

EARLY DETECTION OF CYTOKINE RELEASE SYNDROME USING WEARABLE DEVICES AND CYTOKINE

PROFILING FOLLOWING CAR-T THERAPY FOR MYELOMA: RESULTS FROM AN INVESTIGATOR-INITIATED TRIAL

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SUPPLEMENTARY METHODS

Trial Design and Study Procedures

This investigator-initiated, single-center, single-arm pilot study was conducted at Mount Sinai Hospital (MSH) in New York. The protocol was approved by the Institutional Review Board (IRB) (Protocol 21-1626), and all participants provided written informed consent. Thirty patients with relapsed/refractory multiple myeloma (RRMM) receiving idecabtagene vicleucel (ide-cel) or ciltacabtagene autoleucel (cilda-cel) were enrolled.

Participants wore an FDA-approved wearable device (Current Health Gen 2, Best Buy Health) continuously from infusion until discharge, measuring pulse rate, oxygen saturation, respiratory rate, motion, and skin temperature. Axillary temperature was recorded via a separate patch sensor (Feverscout, VivaLnk). Both staff and patients were blinded to the device's outputs, ensuring unbiased comparison to standard nursing care. Blood samples were collected pre-infusion and at predefined intervals post-infusion for cytokine profiling.

Wearable Device and Temperature Analysis

Adherence was defined as the proportion of valid sensor readings during the participant's stay, calculated both overall and during high-risk CRS periods. Temperature-based CRS detection was evaluated using three methods: (1) fixed thresholds, (2) individualized thresholds (baseline mean +2 standard deviations), and (3) a combined approach using both (OR condition). The primary focus was temperature, given its alignment with the ASTCT definition of Grade 1 CRS.

Analysis prioritized the first CRS episode to avoid confounding from treatment effects on subsequent events. A grid search was conducted to determine optimal observation windows (ranging from 5 to 60 minutes) and step sizes (1 to 15 minutes) to maximize sensitivity and specificity. Model selection prioritized specificity ≥ 0.80 to reduce false alarms, which is critical for potential outpatient use.

Cytokine Profiling and Statistical Analysis

Cytokine profiling was performed using the Olink proximity extension assay (PEA) platform (Immuno-Oncology panel, Article number 95310), measuring 92 immune-related proteins. Cytokine expression values were reported as Normalized Protein Expression (NPX) on a log₂ scale.

Statistical analysis was conducted using the DREAM framework, a linear mixed-effects model, to evaluate longitudinal cytokine changes. Each model included time as an independent variable, with patient age as a covariate, and random intercepts for patient ID and assay batch. Comparisons were adjusted for multiple testing using the Benjamini-Hochberg method, with significance defined as **False Discovery Rate (FDR) <0.05** and **fold-change (FCH) >1.3**.

Machine Learning for CRS Prediction

Machine learning models were developed to predict CRS onset using wearable and cytokine data. Data preprocessing was conducted in Python (version 3.8) with pandas and scikit-learn. Cytokine levels were interpolated (linear, spline, and polynomial methods) to align with the higher-frequency wearable data.

Time-series features were generated using rolling windows (6 to 14 hours), with skin temperature selected over axillary temperature due to higher sampling frequency and ease of future collection.

Five classifiers (Logistic Regression, Random Forest, Gradient Boosting, Support Vector Machine, and k-Nearest Neighbors) were evaluated, with hyperparameter tuning via grid search. Combined models incorporating wearable and cytokine data were implemented using scikit-learn's Pipeline framework. Performance was assessed using sensitivity, specificity, accuracy, precision, concordance, and lead time, with leave-one-patient-out cross-validation ensuring model robustness.

Feature importance was evaluated using SHapley Additive exPlanations (SHAP) to determine the contribution of wearable and cytokine predictors. Model comparisons assessed whether cytokine data improved CRS detection beyond wearable temperature alone.

IFN- γ -Based CRS Prediction

A statistical classifier was developed to predict next-day CRS status using daily IFN- γ fold-change from baseline. Logistic regression and decision tree models were trained separately for ide-cel and ciltacel cohorts, encompassing 49 pre-CRS IFN measurements for ide-cel and 90 for ciltacel. Missing baseline IFN values were imputed using the mean baseline across all patients. Leave-one-patient-out cross-validation was performed, and predictions exceeding a 0.5 probability threshold were flagged as "predicted CRS." Once a prediction was made, subsequent observations for that patient were excluded to ensure realistic performance assessment.

