Supplementary Method

Affinity measurements

The affinity of A-319 to the extracellular region of CD19 protein was determined using an OctetQKe instrument (Pall). Briefly, human CD19 with Fc tag in phosphate buffered saline with Tween 20 (PBST) was loaded onto the surface of ProteinA Capture Biosensors (ProA). Immobilization levels between 0.8 and 1.2 nM were reached. Then a 60-s biosensor baseline step was used before the analysis of association of the antigen and antibodies on the biosensor to the testing antibodies and antigen for 180s. Tested molecules were then applied in a 2-fold concentration gradient. Octet data were evaluated using data analysis software version 8.2 (PALL/ForteBio), and a global fit 1:1 modal was used to determine the diffusion constant (Kd) value.

Supplementary Table

Table. S1 Comparison between blinatumomab and A-319 in terms of preclinical data.

	Blinatumomab	**A-319**
Molecule weight (kD)	55	67
Affinity (Kd/M)		
CD3	2.6×10^{-7} [1]	1.95×10^{-8}
CD19	1.49×10^{-9} [1]	2.22×10^{-9}
Half-life (h)		
non-human primate	2.0 (chimpanzee) [2]	1.5 ~ 3.6 (cyno.monkey)
clinical dosing	28-day continuous IV infusion [3]	tiw IV infusion
EC50 values (pM)		
human B-cell	2.4	3.4
B-cell line (Raji)	0.41	0.40
Cytokine release (pg/mL)		
IL-6	19	20
INF-γ	20	50

Notes: [1] the affinity of blinatumomab and A-319 were not tested side by side, data acquired from reference 1

[2] data acquired from reference 2

[3] data acquired from reference 3, IV infusion: intravenous infusion

References

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