

FIG. 1

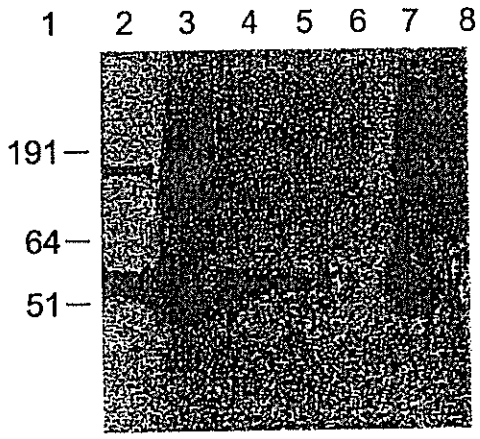
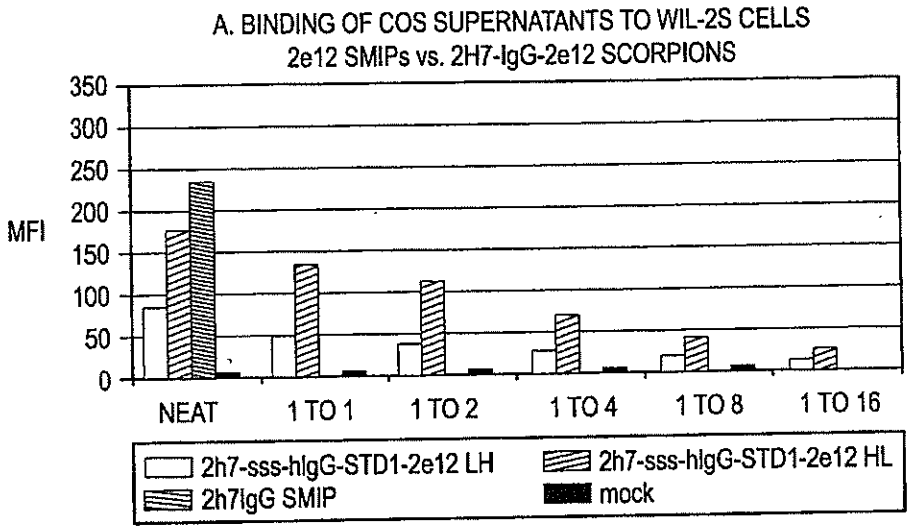


FIG. 2

FIG. 3

BINDING OF PROTEIN EXPRESSED IN COS SUPERNATANTS TO CELLS EXPRESSING TARGET ANTIGENS



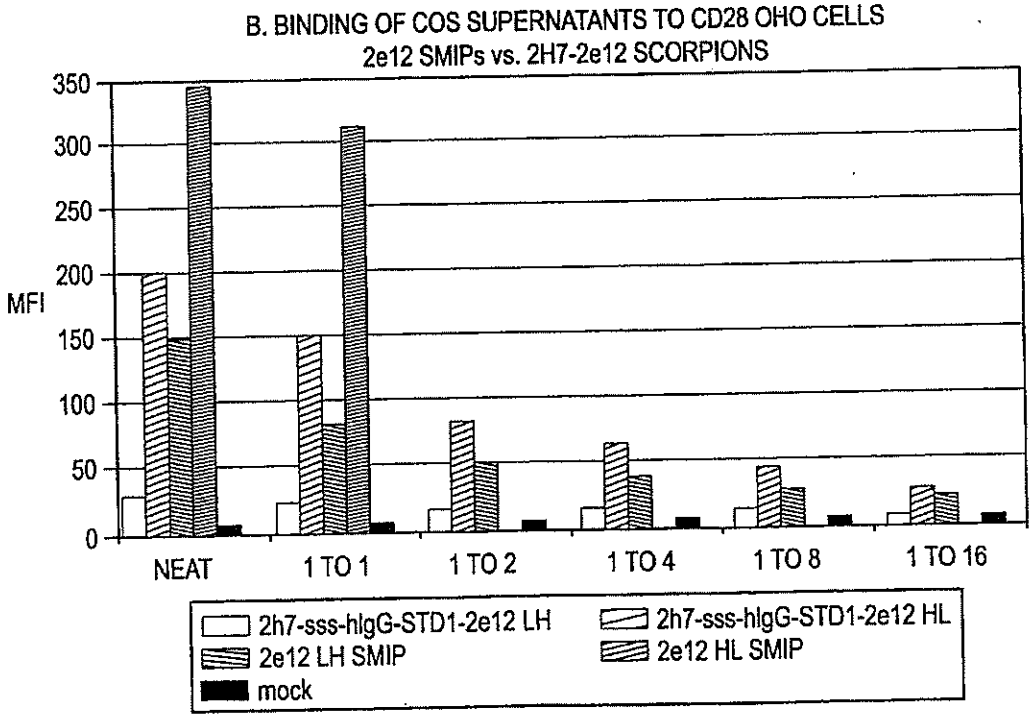


FIG. 4

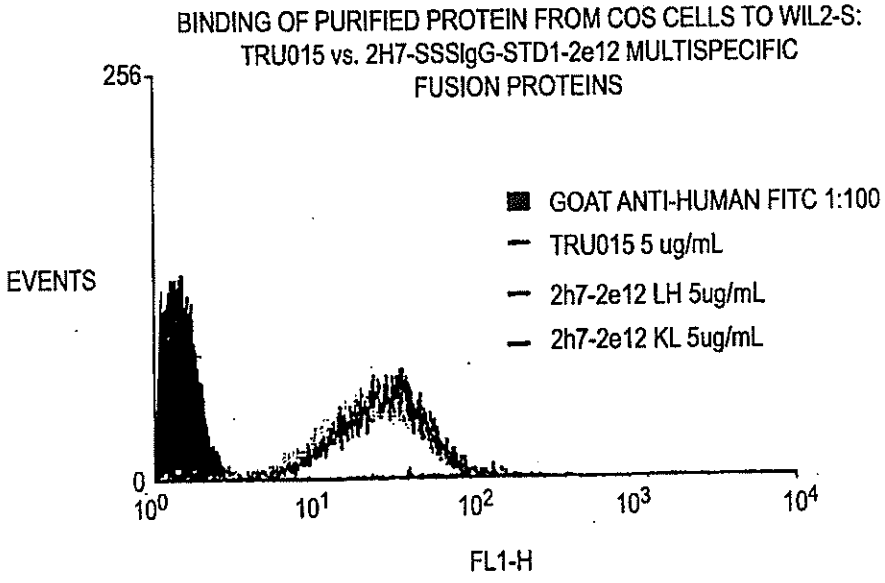

BINDING OF PURIFIED PROTEIN FROM COS CELLS TO WIL2-S:
TRU015 vs. 2H7-SSSIgG-STD1-2e12 MULTISPECIFIC
FUSION PROTEINS
256
EVENTS
0
GOAT ANTI-HUMAN FITC 1:100
TRU015 5 ug/mL
2h7-2e12 LH 5ug/mL
2h7-2e12 KL 5ug/mL
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG. 5

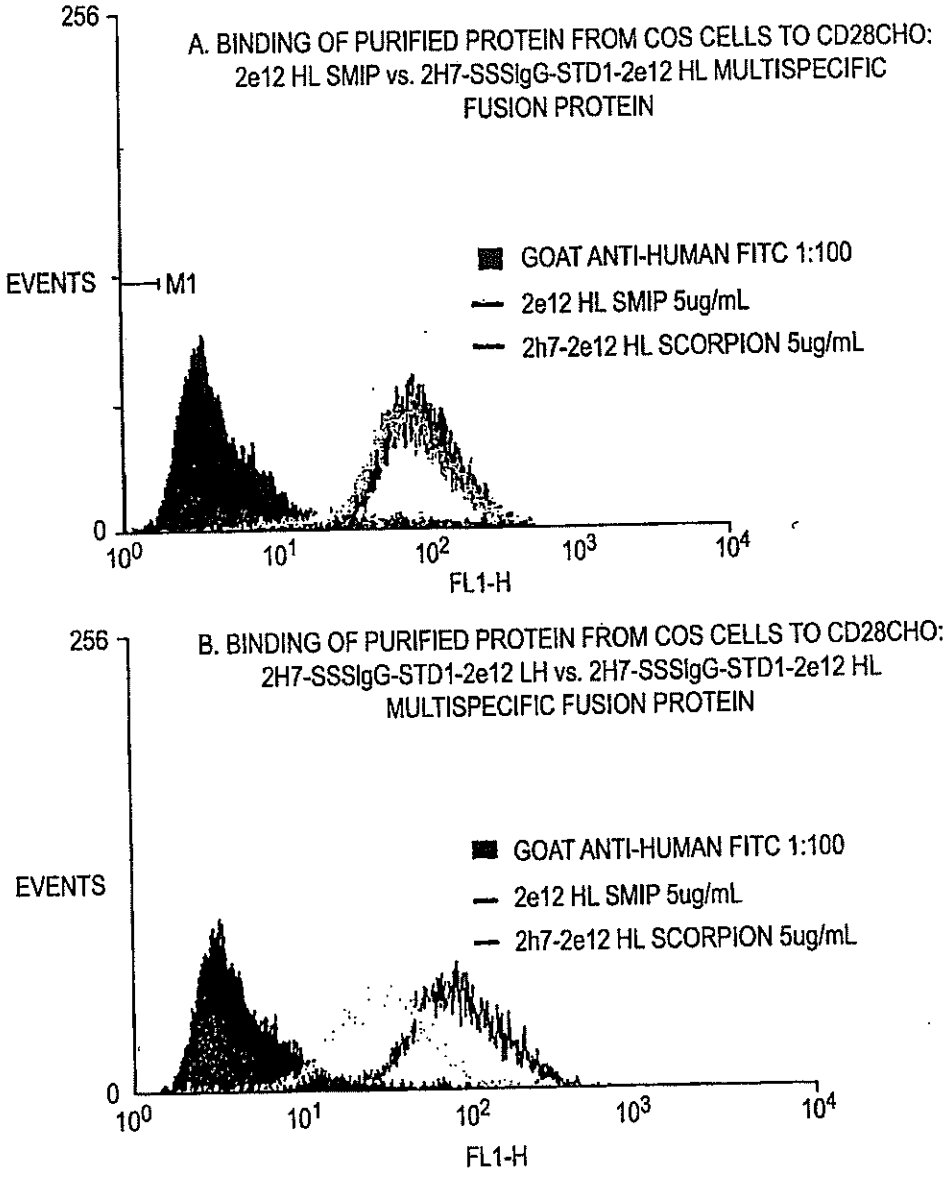

A. BINDING OF PURIFIED PROTEIN FROM COS CELLS TO CD28CHO:
2e12 HL SMIP vs. 2H7-SSSIgG-STD1-2e12 HL MULTISPECIFIC
FUSION PROTEIN
256
EVENTS
M1
0
GOAT ANTI-HUMAN FITC 1:100
2e12 HL SMIP 5ug/mL
2h7-2e12 HL SCORPION 5ug/mL
10^0
10^1
10^2
10^3
10^4
FL1-H
B. BINDING OF PURIFIED PROTEIN FROM COS CELLS TO CD28CHO:
2H7-SSSIgG-STD1-2e12 LH vs. 2H7-SSSIgG-STD1-2e12 HL
MULTISPECIFIC FUSION PROTEIN
256
EVENTS
0
GOAT ANTI-HUMAN FITC 1:100
2e12 HL SMIP 5ug/mL
2h7-2e12 HL SCORPION 5ug/mL
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG. 6A

TABLE IDENTIFYING FUNCTIONAL ELEMENTS OF MULTISPECIFIC FUSION PROTEINS



BD1 Binding Domain 1	Linker 1	EFD Effector Function Domain	Linker 2 ID	Linker Length (aa) Linker Plus R. Site	CH3 COOH	Linker 2 Sequence (- restriction site)	Fusion Junction with BD2 (L2 RS--QVQ 2e12)	BD2 Binding Domain 2
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	STD	20	PGK	NYGGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	STD2	38	PGK	NYGGGGSGGGGSGGGGSG NYGGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H1 L1=2e12 V_L-V_H	2 (RS)	PGK	--	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H2 L2=2e12 V_L-V_H	8	PGK	GGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H3 L3=2e12 V_L-V_H	10	PGK	NYGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H4 L4=2e12 V_L-V_H	13	PGK	GGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H5 L5=2e12 V_L-V_H	15	PGK	NYGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H6 L6=2e12 V_L-V_H	18	PGK	GGGGSGGGGSGGGGSG	NS QVQ	2e12 V_H-V_L
2H7 V_L-V_H	Modified hIgGl hinge ccc->sss	hIgGl-CH2-CH3	H7	8	PGK	GCPPCP	NS QVQ	2e12 V_H-V_L

FIG. 6B (1 of 5)

Constructs

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-STD1-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-sss-hIgG-STD1-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VH-VL)
2H7-sss-hIgG-STD2-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-sss-hIgG-STD2-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-csc-hIgG-STD1-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hIgG-STD2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VL-VH)
2H7-csc-hIgG-STD1-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	2e12 (VL-VH)
2H7-csc-hIgG-STD2-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	2e12 (VH-VL)
2H7-sss-hIgG-H1-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-csc-hIgG-H1-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	2e12 (VL-VH)
2H7-sss-hIgG-H1-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-csc-hIgG-H1-2e12 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	2e12 (VH-VL)
2H7-sss-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-csc-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	2e12 (VL-VH)
2H7-sss-hIgG-H2-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-csc-hIgG-H2-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	2e12 (VH-VL)
2H7-sss-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-csc-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	2e12 (VL-VH)
2H7-sss-hIgG-H3-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-csc-hIgG-H3-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	2e12 (VH-VL)
2H7-sss-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-csc-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	2e12 (VL-VH)
2H7-sss-hIgG-H4-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	2e12 (VH-VL)
2H7-csc-hIgG-H4-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	2e12 (VH-VL)

FIG. 6B (2 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-csc-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	2e12 (VL-VH)
2H7-sss-hIgG-H5-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-csc-hIgG-H5-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	2e12 (VH-VL)
2H7-sss-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-csc-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	2e12 (VL-VH)
2H7-sss-hIgG-H6-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-csc-hIgG-H6-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	2e12 (VH-VL)
2H7-sss-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-csc-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	2e12 (VL-VH)
2H7-sss-hIgG-H7-2e12 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-csc-hIgG-H7-2e12 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	2e12 (VH-VL)
2H7-sss-hIgG-STD1-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-sss-hIgG-STD1-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-sss-hIgG-STD2-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VL-VH)
2H7-sss-hIgG-STD2-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hIgG-STD1-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hIgG-STD2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-csc-hIgG-STD1-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G28-1 (VL-VH)
2H7-csc-hIgG-STD2-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G28-1 (VH-VL)
2H7-sss-hIgG-H1-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hIgG-H1-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hIgG-H1-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VL-VH)
2H7-csc-hIgG-H1-G28-1 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G28-1 (VH-VL)
2H7-sss-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VL-VH)

FIG. 6B (3 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-csc-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hIgG-H2-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VL-VH)
2H7-csc-hIgG-H2-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G28-1 (VH-VL)
2H7-sss-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hIgG-H3-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VL-VH)
2H7-csc-hIgG-H3-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G28-1 (VH-VL)
2H7-sss-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hIgG-H4-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VL-VH)
2H7-csc-hIgG-H4-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-csc-hIgG-H5-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G28-1 (VH-VL)
2H7-sss-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-csc-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VL-VH)
2H7-sss-hIgG-H6-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-csc-hIgG-H6-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G28-1 (VH-VL)
2H7-sss-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-csc-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VL-VH)
2H7-sss-hIgG-H7-G28-1 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-csc-hIgG-H7-G28-1 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G28-1 (VH-VL)
2H7-sss-hIgG-STD1-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-sss-hIgG-STD1-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VH-VL)