SUPPLEMENTARY FIGURES

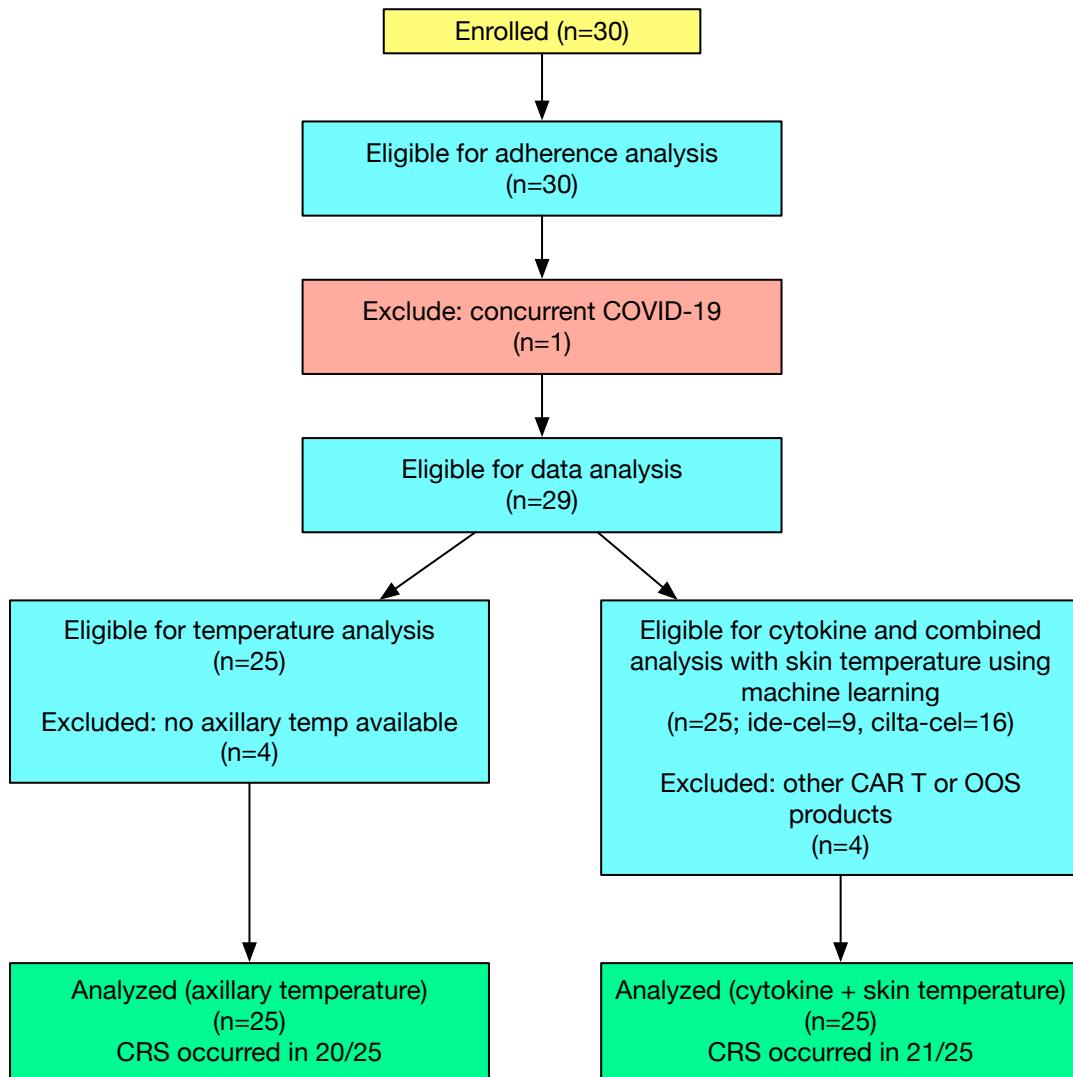


Fig. S1. CONSORT diagram showing the flow of participants through each stage of the study. The OOS (out-of-specification) CAR T cases were excluded from the analysis because they did not meet the predefined quality standards required for clinical use, such as cell viability, purity, or concentration, as set by regulatory guidelines. Including these cases could have introduced variability and potential biases, as they do not represent the standard treatment administered to patients. Our goal was to ensure the analysis reflected the outcomes of CAR T therapies that met regulatory specifications and were deemed safe and effective for clinical application.

