Supplementary Methods.

mLST characterization and immune monitoring details

We used phycoerythrin-, fluorescein isothiocyanate-, peridinin chlorophyll protein-, allophycocyanin-, Alexa Fluor 700-, phycoerythrin cyanin 7-, Pacific Blue- or Krome Orange-conjugated CD3 (clone SK7), CD4 (SK3), CD8 (SK1), CD56 (B159), CD19 (SJ25C1), TCR-ab (T10B9), TCR-gd (B1), CD62L (DREG-56), CD45RA (H100 or 2H4), CD 45RO (UCHL1), CCR7 (3D12) and CD69 (MIH4) (Becton Dickinson [BD], Franklin Lakes, New Jersey, Beckman Coulter, Pasadena, CA). Control samples labeled with appropriate isotype antibodies were included and a "fluorescence minus one" strategy was used for multicolor staining. Cells were analyzed using FACScan equipped with a filter set for 4 fluorescence signals using CellQUEST software, or FACS-Canto II, using DIVA software (Beckton Dickinson). Intracellular cytokine staining (ICS) was performed by stimulating mLSTs with target tumor-associated antigen (TAA) or irrelevant (control) pepmixes in the presence of CD28 and CD49d (1 µg/mL) (BD Biosciences), followed by the addition of BD GolgiStop and BD GolgiPlug, which contain monensin and brefeldin A, respectively. After an overnight incubation, T cells were washed, pelleted, and surface stained with CD8 and CD3, then fixed, and permeabilized with Cytofix/Cytoperm Solution (BD Biosciences). After manufacturer-directed incubations and washes, cells were stained with an IFN-γ antibody (BD Biosciences), then acquired using a Gallios flow cytometer and analyzed with Kaluza software. The cytotoxic activity of mLSTs toward non-malignant patient-derived PHA blasts (alloreactivity) or pepmix-pulsed PHA blasts (tumor reactivity), was measured in a standard ^{51}Cr release assay at varying effector-to-target (E:T) ratios (80:1, 40:1, 20:1, 10:1 and 5:1) and the percentage of specific lysis was calculated as [(experimental release – spontaneous release)/(maxiumum release – spontaneous release)] x 100. To assess the clonal diversity and track the expansion of the infused mLSTs in select lines we used high-throughput deep sequencing of TCRv□ CDR3 regions (Adaptive Biotechnologies, Seattle) to analyze the T cell

repertoire of the infused lines and of patient peripheral blood samples collected pre- and post-infusion.

Tumor antigen profiling details

Clinical grade hematoxylin, eosin, antibodies to AML/MDS antigens such as CD34, CD117, CD33 and in Pt#A1 an additional clinical grade CD3 antibody was used to highlight infiltrating T lymphocytes. The following anti-human primary antibodies were utilized for detection of TAAs/exhaustion markers (research testing): NY-ESO1 mouse mAb (clone E978, 1:50) (Santa Cruz Biotechnology: Dallas, TX, USA), PRAME rabbit pAb (1:200, Bioss Antibodies: Woburn, MA, USA), WT1 mouse mAb (clone 6F-H2; Agilent: Santa Clara, CA, USA), Survivin rabbit mAb (clone 71G4B7, 1:500; Cell Signaling Technology: Danvers, MA, USA), and PD-L1 rabbit mAb (clone SP142, dilution 1:100; Abcam: Cambridge, MA, USA). Standard deparaffinization, rehydration, heat antigen retrieval with citrate buffer (pH 6), dual endogenous peroxidase block (Dako: Carpinteria, CA, USA), and non-specific normal horse serum (2.5%) block were performed with 1X TBST washes. Slides were incubated with primary TAA/ (Programmed death Ligand-1) PD-L1-directed antibodies at 4°C, followed (the next day) by an anti-mouse/rabbit secondary antibody (MP-7402, Vector Labs: Burlingame, CA, USA) and then AEC (red) or DAB (brown). The substrate was added prior to counterstaining, rinsing in deionized water, and cover slipping in aqueous mounting medium. Hematoxylin and eosin-stained slides served as positive and unstained slides as negative controls, respectively. TAA/PD-L1 expression was scored as a percentage of positive cells .All scoring was performed by an HMH pathologist who was blinded to the clinical outcome as well as to the demographics of individual patients. Any level of TAA detection by IHC was considered positive in that given tumor sample.