FIG. 6B (4 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-sss-hIgG-STD2-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VL-VH)
2H7-sss-hIgG-STD2-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hIgG-STD1-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hIgG-STD2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-csc-hIgG-STD1-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD1	G19-4 (VL-VH)
2H7-csc-hIgG-STD2-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	STD2	G19-4 (VH-VL)
2H7-sss-hIgG-H1-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hIgG-H1-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hIgG-H1-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VL-VH)
2H7-csc-hIgG-H1-G19-4 HL	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H1	G19-4 (VH-VL)
2H7-sss-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-sss-hIgG-H2-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-csc-hIgG-H2-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H2	G19-4 (VL-VH)
2H7-sss-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hIgG-H3-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VL-VH)
2H7-csc-hIgG-H3-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H3	G19-4 (VH-VL)
2H7-sss-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VL-VH)
2H7-csc-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hIgG-H4-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VL-VH)
2H7-csc-hIgG-H4-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H4	G19-4 (VH-VL)
2H7-sss-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VL-VH)
2H7-csc-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hIgG-H5-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VL-VH)

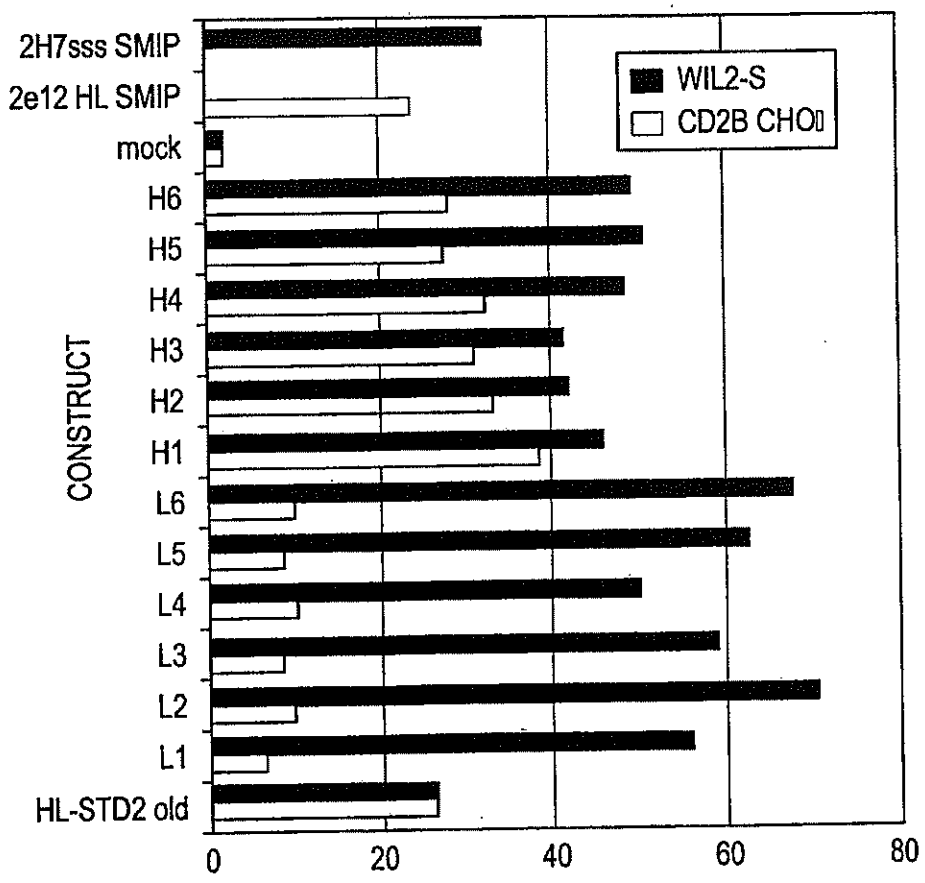
FIG. 6B (5 of 5)

Name	BD1	Hinge	EFD	Linker	BD2
2H7-csc-hIgG-H5-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H5	G19-4 (VH-VL)
2H7-sss-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-csc-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VL-VH)
2H7-sss-hIgG-H6-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-csc-hIgG-H6-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H6	G19-4 (VH-VL)
2H7-sss-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-csc-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VL-VH)
2H7-sss-hIgG-H7-G19-4 HL	2H7 (VL-VH)	hIgG1 -SSS	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VH-VL)
2H7-csc-hIgG-H7-G19-4 LH	2H7 (VL-VH)	hIgG1 -csc	hIgG or hIgG (P238S/P331S)	H7	G19-4 (VH-VL)

FIG. 7

ALTERING THE LINKER AFTER THE EFD AND THE ORIENTATION OF V REGIONS IN BD2 AFFECTS BINDING EFFICIENCY OF MULTISPECIFIC FUSION PROTEINS

BINDING OF 2H7-SSShlgG-L/Hx-2e12 MULTISPECIFIC FUSION PROTEINS WITH VARIOUS LINKERS TO WIL2-S AND CD28 CHO



**ALL FUSION PROTEINS USED AT 0.72 ug/mL, PROTEIN A PURIFIED, GENERATED FROM TRANSIENT COS SUPERNATANTS.

FIG. 8

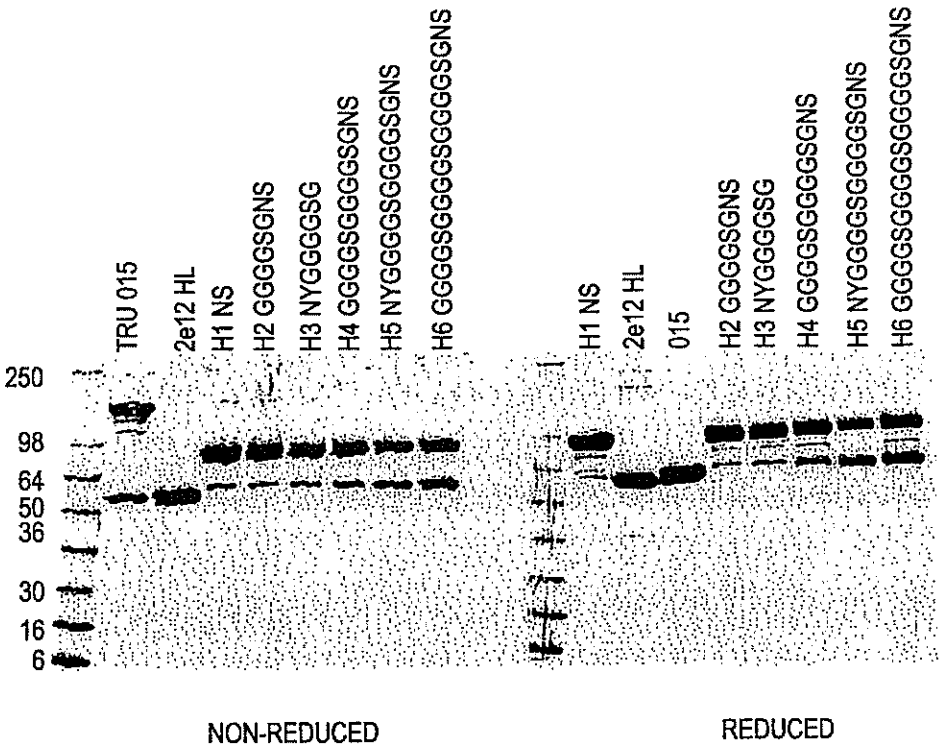

250
98
64
50
36
30
16
6
TRU 015
2e12 HL
H1 NS
H2 GGGGSGNS
H3 NYGGGGSG
H4 GGGGSGGGGSGNS
H5 NYGGGGSGGGGSGNS
H6 GGGGSGGGGSGGGGSGNS
H1 NS
2e12 HL
015
H2 GGGGSGNS
H3 NYGGGGSG
H4 GGGGSGGGGSGNS
H5 NYGGGGSGGGGSGNS
H6 GGGGSGGGGSGGGGSGNS
NON-REDUCED
REDUCED

FIG. 9

Western Blots of Multispecific Fusion Proteins With H6 Linker
A. Absence of SMIP or smaller CD28 detectable forms
B. Presence of a SMIP sized form using CD20 anti-id Fab

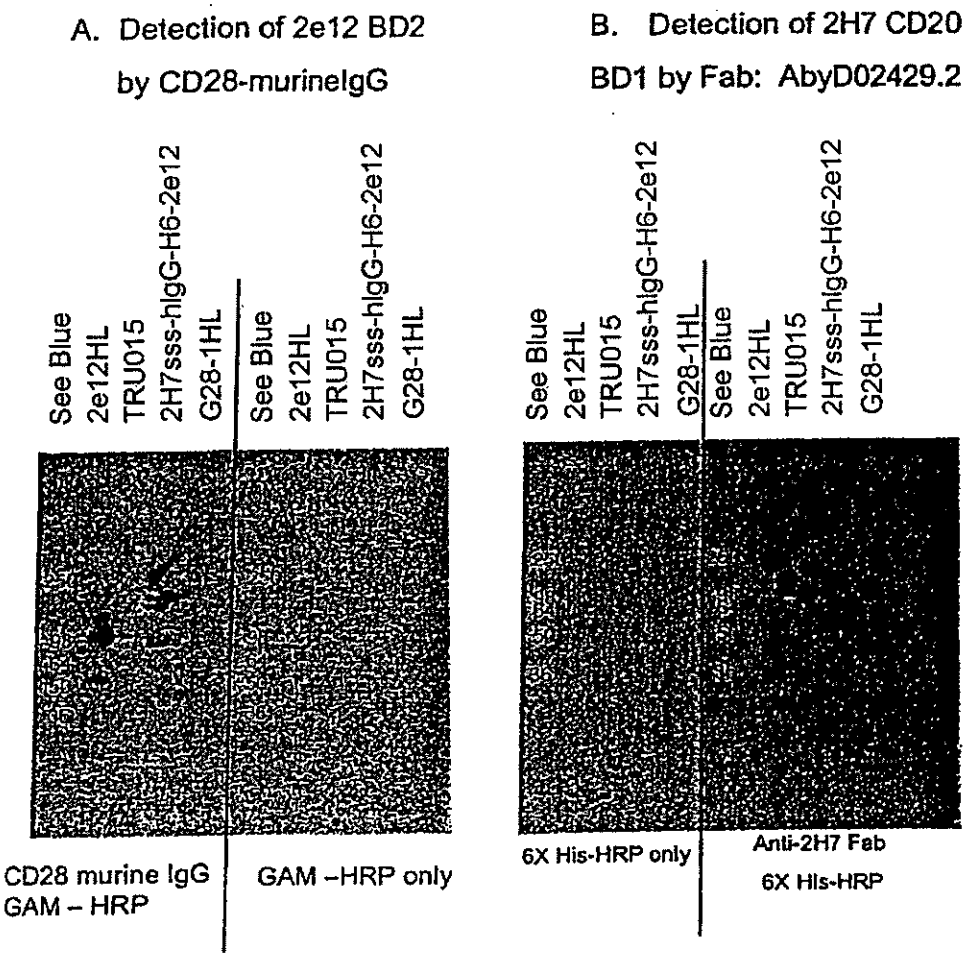
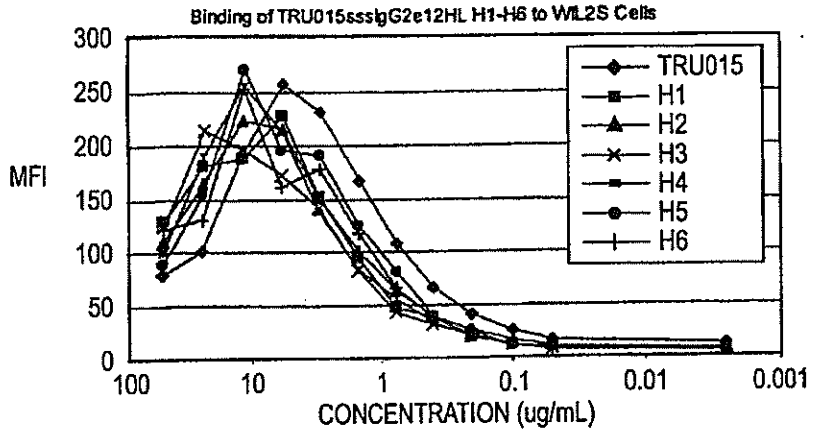


FIG. 10

A. Binding of Multispecific Fusion Proteins With Variant Linkers to WIL-2S Cells



B. Binding of Multispecific Fusion Protein With Variant Linkers to CD28 CHO Cells

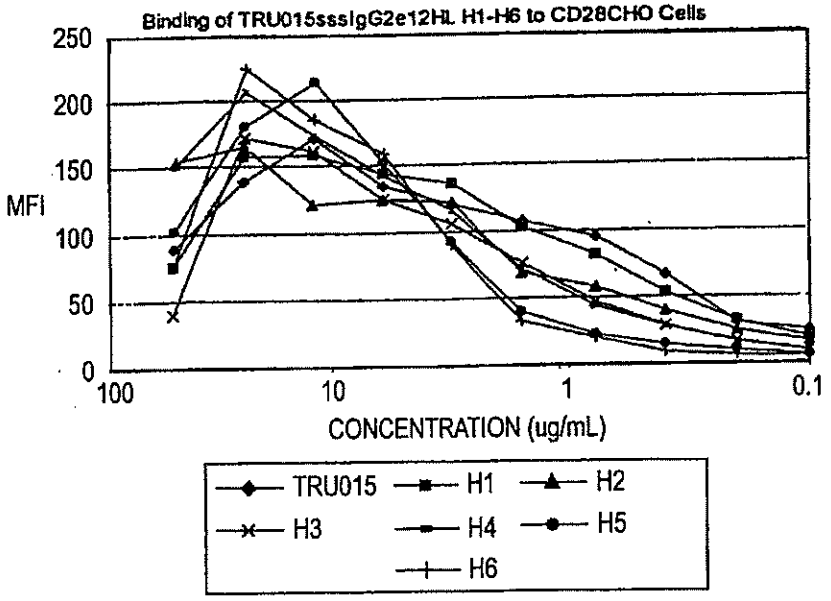


FIG. 11

Summary of SEC Fractionation of 2H7-sssIgG-2e12 HL Multispecific Fusion Proteins with Variant Linkers

Linker ID	Retention Time for POI	% POI	% Other Forms	SDS-PAGE Analysis of Cleavage at EFD/BD2 junction
H1	7.620	70.9	29.0	(Yes)
H2	7.589	67.5	32.5	(Yes)
H3	7.605	68.7	31.4	Yes
H4	7.622 (shoulder)	68.0	32.0	Yes
H5	7.933/7.680 (doublet with shoulder)	32.42/33.94	33.63	YES
H6	7.901/7.69 (doublet with shoulder)	34.5/29.3	36.1	YES
H7	7.788	84.2	15.8 (HMW)	NO

FIG. 12

Binding of [2H7-sss-hIgG-H_X-2e12 HL] Fusion Proteins with Different Linkers to Cells Expressing Target Antigen for BD1 or BD2