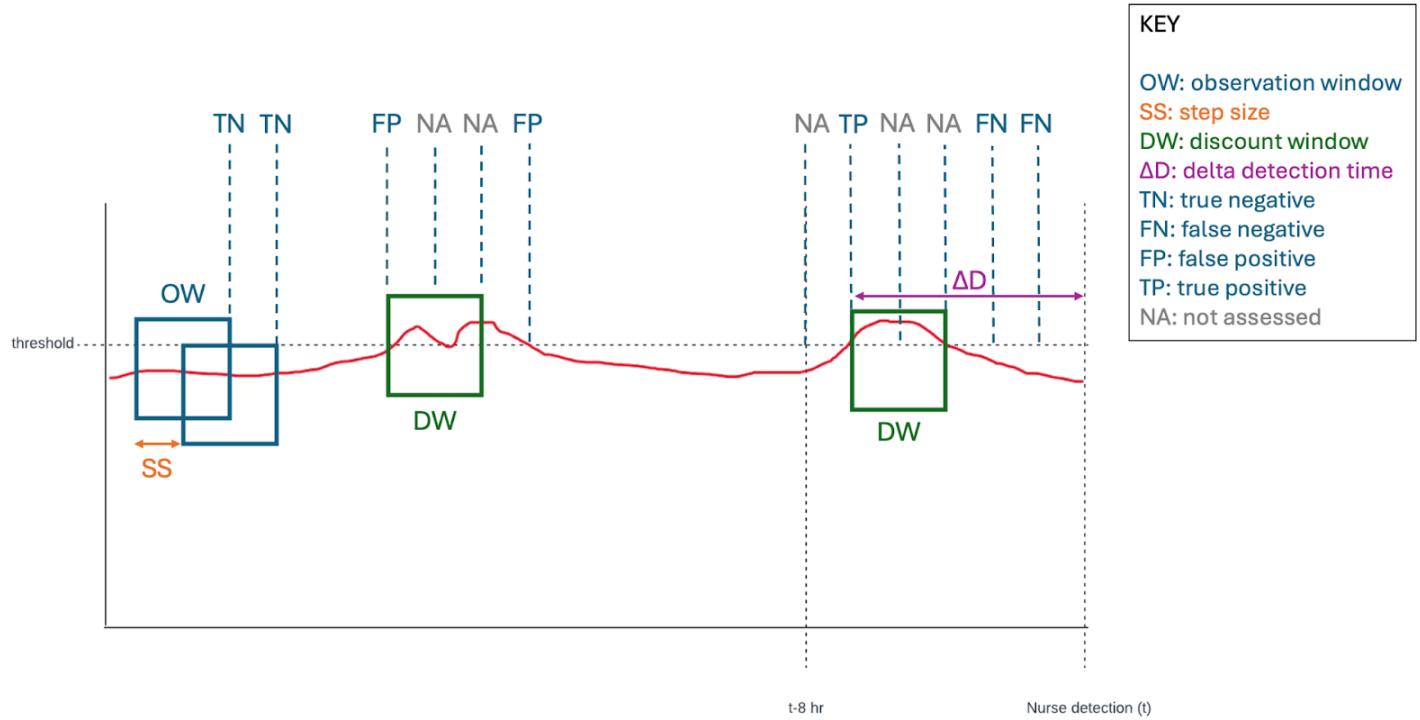
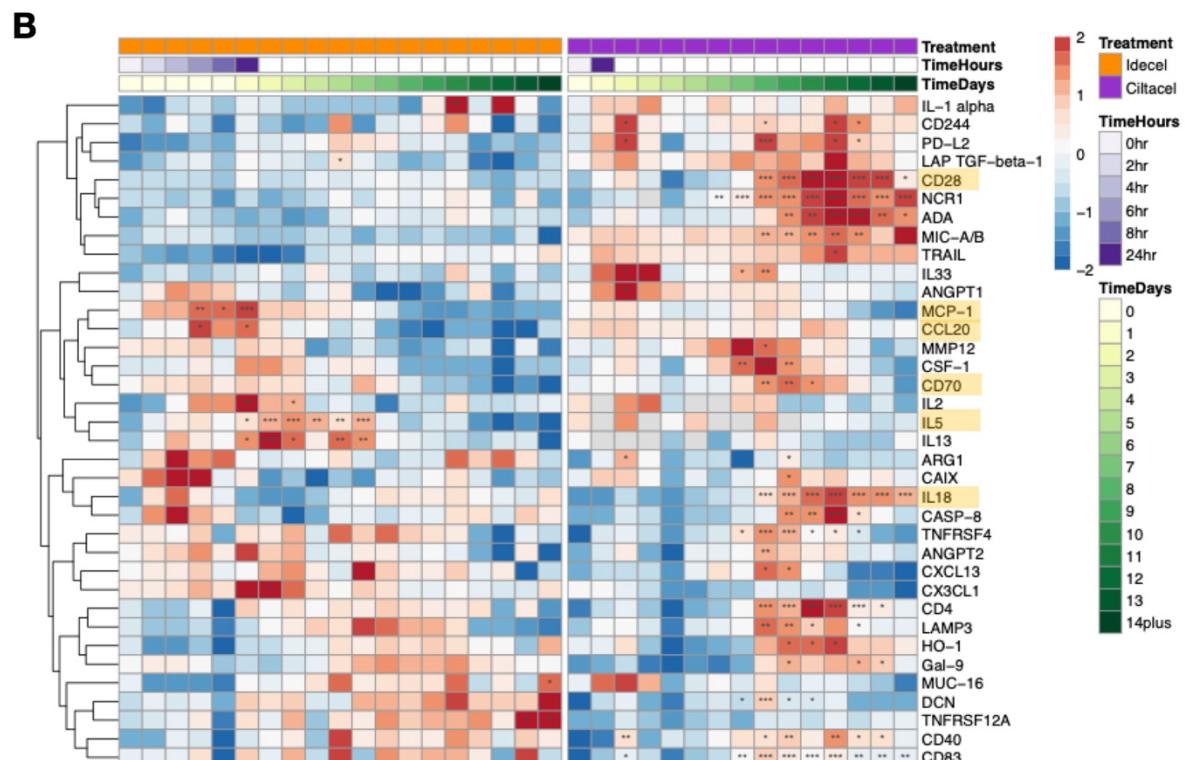
