Background Chimeric antigen receptor (CAR)-T cells are engineered T cells that can identify and eliminate cells expressing a target antigen. Cryopreservation of CAR-T cells preceding infusion is a widely used practice since it allows flexible scheduling time of patient infusions. Using frozen PBMCs from patients prior to standard therapies could impact the fitness and function of the final CAR-T product but provides flexibility in scheduling both leukapheresis and manufacture. The impact of cryopreserved PBMCs on the quality of the final CAR-T product is incompletely characterized and needs to be further studied.

Methods We performed a prospective study using PBMCs from 20 healthy donors (HDs) to examine if manufacture of CAR-T cells using frozen (2X) vs. fresh (1X) PBMCs affects the quality and functionality of the product. The CAR-T manufacturing process is detailed in figure 1. We analyzed second generation CD19-targeted human CAR-T cells with CD28 (h1928z) co-stimulation for transduction efficiency, product expansion, immune-phenotype, activation induced cell death (AICD) and metabolic fitness (MF). We compared functionality by measuring markers of activation (4–1BB, CD107a and pAKT measured by flow cytometry, and cytokine release measured by ELLA) and their cytotoxic capacity (measured by xCELLigence Real-Time Cell Analysis) against hCD19 target-expressing cells.

Results We found that freezing PBMCs delayed expansion during manufacture, with a doubling time 1.25-fold higher in 2X h1928z compared to 1X h1928z CAR-T cells (figure 2B). Transduction efficiency (figure 2A), CD4/CD8 ratio (figure 2C), CAR-T cell phenotype (figure 2D-E), AICD (figure 2F) and MF (figure 2G) were not affected by freezing PBMCs prior to CAR-T manufacture. Moreover, freezing PBMCs did not affect expression of 4–1BB (figure 2H), CD107a (figure 2I) or pAKT (figure 2J) after CAR engagement. IFN-γ and TNF-α production was reduced in 2X h1928z CAR-T cells after hCD19 mediated activation (figure 2K) without compromising their cytotoxic capacity (figure 2L-M).

Conclusions Freezing PBMCs prior to CAR-T production slows cell expansion during manufacture and decreases IFN-γ and TNF-α production, without disturbing CAR-T cell immune-phenotype, activation, or in vitro anti-tumor function. As CAR-T immunotherapy continues to expand, the necessity to store PBMCs to improve manufacturing logistics as well as to allow collection from patients at an early clinical stage will grow. This study supports the concept that cryopreservation of PBMCs is a valid solution to these issues without compromising the quality of the final product.

Acknowledgements Dr. Locke is supported by the Leukemia and Lymphoma Society as a Clinical Scholar. This study was supported by research funding from Kite Pharma to F.L. Locke. The authors thank the team at Kite Pharma for helpful and critical scientific discussions. We would like to thank the Flow Cytometry Core at Moffitt Cancer Center.

REFERENCES
Abstract 193 Figure 2  Comparison between CAR-T cells manufactured from fresh (1X) or cryopreserved (2X) PBMC. A. CAR expression was determined by flow cytometry using anti-FMC63 mAb. *# B. Doubling time was calculated as day 7/day 1 ratio.* C. CD4/CD8 ratio on live CD3+CAR+ cells determined by flow cytometry.*# D-E. CD4+ (D) and CD8+ (E) CAR-T cells immune-phenotype determined by flow cytometry.*# Naive (CCR7+CD45RO-), CM: central memory (CCR7+CD45RO+), EM: effector memory (CCR7-CD45RO+), Eff: terminally differentiated effector (CCR7-CD45RO-). F-H. CAR-T cells were stimulated with irradiated hCD19 target cells for 72h. Cells were then collected, and activation induced cell death (F), metabolic fitness (G) and 4–1BB expression (H) were analyzed by flow cytometry.*# I. Percentages of phosphorylated AKT cells within CD3+CAR+ cells. CAR-T cells were stimulated with OCI-LY3 target cells for 20 minutes and analyzed by flow cytometry.* J. Percentages of CD107a cells within CD3+CAR+ cells. Degranulation was measured by CD107a staining upon incubation of h1928z CAR-T cells with OCI-LY3 target cells for 4h. * K. CAR-T cells were stimulated with hCD19-expressing 3T3 target cells for 24 h before supernatant was collected and analyzed by ELLA immunoassay to quantify IFN-γ, TNF-α, GM-CSF and IL-2.* L-M. The xCELLigence real-time cell analysis system monitored real-time cytotoxicity of CAR-T cells co-cultured with irradiated hCD19-expressing 3T3 target cells at 1:1 E:T ratio. (L) Representative RTCA comparing killing activity of untransduced (UT), 1X and 2X CAR-T cells. (M) Comparison of the killing activity of 1X and 2X CAR-T cells at 12h and 24h.*

*A paired t test was used. Each symbol represents an individual healthy donor, and the p values are indicated in each graph. #The middle line denoted the median.