7th POSTGRADUATE
Lymphoma
Conference

CAR-T in NHL: Other products and next generation CAR-T

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University of Pennsylvania, Philadelphia, PA, USA

Rome,
March 16-17 2023

Donna Camilla Savelli Hotel

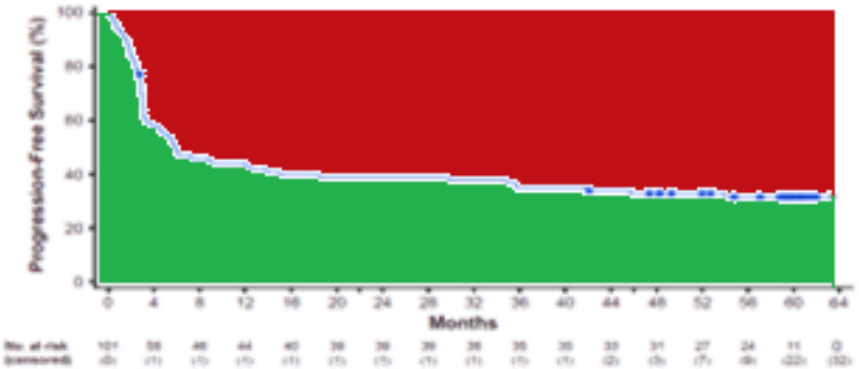
President:
P.L. Zinzani

Disclosures

Disclosures of Prof. Stephen J. Schuster

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
AbbVie						X	
AstraZeneca						X	
BeiGene						X	
Caribou Biotech						X	Steering committee
Fate Therapeutics							Safety DSMB
Genentech/Roche	X					X	Steering committee
Genmab	X					X	Steering committee
Incyte/Morphosys						X	Honoraria for presentation
Kite Pharmaceuticals						X	
Legend Biotech						X	Steering committee
Novartis						X	Steering committee
Mustang Biotech						X	
Nordic Nanovector						X	Steering committee
Takeda							Honoraria for presentation

CAR-T saves lives but there is room for improvement



ZUMA-1 Trial[1]

n = 101

Median follow-up: 63.1 months

Best ORR: 83%, CR: 58%

5-year PFS: 31.8% (95% CI: 22.9-41.1)

[1]*adapted from* Neelapu SS, et al. Blood. 2023; Epub ahead of print.

Ibrutinib Before Apheresis May Improve Tisagenlecleucel Manufacturing in Relapsed/Refractory Large B-Cell Lymphomas: a Phase 1b Study

Julio C. Chavez,[1] Frederick L. Locke,[2] Ellen Napier,[3,4] Carl Simon,[5] Andrew Lewandowski,[5] Rakesh Awasthi,[6] Boris Engels,[5] Petrina Georgala,[7] Attilio Bondanza,[8] Stephen J. Schuster [4]

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Poster Presentation: ASH 2020

Ibrutinib, a BTK inhibitor, may improve CAR-T cell manufacturing, in vivo cellular kinetics, and antitumor efficacy

Plenary Paper

LYMPHOID NEOPLASIA

Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes

Jason A. Dubovsky,[1] Kyle A. Beckwith,[1,2] Gayathri Natarajan,[3] Jennifer A. Woyach,[1] Samantha Jaglowski,[1] Yiming Zhong,[1] Joshua D. Hessler,[1] Ta-Ming Liu,[1] Betty Y. Chang,[4] Karilyn M. Larkin,[1] Matthew R. Stefanovski,[1] Danielle L. Chappell,[1] Frank W. Frissora,[1] Lisa L. Smith,[1] Kelly A. Smucker,[1] Joseph M. Flynn,[1] Jeffrey A. Jones,[1] Leslie A. Andritsos,[1] Kami Maddocks,[1] Amy M. Lehman,[5] Richard Furman,[6] Jeff Sharman,[7] Anjali Mishra,[1] Michael A. Caligiuri,[1] Abhay R. Satoskar,[8] Joseph J. Buggy,[4] Natarajan Muthusamy,[1] Amy J. Johnson,[1,9] and John C. Byrd[1,9]

[1]Department of Internal Medicine, Division of Hematology, [2]Medical Scientist Training Program, and [3]Department of Microbiology, The Ohio State University, Columbus, OH; [4]Pharmacyclics, Inc., Sunnyvale, CA; [5]Center for Biostatistics, The Ohio State University, Columbus, OH; [6]Department of Medicine, Division of Hematology-Oncology, Weill Cornell Medical College, New York, NY; [7]Willamette Valley Cancer Institute/US Oncology, Springfield, OR; and [8]Department of Pathology, and [9]Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH

- Ibrutinib is a clinically viable irreversible ITK inhibitor[1]
- Ibrutinib inhibits the formation of Th2 but not Th1 immunity[1]

Regular Article

IMMUNOBIOLOGY

Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia

Joseph A. Fraietta,[1,2,*] Kyle A. Beckwith,[3,*] Prachi R. Patel,[1,2] Marco Ruella,[1,2] Zhaohui Zheng,[1,2] David M. Barrett,[4] Simon F. Lacey,[1,2] Jan Joseph Melenhorst,[1,2] Shannon E. McGettigan,[1,2] Danielle R. Cook,[1,2] Changfeng Zhang,[1,2] Jun Xu,[1,2] Priscilla Do,[3] Jessica Hulitt,[4] Sagar B. Kudchodkar,[1,2] Alexandria P. Cogdill,[1,2] Saar Gill,[1,5] David L. Porter,[1,2,5] Jennifer A. Woyach,[3] Meixiao Long,[3] Amy J. Johnson,[3] Kami Maddocks,[3] Natarajan Muthusamy,[3] Bruce L. Levine,[1,2,6] Carl H. June,[1,2,6] John C. Byrd,[3,*] and Marcela V. Maus[7,*]

- Ibrutinib treatment of CLL enhances the generation of CAR-T cells for adoptive immunotherapy[2]
- Concurrent ibrutinib therapy improves the engraftment and therapeutic efficacy of anti-CD19 CAR T cells in mouse models[2]

[1]Dubovsky, *et al.* Blood. 2013;122:2539-2549; [2]Fraietta, *et al.* Blood. 2016;127(9):1117-1127.