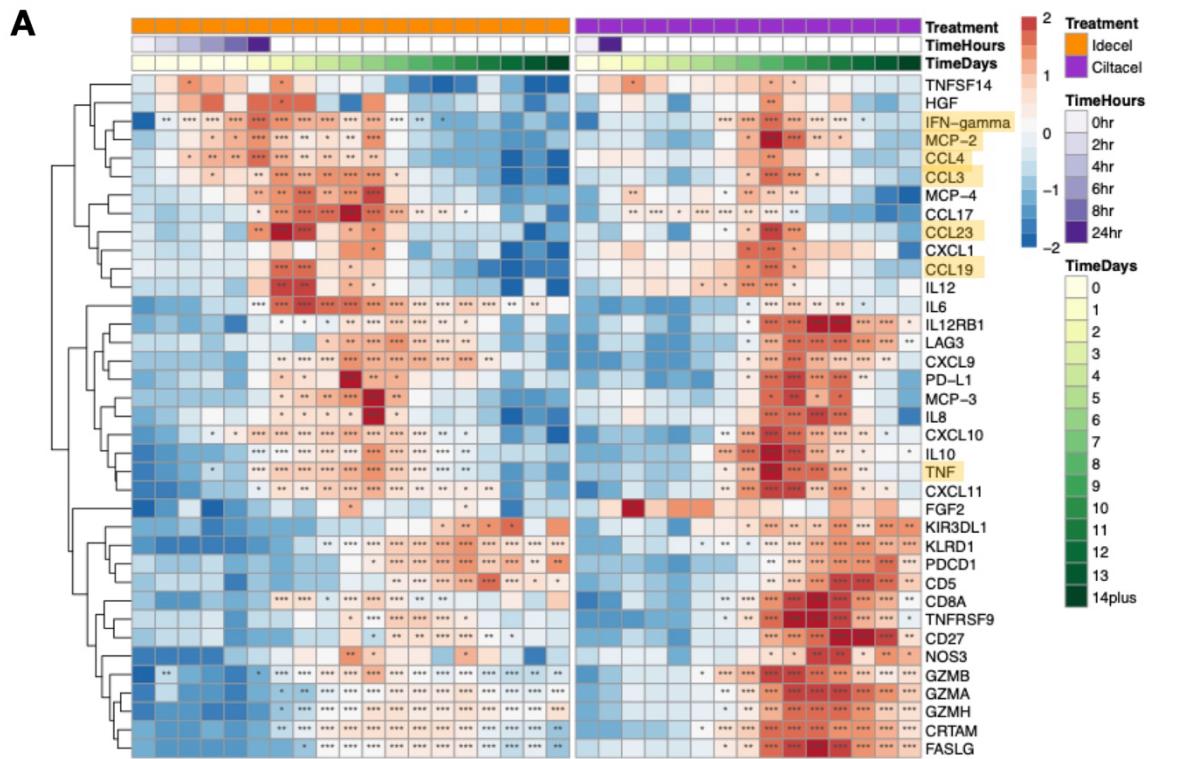