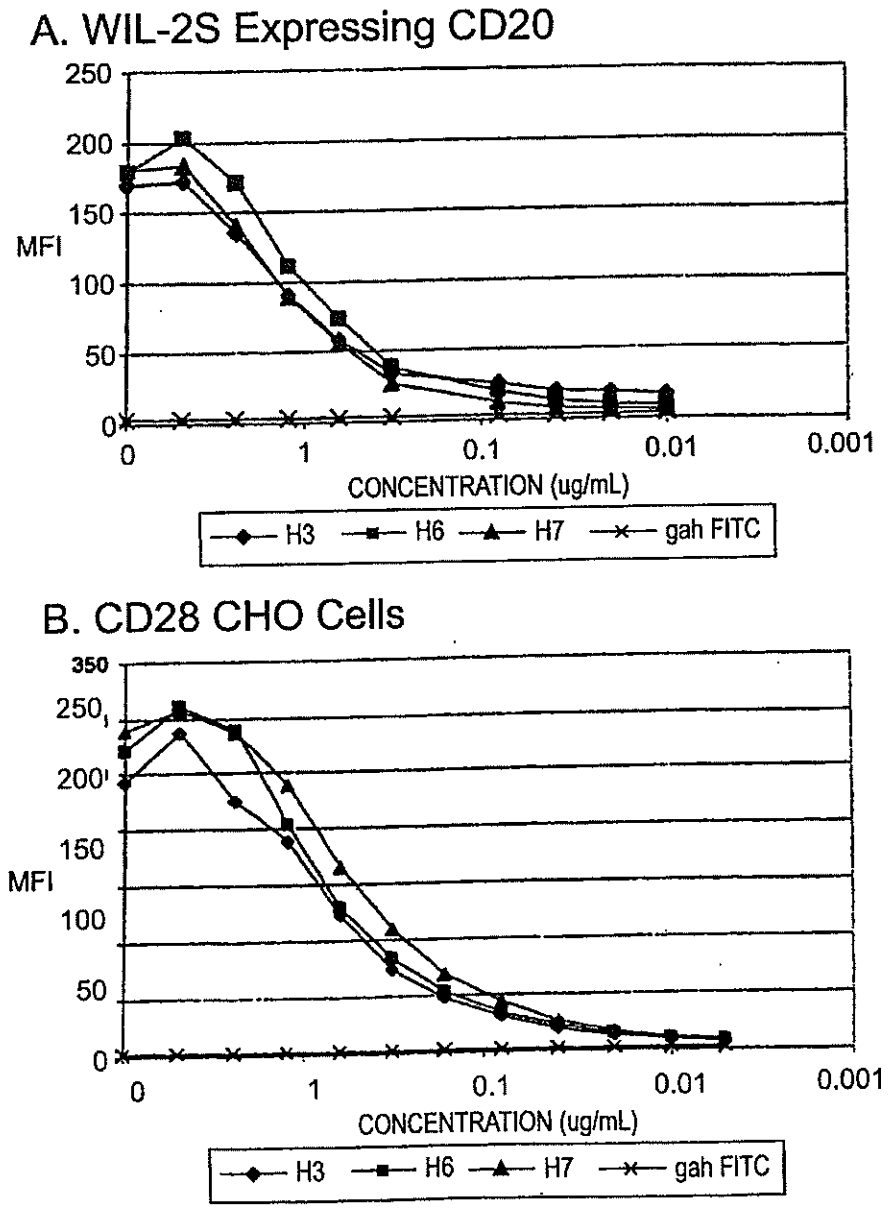


FIG. 13

Simultaneous Binding of BD1 and BD2:

Binding of [2H7-sss-hIgG-H_x-2e12 HL] Fusion Proteins with H3, H6, and H7 Linkers to WIL-2S Cells can be detected with CD28mIgG + FITC anti-mouse

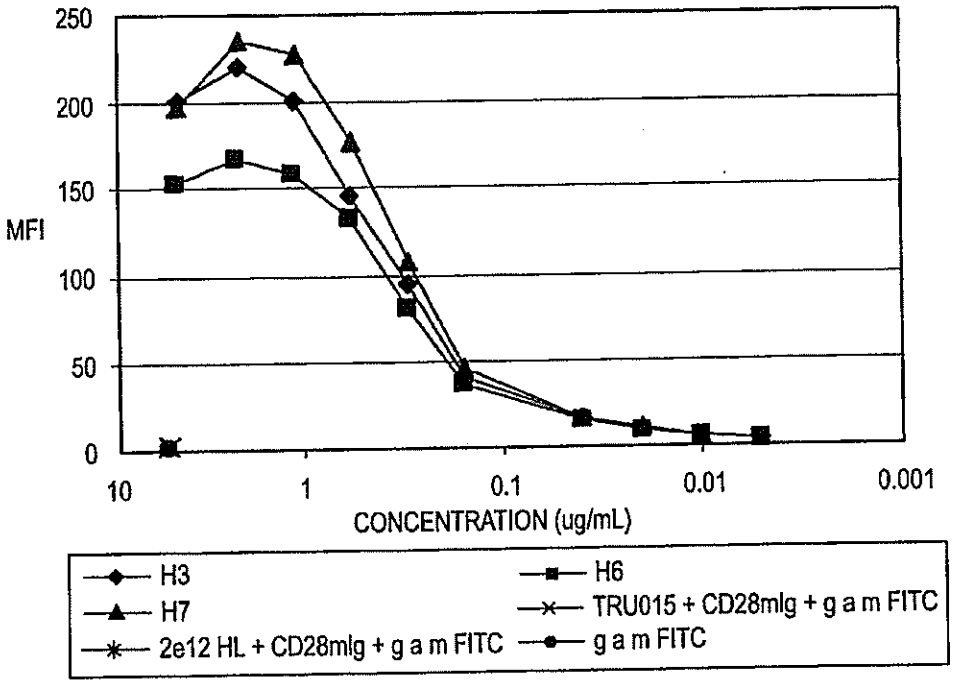
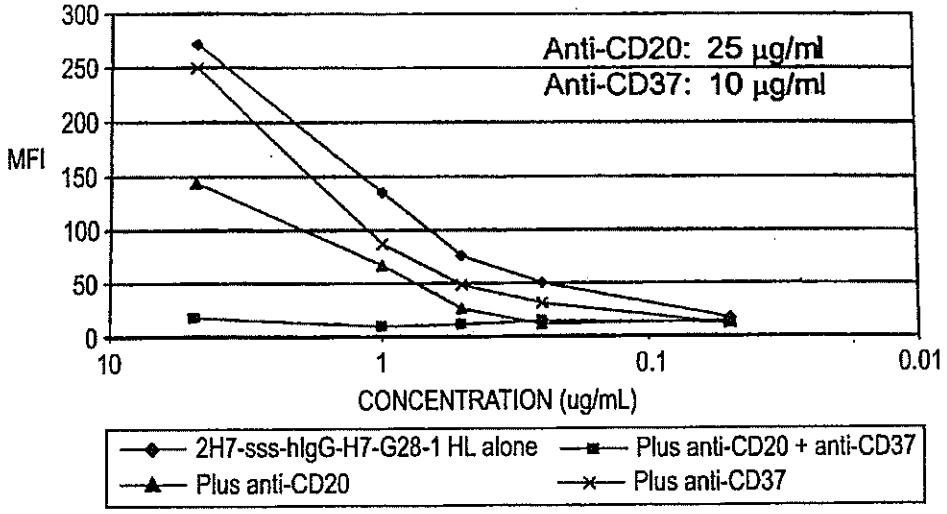


FIG. 14

A. Blocking of Binding of 2H7-sss-hIgG-H7-G28-1 HL Protein to Ramos Cells by CD20 and/or CD37 Targeted Antibodies



B. Blocking of Binding of 2H7-sss-hIgG-H7-G28-1 HL to BJAB Cells by CD20 and/or CD37 Targeted Antibodies

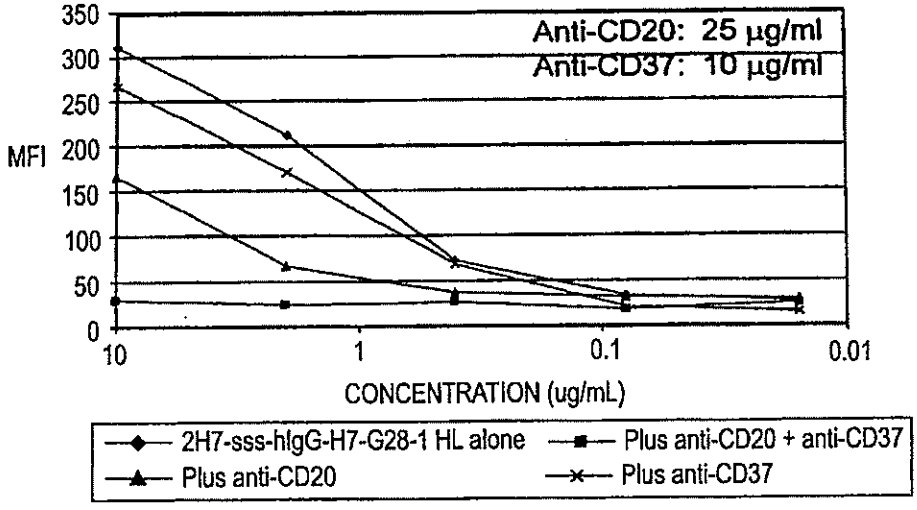


FIG. 15

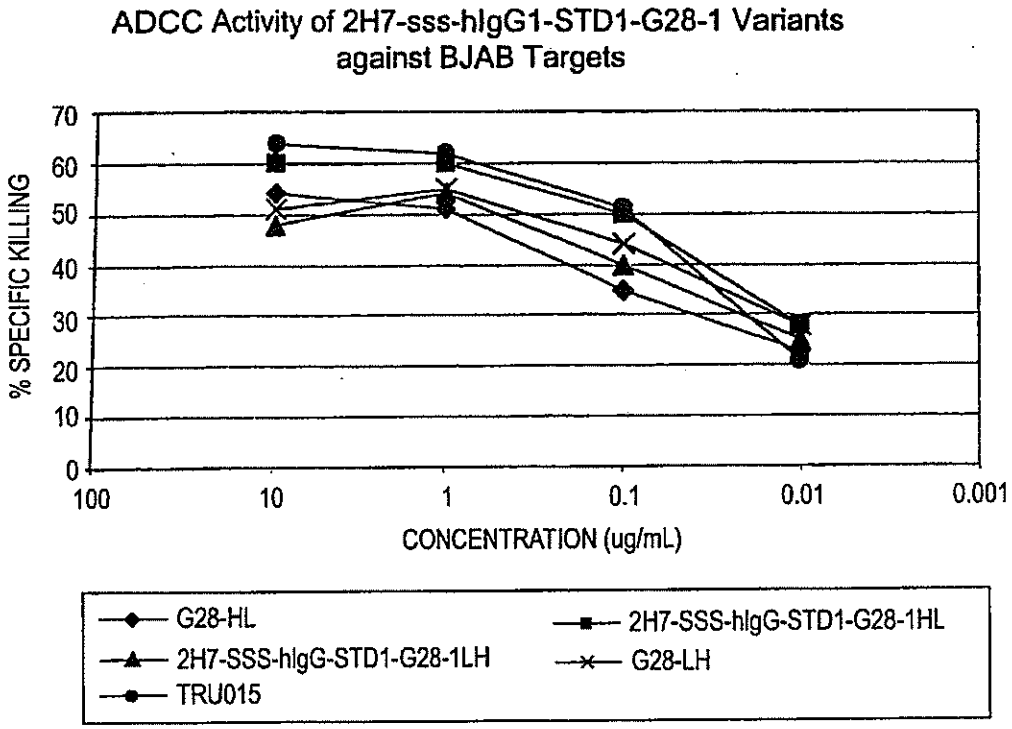

ADCC Activity of 2H7-sss-hIgG1-STD1-G28-1 Variants against BJAB Targets
% SPECIFIC KILLING
70
60
50
40
30
20
10
0
100
10
1
0.1
0.01
0.001
CONCENTRATION (ug/mL)
G28-HL
2H7-SSS-hIgG-STD1-G28-1HL
2H7-SSS-hIgG-STD1-G28-1LH
G28-LH
TRU015

FIG. 16

The Percent CD3, CD19, and CD40 Positive Lymphocytes Present in Culture after Incubation with TRU-015, G28-1 SMIP, TRU-015+G28-1 SMIP, or 2H7-sss-hIgG1-H7-G28-1 HL

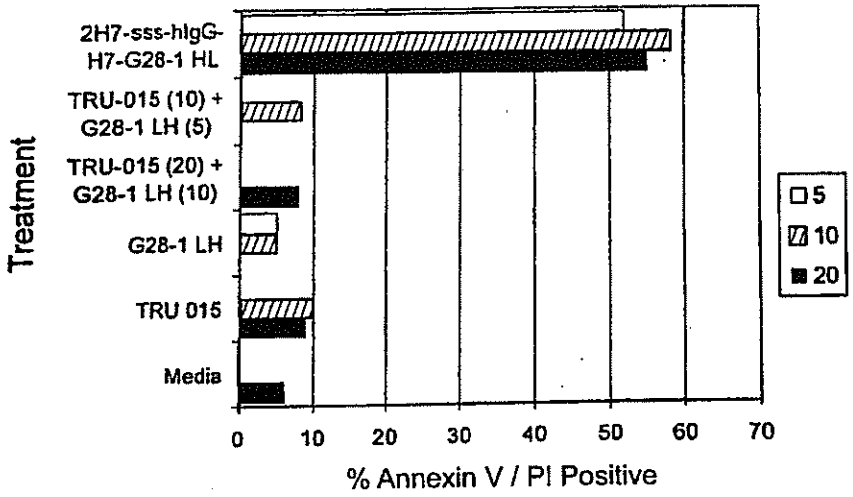
24 Hours	$CD3^+$	$CD19^+$	$CD40^+$
Media	77%	8%	7%
TRU015	76%	8.5%	7.9%
G28-1 SMIP	78%	8. 9%	7.8%
TRU015+G28-1 SMIP	76.3%	8.1%	7.5%
2H7-ssshIgG1-G28-1HL (H7)	78.4%	8.9%	7.9%

72 Hours	$CD3^+$	$CD19^+$	$CD40^+$
Media	78.5%	7.9%	7.7%
TRU015	85.2%	2.3%	2.7%
G28-1 SMIP	86.7%	2.2%	2.3%
TRU015+G28-1 SMIP	87.1%	1.1%	1.1%
2H7-ssshIgG1-G28-1 HL (H7)	96.7%	.08%	.77%

FIG. 17

The Percent Annexin and/or PI Positive B Cells in Culture after 24 Hour Incubation with Single or Multispecific Fusion Proteins.