Ibrutinib Improves T Cell Number and Function in CLL

CLINICAL MEDICINE
The Journal of Clinical Investigation

Ibrutinib treatment improves T cell number and function in CLL patients

Meixiao Long,[1,2] Kyle Beckwith,[1,2,3] Priscilla Do,[1,2,3] Bethany L. Mundy,[1,2] Amber Gordon,[1,2] Amy M. Lehman,[2,4] Kami J. Maddocks,[1,2] Carolyn Cheney,[2] Jeffrey A. Jones,[1,2] Joseph M. Flynn,[1] Leslie A. Andritsos,[1,2] Farrukh Awan,[1,2] Joseph A. Fraietta,[5] Carl H. June,[5] Marcela V. Maus,[6] Jennifer A. Woyach,[1,2] Michael A. Caligiuri,[1,2] Amy J. Johnson,[1,2] Natarajan Muthusamy,[1,2] and John C. Byrd[1,2]

Ibrutinib treatment:

- **increases *in vivo* persistence of activated CD4+ and CD8+ T cells, via diminished activation-induced cell death through ITK inhibition**
- **decreases the Treg/CD4+ T cell ratio**
- **diminishes the immune-suppressive properties of CLL cells through BTK-independent and BTK-dependent mechanisms:**
  1. ***decreased PD-1 expression by T cells***
  2. ***decreased CTLA-4 expression by T cells***
  3. ***decreased CD200 (OX-2) expression by CLL cells***
  4. ***decreased BTLA expression by CLL cells***
  5. ***decreased IL-10 production by CLL cells***

Long, *et al*. J Clin Invest. 2017;127:3052-3064.

Ibrutinib Improves T Cell Number and Function in CLL

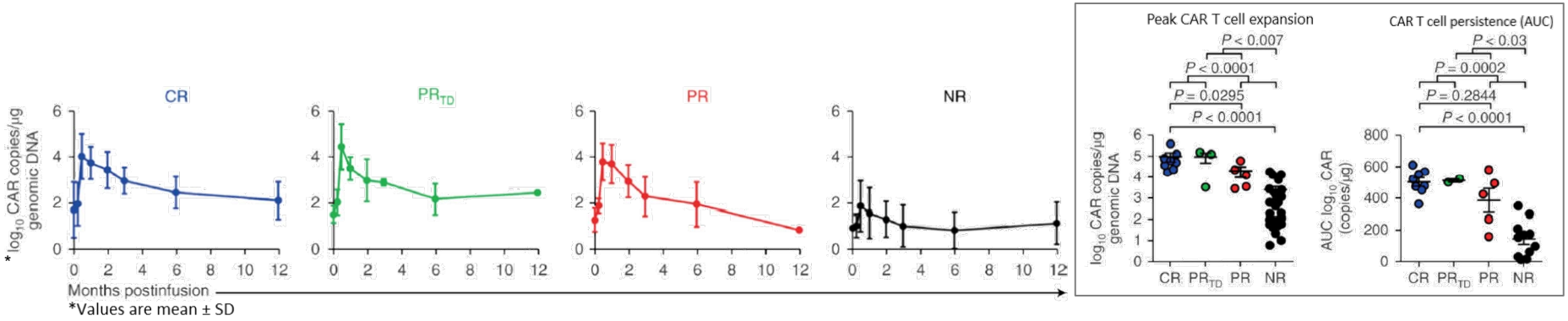
T Cells						
	CD4#	CD8#	PD-1	CTLA-4	Treg:CD4	Th17#
ibrutinib	↑	↑	↓	↓	↓	↑
acalabrutinib	–	–	↓	↓	–	–
MOA	ITK mediated		Indirect via BTKi		Inc. CD4	Non-BTK

CLL Cells			
	CD200	BTLA	IL10 production
Ibrutinib	↓	↓	↓
acalabrutinib	↓	↓	↓
MOA	BTKi-dependent		

Fraietta, *et al.* Blood. 2016; 127:1117-1127; Dubovsky, *et al.* Blood. 2013;122:2539-2549; Long, *et al.* J Clin Invest. 2017;127:3052-3064.

Functional T Cell Subsets May Determine CAR T Cell Responses

- **CAR T expansion kinetics and response in CLL patients**



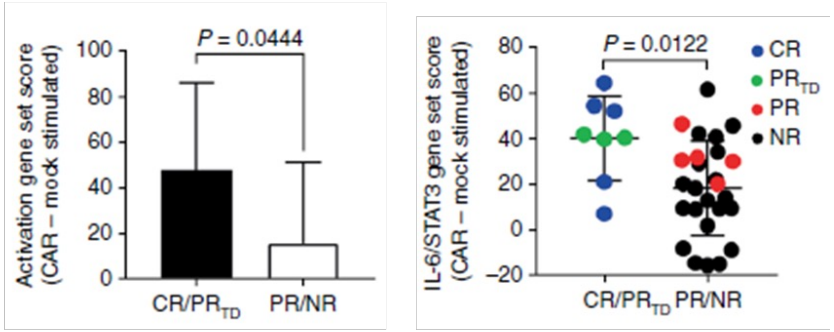
CR, complete remission; PR_{TD}, partial remission with late relapse of transformed disease; PR, partial response; NR, no response

Fraietta, *et al*. Nat Med 2018; 24:563–571.

