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Supplemental Methods

CAR T-cell constructs utilized

Patients received either the FDA-approved commercial product, tisagenlecleucel, or another murine-based CD19-CAR T-cell therapy available on a clinical trial (NCT01626495, NCT02906371, NCT02028455, NCT02625480, NCT02435849, NCT01593696) for r/r B-ALL across 7 centers in the US. CAR constructs included two CD19/4-1BB constructs (CTL019/tisagenlecleucel and SCRI-CAR19) and a CD19/CD28 construct, all of which have been previously described.[1-4]

Definitions for disease assessment

All disease assessments were conducted per protocol or institutional practice. The pre-CD19-CAR assessment was typically performed within 14 days of initiating lymphodepleting chemotherapy. First disease assessment post-CD19-CAR infusion was generally performed 21-28 days after infusion. Complete remission (CR) was defined as a bone marrow aspirate containing <5% blasts by morphologic analysis and without evidence of extramedullary disease. Partial response (PR) was defined as a bone marrow aspirate with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate. Progressive disease was defined as an increase in bone marrow blasts of at least 50% from nadir or development of extramedullary disease. Stable disease (SD) was defined as any evaluation not meeting the definition of CR, PR, or SD. MRD negativity was analyzed by multiparameter flow cytometry and was defined as <0.01% of bone marrow mononuclear cells consistent with B-ALL.

Definitions utilized for patterns of relapse immunophenotype

CD19 expression was defined as follows: $CD19^{pos}$ included moderate to bright expression with >50% blasts expressing CD19 and was inclusive of CD19 dim populations (the latter of which was defined by having a positive population that was $CD19^{pos}$, but with an intensity less than what is seen when compared to normal intensity of CD19 on mature B-cells) [5]. Further delineation between CD19 normal or bright was not performed. $CD19^{neg}$ negative was defined as having an ALL population where the majority of blasts (>50%) lacked CD19 expression. Patients with bimodal populations where the majority of the whole blasts were CD19 negative were also categorized as CD19 negative. Blasts were categorized as having undergone lineage switch when cell surface immunophenotyping was consistent with myeloid

markers with retention of original cytogenetics. Institutional and/or central flow cytometry was used at centers validated to evaluate for measurable residual disease (MRD) in ALL. Re-review of all cases where CD19 modulation occurred was also performed.

Definitions utilized for B-cell aplasia

B-cell aplasia (BCA), and loss thereof, was based on local assessment, and was generally defined by one of the following parameters: a) > 1% bone marrow (BM) CD19+ hematogones; b) > 1% increase in CD19+ cells in BM or peripheral blood (PB), or c) > 3% CD19+ B cells of total PB lymphocytes or > 50 CD19+ cells/μL—verified by two consecutive timepoints. Patients with data available had duration of BCA calculated and any patient with BCA at the timepoint most proximal to relapse, typically within 30 days, was defined as having ongoing BCA at relapse.

Supplemental Results

Additional characterization of patients with infant ALL

Amongst the 6 patients with infant ALL experiencing a LS, 4 occurred in patients achieving an MRD-negative CR post-CAR. Otherwise, 1 event occurred in a patient who achieved an MRD-positive CR post-CAR and 1 event occurred in a patient who experienced a LS at the first disease restaging timepoint. Of the infants with initial induction response data available, 15 of 21 were able to achieve an MRD-negative CR. Four of these responding patients went on to eventually experience a LS. In contrast, only 1 of the 6 non-responding patients eventually experienced a LS.

Supplemental tables

Supplemental Table 1. Overall survival following relapse and Cumulative incidence of relapse post CD19 CAR

Overall survival (OS) following relapse					
		Median OS, months (95% CI)	6 month (95% CI)	12 month (95% CI)	24 month (95% CI)
All relapse (n=166)		11.9 (9.0-17.0)	69.8% (62.0-76.3%)	49.4% (41.1-57.2%)	34.0% (25.7-42.5%)
By relapse phenotype (n=163)	$CD19^{pos}$	18.9 (11.2-27.0)	78.0% (67.0-85.8%)	59.9% (47.5-70.3%)	42.7% (30-54.8%)
	$CD19^{neg}$	9.7 (6.9-15.9)	65.5% (52.8-75.6%)	44.2% (31.8-55.9%)	30% (18-42.9%)
	LS	3.7 (1.2-7.0)	33.3% (10.3-58.8%)	8.3% (0.5-31.1%)	Not-reached
Cumulative incidence of relapse (CIR) from CD19 CAR infusion, by relapse phenotype (n=163)					
		12 month (95% CI)	24 month (95% CI)	36 month (95% CI)	48 month (95% CI)
$CD19^{pos}$		13.8% (10.5-17.5%)	22.0% (17.7-26.5%)	24.2% (19.6-29%)	26% (21-31.4%)

