

# Abstract 192 Pediatric Preclinical Testing Program (PPTP) evaluation of the anti-CD19-DM4 conjugated antibody SAR3419



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## Abstract

**Background:** SAR3419 is composed of the humanized anti-CD19 antibody huB4 conjugated with the potent cytotoxic maytansinoid, DM4, a tubulin polymerization inhibitor. SAR3419 has shown preclinical *in vivo* activity against human B-cell lymphomas, and was selected for evaluation against the Pediatric Preclinical Testing Program (PPTP) *in vitro* and *in vivo* panels of B-lineage acute lymphoblastic leukemia (ALL), which express high levels of cell surface CD19.

**Methods:** The PPTP includes a molecularly characterized *in vitro* panel of leukemia cell lines (n=7) and *in vivo* panel of ALL xenografts (n=10), representing the common subtypes of pediatric ALL. SAR3419 was tested *in vitro* against the RS4;11 (CD19+) and MV4;11 (CD19-) cell lines at concentrations from 0.01 nM to 10 nM, and against the PPTP *in vivo* panel (n=7) at a dose of 10 mg/kg administered weekly x 3 via intraperitoneal injection to NOD/SCID mice. Three measures of anti-leukemic activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) proportion of human CD45+ cells in the murine peripheral blood (%huCD45+) at day 21; and 3) a time to event (25% huCD45+ cells) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in the %huCD45+ cells at the end of the experiment).

**Results:** SAR3419 was ineffective against MV4;11 cells, but potently killed the RS4;11 cell line with IC50 < 2 nM. SAR3419 was unable to prevent the *in vivo* progression of the CD19- Ewing sarcomas (CHLA-258 & TC-71) and two T-lineage ALL xenografts (ALL-8 & ALL-16, both CD19-). However, SAR3419 significantly increased the event-free survival of mice engrafted with 5/5 B-lineage ALL xenografts (all CD19+), with regressions achieved in four xenografts (one partial response, three complete response). The progression of these 4 xenografts was well controlled during the 3-week course of treatment, with leukemia re-growth within 1-3 weeks of treatment cessation.

**Conclusions:** SAR3419 showed little activity against CD19- cell lines and xenografts, but exerted potent *in vitro* cell killing and effectively induced regressions and/or delayed the *in vivo* progression of CD19+ leukemia cells. Further investigations into the anti-leukemic activity of combinations of SAR3419 with other anticancer drugs, as well as its killing effects on putative leukemic stem cells, are anticipated. (Supported by NCI N01CM42216)

## Methods for PPTP In Vivo Testing

Stage 1 testing involves testing an agent across the entire PPTP panel of childhood cancer xenograft lines at its MTD or at a dose selected based on PK/PD studies using adult preclinical models.

**Solid tumor testing:** For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2-0.5 cm<sup>3</sup>. Two perpendicular tumor diameters were measured at once weekly intervals with digital vernier calipers. Assuming tumors to be spherical, volumes were calculated from the formula (π/6)\*d<sup>3</sup>, where d represents the mean diameter.

**Acute lymphoblastic leukemia testing:** For each xenograft line, 8 mice were inoculated with 3.5 x 10<sup>6</sup> mononuclear cells purified from the spleens of secondary recipient mice. Engraftment was monitored weekly by flow cytometry, and treatment was initiated when the proportion of human CD45+ cells in the peripheral blood reached 1%. The proportion of human CD45+ cells in the peripheral blood was monitored weekly throughout the course of treatment.

**Drug:** SAR3419 was provided by Sanofi-Aventis (Vitry-sur-Seine, France). SAR3419 was diluted in PBS (w/o calcium or magnesium) and administered intraperitoneally weekly for 3 consecutive weeks at a dose of 10 mg/kg. SAR3419 was provided at each testing site in coded vials for blinded testing according to the PPTP's standard operating procedures.

### Solid Tumor Response Criteria:

Response	Definition	Score
PD1 (Progressive Disease 1)	>25% ↑ in tumor volume, TGD value ≤1.5	0
PD2 (Progressive Disease 2)	>25% ↑ in tumor volume, TGD value >1.5	2
SD (Stable Disease)	<25% ↑ in tumor volume, <50% regression	4
PR (Partial Response)	≥50% regression, but no CR	6
CR (Complete Response)	<0.1 cm <sup>3</sup> tumor volume	8
MCR (Maintained CR)	<0.1 cm <sup>3</sup> tumor volume at the end of study	10