Fig. S2. Schematic depiction of the measured intervals and the approach to CRS detection classification in the wearable analysis. The red line depicts temperature, the 'threshold' is the temperature at which a positive, either true or false, was assigned at each point of evaluation.



C

		Idecel	
		Change	No Change
Ciltacel	Change	37	26
	No Change	10	19

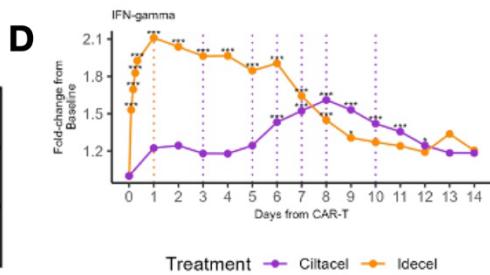


Figure S3: OLINK analysis reveals distinct timing of peak inflammation following ide-cel and ciltacel. (A, B).

Heatmaps shows scaled mean expression of inflammatory biomarkers following treatment with ciltacel or ide-cel. Markers showing significant increase in both treatment groups ($n=45$) are shown in A, markers showing treatment-specific changes ($n=29$) are shown in B. **(C)** Contingency table showing the number of significantly up-regulated inflammatory markers following each treatment. **(D)** Plots show fold-change from baseline for IFN-gamma following each CAR-T treatment. Stars indicate significant ($FCH>1.3$, $FDR<0.05$) difference from baseline. Vertical lines indicate days in which patients experienced CRS.

