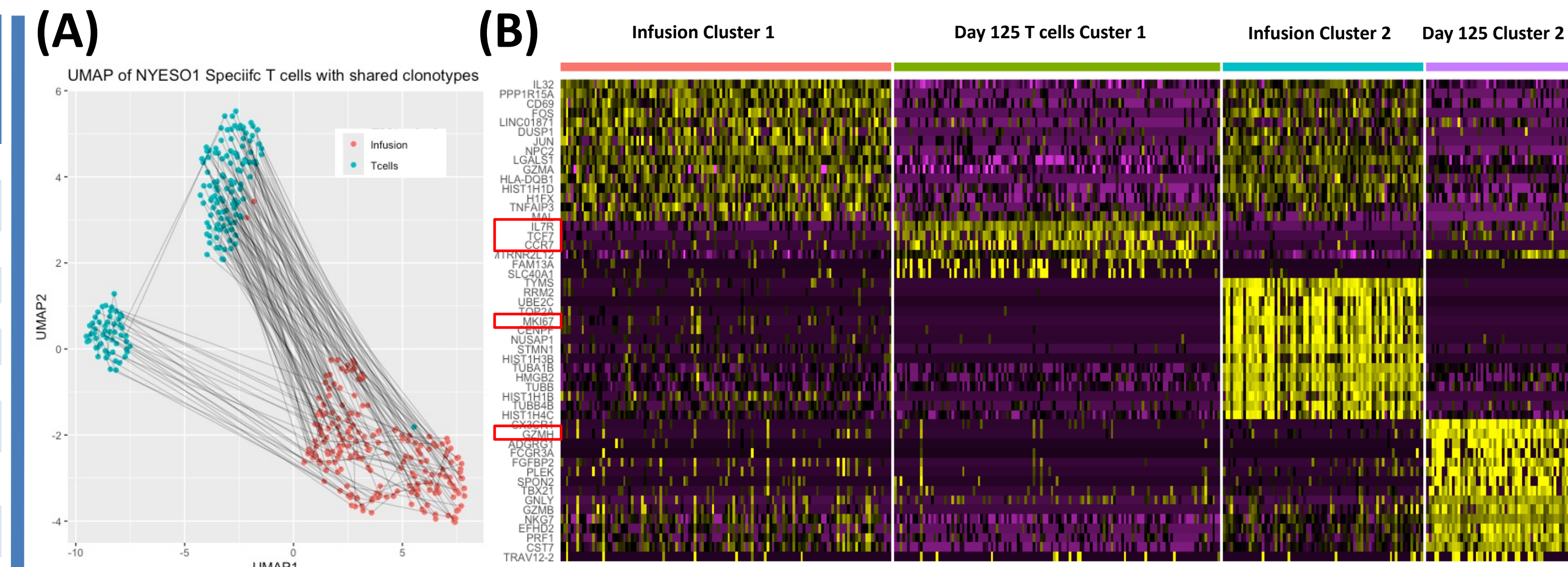


Figure 3. scRNAseq, integrated with T-cell receptor (TCR) sequencing was performed on both the infusion product and peripheral PBMCs collected from patient 1301-368 on Day 125 post-infusion. TCR clonotype analysis identified overlapping T-cell clones present in both the infusion product and the Day 125 sample. These shared clonotypes were clustered and visualized using UMAP, with lines connecting identical clonotypes (A). The comparative analysis revealed that T cells from Day 125 exhibited higher expression of memory-associated markers, including **IL7R**, **CCR7**, and **TCF7**, indicating a shift towards a memory phenotype over time.

