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TANDEM MEETINGS

Transplantation & Cellular Therapy Meetings *of* ASTCT® and CIBMTR®

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Hawai‘i Convention Center, Honolulu, HI

Disclosure

Soumya Poddar
Principal Scientist at Kite Pharma, A Gilead Company

Employment at Kite, a Gilead Company, and equity ownership in Gilead Sciences, Inc.

Patents and Royalties, University of California, Los Angeles and Gilead Sciences, Inc

In vitro and in vivo Characterization of Axicabtagene Ciloleucel Identifies Features Associated with Treatment Resistance in Patients, including a Dysfunctional CD8+ T Cell State Characterized by the GATA3 overexpression

Soumya Poddar, Gabriela Balderrama-Gutierrez, Chad Williams, Subing Cao, Tan Trinh, Sunanda Kumar, Quinn Walker, Martin Gomez, Madison Davis, Alessandro Calo, Stacey Valny, Sean Yoder, Lei Huang, Justin Budka, Jenny J. Kim, Rhine Shen, Jorge Andrade, Bhargavi Rajan, Simone Filosto, Sophie Viaud, Davide Bedognetti, Mike Mattie

Affiliations: Kite, a Gilead Company, Santa Monica, CA

Background and Objective of the study

- Autologous anti-CD19 CAR T cell therapy is a curative-intent treatment for patients with B cell malignancies. Still, more than 50% of R/R patients either do not respond or relapse after an initial response to the treatment[4].
- Axicabtagene ciloleucel (axi-cel) is an autologous anti-CD19 CAR T-cell therapy approved for the treatment of relapsed/refractory (R/R) large B-cell lymphoma (LBCL)[1,2] and Follicular lymphoma[3]
- Tumor features including elevated disease burden, low antigen expression, or an immune suppressive microenvironment have been associated with disease progression[5,6]
- A less differentiated, naïve-like, product T cell phenotype and CAR T cell expansion have been associated with favorable outcome[7,8]

However, our understanding of the product features linked to and potentially predictive of the treatment resistance remains limited.

Objective: Explore the association between product functional attributes and clinical outcome by analyzing products available from ZUMA1 patients

1. Neelapu et al., N Engl J Med 2017;377:2531-2544; 2. Locke et al., N Engl J Med 2022;386:640-654; 3. Jacobson et al., The Lancet Oncology Volume 23, Issue 1P91-103 January 2022; 4. Cappell and Kochenderfer, Nat Rev Clin Oncol 20, 359–371 (2023); 5. Scholler et al., Nature Medicine volume 28, pages1872–1882 (2022); 6. Locke, Filosto et al., 2024 Feb;30(2):507-51; 7. Filosto et al., Blood Cancer Discov. 2024 Jan 8;5(1):21-33; 8. Locke et al., Blood Adv (2020) 4 (19): 4898–4911

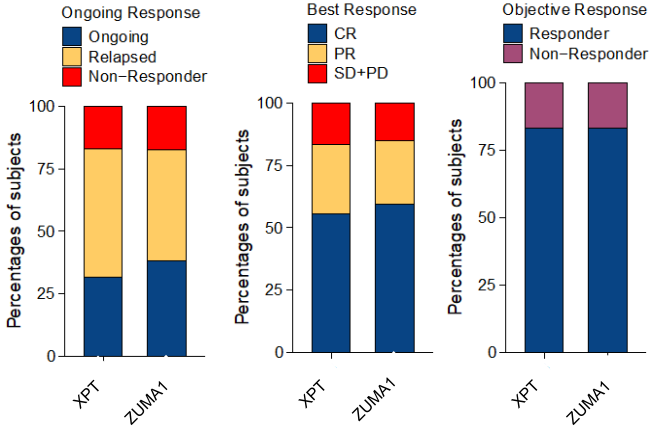
CAR T cell products analyzed in this study are representative of clinical response in ZUMA1

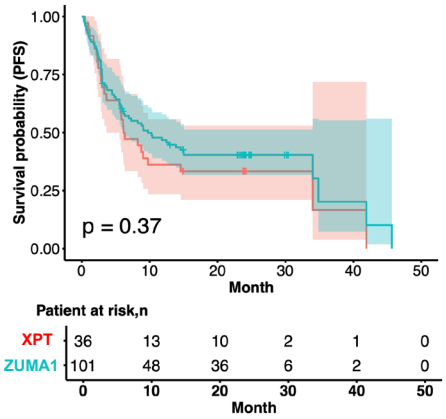
Evaluated in this study

ZUMA1 patients | CAR T product

- Ongoing responders (n=11)
- Relapsed (n=19)
- Non-responders (n=6)

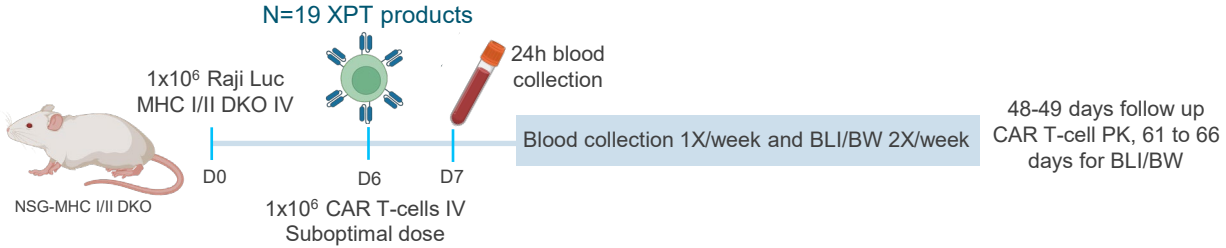
ZUMA1 this study (XPT): 36 subjects
ZUMA1[1] Cohort 1 & 2: 101 subjects