A. Ramos Cells:



B. Daudi Cells:

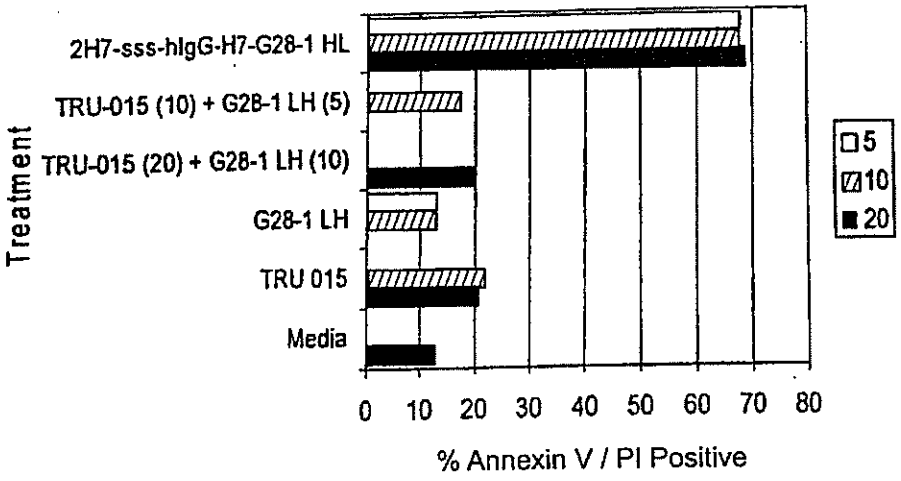


FIG. 18

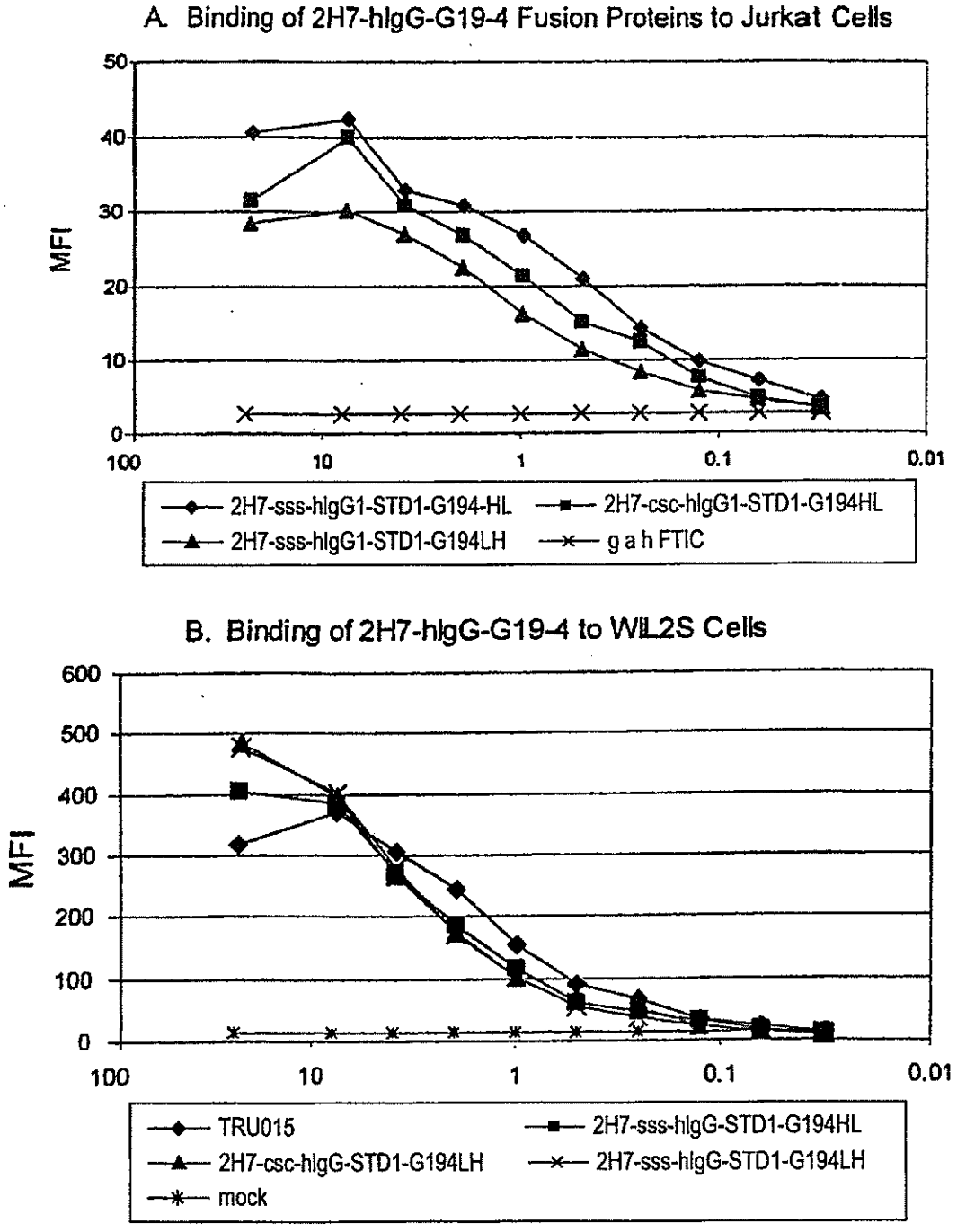
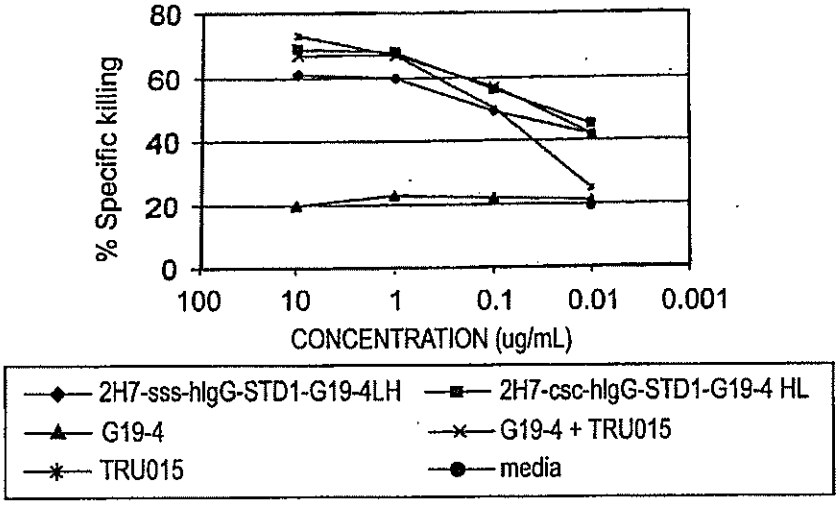

A. Binding of 2H7-hIgG-G19-4 Fusion Proteins to Jurkat Cells
MFI
50
40
30
20
10
0
100
10
1
0.1
0.01
2H7-sss-hIgG1-STD1-G194-HL
2H7-csc-hIgG1-STD1-G194HL
2H7-sss-hIgG1-STD1-G194LH
g a h FTIC
B. Binding of 2H7-hIgG-G19-4 to WIL2S Cells
MFI
600
500
400
300
200
100
0
100
10
1
0.1
0.01
TRU015
2H7-sss-hIgG-STD1-G194HL
2H7-csc-hIgG-STD1-G194LH
2H7-sss-hIgG-STD1-G194LH
mock

FIG. 19

A. ADCC Activity of 2H7-G19-4 Multispecific Fusion Proteins Against BJAB Targets Using Human PBMC Effectors at 25:1



B. ADCC Activity in NK Cell Depiated PBMC Effector Cultures Using BJAB Targets (30:1, E:T) and 2H7-G19-4 Fusion Proteins

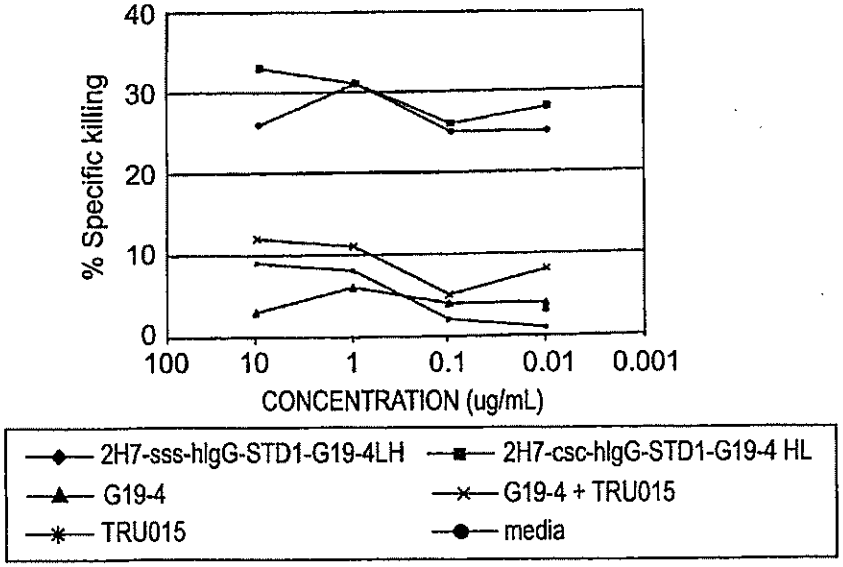


FIG. 20

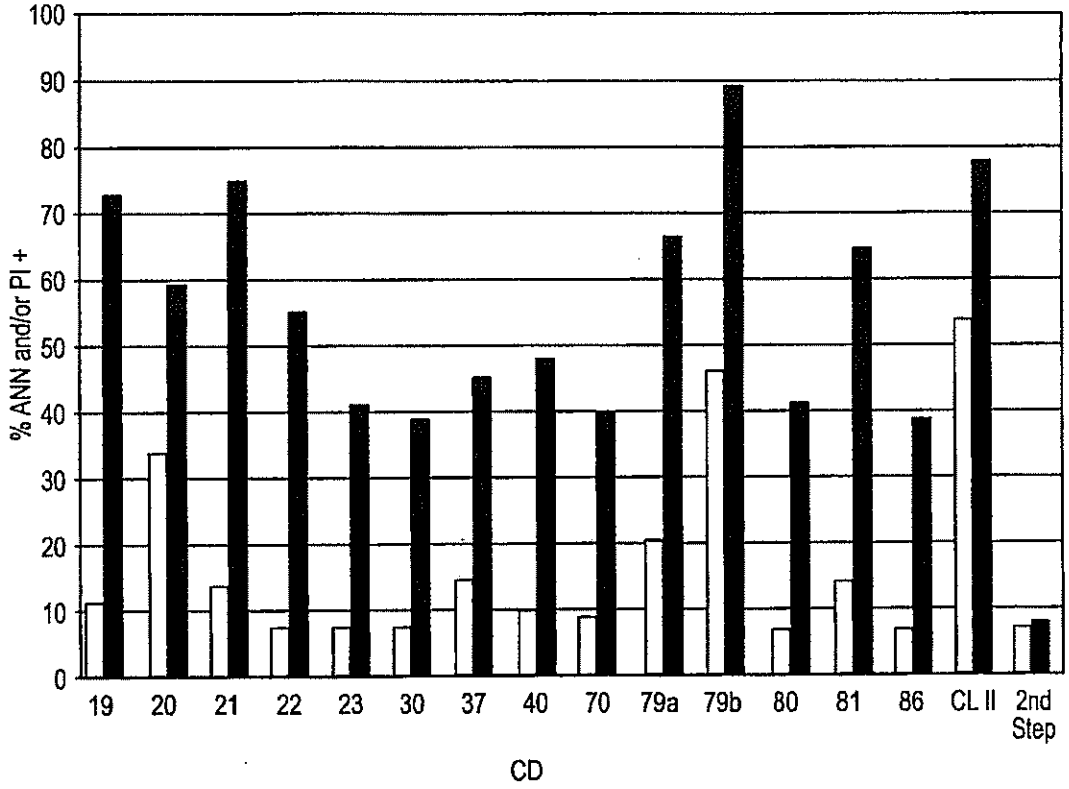

100
90
80
70
60
50
40
30
20
10
0
% ANN and/or PI +
19
20
21
22
23
30
37
40
70
79a
79b
80
81
86
CL II
2nd Step
CD

FIG. 21

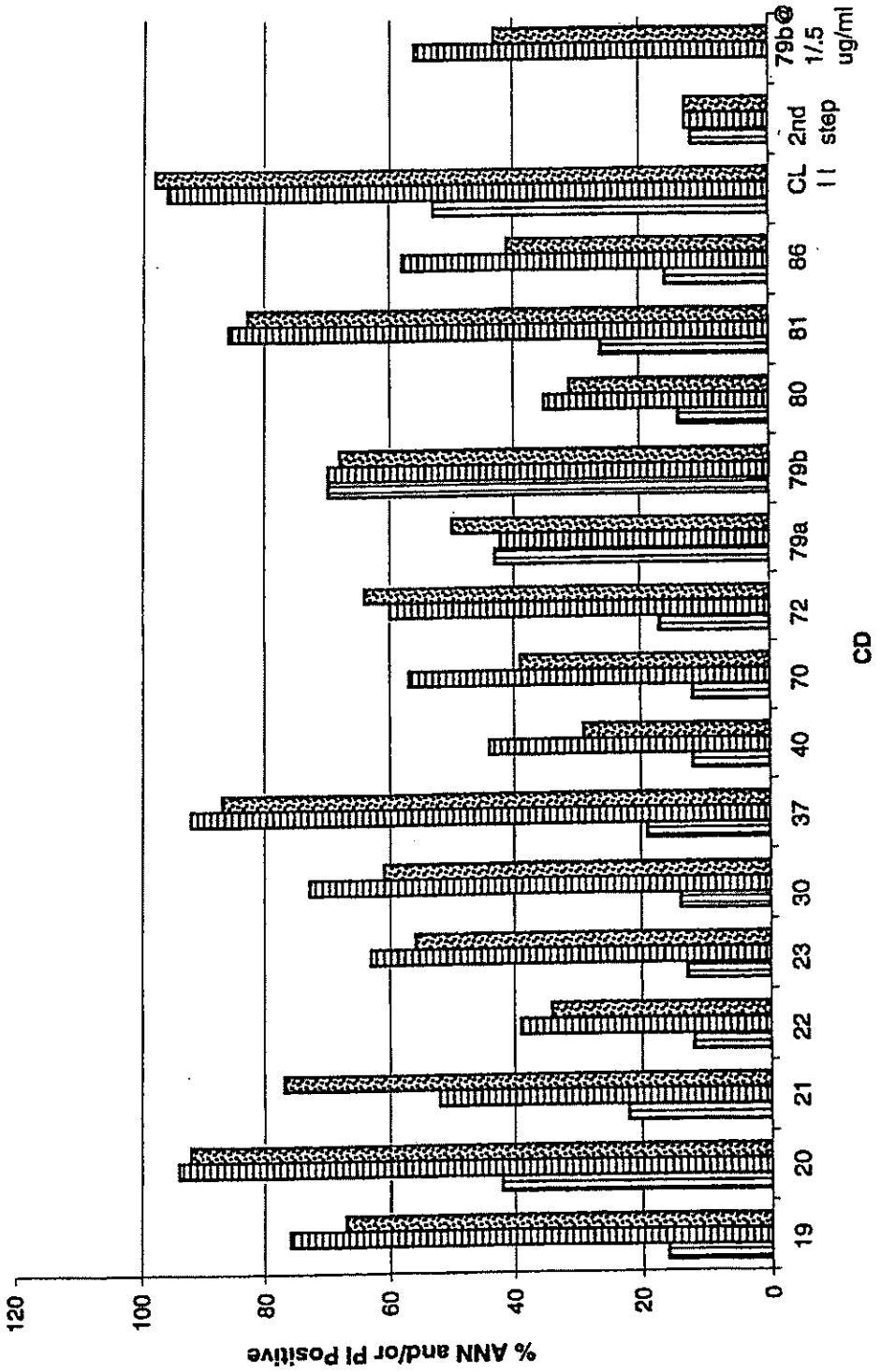

% ANN and/or PI Positive
0
20
40
60
80
100
120
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL 2nd step 1/5 ug/ml
79b@
CD