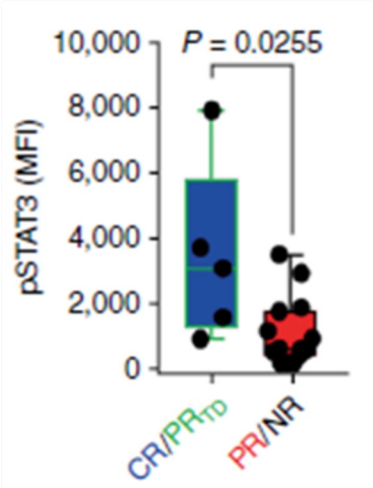
Functional T Cell Subsets May Determine CAR T Cell Responses

- **Genomic and phenotypic evaluation of CLL patient-derived CAR T cells**

Change in expression of T cell–activation gene set signatures in pre-infusion CAR-T cells from CR and non-CR patients



Change in pSTAT3 levels of pre-infusion CAR-T cells from CR and non-CR patients



→ **Responders upregulate memory-related gene and IL-6/STAT3 signatures**

→ **Non-responders upregulate programs involved in effector T cell differentiation, glycolysis, exhaustion and apoptosis**

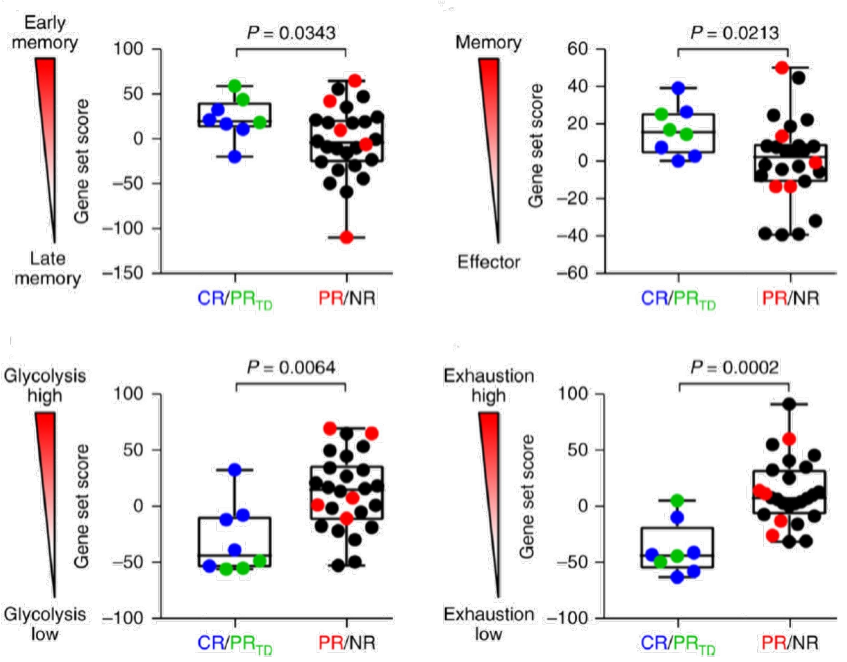
Fraietta, *et al*. Nat Med 2018; 24:563–571.

Functional T Cell Subsets May Determine CAR T Cell Responses

- **Genomic evaluation of CLL patient-derived CAR T cells**

Genes Significantly Up- or Down-regulated

Early memory T cell
Nonexhausted T cell
Naive vs. activated T_H2 $Cd4^+$ T cell
Unstimulated vs. stimulated memory T cell
Resting vs. bystander activated $CD4^+$ T cel
Conventional vs. effector memory T cell
Multipotent vs. progenitor $CD4^+$ T cell
Memory vs. effector $CD8^+$ T cell
Exhausted vs. effector T cell
Exhausted T cell
Activated T_H2 vs. naive $CD4^+$ T cell
Stimulated vs. unstimulated memory T cell
Glycolysis
Hypoxia
Effector vs. memory $CD8^+$ T cell
Apoptosis



CR, complete remission; PR_{TD}, partial remission with late relapse of transformed disease; PR, partial response; NR, no response

Fraietta, *et al*. Nat Med 2018; 24:563–571.