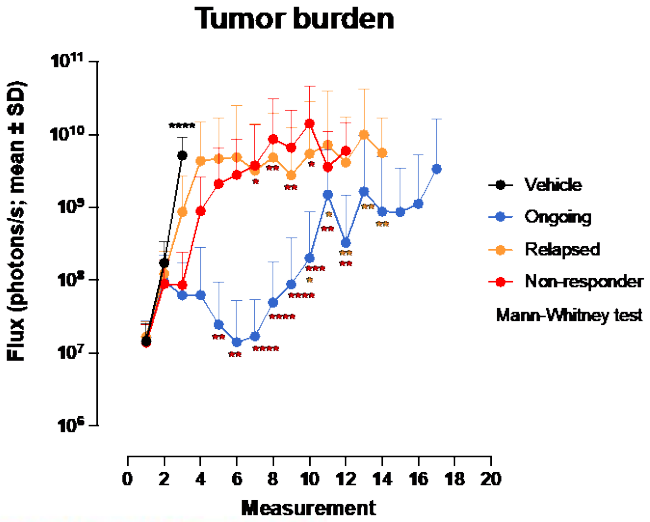


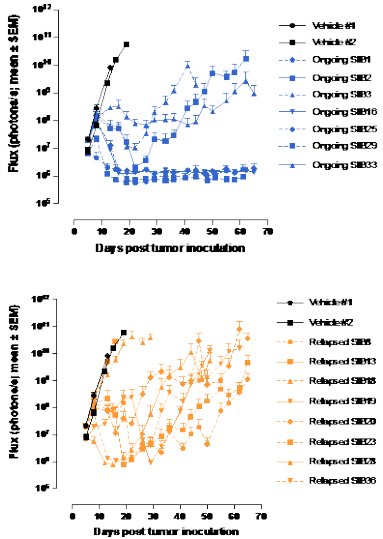


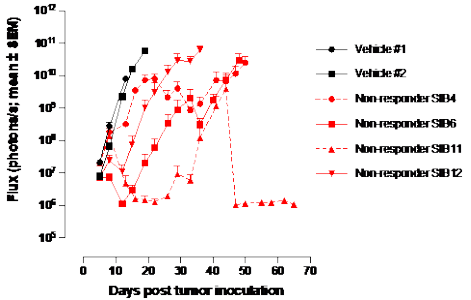
1. Neelapu et al., N Engl J Med 2017;377:2531-2544

In vivo efficacy of ZUMA1 products in a systemic human B-cell lymphoma model re-capitulates clinical outcome