FIG. 22

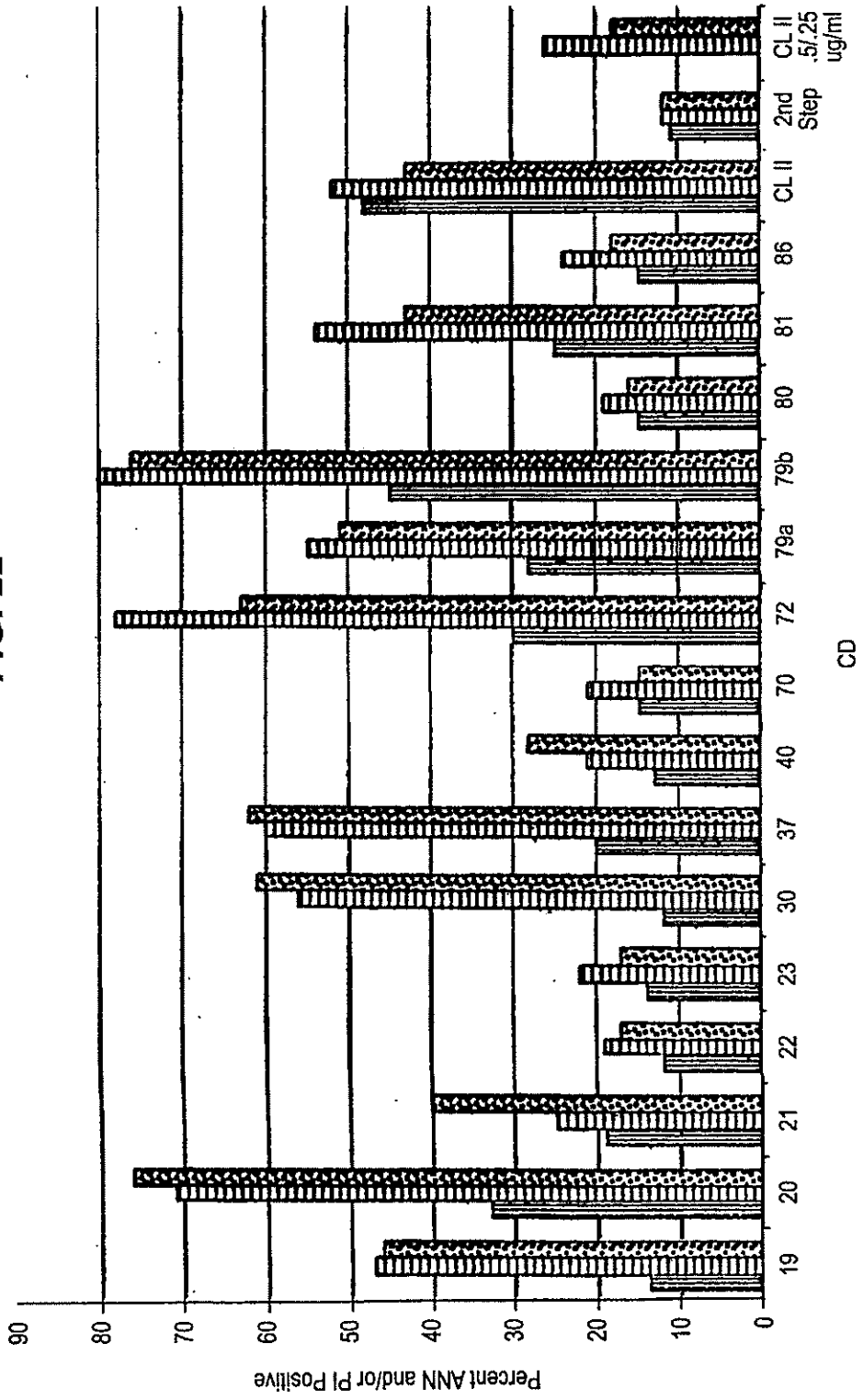

Percent ANN and/or PI Positive
0
10
20
30
40
50
60
70
80
90
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL II
2nd Step
CL II .5/.25 ug/ml
CD

FIG. 23

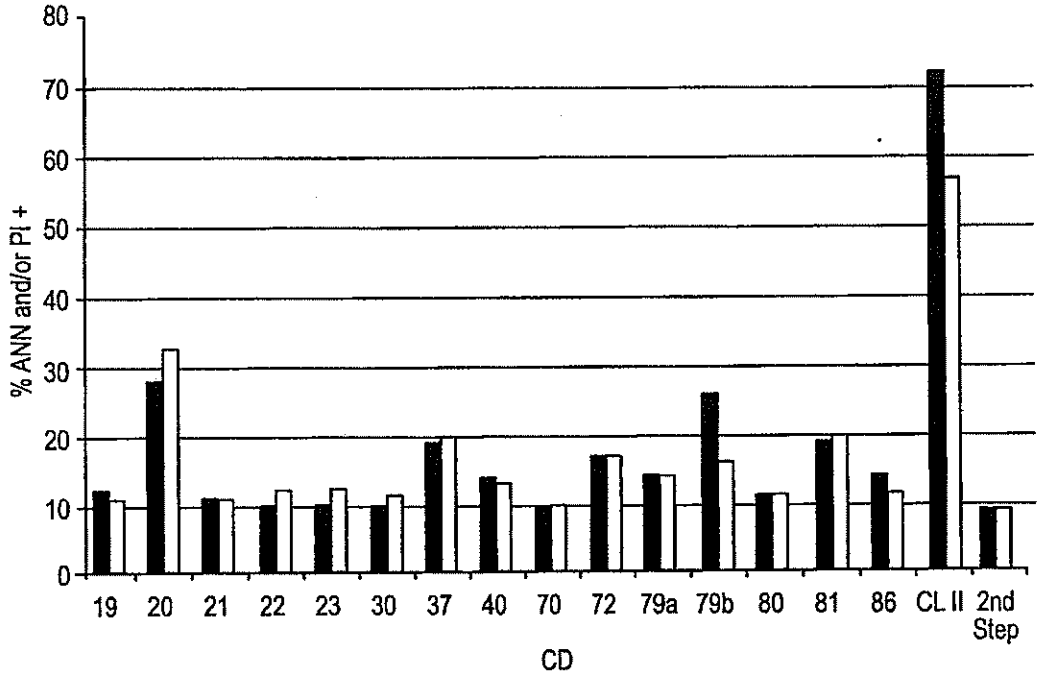

80
70
60
50
40
30
20
10
0
% ANN and/or PI +
19
20
21
22
23
30
37
40
70
72
79a
79b
80
81
86
CL II
2nd Step
CD

FIG. 24

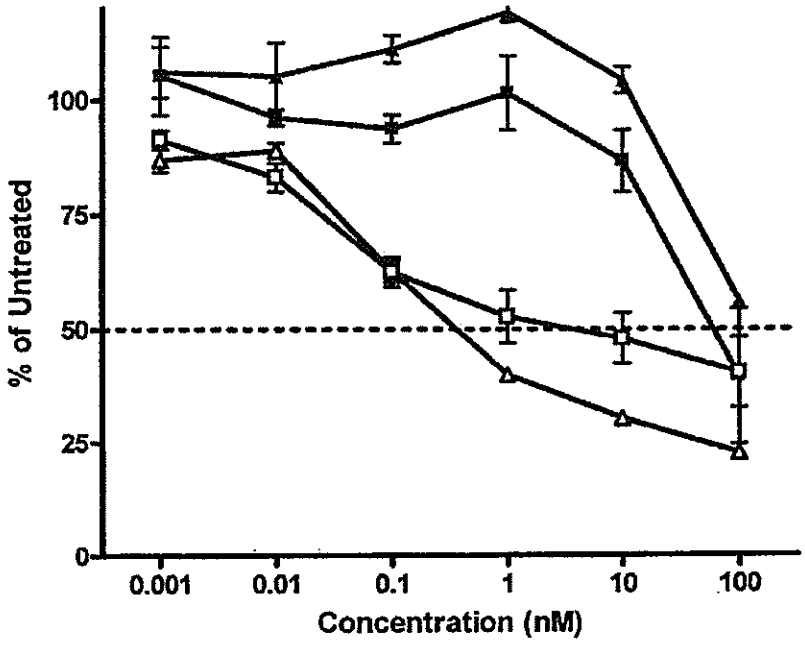

% of Untreated
100
75
50
25
0
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 25

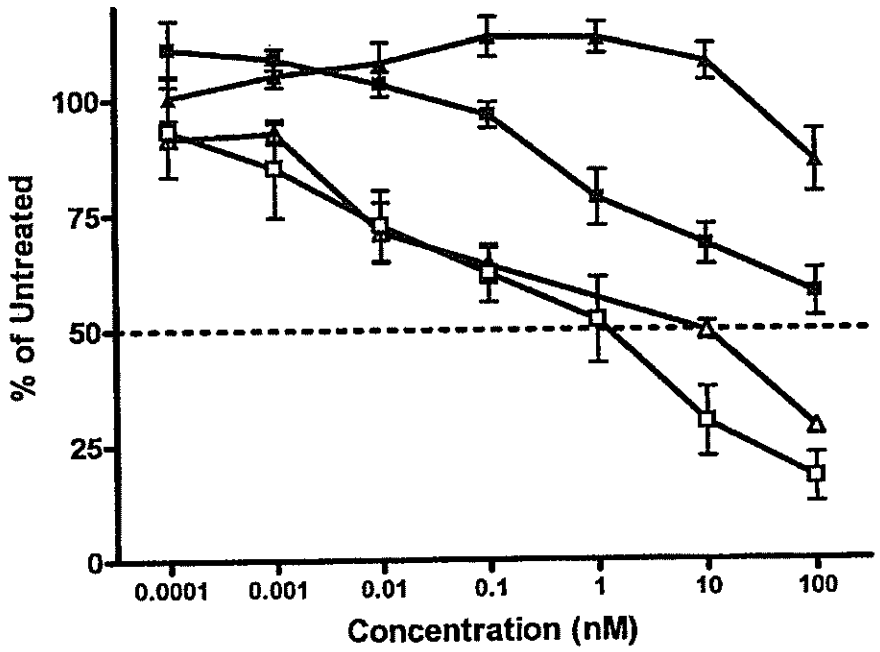

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 26

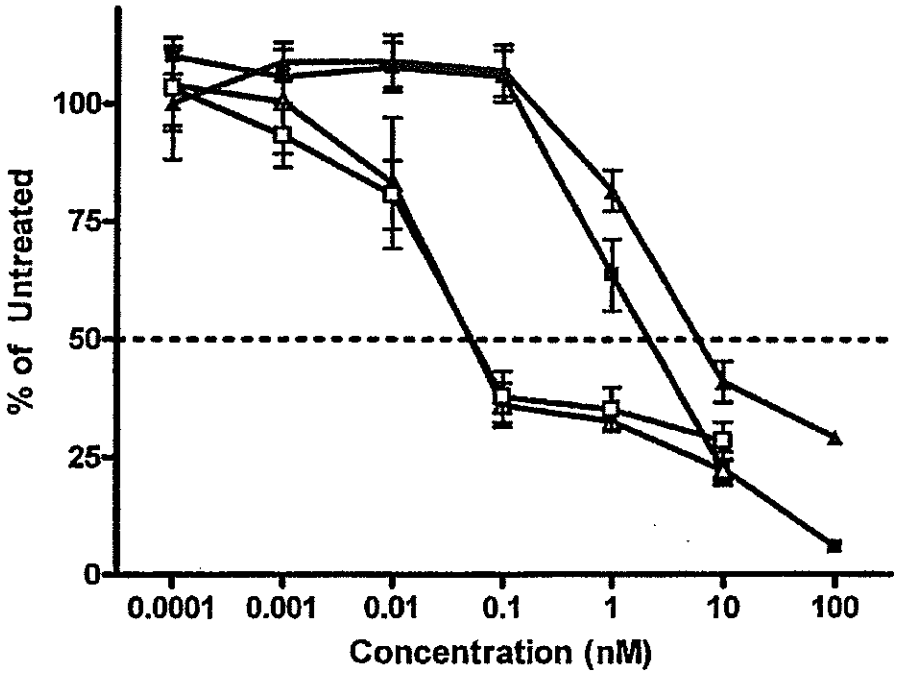

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)

FIG. 27

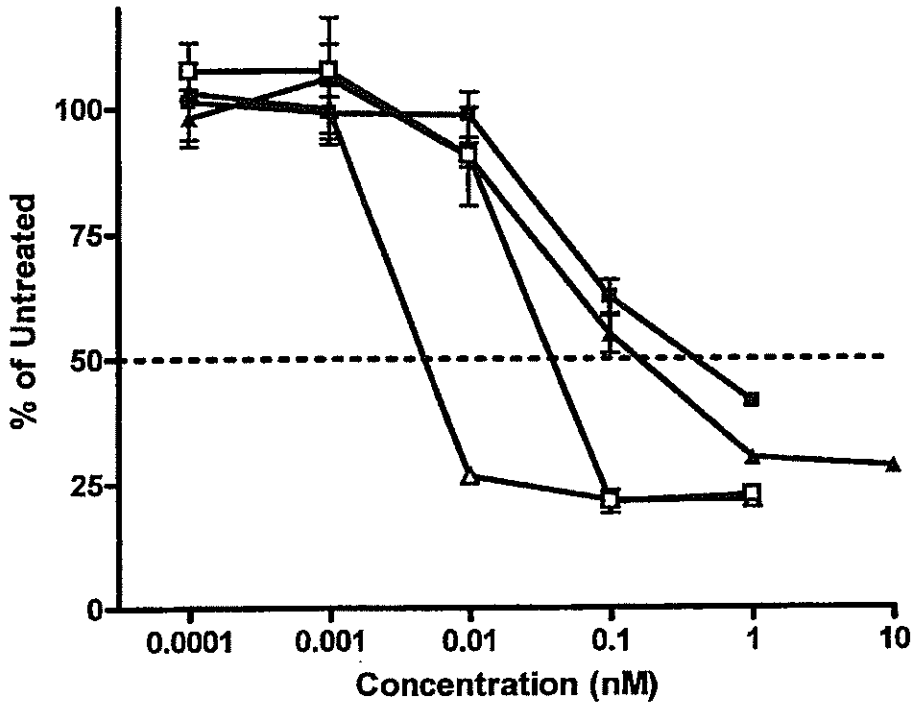

% of Untreated
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
Concentration (nM)