Ibrutinib Before Apheresis May Improve Tisagenlecleucel Manufacturing in Relapsed/Refractory Large B-Cell Lymphomas: a Phase 1b Study

Objectives

- To assess the safety, tolerability, and antitumor activity of tisagenlecleucel in combination with ibrutinib in adult patients with r/r DLBCL
- To describe the T-cell immunophenotype and functional activity of the leukapheresis and final tisagenlecleucel product in patients who had initiated ibrutinib *prior to* or *post* apheresis, arm 1 and arm 2, respectively

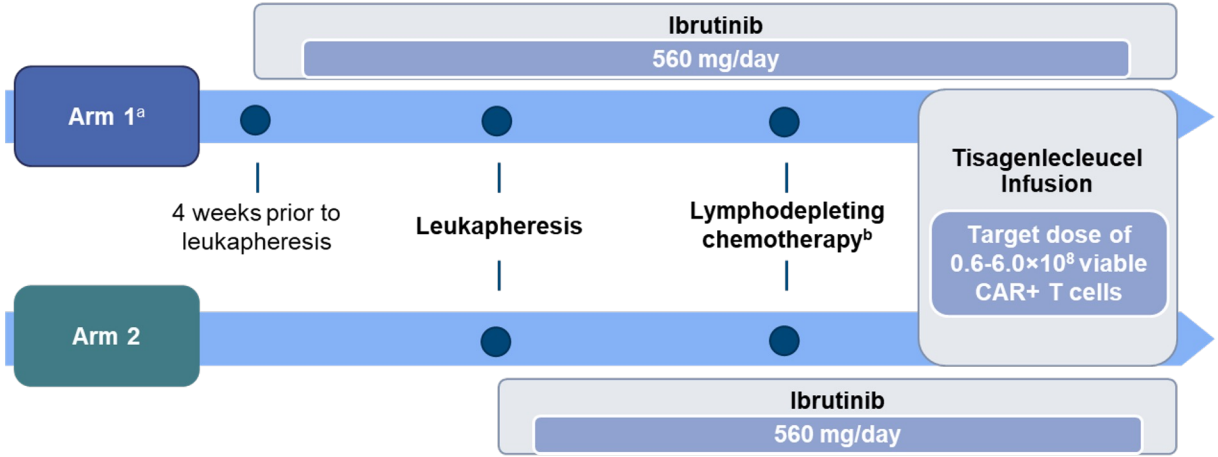
Study Design: Phase Ib, Multicenter, Nonrandomized, Open-Label

Patients With r/r DLBCL

- ≥18 years old
- ≥2 prior lines of therapy, including anti-CD20 and an anthracycline
- Relapsed after or ineligible for autoSCT

Patients who received prior anti-CD19, prior alloSCT, ibrutinib within 30 days prior to screening, or had active CNS involvement were excluded

ClinicalTrials.gov Identifier: **NCT03876028**



Ibrutinib was continued throughout lymphodepleting chemotherapy, tisagenlecleucel infusion, and post infusion for up to 24 months in both arms.[c]

Primary Endpoints:
Incidence and severity of adverse events, ibrutinib dose interruptions/modifications
Secondary Endpoints Include:
BOR per Lugano, progression-free survival

[A] Patients in Arm 1 were enrolled after completion of enrollment of Arm 2. [b] Lymphodepleting chemotherapy, ending at least 2 days before tisagenlecleucel infusion, was either fludarabine (25 mg/m^2) and cyclophosphamide (250 mg/m^2) daily for 3 days or bendamustine (90 mg/m^2) daily for 2 days. [C] Patients in complete response at 12 months post infusion were discontinued from ibrutinib.

alloSCT, allogeneic stem cell transplant; autoSCT, autologous stem cell transplant; BOR, best overall response; CAR, chimeric antigen receptor; CD, cluster of differentiation; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; r/r, relapsed or refractory.

Patient Demographics and Baseline Clinical Characteristics

	Arm 1 (N=4)	Arm 2 (N=6)
Age, median, (range)	59 (32-67)	64 (58-76)
Sex		
Male/female	4 (100)/0	4 (67)/2 (33)
ECOG performance status		
0/1	3 (75)/1 (25)	1 (17)/5 (83)
Lines of prior therapy		
2	2 (50)	4 (67)
3	0	2 (33)
4-6	2 (50)	0
Cells of origin of cancer		
Germinal center B-cell type	2 (50)	4 (67)
Activated B-cell type	1 (25)	2 (33)
T-cell/histiocyte-rich	1 (25)	0
Disease stage at study entry		
Stage I	0	0
Stage II	2 (50)	0
Stage III	2 (50)	0
Stage IV	0	6 (100)
Previous autologous HSCT	1 (25)	2 (33)
LDH at screening (U/L), median (range)	198 (146-234)	217 (178-303)

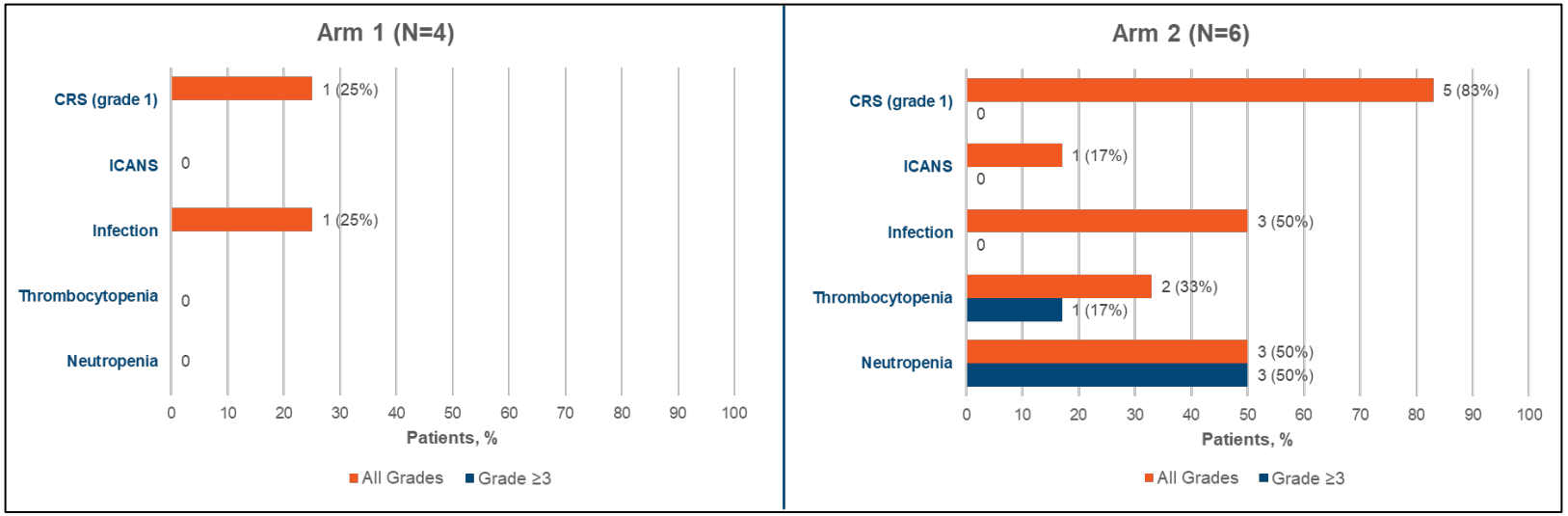
As of September 14, 2020, 10 patients had been treated and observed through at least the Month 3 assessment

