YMTHE, Volume *25*

Supplemental Information

Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma

Frederick L. Locke, Sattva S. Neelapu, Nancy L. Bartlett, Tanya Siddiqi, Julio C. Chavez, Chitra M. Hosing, Armin Ghobadi, Lihua E. Budde, Adrian Bot, John M. Rossi, Yizhou Jiang, Allen X. Xue, Meg Elias, Jeff Aycock, Jeff Wiezorek, and William Y. Go

Supplementary Materials

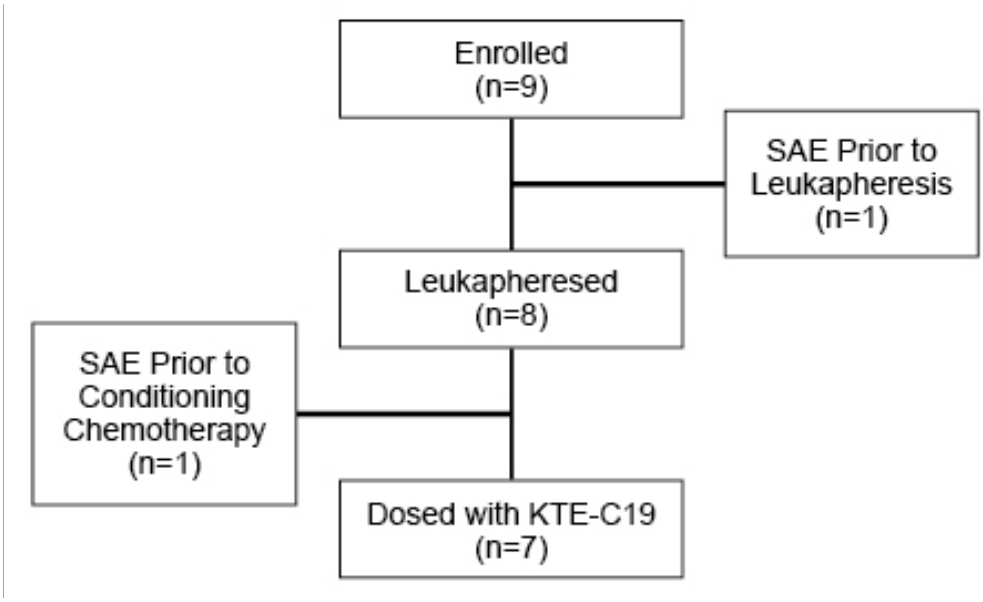
Supplementary Methods

Co-culture of Anti-CD19 CAR Product Samples with Target Cells

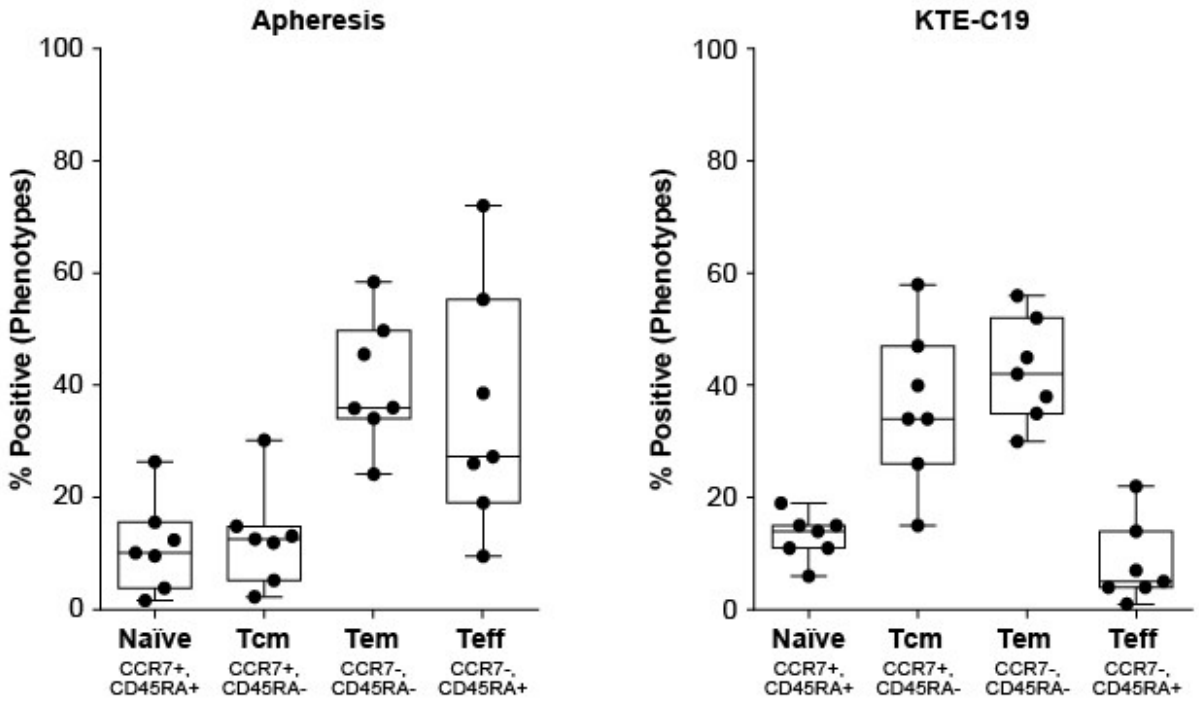
KTE-C19 and target cell lines Toledo (ATCC® CRL2631™) and CEM (ATCC® CCL-119™) viability and density were assessed using a Nucleocounter (ChemoMetec A/S, Denmark). Cell densities were adjusted to 1×10^6 viable cells per mL. Final cell density was adjusted to 2.5×10^5 viable cells/mL for both target cells and KTE-C19. Viable target cells (2.5×10^4) and viable KTE-C19 (2.5×10^4) were mixed into individual wells of a 96-well U bottom plate in triplicate. As controls, KTE-C19–only and target-only cultures were run in parallel at the same densities as co-culture replicates. Cultures were incubated for 24 hours at 37°C and 5% CO_2. After incubation, supernatants were harvested by centrifugation and transferred into a fresh 96-well U bottom plate. Analysis was performed using a qualified ELISA (R&D systems, Minneapolis, MN).

