A novel, native-format bispecific antibody triggering T-cell killing of B-cells is robustly active in mouse tumor models and cynomolgus monkeys

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Supplementary Information

Supplementary Table 1

A panel of anti-CD20 heavy chains were co-expressed with a panel of anti-CD3 light chains, and supernatants from transient transfections were evaluated for binding to Raji (CD20+) cells by flow cytometry. Antibodies showing at least a 3-fold signal above background binding to control CD20-negative cells (Jurkat, HEK293) were scored as +, while those showing lower levels of binding were scored +/-, and those showing no binding above background were scored as negative (NT = not tested). We observed that several heavy chains (CD20-1 and CD20-3) retained the ability to bind CD20 when paired with most of the non-cognate light chains tested, and we therefore selected these heavy chains for further characterization.

Supplementary Figure 1

The CD20-1 /CD3-11 heavy chain/light chain pair identified as described in Supplementary Table 1 was purified and compared to the purified parental anti- CD20 antibody by flow cytometry, as described in Methods. This non-cognate heavy/light chain pairing demonstrated similar binding affinity to Raji (CD20+) cells by FACS when compared to the parental. As expected, no binding was observed with the parental anti-CD3 antibody. The CD20-1 heavy chain, combined with the CD3-11 heavy and light chains, were therefore chosen to generate a CD20xCD3 bispecific.

Supplementary Methods

Characterization of CD20 Heavy Chain/CD3 Light Chain hybrid antibodies

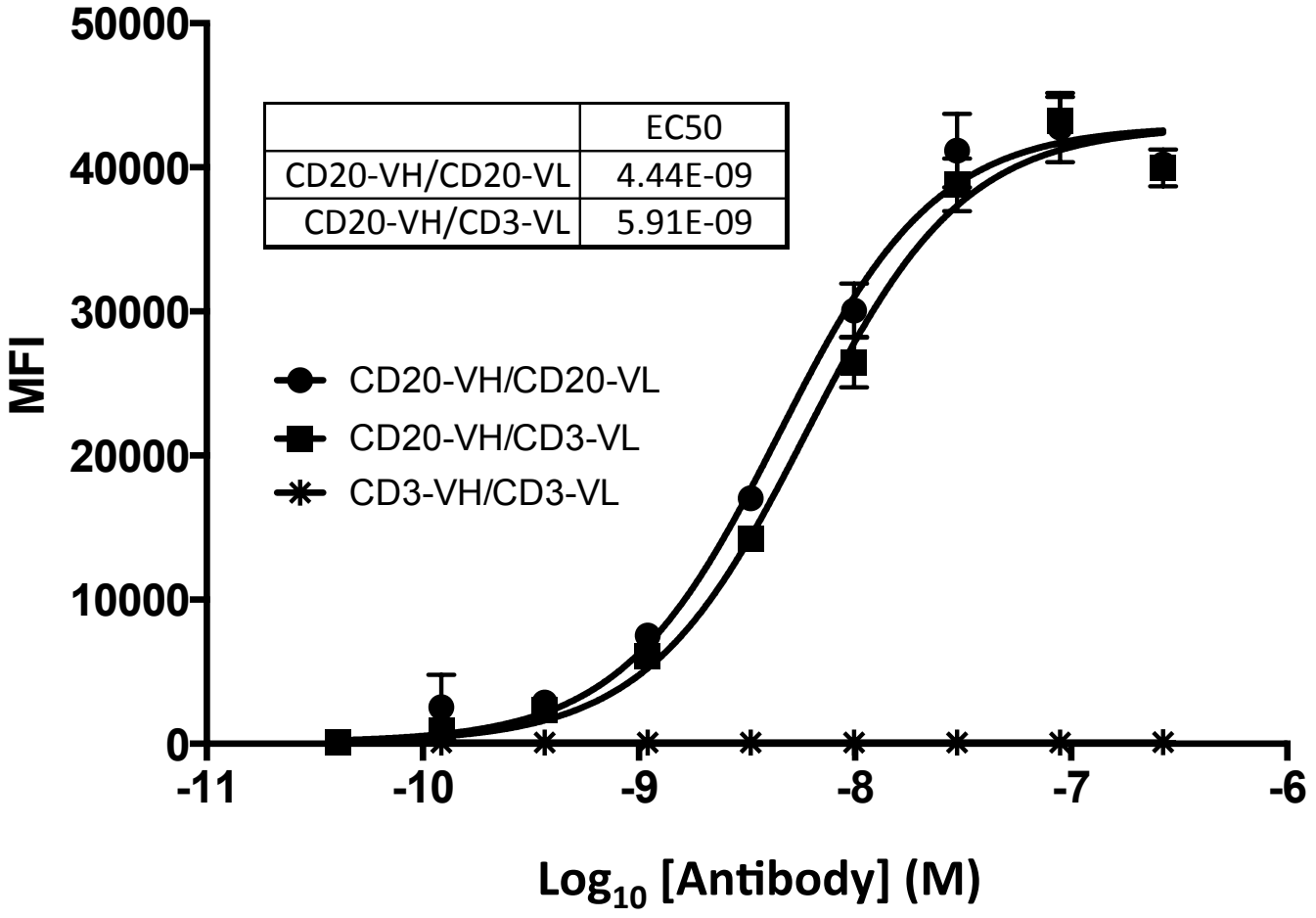
To generate hybrid antibodies, CHO-K1 cells were transiently transfected with a ratio of 3:1 light chain to heavy chain plasmid DNA, using standard Lipofectamine protocols. After culturing for an additional 72-96 hr, supernatants were collected.