Efficacy and Safety Outcomes

	Patient No.	DLBCL Subtype	Bridging (Yes or No)	CAR-T Cell Dose ($\times 10^8$)	CRS, Grade[a]	ICANS, Grade[a]	BOR (Assessment)[b]	PFS, Median (95% CI)
Arm 1	1	ABC	No	3.4	1	0	**CR (Month 6)**	NE (NE-NE)
	2	TCHR	No	3.6	0	0	**CR (Month 6)**	
	3	GCB	No	4.1	0	0	PR (Day 28)	
	4	GCB	No	4.6	0	0	**CR* (Month 3)**	
Arm 2	5	ABC	No	2.2	1	0	**CR (Month 12)**	2.5 months (1.0-NE)
	6	GCB	No	1.6	0	0	PD (Day 28)	
	7	GCB	No	1.2	1	0	PD (Day 28)	
	8	GCB	No	1.4	1	1	PD (Day 28)	
	9	GCB	Yes (rituximab)	1.9	1	0	PD (Month 2)	
	10	ABC	No	3.0	1	0	**CR* (Month 6)**	

- Six of 10 patients (60%) across both treatment arms had grade 1 CRS; no other instances of CRS were observed, and no patients required tocilizumab or were admitted to the intensive care unit
- One patient in Arm 2 (17%) had grade 1 ICANS; no other instances of ICANS were reported
- **Three of 4 patients (75%) in Arm 1 and 2 of 6 patients (33%) in Arm 2 achieved a BOR of CR**
  - ***Two patients responded to ibrutinib alone: Patient No. 4 in Arm 1 and patient No. 10 in Arm 2**

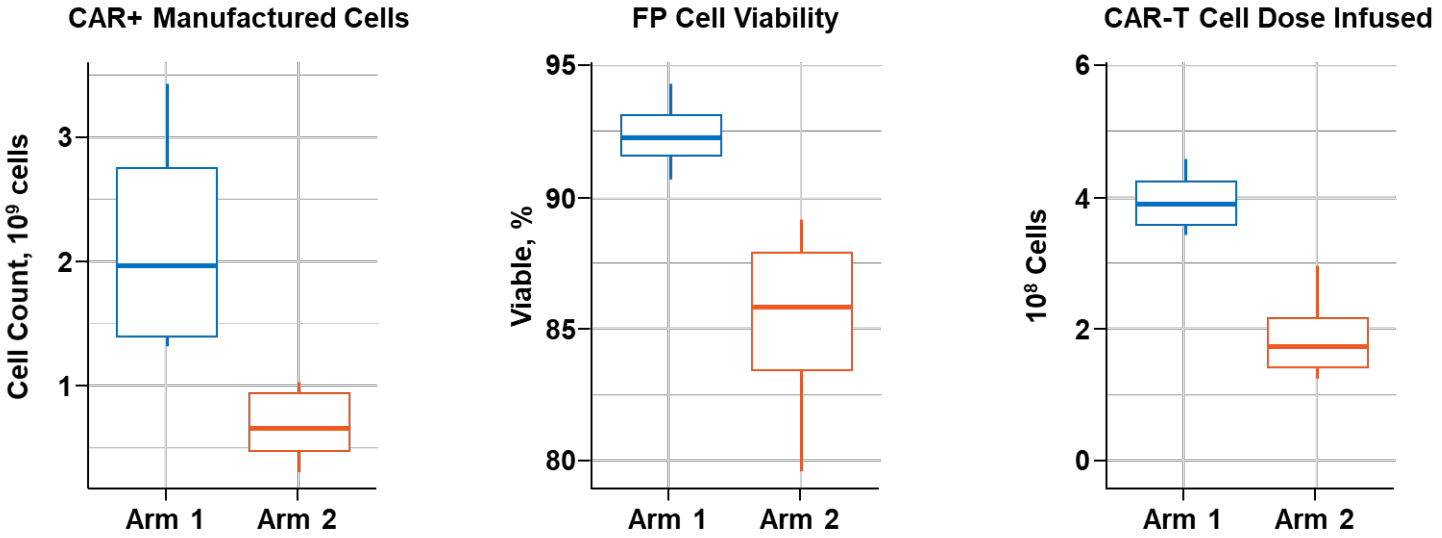
Summary of CAR-T Treatment-Emergent AEs



- One patient in Arm 2 (17%) had grade 3 neutropenia lasting >28 days post tisagenlecleucel infusion; no other patients had grade 3 or 4 neutropenia or thrombocytopenia lasting >28 days
- Ibrutinib-related bradycardia and atrial fibrillation (both grade 2) were each observed in 1 patient in Arm 1[a]; supraventricular tachycardia (grade 1) related to tisagenlecleucel was observed in 1 patient in Arm 2. One patient in Arm 1 with low platelet levels at baseline (grade 1) had a decrease in platelet count (grade 2) related to ibrutinib. No major bleeding events were observed