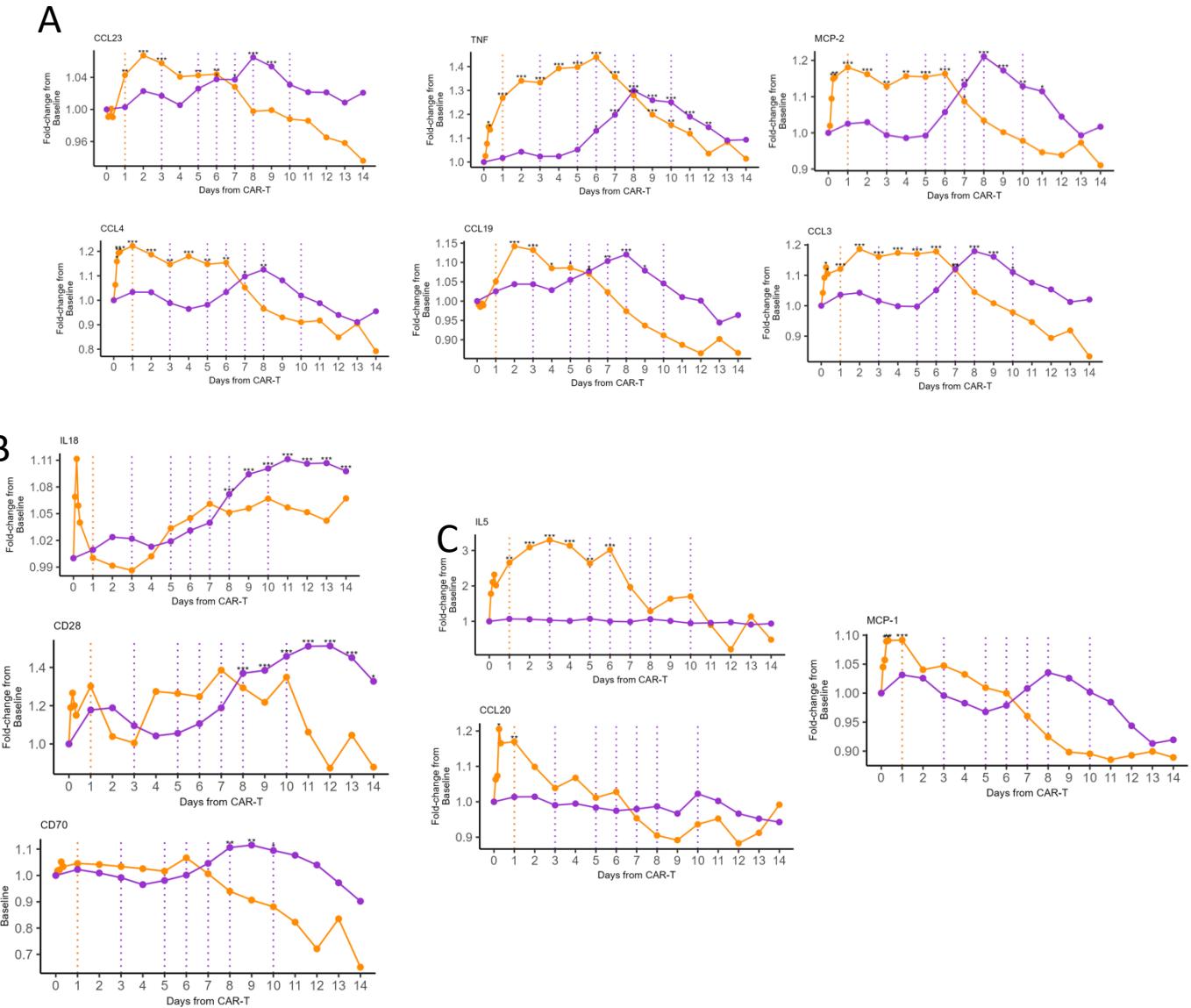
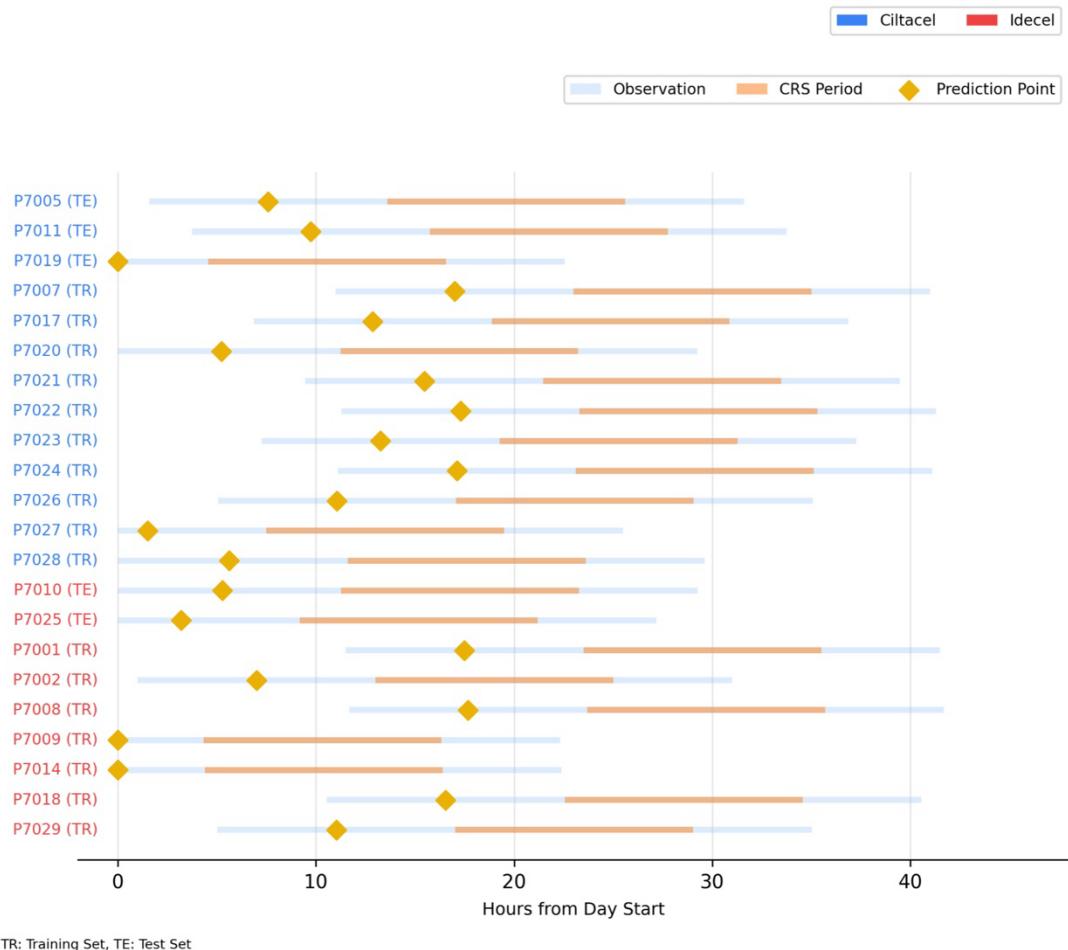
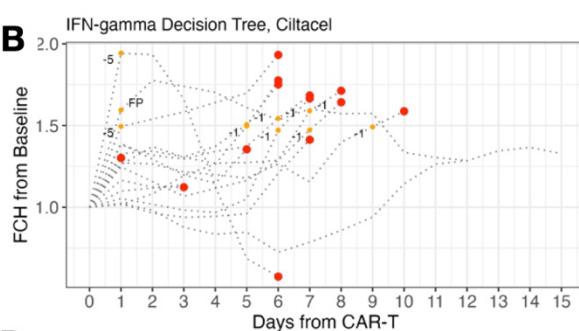
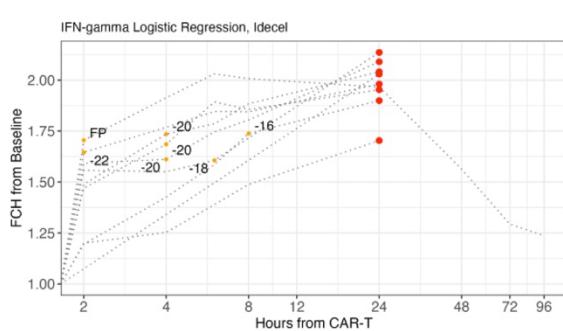