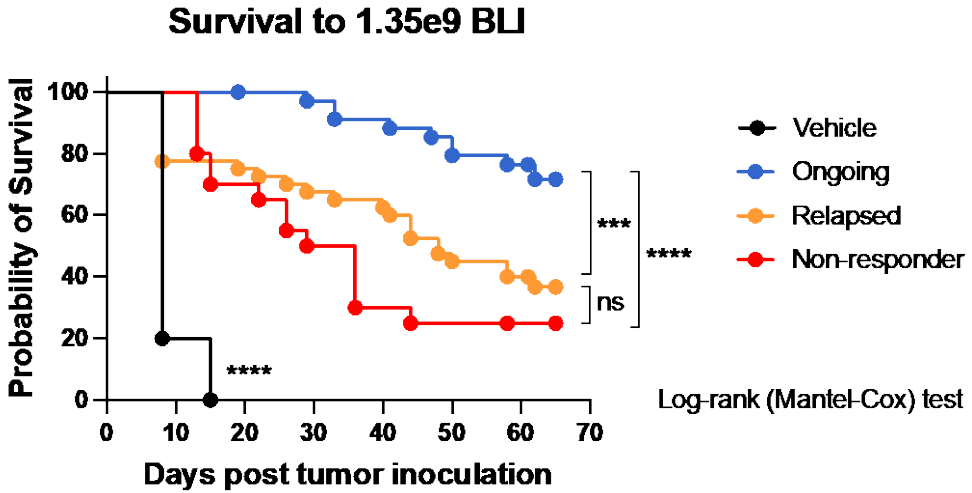






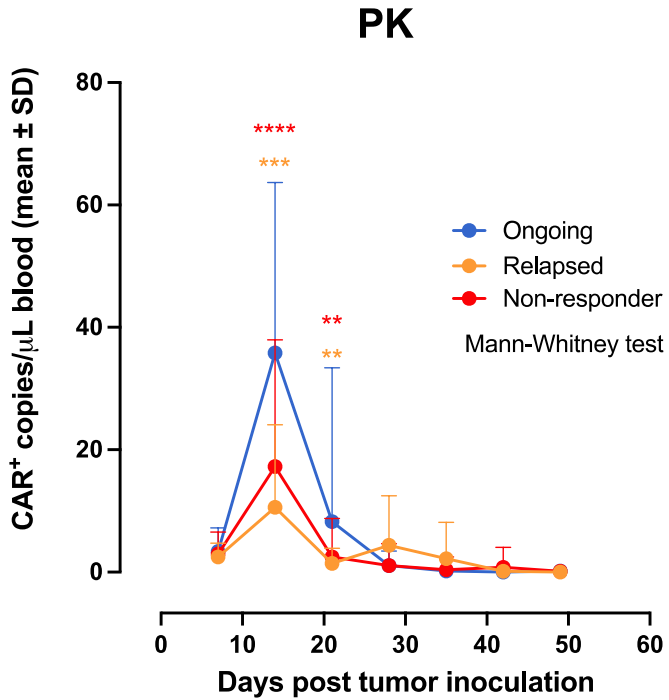


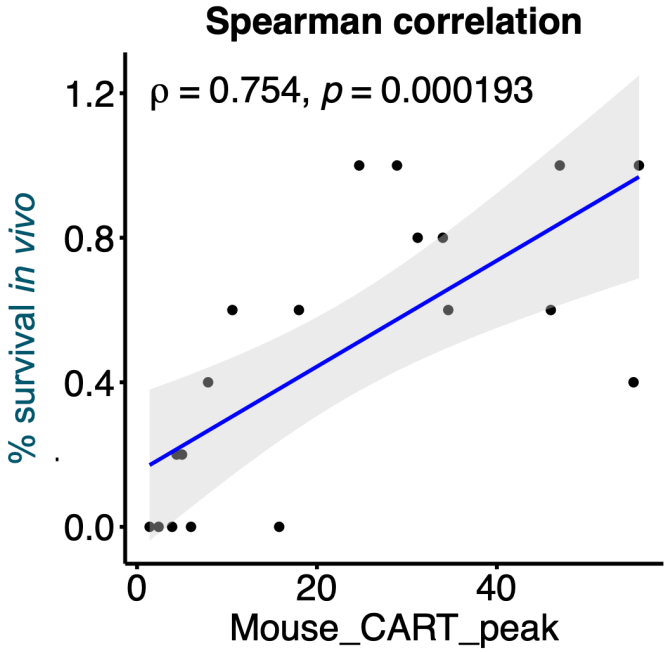
Survival analysis over time of tumor-bearing mice treated with axi-cel products



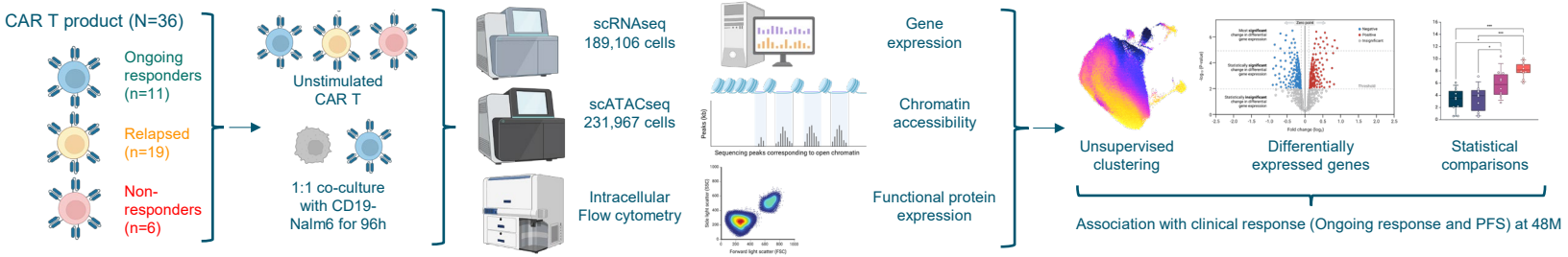
Kaplan-Meier survival analysis set at a tumor burden < 1.35 x 10^9 photons/second bioluminescence. Statistical analysis performed with the Mantel-Cox log-rank test

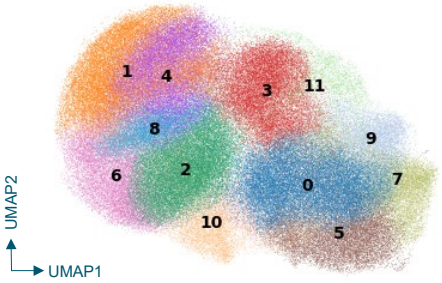
Expansion of CAR-T cells in vivo (in mice) correlated with response in mice and ZUMA-1 clinical outcome