### Leukemia Response Criteria:

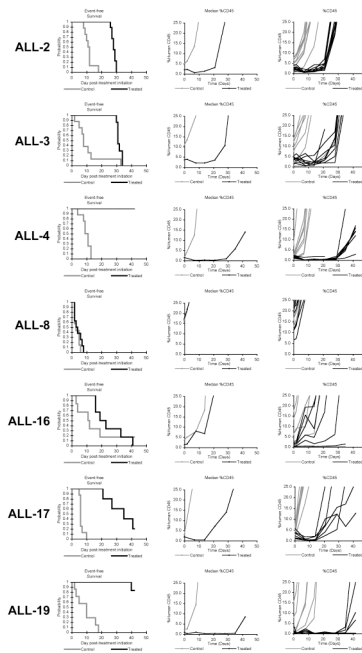
Response	Definition	Score
PD1 (Progressive Disease 1)	No PR & TGD value of ≤1.5 & events at EOS	0
PD2 (Progressive Disease 2)	No PR & TGD value >1.5 & events at EOS	2
SD (Stable Disease)	No PR and no events at EOS	4
PR (Partial Response)	CD45% <1% for only 1 week	6
CR (Complete Response)	CD45% <1% for 2 consecutive weeks	8
MCR (Maintained CR)	CD45% <1% for last 3 weeks of study	10

**Median Group Response:** Each individual mouse in the treatment group was assigned a response score (see Tables above) and a median score for the treatment group was calculated and then each treatment group was assigned an overall response according to the table below.

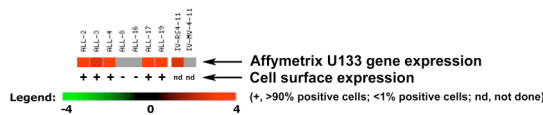
If Median Score (MS) from (1):	Overall Group Response
0 < MS ≤1	PD1
1 < MS ≤3	PD2
3 < MS ≤5	SD
5 < MS ≤7	PR
7 < MS ≤9	CR
9 < MS	MCR

**Statistical Methods:** Event-free survival (EFS) distributions of each treatment group were compared to the EFS distribution of the respective control group using the exact log-rank test. P-values were 2-sided & were not adjusted for multiple comparisons given the exploratory nature of this study. P-values < 0.05 were considered to be significant.

## SAR3419 In Vivo Activity



## CD19 Gene Expression & Cell Surface Levels



## ALL Xenograft Demographics

Xenograft	Age at diagnosis (mo)/sex	ALL Subtype	Disease status at biopsy	Length of CR1 (mo)	Site of Relapse	Survival after first relapse (mo)	Current clinical status
ALL-2	65/F	c-ALL	Relapse 3	30	BM/CNS	46	DOD
ALL-3	154/F	Pre-B	Diagnosis	38	BM	126*	CR2
ALL-4	105/M	Ph <sup>+</sup> c-ALL	Diagnosis	10	BM	1	DOD
ALL-8	152/M	T-ALL	Relapse 1	17	BM	1	DOD
ALL-16	122/F	T-ALL	Diagnosis	125*	-	-	CR1
ALL-17	107/F	c-ALL	Diagnosis	25	CNS	81*	CR2
ALL-19	194/M	c-ALL	Relapse 1	4	BM	7	DOD

BM, bone marrow; CNS, central nervous system; c-ALL, common (CD10<sup>-</sup>) ALL; CR2 alive in second complete remission; DOD, dead of disease; Ph<sup>+</sup>, Philadelphia chromosome positive ALL; Biphem, biphenotypic; CR1, alive in first complete remission. \*No event (censored).

## SAR3419 In Vivo Activity

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	Heat Map
TC-71	Ewing sarcoma	0.974	1	>4	1.09	PD1
CHLA258	Ewing sarcoma	0.952	0.8	>4	1.18	PD1
ALL-2	ALL B-precursor	<0.001	2.5	>25	-	PR
ALL-3	ALL B-precursor	0.012	3.9	>25	-	PD2
ALL-4	ALL B-precursor	<0.001	> 4.2	13.9	-	CR
ALL-8	ALL T-cell	0.466	1	>25	-	PD1
ALL-16	ALL T-cell	0.58	1.8	>25	-	PD2
ALL-17	ALL B-precursor	<0.001	5.5	>25	-	CR
ALL-19	ALL B-precursor	<0.001	> 4.2	8.4	-	CR

Shading in the EFS column indicates xenografts that have either high (dark blue), intermediate (light blue), or indeterminate (grey) activity.

## CONCLUSIONS

- SAR3419 was effective in delaying leukemia in 5 ALL xenografts of B lineage with high CD19 expression and did not induce a significant delay in two ALL T-cell xenografts or two Ewing sarcomas (all CD19-). These data confirm the specificity provided by the antibody molecule in the drug conjugate.
- SAR3419 induced objective responses in four out of five B-lineage ALL xenografts (one partial response and three complete responses) and leukemia growth was well contained during the three week treatment period. Good anti-leukemic responses were observed against aggressive and chemoresistant xenografts derived from patients at relapse (ALL-2, ALL-19) or with Ph<sup>+</sup>-ALL (ALL-4), and the dosing regimen (3 x 10 mg/kg, weekly) did not induce adverse or toxic effects.
- Additional investigations to elucidate the mechanistic basis for the differential responses observed between xenografts, as well as to optimize combinations of SAR3419 with established chemotherapeutic drugs, are warranted.

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