[a] Ibrutinib was discontinued in the patient with bradycardia and was dose-reduced to 140 mg/day in the patient with atrial fibrillation.

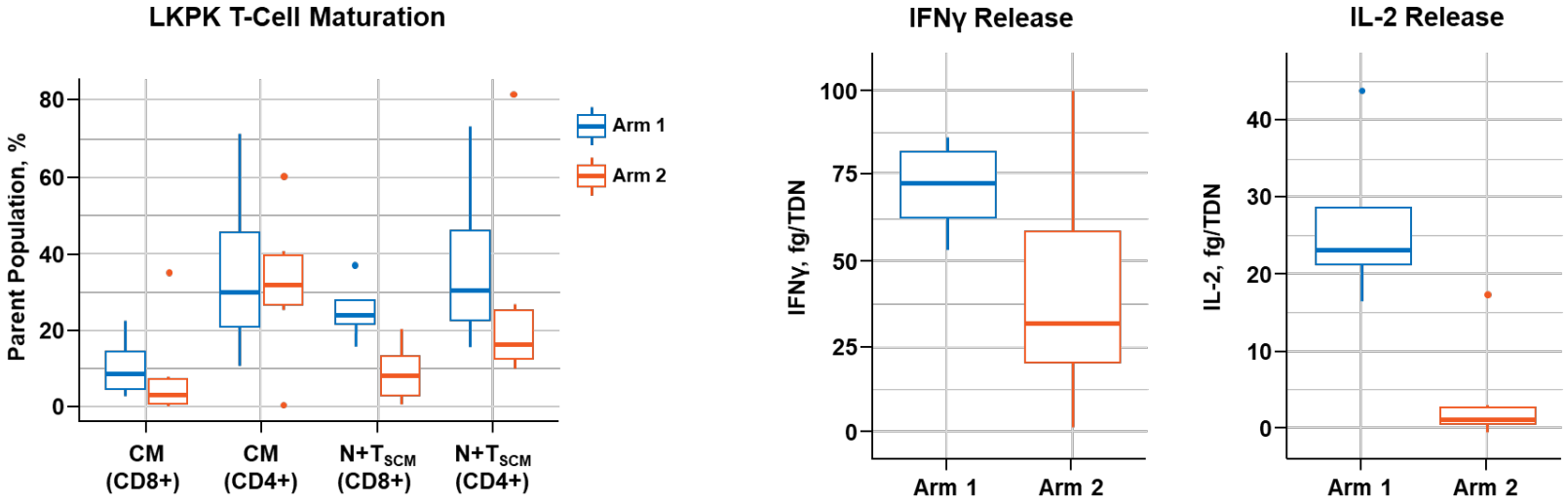
Impact of Ibrutinib on CAR-T Cell Manufacturing



- Arm 1 was associated with higher total CAR+ manufactured cells and higher viability of the final product compared with Arm 2
- The median dose of tisagenlecleucel infused was moderately higher in Arm 1 compared with Arm 2: 3.9 (range, 3.4-4.6) vs 1.7 (range, 1.2-3.0) × 10^8 CAR+ viable T cells, respectively

- FP, final product

Impact of Ibrutinib on T-Cell Phenotype in Apheresis Product



- Arm 1 was associated with an increased percentage of naïve/T_{SCM} cells in the leukapheresis material compared with Arm 2
- Arm 1 was associated with a final product characterized by preserved production of IFNγ (effector cytokine considered as a biomarker for potency) and increased production of IL-2 (proliferative cytokine considered a marker of self-renewal) upon antigen-specific stimulation

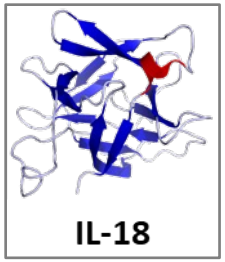
Data pertaining to IFNγ and IL-2 release are normalized to a non–CD19-expressing cell line: K562 meso cells.

CD, cluster of differentiation; CM, central memory (CD45RA-/CCR7+); N+T_{SCM}, naïve/stem cell-like central memory (CD45RA+/CCR7+); IFNγ, interferon gamma; IL, interleukin; LKPK, leukapheresis starting material; TDN, transduced number

Conclusions

- These results support the feasibility of administering ibrutinib to patients with DLBCL throughout tisagenlecleucel therapy
- Ibrutinib may improve CAR-T cell manufacturing when given prior to apheresis: T-cell phenotype and function were associated with less differentiated cells and preserved production of IFNγ and IL-2. Further studies are needed to confirm these findings
- An increased safety risk was not observed in patients who were administered ibrutinib prior to apheresis
- Results from patients administered ibrutinib prior to apheresis are promising. However, efficacy claims are limited by the small, non-randomized nature of this study. Further studies will be needed to characterize the impact of ibrutinib pre-treatment in patients with DLBCL