Flow cytometry on AML/MDS cells in fresh blood or aspirate was performed as per standard of care at US Clinical Laboratory Improvement Act (CLIA)-certified clinical laboratories. At HMH (where the majority of flow cytometry studies were performed), samples were run on BD

FacsCanto II flow cytometers after staining with the following panel of antibodies: CD38-Pacific blue, CD45-V500, CD15-FITC, CD13-PE, CD117-PERCP cy5.5, CD33-APC, HLA-DR-APC C7, CD34-PE Cy7, HLA-DR-Pacific blue, CD14-FITC, CD11b-PE, CD123-PERCP cy5.5, CD34-APC, CD16-APC C7, CD13-PE Cy7, CD36-FITC, CD64-PE, CD7-PERCP cy5.5, CD56-CDAPC C7, CD33-PE Cy7. Blasts were identified as CD45 dim, low-to-intermediate side scatter events. Internal and/or isotype controls were used to determine intensity (mean fluorescent intensity - MFI) as well as the percent of blasts that expressed surface antigens.

Supplementary Results.

Patient referral patterns.

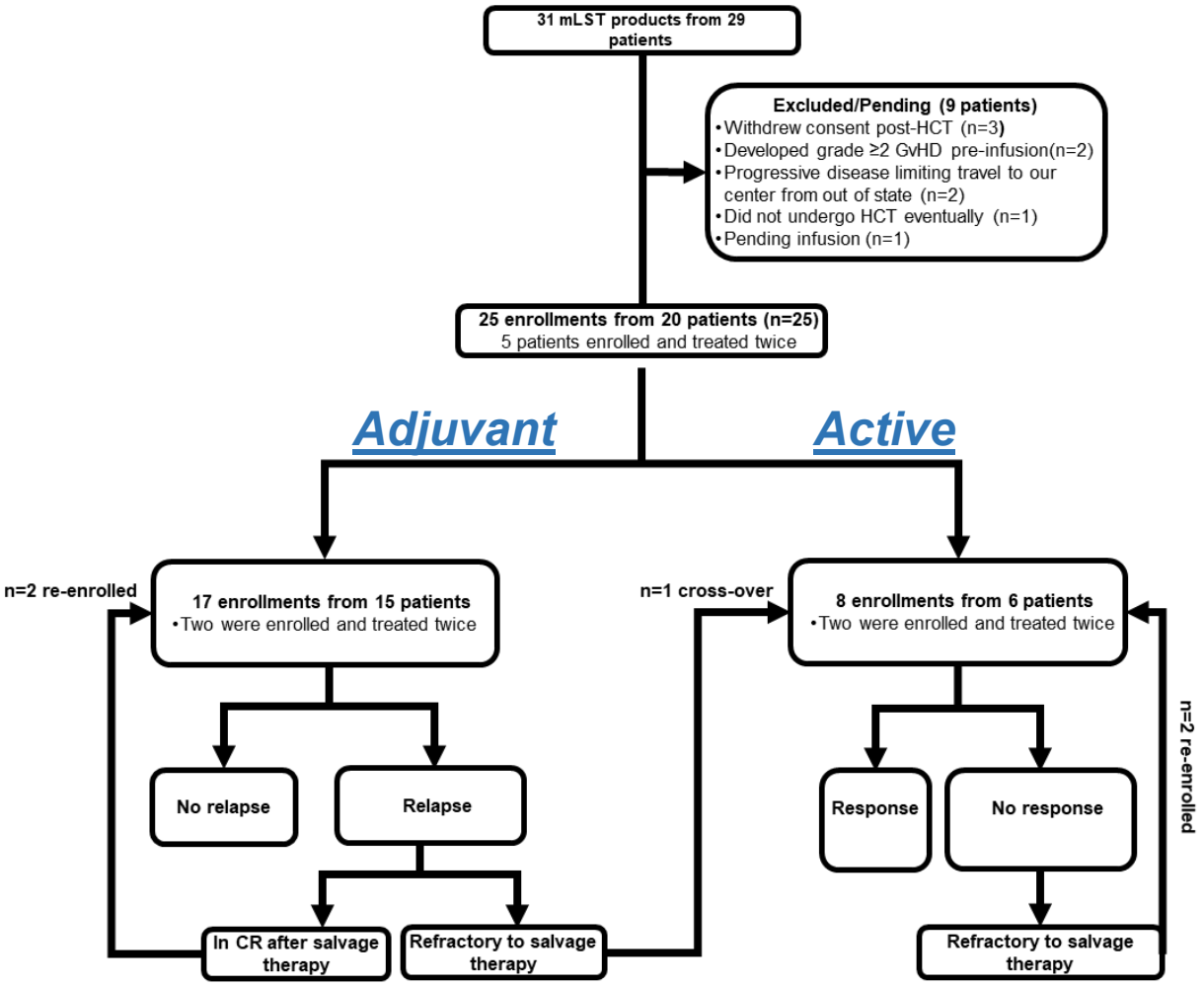
We conducted a post-hoc analysis to determine what proportion of all potentially eligible patients at our site were eventually referred to our study. So we conducted a chart review of all first sibling or related donor transplants performed at our center for AML or MDS between May 2016 and May 2019. Of the 36 consecutive sibling/related donor recipients at our site during the specified timeframe, 30 were potentially eligible to participate on our Phase I clinical trial. Of these the majority [18 of 30 (60%)] were referred to our trial. Reasons for not referring the remaining 12 included: n = 3 considered to be in a better prognosis category and thus less favorable risk/benefit ratio; n = 9 no discernable reason based on chart review [unsubstantiated possibilities include: patients or treating team not aware of the trial (mostly an issue in early phase of the trial), treating physician preference or participation on competing clinical trials)].