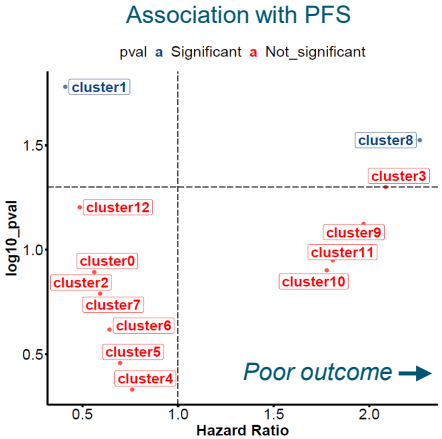


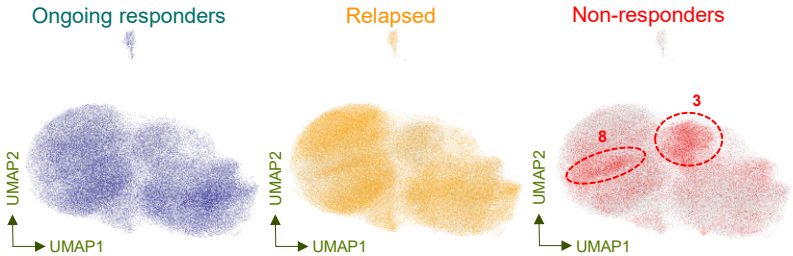


Single-cell analysis of ZUMA1 products identifies cell clusters associated with clinical outcomes









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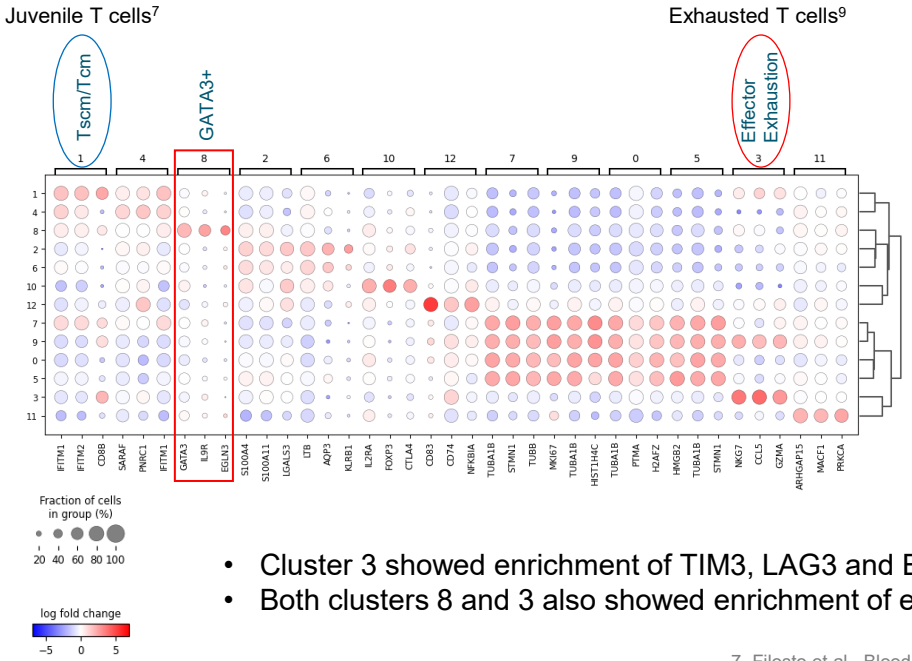
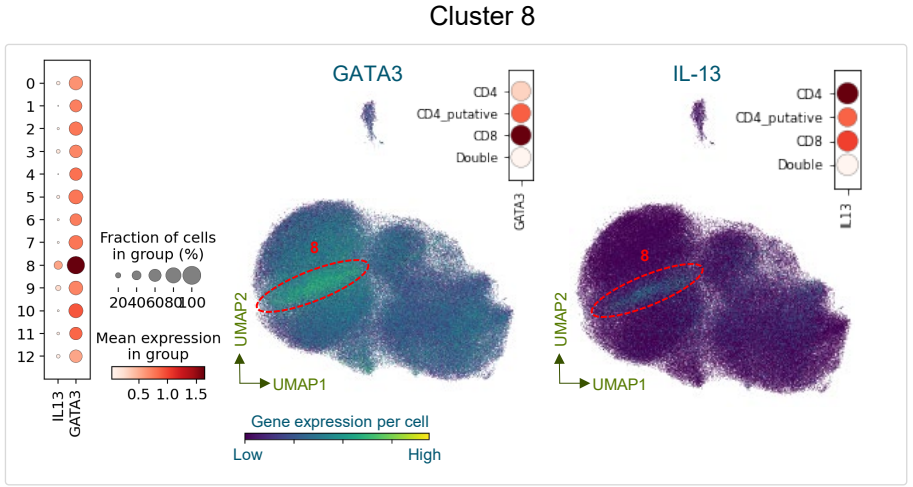
Non-responders enriched clusters show increased GATA3+CD8+ T cells and/or exhaustion markers

Top 3 differentially expressed gene in each cluster



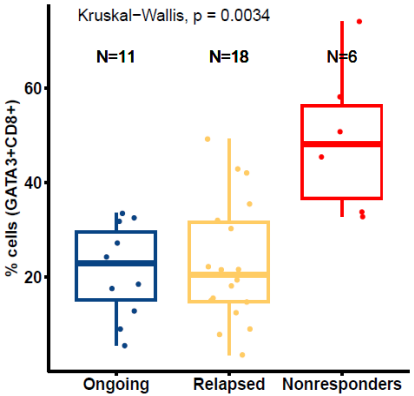


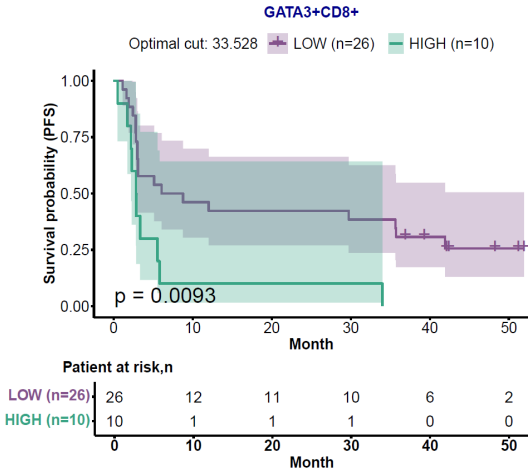
- Cluster 3 showed enrichment of TIM3, LAG3 and EOMES
- Both clusters 8 and 3 also showed enrichment of exhaustion marker, TIGIT

