Supplementary Materials for

Dynamic Targetable Extracellular Vesicle Surface Proteins Monitor Depth of Response to CAR T Therapy

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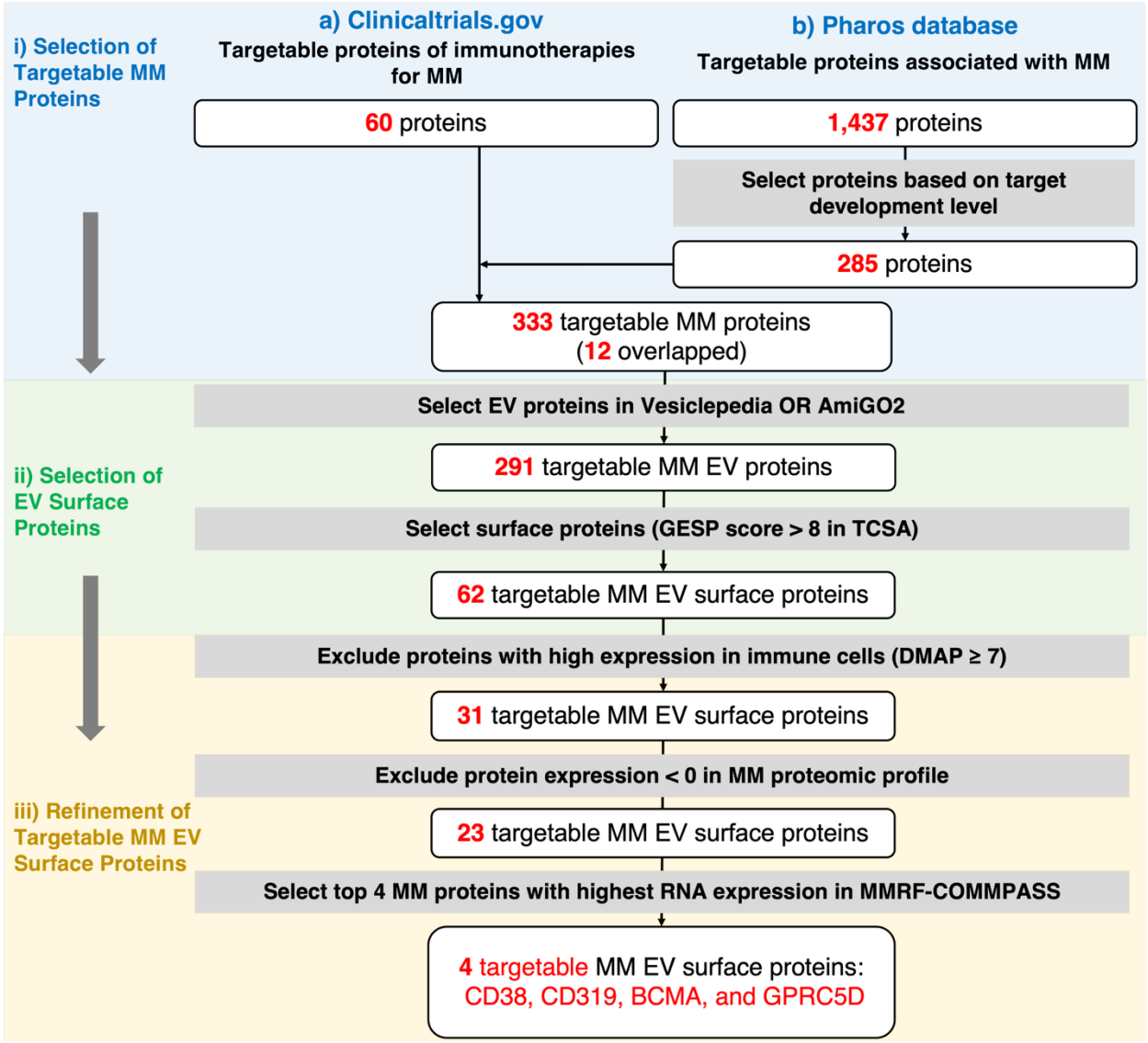