Timing of T cell infusion in the adjuvant, never relapsed cohort.

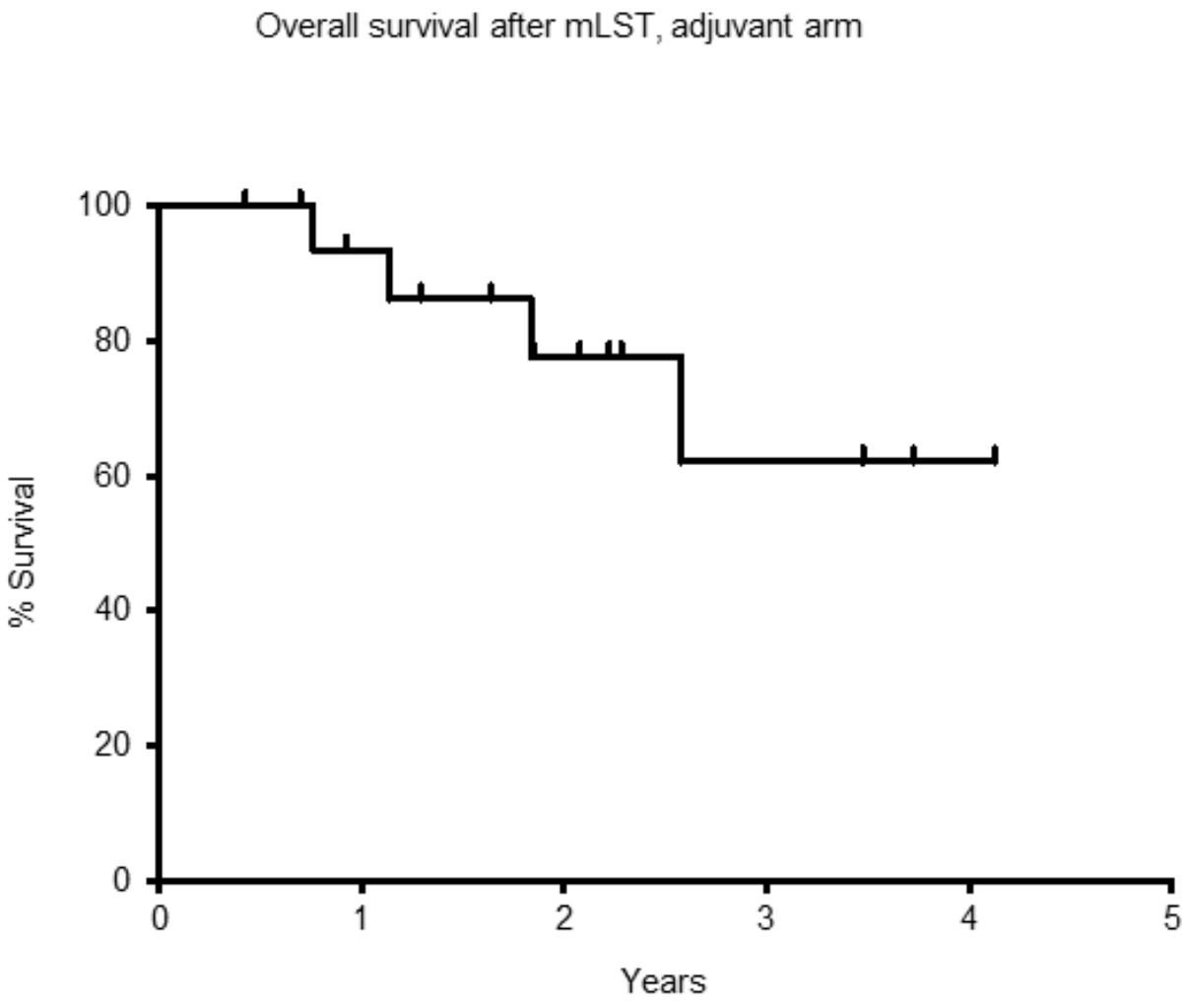
D+30 was the minimum timeframe before mLSTs could be administered to patients post-HCT. However, there was no timeline set for procurement of starting material from their donors. Since procurement timing was not controlled, mLST production (including completion of product release testing) ended at variable timepoints post-HCT, accounting for the median time to infusion of 117