FIG. 28

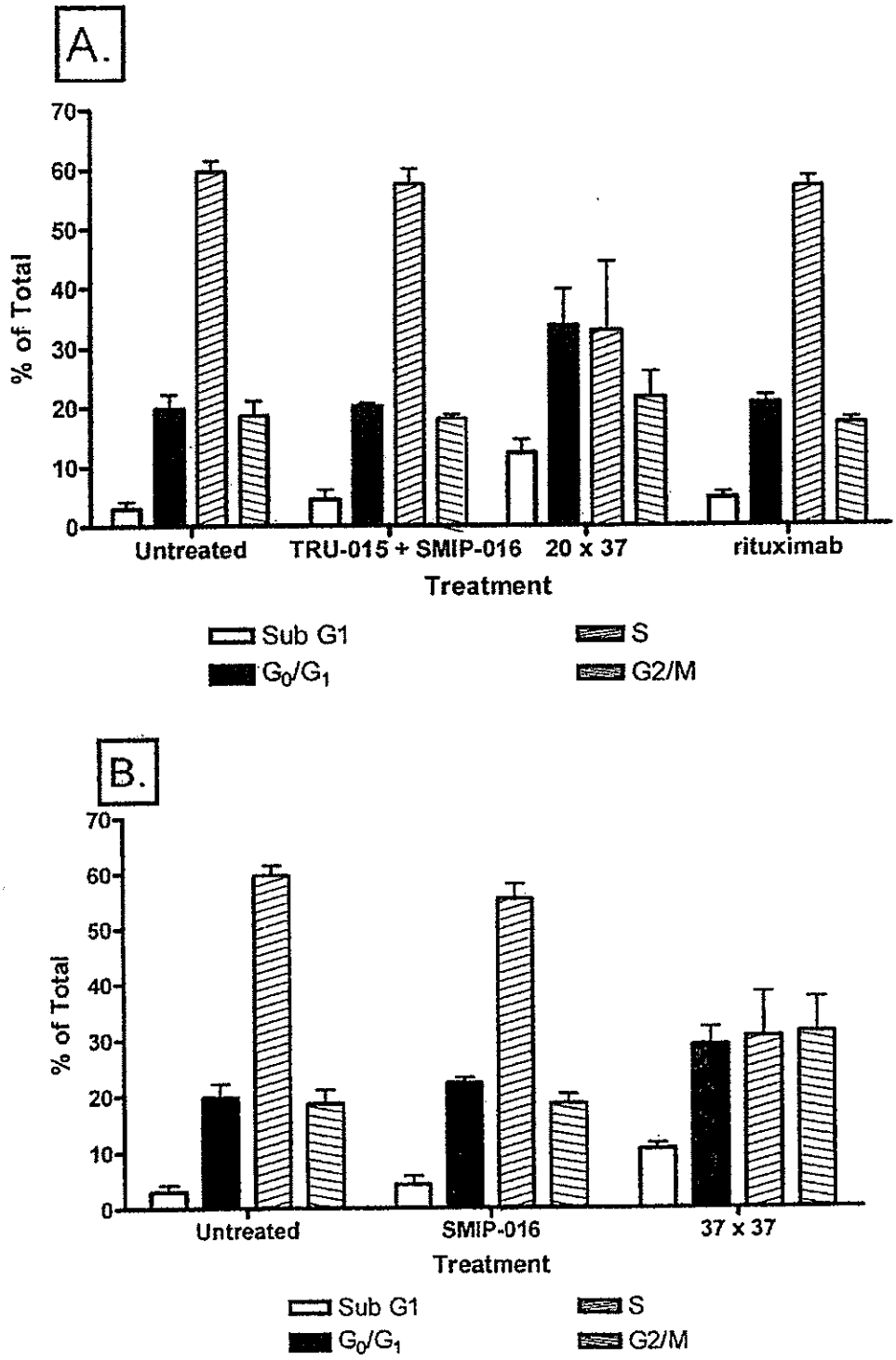

A.
70
60
50
40
30
20
10
0
% of Total
Untreated
TRU-015 + SMIP-016
20 x 37
rituximab
Treatment
Sub G1
S
G_0/G_1
G2/M
B.
70
60
50
40
30
20
10
0
% of Total
Untreated
SMIP-016
37 x 37
Treatment
Sub G1
S
G_0/G_1
G2/M

FIG. 29

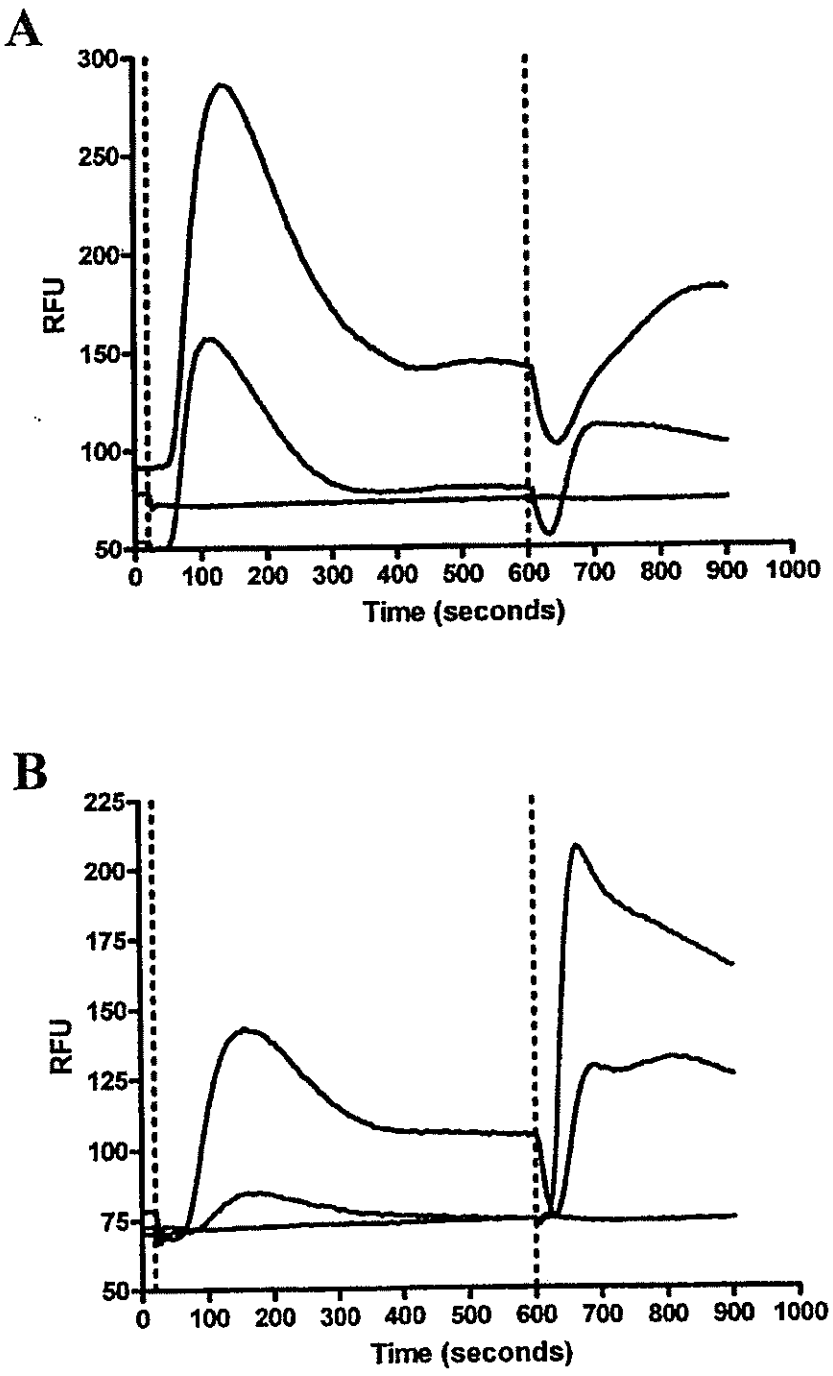

A
300
250
200
150
100
50
RFU
0
100
200
300
400
500
600
700
800
900
1000
Time (seconds)
B
225
200
175
150
125
100
75
50
RFU
0
100
200
300
400
500
600
700
800
900
1000
Time (seconds)

FIG. 30

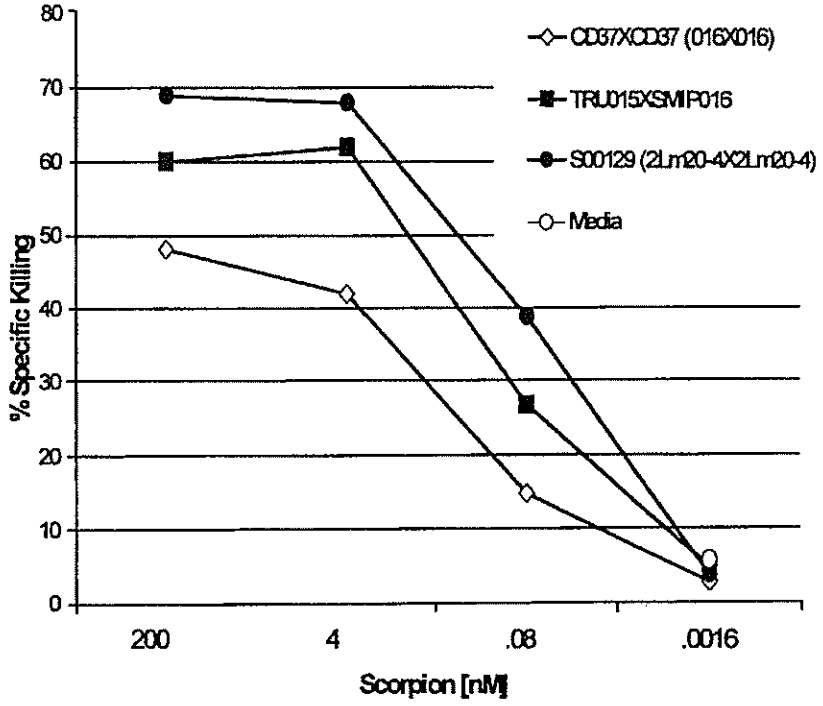

80
70
60
50
40
30
20
10
0
% Specific Killing
200
4
.08
.0016
Scorpion [nM]
CD37XCD37 (016X016)
TRU015XSMIP016
S00129 (2Lm20-4X2Lm20-4)
Media

FIG. 31

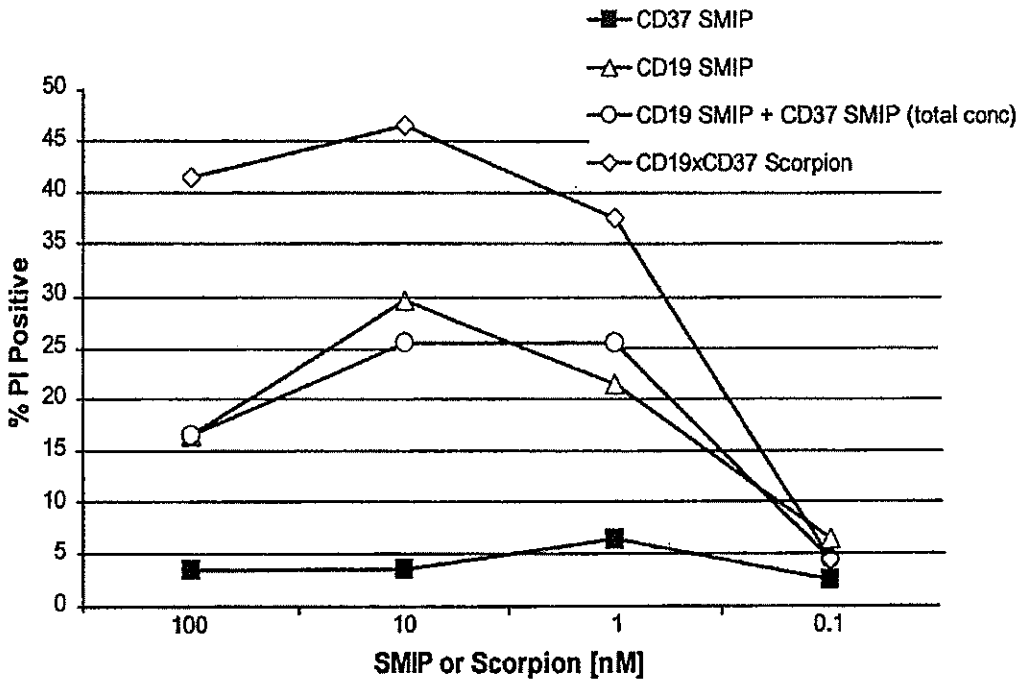

CD37 SMIP
CD19 SMIP
CD19 SMIP + CD37 SMIP (total conc)
CD19xCD37 Scorpion
% PI Positive
50
45
40
35
30
25
20
15
10
5
0
100
10
1
0.1
SMIP or Scorpion [nM]

FIG. 32A

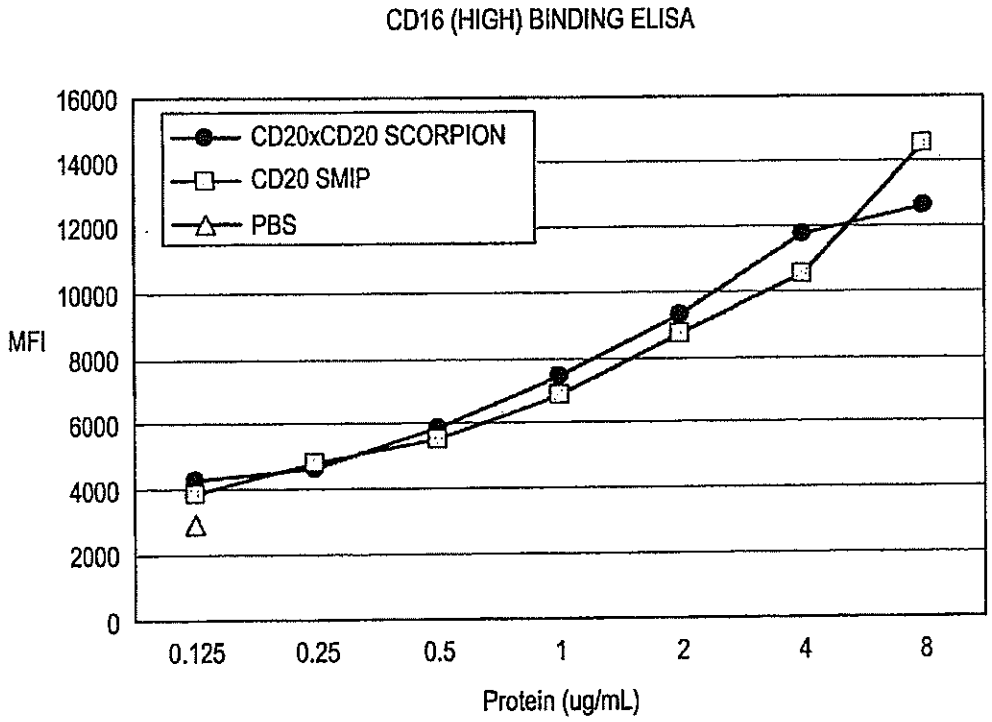

CD16 (HIGH) BINDING ELISA
CD20xCD20 SCORPION
CD20 SMIP
PBS
MFI
16000
14000
12000
10000
8000
6000
4000
2000
0
0.125
0.25
0.5
1
2
4
8
Protein (ug/mL)