Fig. S1. An integrated bioinformatic framework employed for identifying 4 Targetable MM EV surface proteins. The framework employed sequential filtering steps: i) Selection of targetable MM proteins from two publicly available databases: a) Clinicaltrials.gov, which identified 60 surface proteins (ADC, CAR-T, monoclonal antibodies, and bispecific antibodies); b) The Pharos database (https://pharos.nih.gov/), which initially identified 1,437 surface proteins. The list was then refined using Target Development Levels 'Tclin' and 'Tchem', representing clinically validated and preclinical targets respectively, narrowing it down to 285 surface proteins. ; ii) MM EV surface proteins were selected through TCSA data (GESP score > 8), and Vesiclepedia or AmiGO2 data (https://amigo.geneontology.org/); and iii) Refinement of targetable MM EV surface proteins by analyzing their RNA expression levels using Differentiation Map (DMAP) data and expression in MM proteomic profile, where MM proteins with high expression in immune

cells (DMAP ≥ 7) and protein expression less than 0 in MM proteomic profile were excluded. The top four proteins with the highest RNA expression in MMRF-COMMPASS were selected: CD38, CD319, BCMA, and GPRC5D.

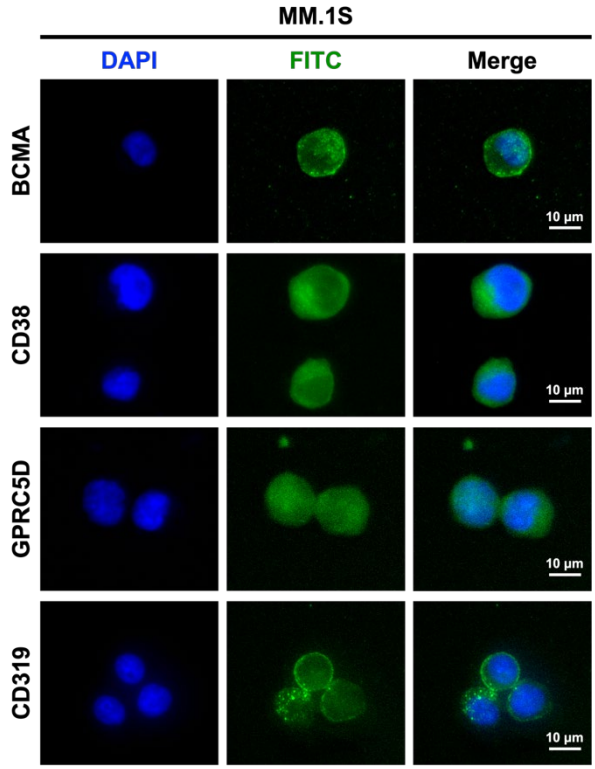

Fig. S2. Validation of the four targetable MM EV surface proteins using MM cell lines. Representative IF micrographs illustrating the expression of BCMA, CD38, GPRC5D and CD319 in MM.1S cell lines. Blue: DAPI; green: FITC. Scale bar, 10μm.

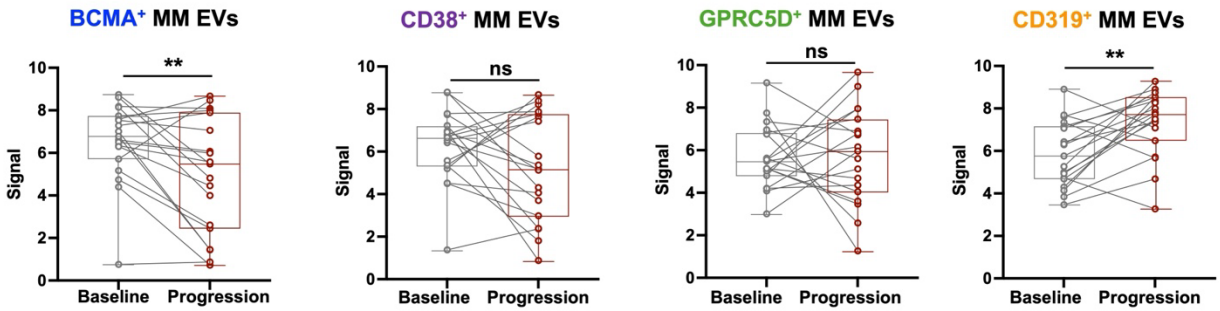

Fig. S3. Boxplot showing signals of four MM EV subpopulations from baseline to first documented progression in 19 patients with PD. Statistical significance was assessed using a paired Student's t-test (** $p < 0.01$, ns: no significance, $n = 19$).