Fig. S4. Olink analysis revealed cytokines significantly induced by specific treatments. (A-C) Plots show expression of example inflammatory markers inflammatory following cilt-a-cel and ide-cel. **(A)** Shows markers that change following both treatments, **(B)** shows markers that are specific to cilt-a-cel, and **(C)** shows markers specific to ide-cel.

A**B****C****D**

Treatment	Model	Sensitivity	Specificity	Accuracy	Precision	F1	Mean Lead Time
Ciltacel	Tree	75%	67%	73%	90%	82%	40 hours
	Logistic	83%	33%	73%	83%	83%	72 hours
Idecel	Tree	63%	0%	57%	83%	71%	14 hours
	Logistic	75%	0%	67%	86%	80%	15 hours

Figure S5. Machine learning approaches to predict CRS. **(A)** Swimmers plot for combined skin temperature + cytokine machine learning model. **(B,C)** Line plots show fold-change from baseline of IFN- γ for each patient following treatment with ciltacel (B) or idecel (C). The red points indicate the day that CRS occurred for each patient, orange labels indicate lead time for each patient, ‘FP’ indicates False Positive (i.e. patient never experienced CRS during the study). **(D)** Table shows predictive performance of each model, as well as mean lead time.

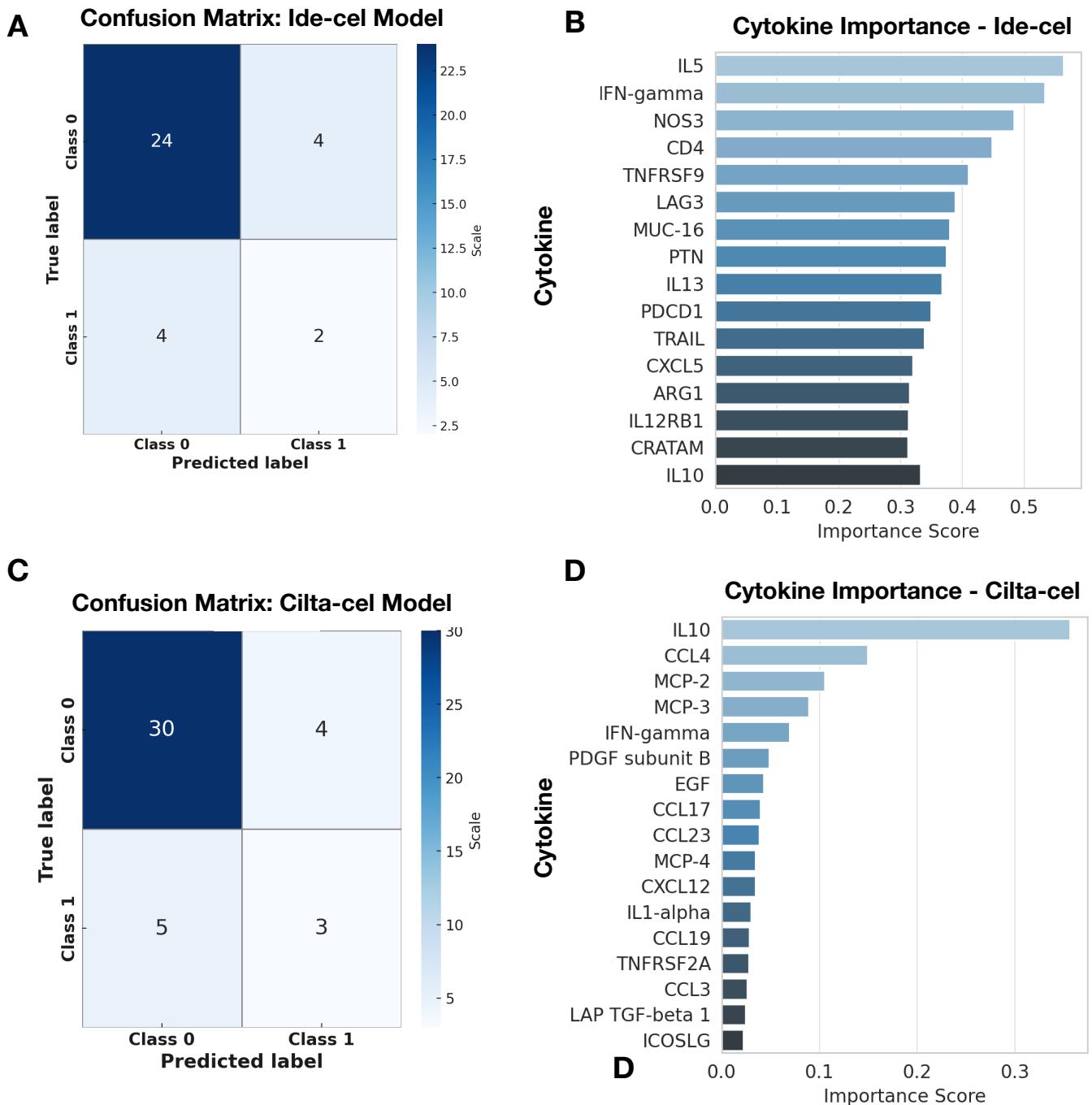


Fig. S6. Performance and key cytokines in ML analysis. (A,C) Confusion matrices for ide-cel and cilta-cel cytokine models. (B,D) Top cytokines for ide-cel and cilta-cel ranked by importance as calculated using the scikitlearn package.

A

Treatment	Model	Sensitivity	Specificity	Accuracy	Precision	F1	Mean Lead Time
Ciltacel	IFN-Tree	77%	67%	82%	91%	83%	40 hours
	Multi-RF	69%	67%	69%	90%	78%	91 hours
Idecel	IFN-Logistic	78%	0%	70%	88%	82%	14 hours
	Multi-Elastic-Net	56%	0%	50%	83%	67%	8 hours

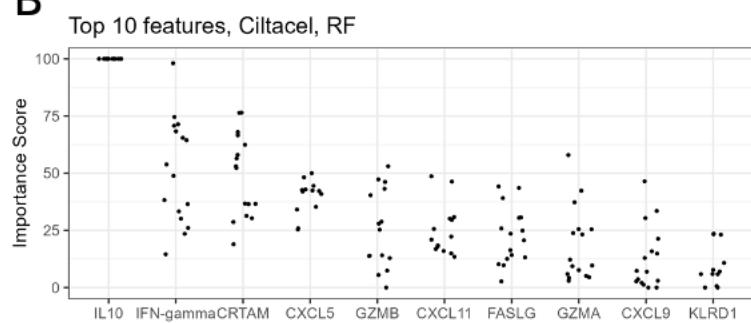
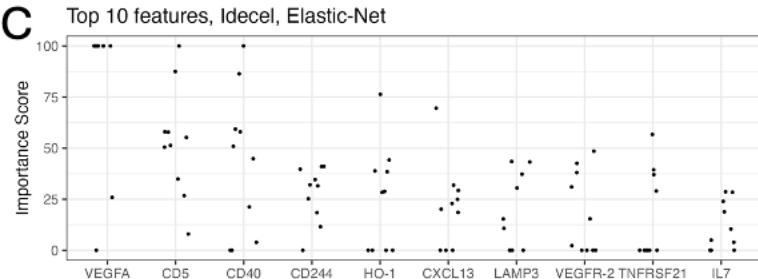
B**C**

Fig. S7. Performance of IFN classifier. **(A)** Table shows performance metrics for the IFN-only classifier and the corresponding classifier trained using all cytokines. **(B, C)** Plots show importance scores for the top10 most important genes. Each point represented a separate model, trained in each iteration of our leave-one-patient-out validation approach.