7. Filosto et al., Blood Cancer Discov. 2024 Jan 8;5(1):21-33; 9. Delgoffe, Greg M. et al. Cancer Cell, Volume 39, Issue 7, 885 - 888

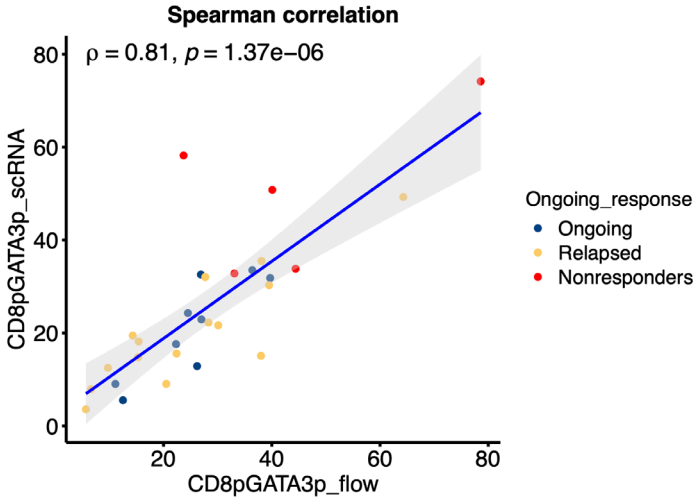
GATA3+ CD8+ T cells associated with poor clinical outcome