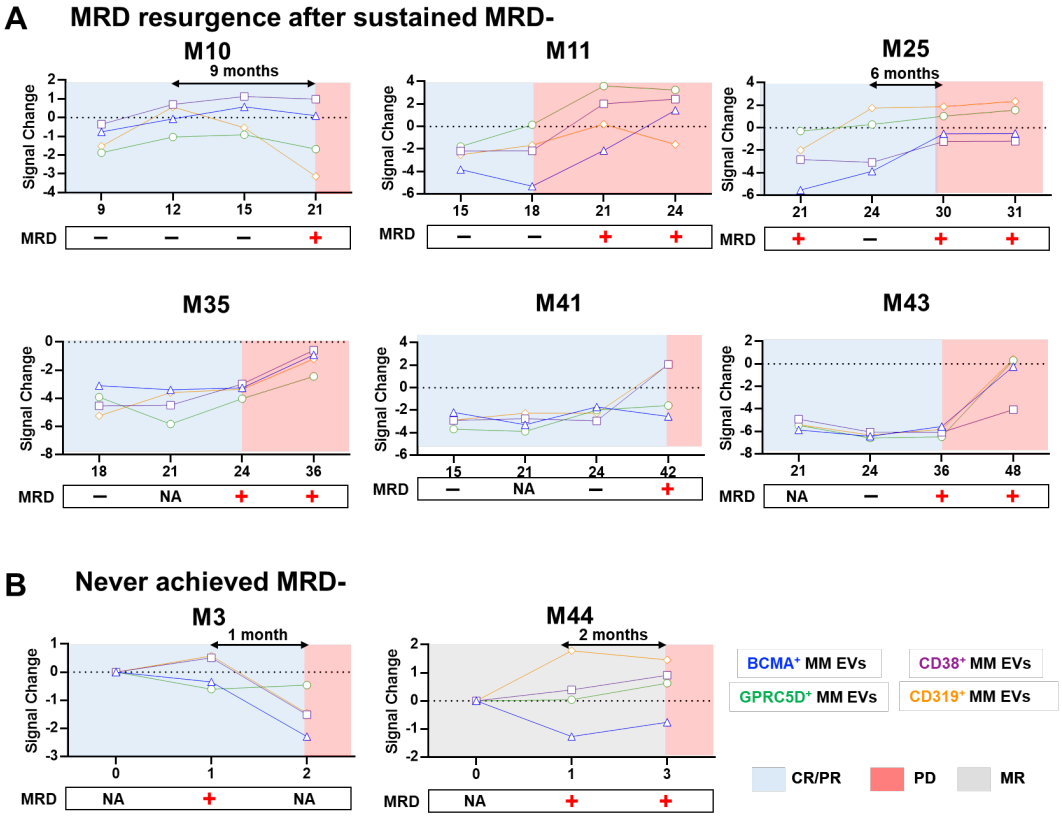

Fig. S4. Longitudinal dynamics of MM EV subpopulations in relation to minimal residual disease (MRD) dynamics and clinical outcome in patients with progressed disease in subgroups of **(A)** MRD resurgence after sustained MRD negativity, and **(B)** Never achieved MRD negativity. MM EV signal changes were computed as follow-up signal subtract baseline signal. Color-coded dots represent EV signals: BCMA$^+$ MM EVs (blue), CD38$^+$ MM EVs (purple), GPRC5D$^+$ MM EVs (green), and CD319$^+$ MM EVs (orange). MRD status at each time point is indicated as "+" (positive) or "–" (negative). Shaded regions correspond to the clinical progression phase (red), or response period (blue). Lead time prior to clinical progression is indicated as (⟷), and x-axis indicates time in months after CAR T-cell infusion. CR/PR, complete or partial response; PD, progressive disease; MR, minor response.