SUPPLEMENTARY TABLES

Patient_ID	Age	Sex	CAR T product	CRS (0 = No, 1= Yes)	CRS grade	Included in Temp Analysis	Included in Cytokine and ML analysis
WEAR_7001	83	F	ide-cel	1	1	Y	Y
WEAR_7002	68	M	ide-cel	1	1	Y	Y
WEAR_7003	67	M	Other investigational	1	1	Y	N
WEAR_7004	59	M	cilta-cel	0	NA	Y	Y
WEAR_7005	59	F	cilta-cel OOS	1	1	Y	N
WEAR_7006	48	M	ide-cel	0	NA	Y	Y
WEAR_7007	65	M	cilta-cel	1	1	Y	Y
WEAR_7008	68	F	ide-cel OOS	1	1	Y	N
WEAR_7009	75	M	ide-cel	1	3	Y	Y
WEAR_7010	50	F	ide-cel	1	1	Y	Y
WEAR_7011	51	M	cilta-cel	1	1	N	Y
WEAR_7012	66	M	cilta-cel	0	NA	Y	Y
WEAR_7013*	67	F	Other investigational	1	1	N	N
WEAR_7014	73	M	ide-cel	1	1	Y	Y
WEAR_7015	58	M	Other investigational	0	NA	Y	N
WEAR_7016	78	F	cilta-cel	0	NA	Y	Y
WEAR_7017	65	M	cilta-cel	1	1	Y	Y
WEAR_7018	82	M	ide-cel	1	1	Y	Y
WEAR_7019	67	F	cilta-cel	1	2	Y	Y
WEAR_7020	60	F	cilta-cel	1	4	Y	Y
WEAR_7021	54	F	cilta-cel	1	1	Y	Y
WEAR_7022	40	F	cilta-cel	1	1	Y	Y
WEAR_7023	55	F	cilta-cel	1	1	Y	Y
WEAR_7024	60	M	cilta-cel	1	1	N	Y
WEAR_7025	50	F	ide-cel	1	1	Y	Y
WEAR_7026	62	M	cilta-cel	1	NA	N	Y
WEAR_7027	62	M	cilta-cel	1	1	Y	Y
WEAR_7028	74	F	cilta-cel	1	1	Y	Y
WEAR_7029	88	M	ide-cel	1	1	Y	Y
WEAR_7030	53	F	cilta-cel	1	1	N	Y

Table S1. Patient characteristics. WEAR_7013 was excluded for concurrent COVID-19.

Metric	Overall	Ide-cel	Cilta-cel	Other experimental products
Total patients	50	10	17	3
Patients included in the temperature analysis	25	10	13	2
Patients who developed CRS	20	9	10	1
CRS episodes detected	18	9	8	1
Sensitivity	0.72	0.91	0.55	1
Specificity	0.8	0.73	0.82	0.74
Delta time to nurse detection Mean, Median [IQR], (hr:min)	6:00, 7:01 [3:17]	5:54, 7:21 [4:30]	6:08, 7:15 [2:57]	5:40, 5:40 [0]

Table S2. Best performing balanced model (axillary temperature; “Combined”: fixed (36.4 C) or individual threshold, observation window 60 mins, step size 10 mins) in the 25 patients included in the analysis; ‘ide-cel’: idecabtagene vicleucel; ‘cilta-cel’: ciltacabtagene autoleucel; ‘Other experimental products’: GPRC5D CAR-T, Caribou AlloCAR-T, Next-Gen CAR-T.

Olink Immuno-Oncology panel				
		PDGF subunit B		
IL8	CXCL9	PDGF subunit B	IL10	ICOSLG
TNFRSF9	CD8A	PDCD1	TNFRSF12A	MMP12
TIE2	CAIX	FASLG	CCL23	CXCL13
MCP-3	MUC-16	CD28	CD5	PD-L2
CD40-L	ADA	CCL19	CCL3	VEGFA
IL-1 alpha	CD4	MCP-2	MMP7	IL4
CD244	NOS3	CCL4	ARG1	LAG3
EGF	IL2	IL15	NCR1	IL12RB1
ANGPT1	Gal-9	Gal-1	DCN	IL13
IL7	VEGFR-2	PD-L1	TNFRSF21	CCL20
PGF	CD40	CD27	TNFRSF4	TNF
IL6	IL18	CXCL5	MIC-A/B	KLRD1
ADGRG1	GZMH	IL5	CCL17	GZMB
MCP-1	KIR3DL1	HGF	ANGPT2	CD83
CRTAM	LAP TGF-beta-1	GZMA	PTN	IL12
CXCL11	CXCL1	HO-1	CXCL12	CSF-1
MCP-4	TNFSF14	CX3CL1	IFN-gamma	
TRAIL	IL33	CXCL10	LAMP3	
FGF2	TWEAK	CD70	CASP-8	

Table S3. Olink immuno-oncology cytokine panel.