days post-HCT. Indeed, 4 out of 12 patients treated with mLSTs immediately post-HCT ("Never relapsed post-HCT" subset in **Table 1**) had their donor products procured after the recipients had undergone an HCT and in all 12, donor procurements were performed after the stem cell donation. mLSTs were infused within 30 days of manufacture to all but 3 patients (to allow for scheduling issues and pre-infusion protocol mandated procedures such as bone marrow exams, etc.). Delayed infusions in these 3 patients were due to acute GVHD in one, CMV reactivation in another and CMV disease in the third. All were eventually infused with mLSTs within 120 days of manufacture.

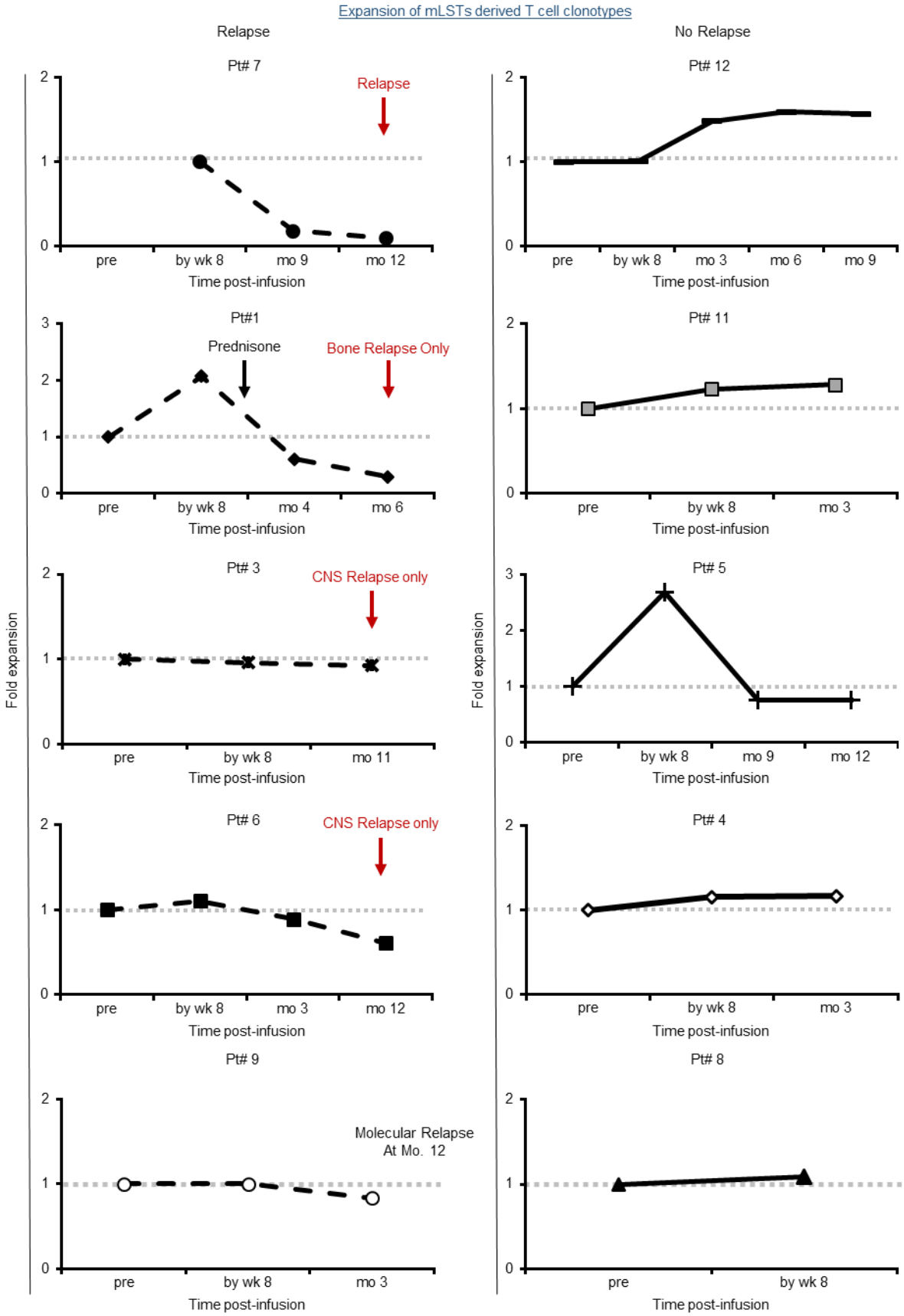
Supplementary Figures.