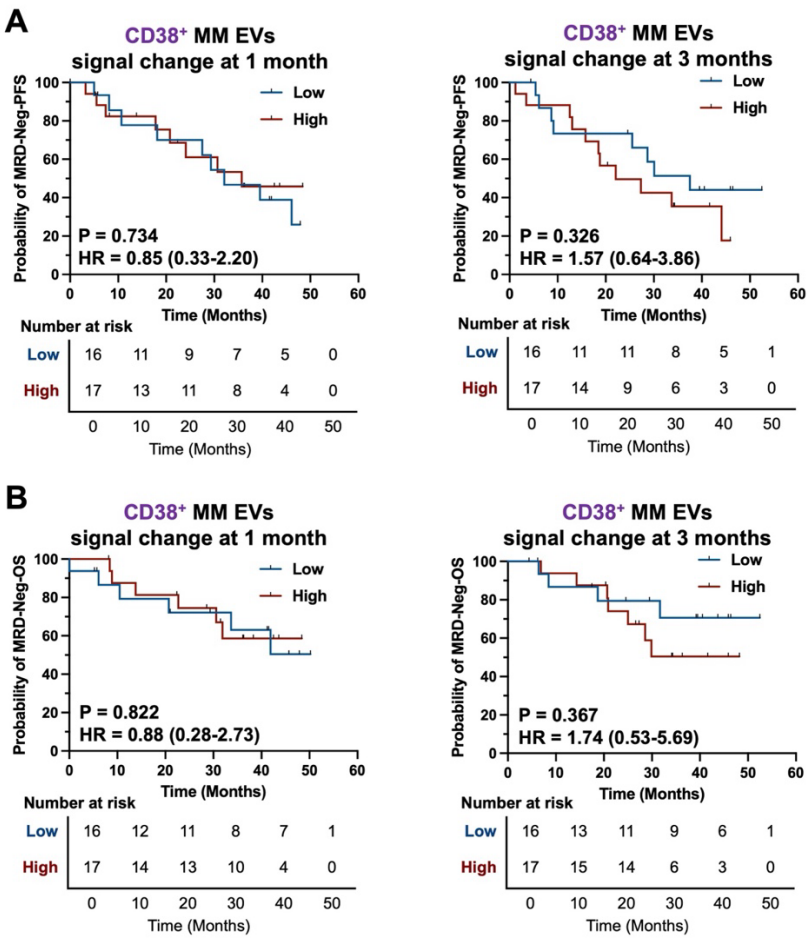

Fig. S5. Prognostic value of CD38$^+$ MM EVs for predicting progression-free survival and overall survival in MRD-negative RRMM patients after anti-BCMA CAR T-cell therapy. Kaplan-Meier survival curves for (**A**) PFS and (**B**) OS stratified by CD38$^+$ MM EVs signal changes at multiple follow-up time points (1 month and 3 months post-therapy) in MRD-negative patients. Patients were grouped as "low" or "high" using the median value as a cutoff. Prognostic significance was assessed using log-rank tests, and hazard ratios (HR) with 95% confidence intervals (CI) are provided.

Table S1. Demographic and clinical characteristics of the participants of Tx-naïve MM and HD in the feasibility study.

1. Treatment-naïve MM	
Characteristics	**Total (n=31)**
Sex = M (%)	14 (45.2)
Age (years) (median [IQR])	68(60-76)
Hb (g/dL) (median [IQR])	10.10 (8.30-11.78)
Platelets (10^9/L) (median [IQR])	234.50 (198.25-265.00)
WBC (10^9/L) (median [IQR])	6.31 (5.21-7.87)
Lymphocytes (10^9/L) (median [IQR])	1.98 (1.38-2.60)
Neutrophils (10^9/L) (median [IQR])	3.76 (2.96-4.72)
Monocytes (10^9/L) (median [IQR])	0.49 (0.40-0.62)
Plasma (BM%) (median [IQR])	14 (4-35)
Smoking = Yes (%)	3 (9.7)
Height (cm) (median [IQR])	165.00 (162.00-173.00)
Weigh (kg) (median [IQR])	72.00 (63.00-82.00)
BMI (median [IQR])	25.00 (22.02-27.75)
2. Healthy Donor (HD)	
Characteristics	**Total (n=32)**
Sex = M (%)	16 (50)
Age (years) (median [IQR])	67.00 (35-81)

Table S2. Comparison of MM EV subpopulations and MRD assessment in Detecting Early Relapse and Disease Progression.

MRD dynamic patterns	Early relapse (detected before clinical progression)		Disease progression (detected at clinical progression)	
	MM EVs	**MRD**	**MM EVs**	**MRD**
i) Achieved MRD negativity but not sustained (n=6)	5	0	6	5
ii) Sustained MRD negativity but progressed (n=5)	3	0	5	0
iii) MRD resurgence after sustained MRD negativity (n=6)	2	1	4	5
iv)Never achieved MRD negativity (n=2)	2	2	1	1
Overall detection rate	**12/19 (63.2%)**	**3/19 (15.8%)**	**16/19 (84.2%)**	**11/17* (64.7%)**
Statistical analysis with Chi-square test				
Detection Context	MM EVs Positive (%)	MRD Positive (%)	χ^2 (df=1)	p-value
Early Relapse (n=19)	12 (63.2%)	3 (15.8%)	7.05	0.008 **
Disease Progression (n=19/17*)	16 (84.2%)	11 (64.7%)	0.93	0.335

* MRD data was not available in 2 cases.