Association of GATA3+CD8+ T cells (**scRNAseq**) with outcome

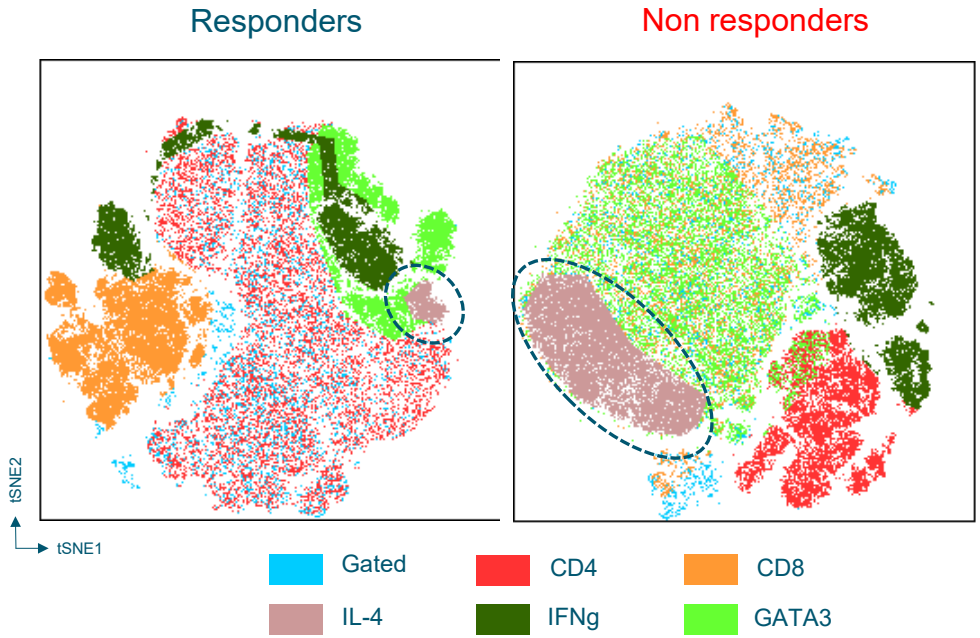


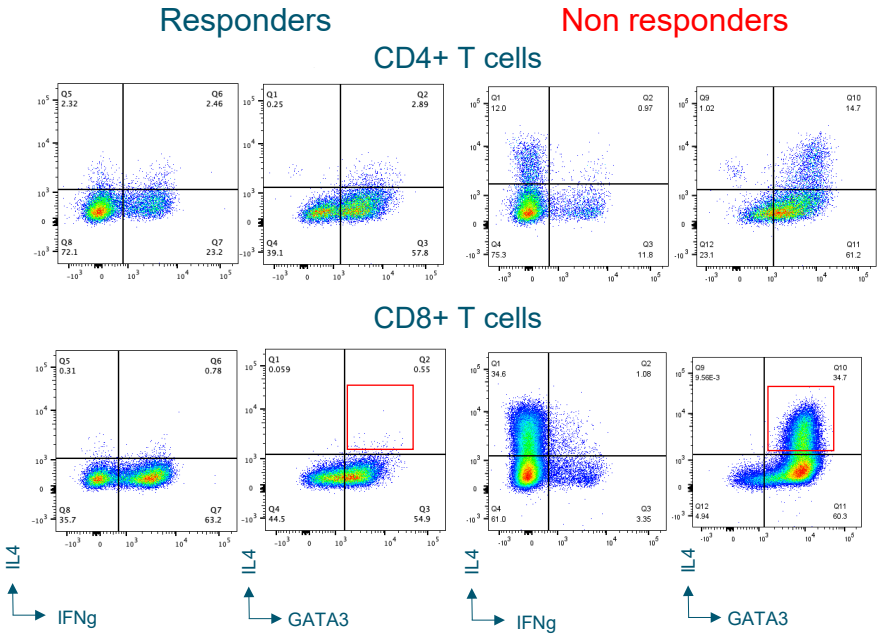


Frequency of GATA3+ CD8+ T cells by Flow and scRNAseq



IL-4 producing GATA3+CD8+ T cells is a major distinguishing factor in non-responders



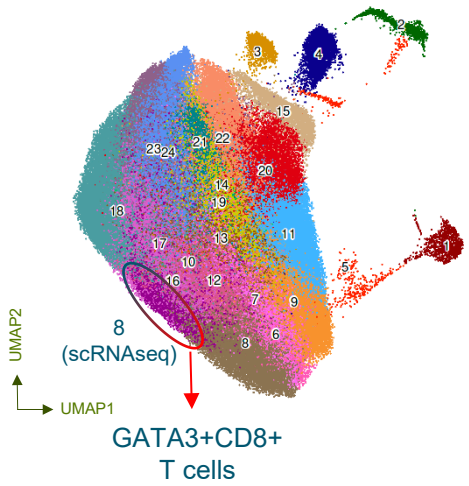


N=8
Ongoing: 3; Relapsed: 3; Non-responders: 2

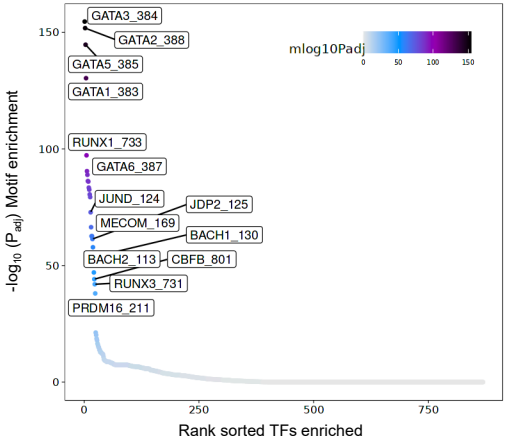
UMAPs comparing products from all 36 subjects are in works

scATACseq validated the findings on GATA3^{high} CD8+ cells and showed GATA3^{high} cluster may rely on promoter/enhancer activity

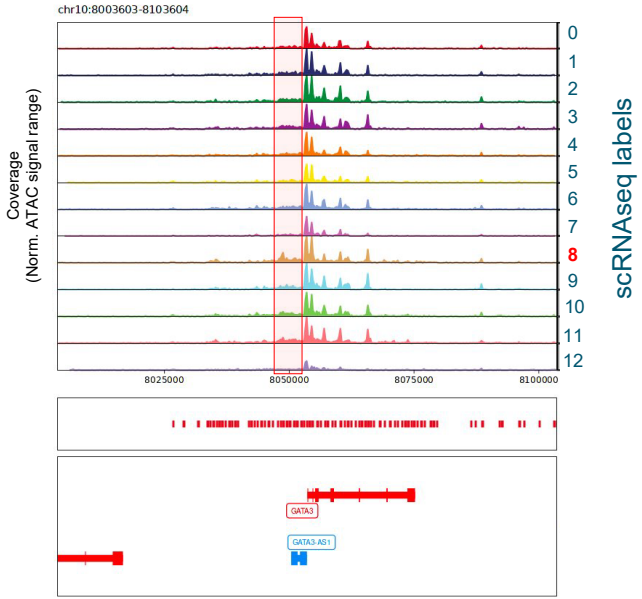
scATACseq clusters



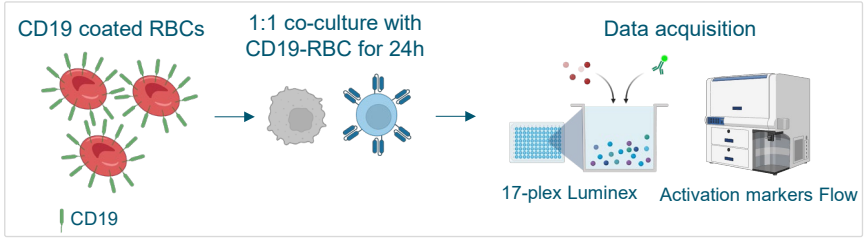
Motif enrichment



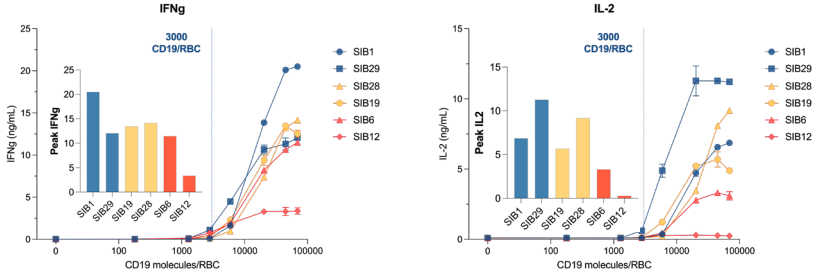
Chromatin accessibility of GATA3 promoter