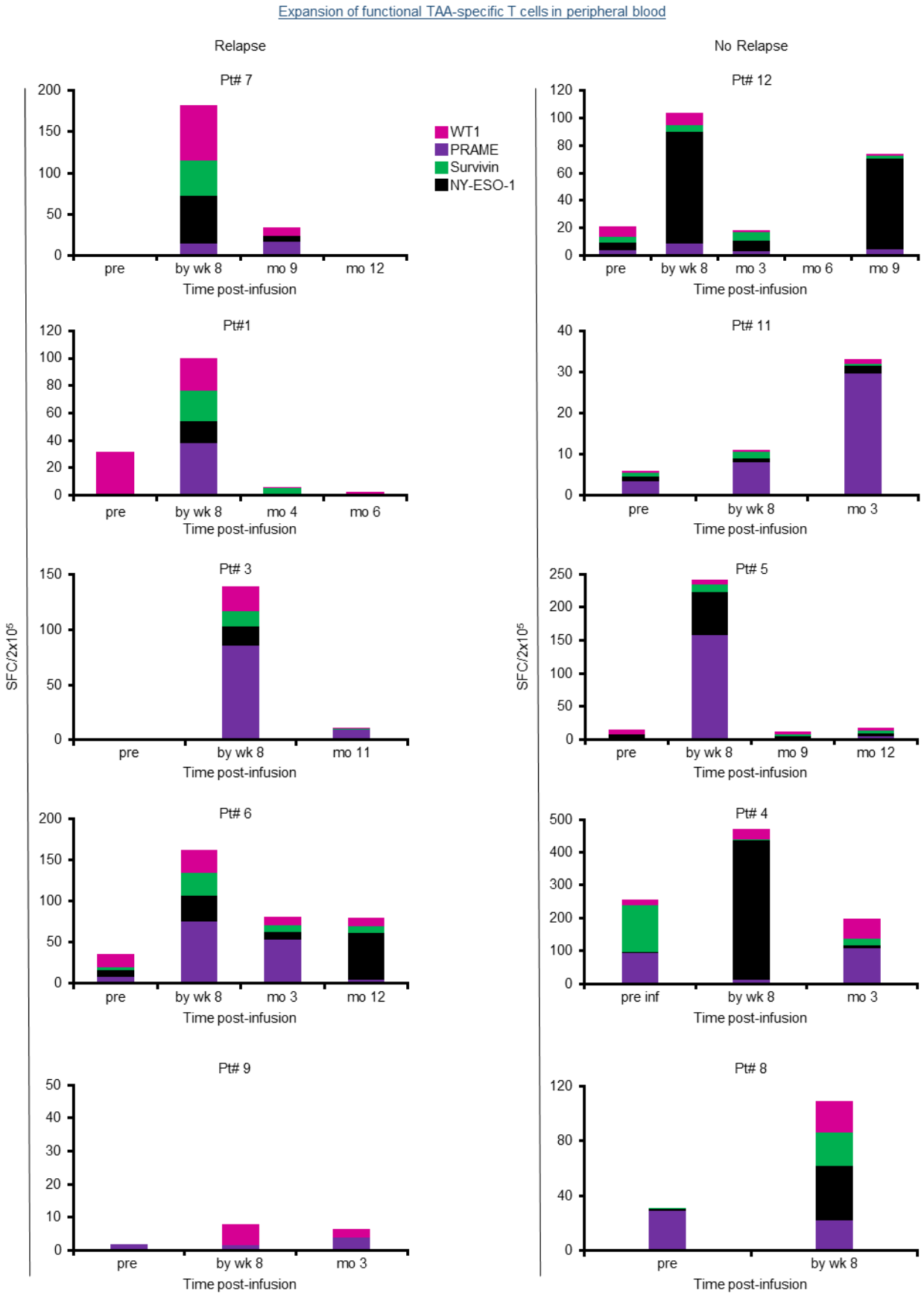
Supplementary Figure 1. CONSORT Flow diagram. Reasons for exclusion, enrollments and re-enrollments to each arm of the clinical trial.