FIG. 32B

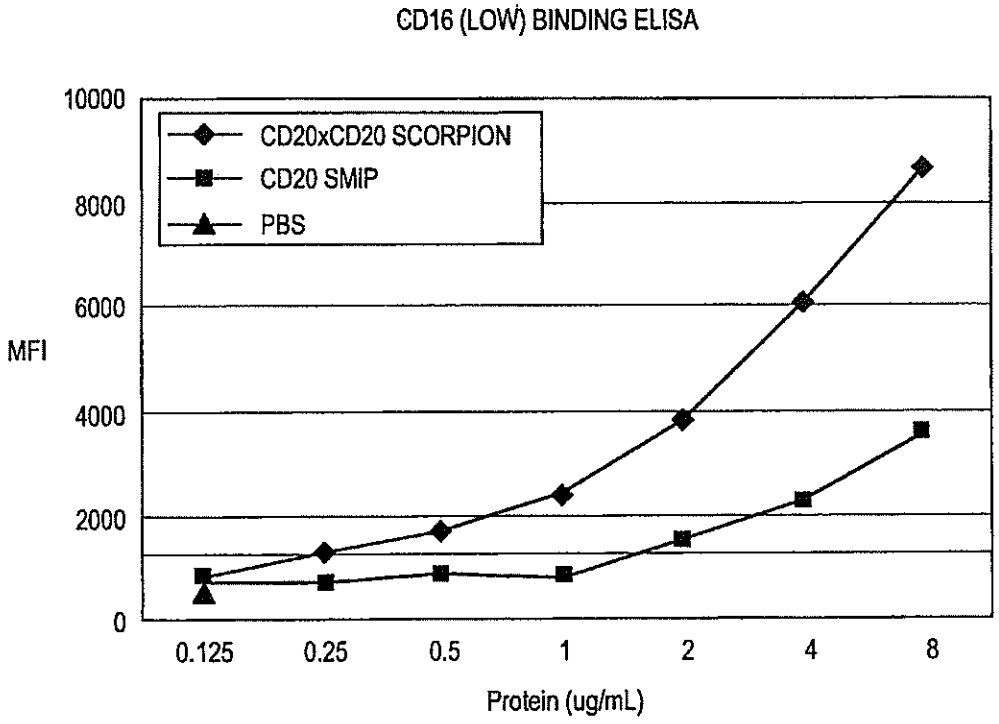

CD16 (LOW) BINDING ELISA
10000
8000
6000
4000
2000
0
MFI
CD20xCD20 SCORPION
CD20 SMIP
PBS
0.125
0.25
0.5
1
2
4
8
Protein (ug/mL)

FIG. 33

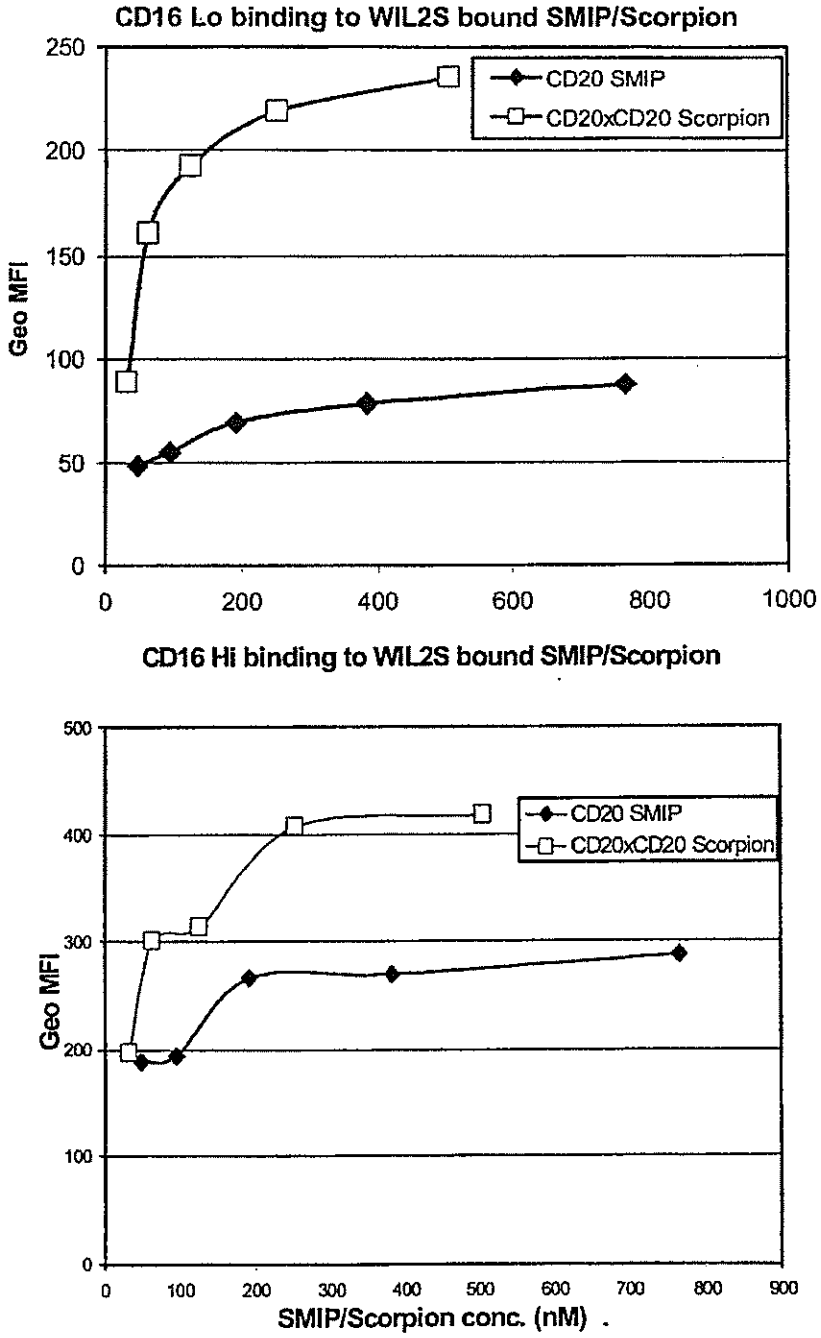

CD16 Lo binding to WIL2S bound SMIP/Scorpion
Geo MFI
250
200
150
100
50
0
0
200
400
600
800
1000
CD20 SMIP
CD20xCD20 Scorpion
CD16 Hi binding to WIL2S bound SMIP/Scorpion
Geo MFI
500
400
300
200
100
0
0
100
200
300
400
500
600
700
800
900
SMIP/Scorpion conc. (nM)
CD20 SMIP
CD20xCD20 Scorpion

FIG. 34

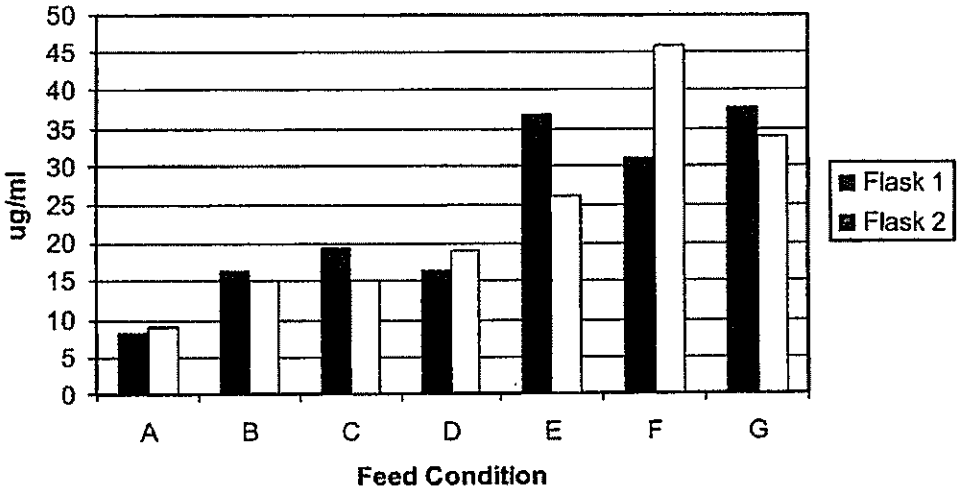

50
45
40
35
30
25
20
15
10
5
0
ug/ml
A
B
C
D
E
F
G
Feed Condition
Flask 1
Flask 2

FIG. 35

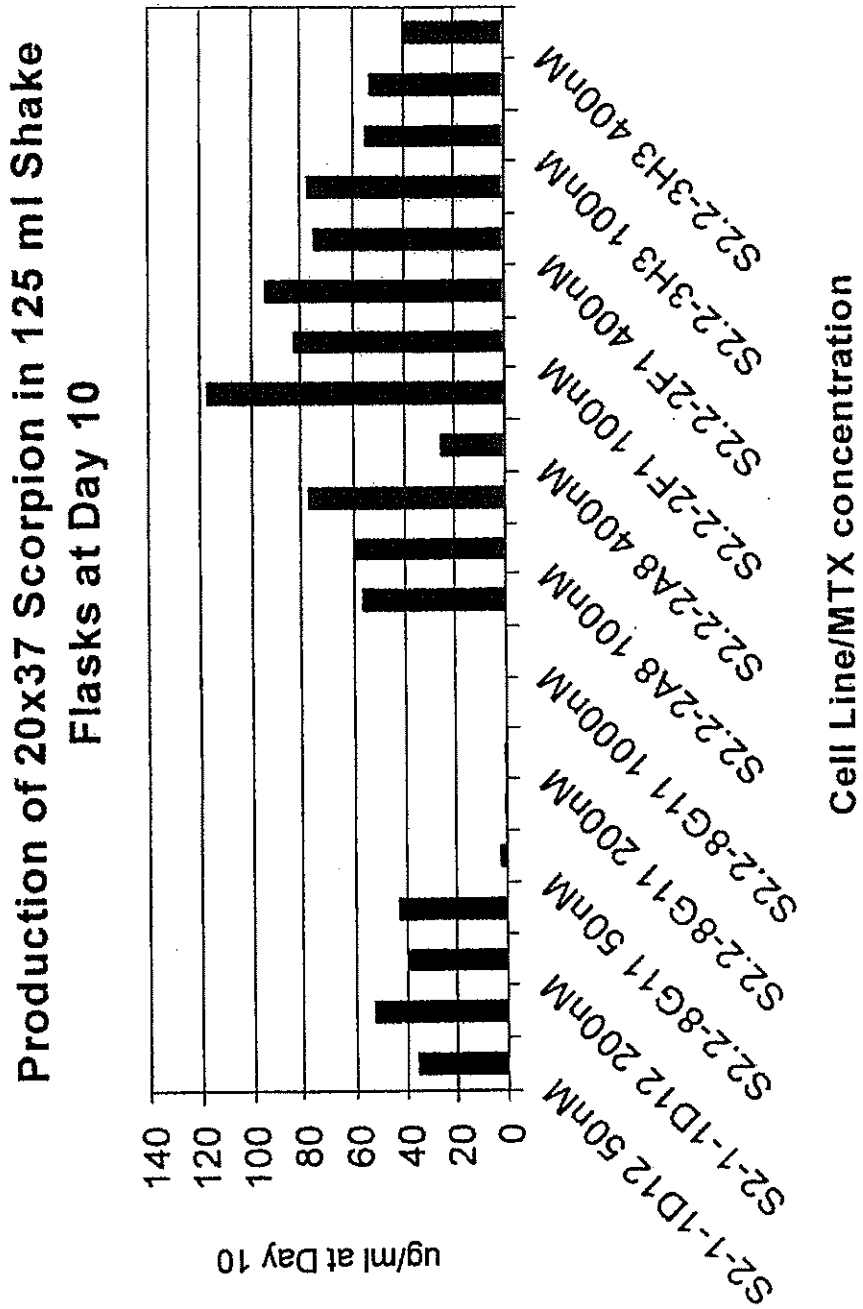

Production of 20x37 Scorpion in 125 ml Shake Flasks at Day 10
ug/ml at Day 10
0
20
40
60
80
100
120
140
S2-1-1D12 50nM
S2-1-1D12 200nM
S2.2-8G11 50nM
S2.2-8G11 200nM
S2.2-8G11 1000nM
S2.2-2A8 100nM
S2.2-2A8 400nM
S2.2-2F1 100nM
S2.2-2F1 400nM
S2.2-3H3 100nM
S2.2-3H3 400nM
Cell Line/MTX concentration

FIG. 36

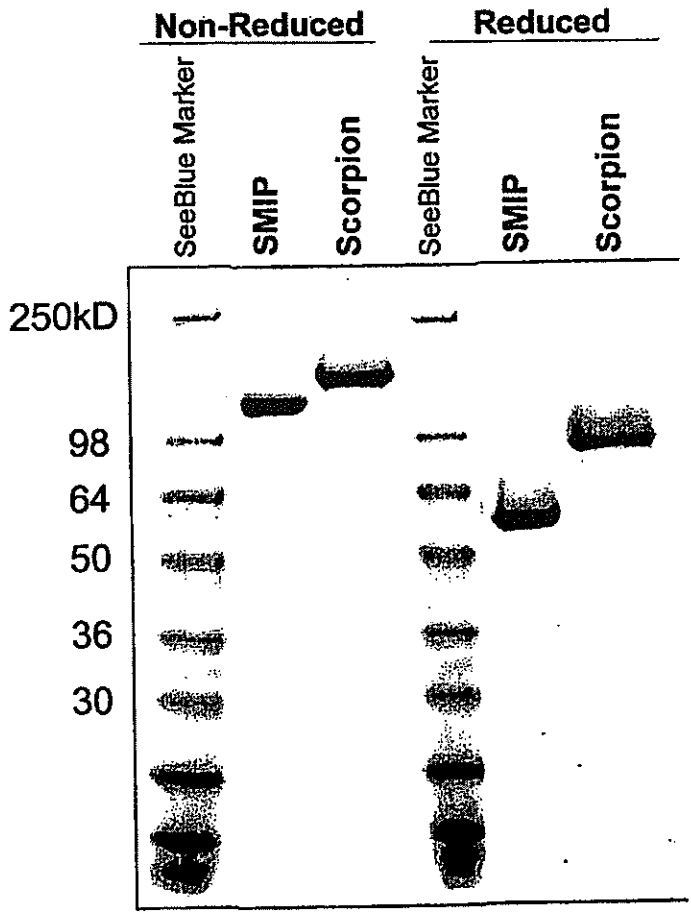

Non-Reduced
Reduced
SeeBlue Marker
SMIP
Scorpion
SeeBlue Marker
SMIP
Scorpion
250kD
98
64
50
36
30

FIG. 37

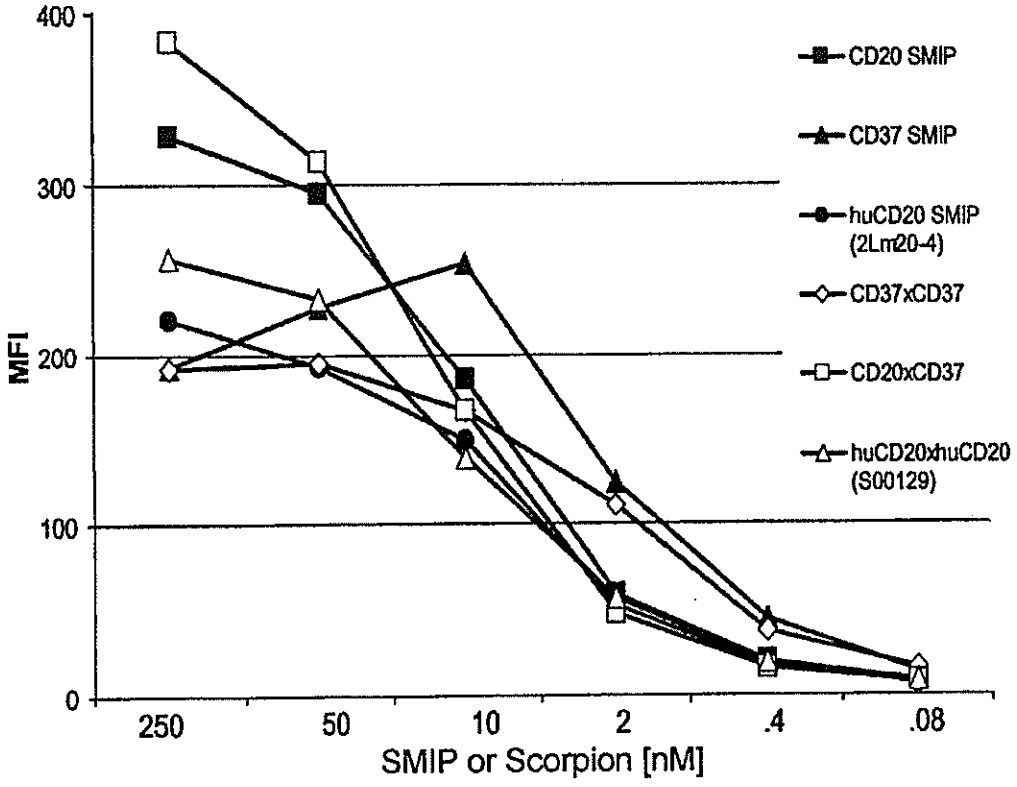

400
300
200
100
0
MFI
250
50
10
2
.4
.08
SMIP or Scorpion [nM]
CD20 SMIP
CD37 SMIP
huCD20 SMIP (2Lm20-4)
CD37xCD37
CD20xCD37
huCD20xhuCD20 (S00129)