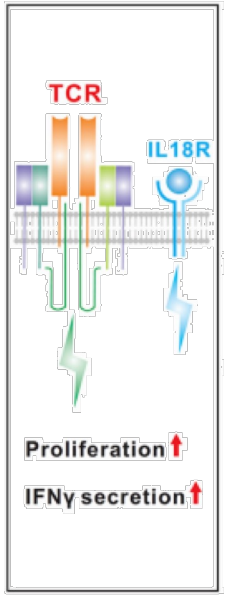
Augmentation of Antitumor Immunity by Human and Mouse CAR T Cells Secreting IL-18



Biliang Hu, Carl June, *et al*., created IL-18-secreting CAR T cells (IL-18-CAR T) to significantly boost CAR T cell proliferation and antitumor activity.

Preclinical studies showed:

- Robust enhancement of proliferation of IL-18-secreting human T cells in a xenograft model, which was dependent on TCR and IL-18R signaling.
- IL-18 augmented IFN-γ secretion and proliferation of T cells activated by the endogenous TCR.
- In a xenograft model, TCR-deficient, human IL-18-expressing CD19 CAR T cells exhibited significantly enhanced CAR T cell proliferation and antitumor activity.

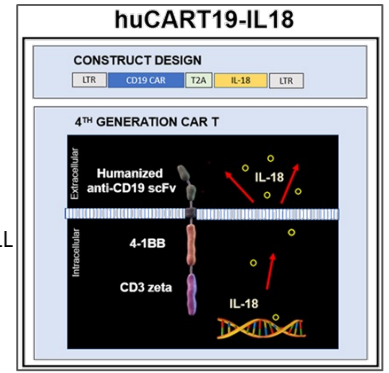


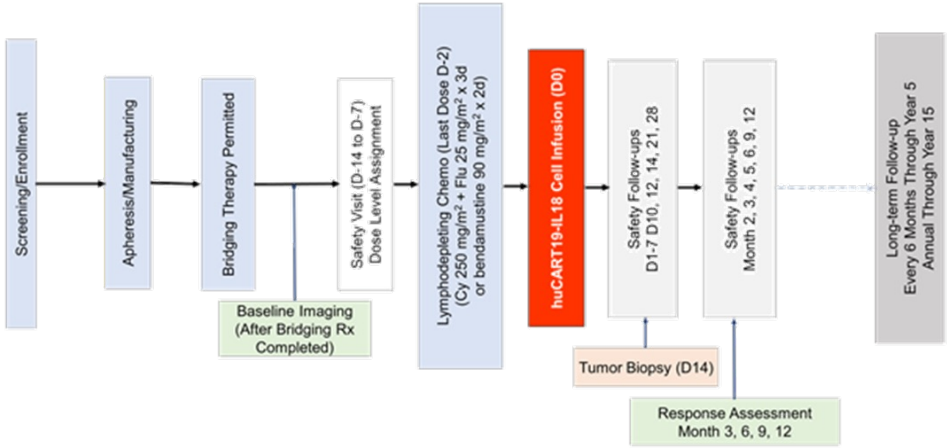
Hu B, et al. Cell Rep. Sep 26; 2017(13)20, 3025–3033.

IL-18 Secreting Anti-CD19 CAR-T Cells after Prior CAR-T Failure

- a Phase 1 UPenn Study

- **IL-18** is a pro-inflammatory cytokine shown to enhance[1]
  - CAR T-cell proliferative potency
  - Anti-tumor activity
- **huCART19-IL18 is a 4th generation autologous CAR T-cell product** transduced by lentiviral vector to co-express humanized anti-CD19 CAR and human IL-18; **3-day manufacturing**
- **First-in-human trial using huCART19-IL18** in patients with relapsed/refractory B-cell non-Hodgkin lymphomas and CLL (NCT04684563)





Key Eligibility Criteria

- **≥ 18 years old with CD19+ relapsed or refractory B-cell NHL or CLL**
- **At least 2 prior lines of therapy; must be relapsed after or refractory to prior CAR T-cell therapy (if indicated by FDA label)**

Primary objective

- **Evaluate safety of huCART19-IL18**

Secondary objectives

- **Determine feasibility, dose-limiting toxicities, efficacy, and pharmacokinetics**
- **Define the recommended phase 2 dose**

- used a Bayesian optimal interval dose titration design exploring doses between 3 and 300 million huCART19-IL18 cells per patient

[1] Hu B. *et al.* Cell Rep. 2017 Sep 26;20(13):3025-3033.

Svoboda, *et al*. ASH 2022: abstract 2016

IL-18 Secreting Anti-CD19 CAR-T Cells after Prior CAR-T Failure

- Patient characteristics, prior CAR-T treatment, and protocol therapy

PATIENT CHARACTERISTICS (N=8)	
Median age (range)	65 yrs (56-75)
Diagnosis	
DLBCL	3
MCL	2
THRBCL	1
HGBL	1
FL	1
p53 mutation by NGS*	3 (50%)*
Median prior Rx (range)	6.5 (4-13)

* p53 status available in 6 patients

PRIOR CAR T-CELLS (N=7**)	
Axi-cel	3
Tisa-cel	3
Brexu-cel	1
Best response to prior CAR-T	
CR	3 (43%)
PR	1 (14%)
PD	3 (43%)

** 1 patient failed manufacturing for brexu-cel twice and was deemed eligible for huCART19-IL18 without prior commercial CAR T-cell