Supplementary Figure 2. Kaplan-Meier estimates of overall survival (LFS) in the adjuvant arm.



Supplementary Figure 3. Expansion of infused mLST clones. Expansion of infused mLST clones (assessed by TCR vβ deep sequencing) in individual patients (Pt#s 7, 1, 3, 6, 9, 12, 11, 5, 4 and 8) treated on the adjuvant arm represented as fold change from baseline repertoire frequency. Where applicable, mLST clone frequency at the time of relapse is depicted. Left panel: Patients who relapsed, Right panel: Patients who did not. "By week 8" denotes a time-point between week 1 and week 8 post-infusion where follow-up material was available to perform simultaneous TCR sequencing and ELISpot (Supplementary Figure 4).



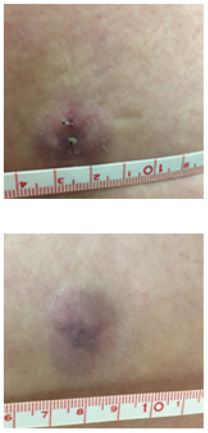
Supplementary Figure 4. Expansion of functional TAA-specific T cells in peripheral blood. Expansion of LA-reactive T cells (as measured by IFNγ ELISPOT assay) in individual patients (Pt#s 7, 1, 3, 6, 9, 12, 11, 5, 4, and 8) treated on the adjuvant arm at the corresponding time-points when clone-tracking data (from Supplementary Figure 1) was available. Left panel: Patients who relapsed, right panel: patients who did not. "By week 8" denotes a time-point between week 1 and week 8 post-infusion where follow-up material was available to perform simultaneous TCR sequencing (Supplementary Figure 3) and ELISpot.