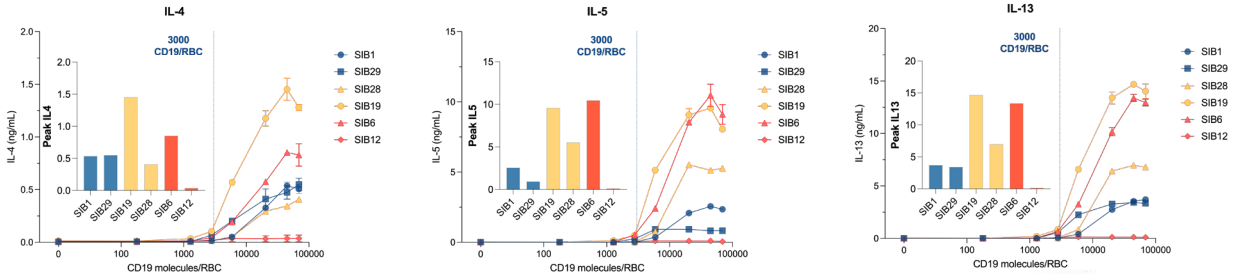
Differential production of Th1 and Th2 cytokines, highlighting difference in Th1 polarization



Th1 cytokines



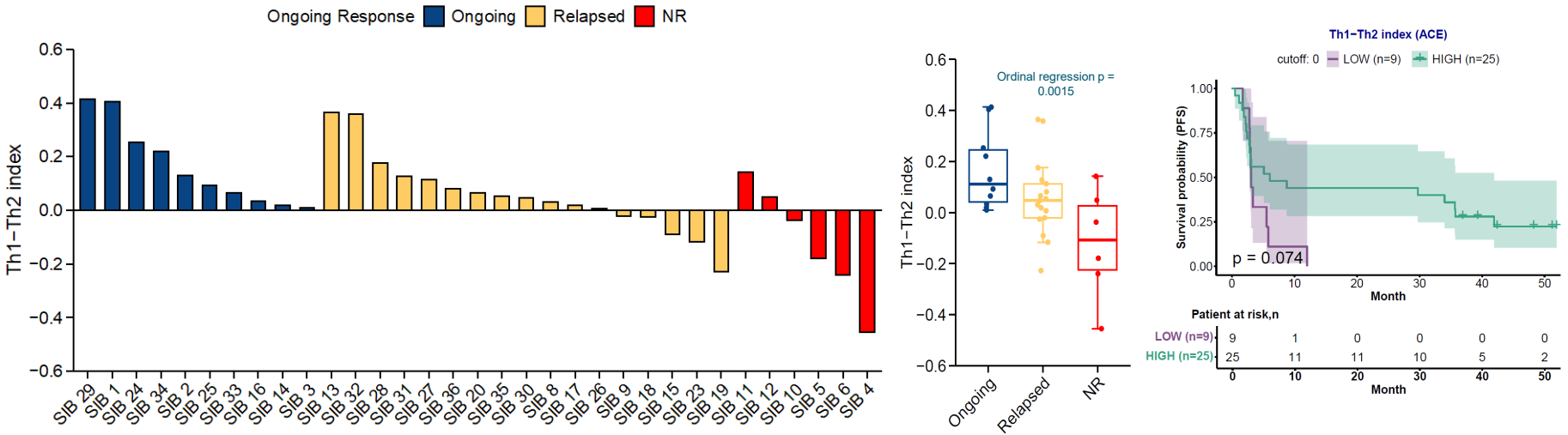
Th2 cytokines



Ongoing
Relapsed
Non-responders

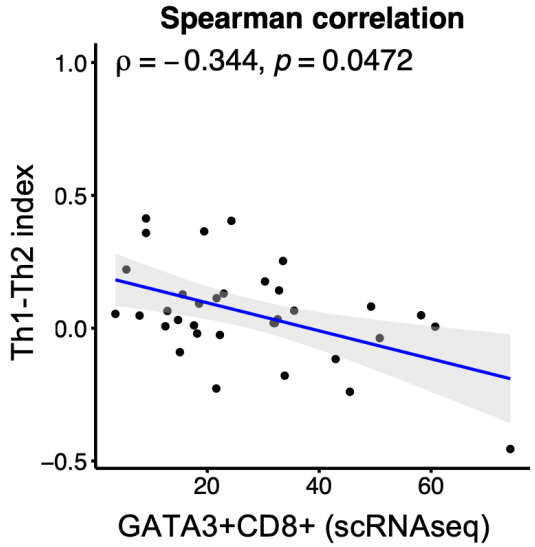
N=6

Th1-Th2 index is strongly linked to clinical outcome

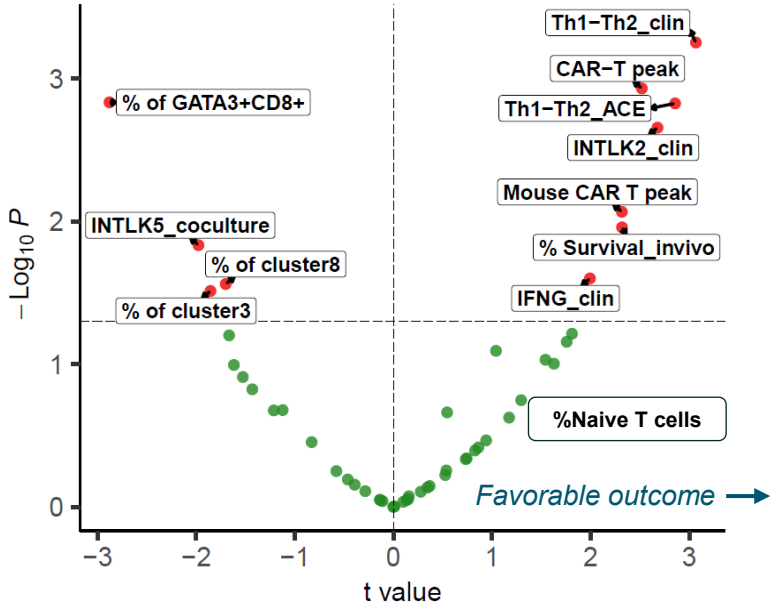


Th1 and Th2 cytokines measured in vivo (in patients and in mice), and Th1-Th2 index showed significant association with clinical efficacy

GATA3+CD8+ T cells and Th1-Th2 index ranked as top covariates to be associated with clinical response



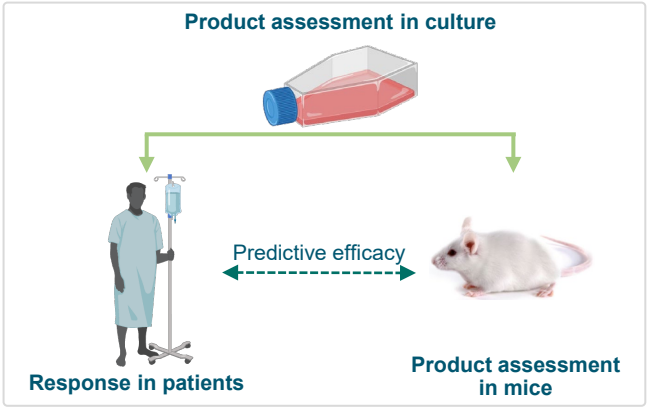
Ongoing Response (Ordinal regression)



t value: 0; p−value cutoff: 0.05

N= All Subjects
Ongoing: 11; Relapsed: 19; Non-responders: 6

Summary of the findings



Product features associated with outcome

Relapse/Non-response
Shorter PFS

Ongoing response
Longer PFS

Known
Naive product phenotype

Novel features
Th1-Th2 index
GATA3+ CD8+ cells

Higher level
Lower level

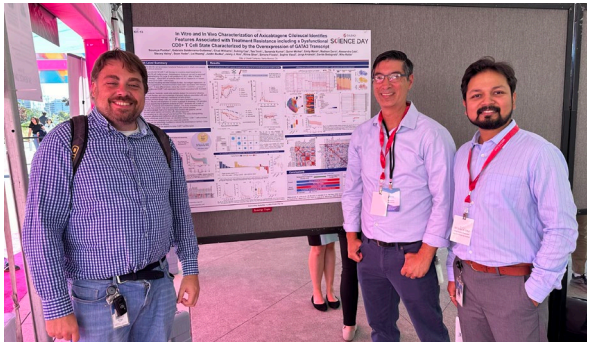
GATA3 CD8 → T_C2 → IL-5 IL-4 IL-13

GATA3+CD8+ T cells identified in complementary assays in NR