Supplementary Tables and Figures

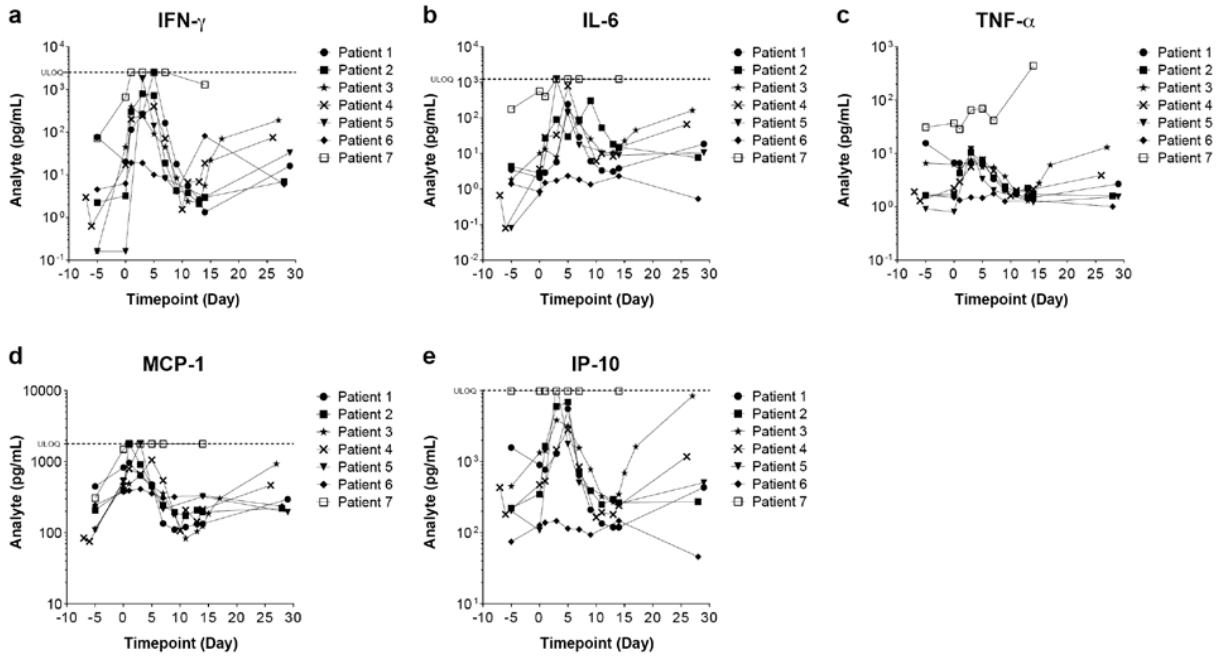
Supplementary Figure S1. Study CONSORT diagram.



Supplementary Figure S2. KTE-C19 product exhibited a less differentiated phenotype based on CCR7 and CD45RA markers relative to apheresis material and was characterized by a higher proportion of naïve and central memory (Tcm) T cells and comparable or lower proportion of effector memory (Tem) and effector (Teff) T cells. The bars and boxes show the minimum, maximum, median and interquartile range.



Supplementary Figure S3. Analysis of patient serum reveals a biomarker profile composed of specific cytokines, chemokines and effector proteins associated with KTE-C19 treatment. Expansion of CD19 CAR T cells was mirrored by induction and elevation of a range of cytokines and chemokines that regulate proliferation, activation and effector function. Induction of IFN-γ (a) begins during conditioning chemotherapy and levels begin to decline following KTE-C19 infusion. Inflammatory cytokines IL-6 (b) and TNF-α (c) levels peak 3-5 days post-infusion, during peak anti-CD19 CAR T cell expansion. MCP-1 (d) and IP-10 (e) levels increase up to day 5 post-infusion and provide evidence for CAR T cell homing.



Supplementary Table S1. Time to Progression and Duration of Response in Patients Relapsing After Prior ASCT.

Patient	Response to Prior ASCT	Time to Progression After Prior ASCT (Months)	Best Overall Response on Study	Time from KTE-C19 Infusion and Ongoing Response (months)
1	CR	6.3	PR	3.1
4	CR	5.8	CR	12.0+[a]
5	CR	3.2	CR	12.3+[a,b]
6	CR	5.8	CR	12.0+[a,b]

[a]Response ongoing at the time of analysis.
[b]Numbers calculated based on the post-cutoff data as of August 22, 2016.

Supplementary Table S2. Apheresis products reflected diversity among patients enrolled in the study. The table below captures high variability in baseline natural killer (NK), monocyte and T cell populations.

	Baseline proportion of cell population in apheresis product, %		
Patient	**Monocytes**	**NK cells**	**T cells**
1	17.7	11.1	62.0
2	59.8	21.9	2.5
3	26.1	4.3	66.3
4	38.4	3.9	50.7
5	37.5	10.7	36.3
6	32.2	37.2	14.8
7	72.4	8.0	10.0