A

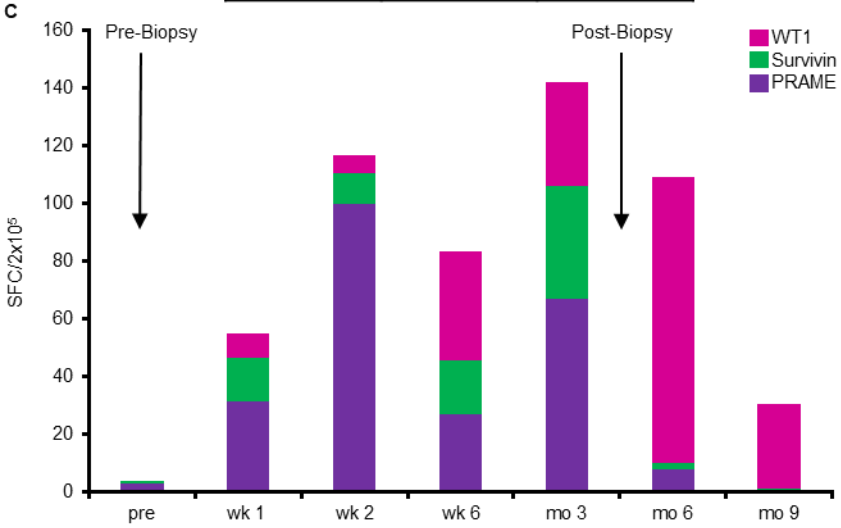
Pt# A2

LA expression	Pre-Biopsy % cells	Post-Biopsy % cells
WT1	Failed	Unknown
Survivin	25%	0%
NYESO1	0%	0%
PRAME	25%	0%

B



C

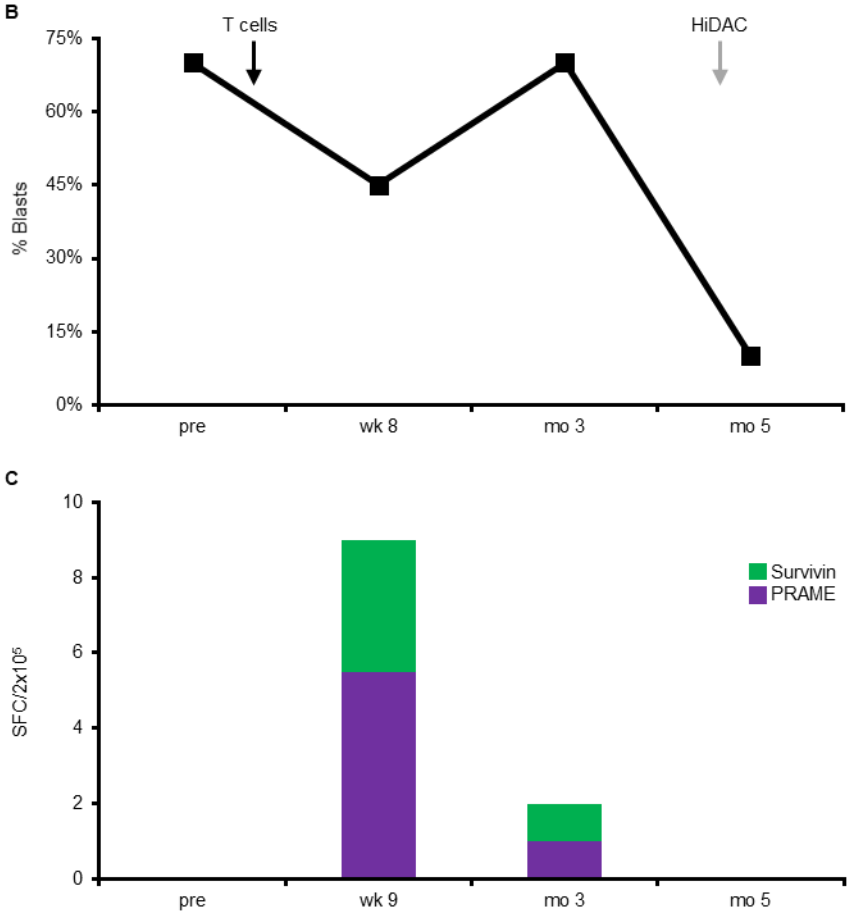


Supplementary Figure 5. TAA-antigen changes post-infusion in leukemia cutis. Decline in TAA-expression post-infusion (**A**) in leukemia cutis lesions (**B**) in a patient treated on the active disease arm (Pt#A2). Change in frequency of WT1-, PRAME- and Survivin-specific T cells in the circulation post-mLST infusion (**C**).