CD19^{neg}	14.9% (11.4-18.7%)	16.3% (13.2-21.0%)	19.6% (15.4-24.2)	20.5% (16.0-25.4%)
LS	1.7% (1.3-4.7%)	3.0% (1.6-5.2%)	3.0% (1.6-5.2%)	3.9% (2.0-6.8%)

Supplemental Table 2. Treatment of LS and overall outcomes

Age at Dx	Age at CAR Rx	Cytogenetics	# of CR prior to CAR	Prior blina	HSCT status prior to CAR	Disease Burden	Type of CAR	CAR response	HSCT Status post CAR	Outcomes	Days post CAR	BCA Duration	Intervention	Death post LS
Non-*KMT2A*r LS														
19.4	20.4	Trisomy 10	2	Y	Y	High	4-1BB	MRD^{neg} CR	N	LS MDS/AML	93	-	HSCT	28
7.6	11.4	Unknown	2	N	Y	High	4-1BB	MRD^{neg} CR	N	LS AML	43	1 mo (Y)	Unknown	94
5.7	27.1	t(12;19)	6	N	Y	Low	4-1BB	MRD^{neg} CR	N	LS AML (CNS only)	637	21 mo (Y)	Intra-ommaya chemo; aza/ven	594
***KMT2A*r LS**														
0.2	0.8	t(4;11)	1	N	N	High	4-1BB	No CR	N	LS MPAL (B/Myeloid)	21	-	None	36
0.4	9.4	t(1;11)	3	Y	N	Low	4-1BB	MRD^{neg} CR	N	LS AML	162	-	Aza/ven/GO	124
6.4	15.6	t(11;11)	2	N	Y	High	4-1BB	MRD^{neg} CR	N	LS AML (myeloid sarcoma)	1158	38 mo (Y)	HSCT	336
0.6	1.9	ins(11;10)	2	N	N	High	4-1BB	MRD^{neg} CR	N	LS AML	31	1 mo (Y)	FLAG	212
0.4	2.1	t(4;11)	0	N	N	High	4-1BB	MRD^{pos} CR	N	LS AML	41	-	None	44

0.5	1.8	*KMT2A*	3	N	N	Low	4-1BB	MRD^{neg} CR	N	LS AML	64	2 mo (Y)	Unknown	75
0.9	1.4	t(11;19)	1	N	N	High	4-1BB	MRD^{neg} CR	N	LS AML	67	2 mo (Y)	CD123 CAR T	102
3.7	4.9	*KMT2A*	0	Y	Y	High	4-1BB	No CR	N	LS AML	36	1 mo (Y)	Unknown	202
2.2	8.8	*KMT2A*	2	N	Y	High	4-1BB	MRD^{neg} CR	N	LS AML	362	11.9 mo (Y)	CPX-351	154

Abbreviations: LS, lineage switch; Dx, diagnosis; CAR, chimeric antigen receptor; Rx, infusion; CR, complete remission; blina, blinatumomab; HSCT, hematopoietic stem cell transplant; BCA, B-cell aplasia; *KMT2A*r, *KMT2A* rearrangement; High disease burden; >5% tumor blasts; Low disease burden, <5% tumor blasts; MRD, minimal residual disease; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CNS, central nervous system; Aza, azacytidine; Ven, venetoclax; GO, gemtuzumab ozogamicin; MPAL, mixed-phenotype acute leukemia

Supplemental Table 3. Relapse Pattern Based on Cytogenetics

	Relapse (n=163)		
	CD19^{pos} (n=83)	**CD19^{neg} (n=68)**	**LS (n=12)**
Normal	6 (7.2%)	4 (5.9%)	0
ETV-RUNX1	7 (8.4%)	7 (10.3%)	0
*KMT2A*r	5 (6.0%)	1 (14.7%)	9 (75%)
Ph+	6 (7.2%)	3 (4.4%)	0
Hypodiploid	2 (2.4%)	5 (7.4%)	0
Ph-Like	4 (4.8%)	4 (5.9%)	0
Other	42 (50.6%)	40 (58.8%)	3 (25%)
Unknown	11 (13.3%)	4 (5.9%)	0

Abbreviations: CD19^{pos}, CD19 positive relapse; CD19^{neg}, CD19 negative relapse; LS, lineage switch; KMT2Ar, KMT2A rearrangement; Ph, Philadelphia chromosome

References

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