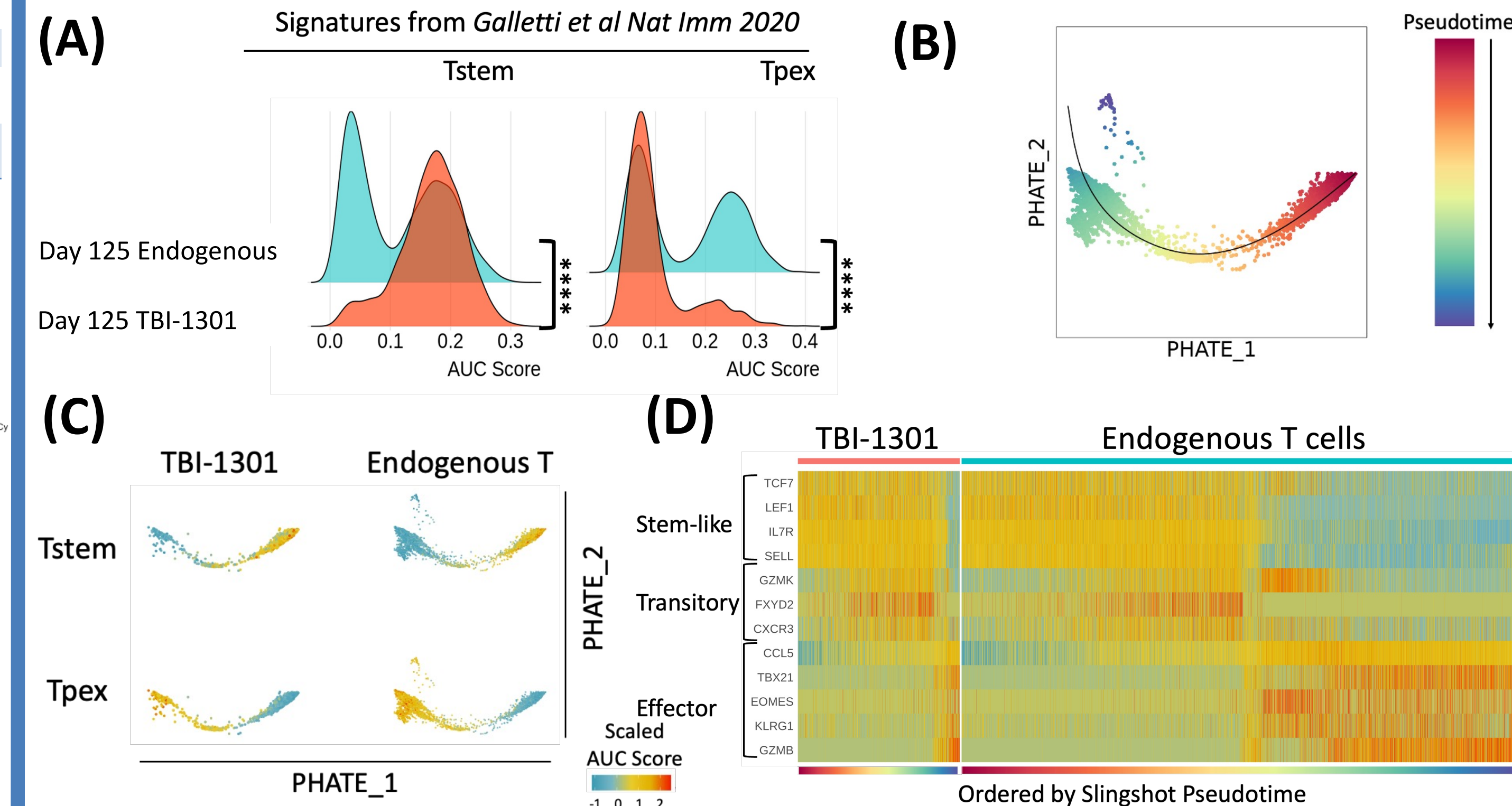


Figure 4. scRNA-seq was performed on Day 125 PBMC samples, focusing on the expression of stem/memory-associated genes. An area under the curve (AUC) score was generated using AUCell to quantify the stem/memory-like phenotype, revealing that a significant proportion of long-surviving NY-ESO1-specific T cells exhibited a memory phenotype compared to endogenous T cells (A). A Slingshot Pseudotime on PHATE dimensionality reduction analysis was performed to assess the trajectory of gene expression changes, showing a marked increase in the expression of memory-associated genes within the NY-ESO1-specific T cell population (B,C). Further ordering of T cells along the pseudotime trajectory indicated that NY-ESO1-specific T cells were enriched in key memory markers, including **TCF7**, **LEF1**, **IL7R**, and **CD62L**. In contrast, endogenous T cells exhibited a more effector-like profile, with higher expression of **EOMES**, **GZMB**, and **CCL5**, which are associated with cytotoxic effector function (D).

Conclusions:

1. Patients who underwent more extensive lymphodepletion exhibited improved long-term engraftment of the infused NY-ESO-1-specific T cells.
2. Single-cell RNA sequencing (scRNA-seq) was employed to trace NY-ESO-1-specific T cells from the infusion product to peripheral blood, with tracking continuing up to Day 125 post-infusion. Clustering analysis revealed a marked upregulation of **TCF7** and **IL7R** in the infused T cells when compared to identical clones from the original infusion product.
3. Using Slingshot pseudotime analysis demonstrated that the infused NY-ESO-1-specific T cells expressed significantly higher levels of stem-cell-associated markers, such as **TCF7** and **LEF1**, indicative of a memory-like phenotype. In contrast, endogenous T cells predominantly expressed effector-associated markers, including **EOMES** and **GZMB**, which are characteristic of effector T cell populations.