scRNAseq, scATACseq, Phenotyping

- *Biomarker: Th1-Th2 index may have the potential to differentiate between efficacious and non-efficacious CAR T products*
- *Actionable product optimization: Dysregulated GATA3+CD8+ T cells are enriched in non responders in axi-cel treated subjects*

Acknowledgements

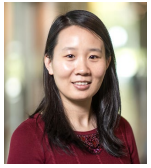
- The patients, families, friends and caregivers
- The study investigators, coordinators and healthcare staff
- This study is funded by Kite, A Gilead Company



Supervision by Mike Mattie and Davide Bedognetti

Gabriela

Chad



Subing

- Leadership: Mike Mattie, Davide Bedognetti, Sophie Viaud, Simone Filosto, Rhine Shen, Jorge Andrade
- scRNAseq and scATACseq: Gabriela Balderrama-Gutierrez, Tarinee Huang, Lei Huang
- In vitro experiments (including ACE assay): Tan Trinh, Chad Williams
- Correlative analyses and Statistics: Subing Cao, Justin Budka
- In vivo experiments and analysis: Sunanda Kumar, Quinn Walker, Sophie Viaud
- Intracellular Flow cytometry: Martin Gomez, Bhargavi Rajan
- Automation: Alessandro Calo, Stacey Valny, Sean Yoder
- Project Management: Abinaya Nathan, Soumyajit Banerjee