To assess binding to CD20, 20 ul of supernatant was incubated with cells (Raji, Jurkat or HEK293 cells) for 45 minutes at 4^0C. The cells were then washed, incubated with the secondary anti-Human PE for an additional 30 minutes at 4^0C, washed again and then resuspended for analysis. The geometric mean fluorescence (MFI) of stained cells was measured using a BD FACS Canto II flow cytometer.

Supplementary Table 1

		anti_CD20 heavy chain											
		CD20-1	**CD20-2**	**CD20-3**	**CD20-4**	**CD20-5**	**CD20-6**	**CD20-7**	**CD20-8**	**CD20-9**	**CD20-10**	**CD20-11**	**CD20-12**
anti_CD3 light chain	**CD3-1**	+	+	+	+	+	+	-	+	+	+	-	-
	CD3-2	+	+	+	+	+	-	-	-	+	+/-	+	-
	CD3-3	+	-	+	+/-	-	+/-	-	-	-	+/-	+	-
	CD3-4	+	-	+	-	-	+/-	-	-	-	NT	-	-
	CD3-5	+	+	+	+	-	-	-	-	+	+	+	-
	CD3-6	+	-	+	+/-	-	+/-	-	-	-	NT	-	-
	CD3-7	+	+	+	+	+	+	-	+	+	+/-	+	-
	CD3-8	+	-	+	-	-	+/-	-	-	-	-	-	-
	CD3-9	+	+	+	+	+	+	-	+	+	+	+/-	-
	CD3-10	+	+	+	+	+	+	-	+	+	+	+	-
	CD3-11	+	+	+	+	+	+	-	+	+	+	-	-
	CD3-12	-	-	-	-	-	-	-	-	NT	NT	-	-
	CD3-13	+	-	-	-	+/-	+/-	-	-	NT	NT	-	-
	CD3-14	+	-	+	+	-	+/-	-	-	NT	NT	-	-
	CD3-15	+	-	-	-	-	-	-	-	NT	NT	-	-

Supplementary Figure 1



	EC50
CD20-VH/CD20-VL	4.44E-09
CD20-VH/CD3-VL	5.91E-09