A

Pt# A6

LA expression	(Pre-biopsy) pre	wk 8	mo 3	(Post-HiDAC) mo 5
WT1	0%	0%	0	0
Survivin	100%	100%	60%	100%
NYESO1	0%	0%	0	0
PRAME	20%	0%	7%	80%



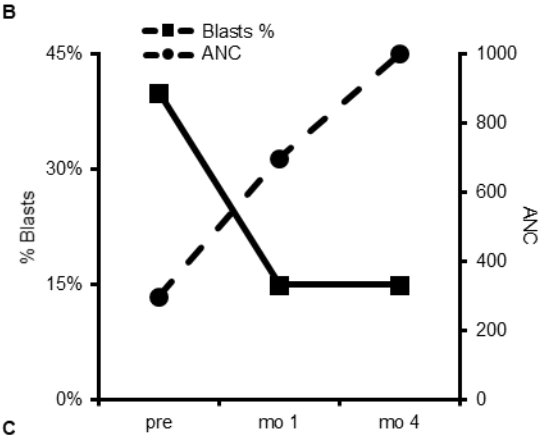
Supplementary Figure 6. TAA-antigen changes post-infusion in marrow blasts. Decline in TAA-expression post-infusion (**A**) in marrow blasts (**B**, HiDAC indicates introduction of high dose cytarabine chemotherapy introduced at disease progression post mLSTs) in a patient treated on the active disease arm (Pt#A6). Change in frequency of PRAME- and Survivin- specific T cells in the circulation post-mLST infusion at corresponding time-points (**C**) where biopsies were performed for TAA expression.

A

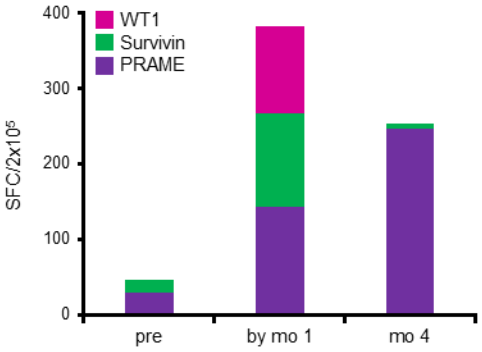
Pt# A4

Pre-Biopsy	
LA expression	% cells
WT1	<10%
Survivin	<10%
NYESO1	0%
PRAME	50-75%

B



C



D

T cell chimerisms	Pre	Mo. 4
Donor	5%	98%*
Recipient	95%	2%*

*Performed on 7 day cultured T cells, as this was the only sample available at this timepoint

Supplementary Figure 7. Partial response in patient (Pt#A4) with active AML after mLST infusion. Decline in AML blasts that expressed WT1, Survivin and PRAME (**A**) post-mLST infusion along with increase in absolute neutrophil counts (ANC) (**B**). Change in the frequency of WT1-, PRAME- and Survivin-reactive T cells post-infusion (**C**). **D** compares the T cell chimerisms pre- and 4 months post-infusion.

Supplementary Table 1

Outcomes of consecutive matched sibling donor HCT or DLIs in recipients with AML/MDS at our center (2012 to 2018)

Totals	**N=17 (mLSTs)**	**N=21 (no mLSTs)**	
Median age	54 (18 – 73)	55 (19 – 70)	
Gender	M=7, F=10	M=13, F=8	
AML	13, 4 MDS/RAEB/CMML	19, 2 MDS	
MAC	9	9	
AML/MDS features:			
Relapsed post-HCT but in CR prior to DLI	5	3	
FLT3-ITD	3	3	
PIF/CR≥2	7	8	
TP53/DNMT3A mut	2	2	
MLL-r	2	2	
Complex cytogenetics	1	2	
Other (t-MDS, other ELN poor risk cyt: Monosomal karyotype)	4	6	
Relapses	**N=6**	**N=12**	
Extra-medullary	3 of 6	1 of 11 (no marrow report available for 1)	P=0.09
Decrease in HLA-DR expression	4 of 5	1 of 9 (no reports available for 3)	P=0.02

Supplementary Table 2

Mechanisms of immune escape at progression

Pt. ID	Anatomic Site	HLA-DR downregulation/loss (flow)	Target TAA expression (IHC)	PD-L1 overexpression
A2	Skin	Not done	Decline post-infusion (see suppl.figure)	Not performed
A3	Marrow	No change	No follow-up biopsy	Not performed
A1	CNS/jaw mass	Not done	Decline post-infusion (WT 75%→0% and Survivin 75%→25%)	Negative
A5	Marrow	Decrease from pre-infusion	No follow-up biopsy obtained for research	Not performed
A4	Marrow	No change	No follow-up biopsy obtained for research	Not performed
A6	Marrow	No change	Decline post-infusion, (see suppl. figure)	Negative