ON-STUDY THERAPIES (N=8)	
Systemic bridging Rx	7 (88%)
Radiation	5 (63%)
LD chemotherapy (bendamustine)	7 (88%)

DOSE LEVELS ADMINISTERED (N=8)	
DL1A $3x10^6$ cells (no LD chemo)	1
DL1B $3x10^6$ cells	2
DL2 $7x10^6$ cells	2
DL2* $2.8x10^7$ cells	1
DL3 $3x10^7$ cells	2

* 2 DL3 products did not meet the target dose but exceeded minimum infusible dose and patients were treated:
1 with DL2 ($7x10^6$ cells)
1 with dose between DL2 and DL3 ($2.8x10^7$ cells)

DLBCL, diffuse large B-cell lymphoma;
MCL, mantle cell lymphoma;
THRBCL, T-cell/histiocyte-rich large B-cell lymphoma;
HGBCL, high-grade B-cell lymphoma;
FL, follicular lymphoma
LD, lymphodepletion chemotherapy

Svoboda, *et al*. ASH 2022: abstract 2016

IL-18 Secreting Anti-CD19 CAR-T Cells after Prior CAR-T Failure

- Safety (N=8) and efficacy (N=7)

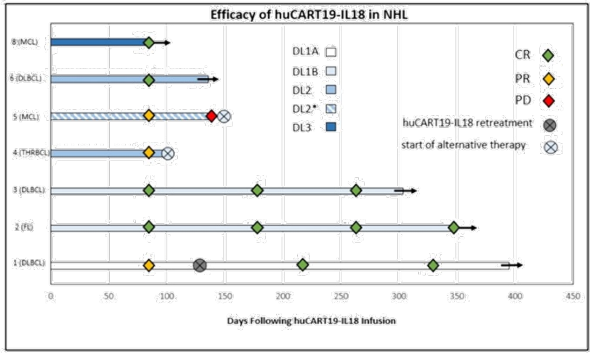
RELATED ADVERSE EVENTS OF SPECIAL INTEREST		
	CRS*	NEURO
Any grade	4 (50%)	2 (25%)
Median onset (range) in days	7.5 (2-8)	14 (8-20)
Median duration (range) in days	5.5 (5-11)	4 (2-6)
Grade 1	2 (25%)	1 (13%)
Grade 2	1 (13%)	1 (13%)
Grade 3	1 (13%)	0

*2 patients received tocilizumab

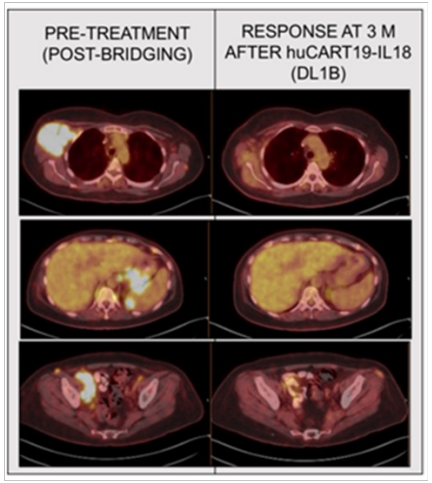
RELATED NON-HEMATOLOGIC ADVERSE EVENTS ≥ GRADE 3	
Total patients with G3/G4 AE	3 (38%)
Infections	2 (25%)
Hypotension	2 (25%)
Pulmonary edema	1 (13%)
AST elevation	1 (13%)
Fibrinogen decreased	1 (13%)

No study-related deaths

RESPONSE AT 3 MONTHS (N=7)	
Overall response rate	**7 (100%)**
Complete response	4 (57%)
Partial response	3 (43%)



Patient with follicular lymphoma refractory to axi-cel

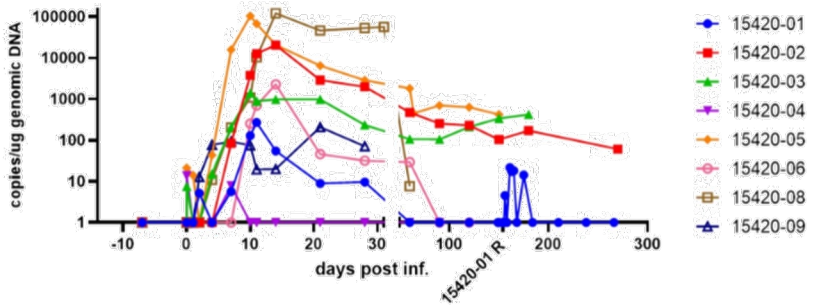


- Median follow-up is 8 months (1.9-14.1)
- All patients in CR at M3 (N=4) remain progression-free
- Patients in PR at M3 (N=3): -01 was re-treated with huCART19-IL18 at M4 and achieved CR
  -04 was taken off study in PR to pursue alternative therapy
  -05 progressed at M5 with CD19$^-$ disease

Svoboda, *et al*. ASH 2022: abstract 2016

IL-18 Secreting Anti-CD19 CAR-T Cells after Prior CAR-T Failure

- huCART19-IL18 expansion and persistence (N=8)



- Conclusions

- **huCART19-IL18 has a manageable safety profile**
- **Expansion and persistence of huCART19-IL18 cells appear adequate**
- **Early efficacy is observed across dose levels in lymphoma patients previously refractory to or relapsing after commercial CAR T-cell products**
- **Enrollment continues**

Molte Grazie

Questions & Comments