FIG. 38

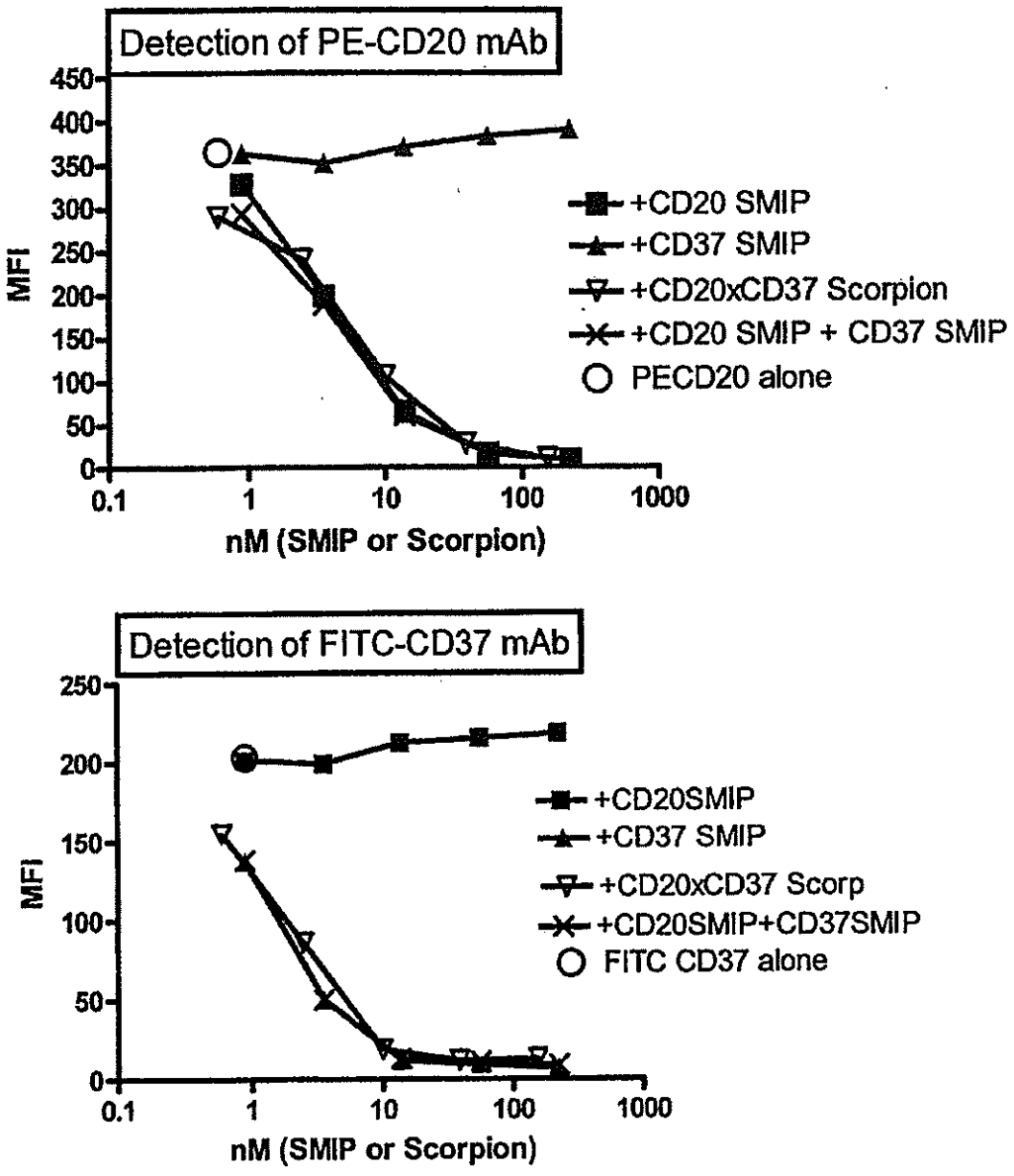

Detection of PE-CD20 mAb
MFI
450
400
350
300
250
200
150
100
50
0
0.1
1
10
100
1000
nM (SMIP or Scorpion)
+CD20 SMIP
+CD37 SMIP
+CD20xCD37 Scorpion
+CD20 SMIP + CD37 SMIP
PECD20 alone
Detection of FITC-CD37 mAb
MFI
250
200
150
100
50
0
0.1
1
10
100
1000
nM (SMIP or Scorpion)
+CD20SMIP
+CD37 SMIP
+CD20xCD37 Scorp
+CD20SMIP+CD37SMIP
FITC CD37 alone

FIG. 39

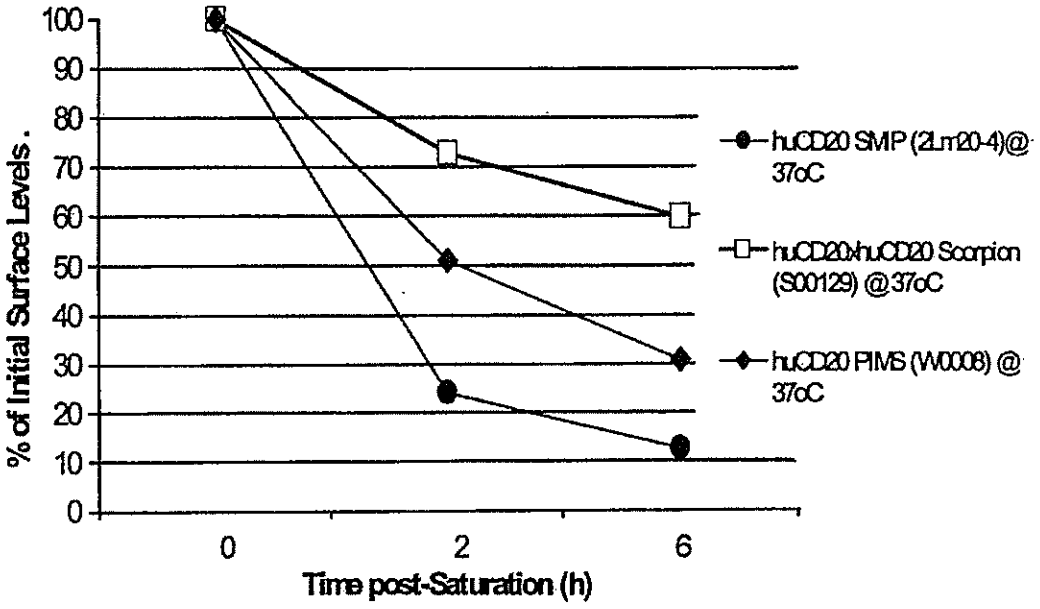

100
90
80
70
60
50
40
30
20
10
0
% of Initial Surface Levels.
0
2
6
Time post-Saturation (h)
huCD20 SMIP (2Lm20-4)@ 37oC
huCD20/huCD20 Scorpion (S00129) @37oC
huCD20 PIMS (W0008) @ 37oC

FIG. 40

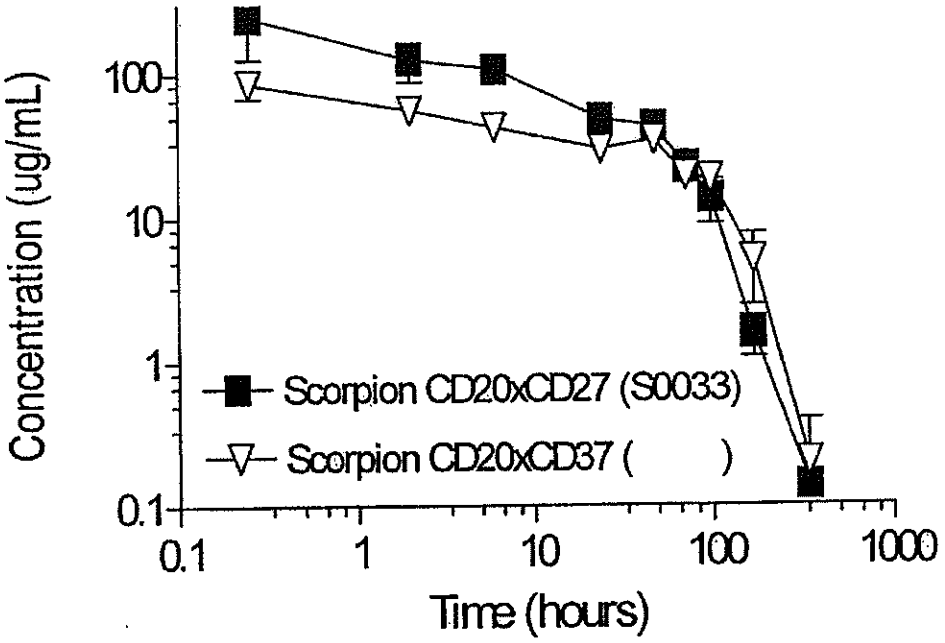

Concentration (ug/mL)
100
10
1
0.1
0.1
1
10
100
1000
Time (hours)
Scorpion CD20xCD27 (S0033)
Scorpion CD20xCD37 ( )

FIG. 41

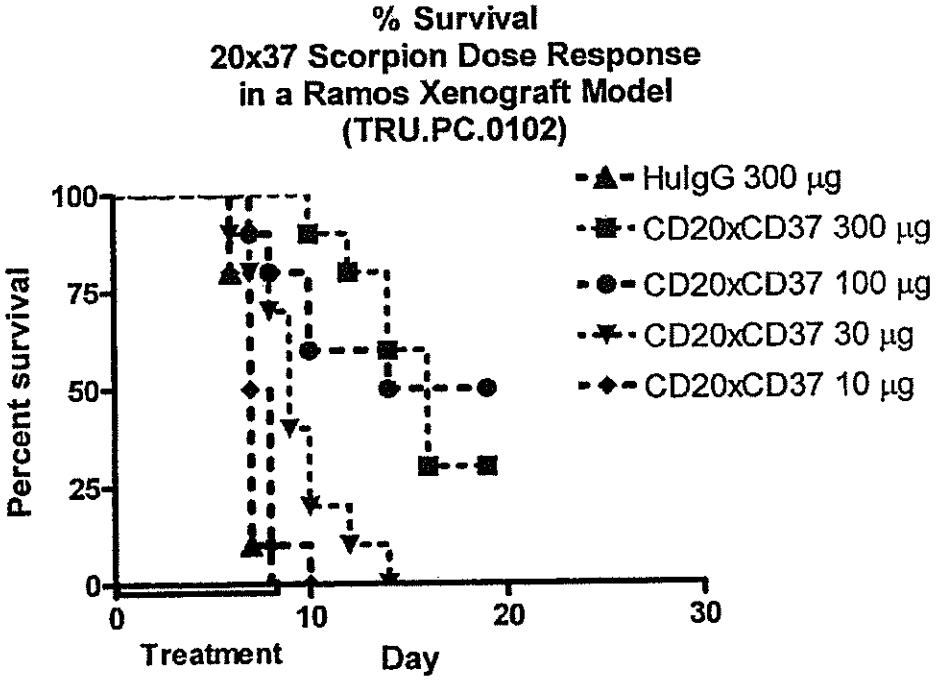
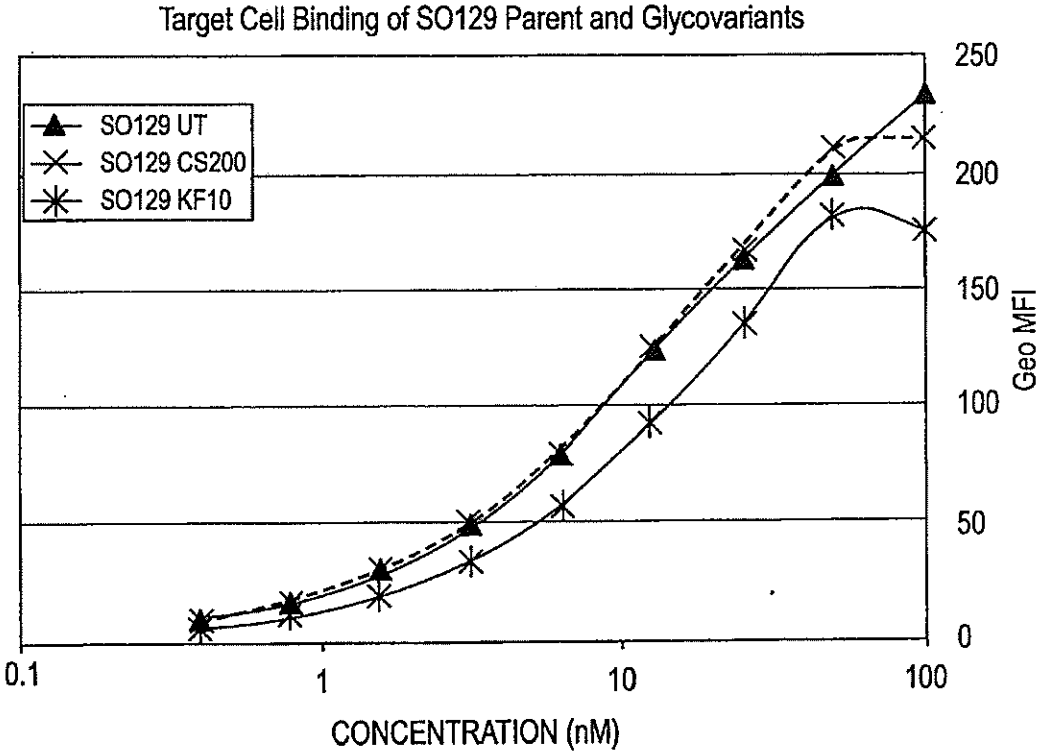

% Survival
20x37 Scorpion Dose Response
in a Ramos Xenograft Model
(TRU.PC.0102)
HulgG 300 µg
CD20xCD37 300 µg
CD20xCD37 100 µg
CD20xCD37 30 µg
CD20xCD37 10 µg
Percent survival
100
75
50
25
0
0
10
20
30
Treatment
Day

FIG. 42



WIL2S staining with 2Im20-4, and SO129 +/- carbohydrate modification
Kifunensine@10 uM (excess), Castanospermine@200 uM

FIG. 43

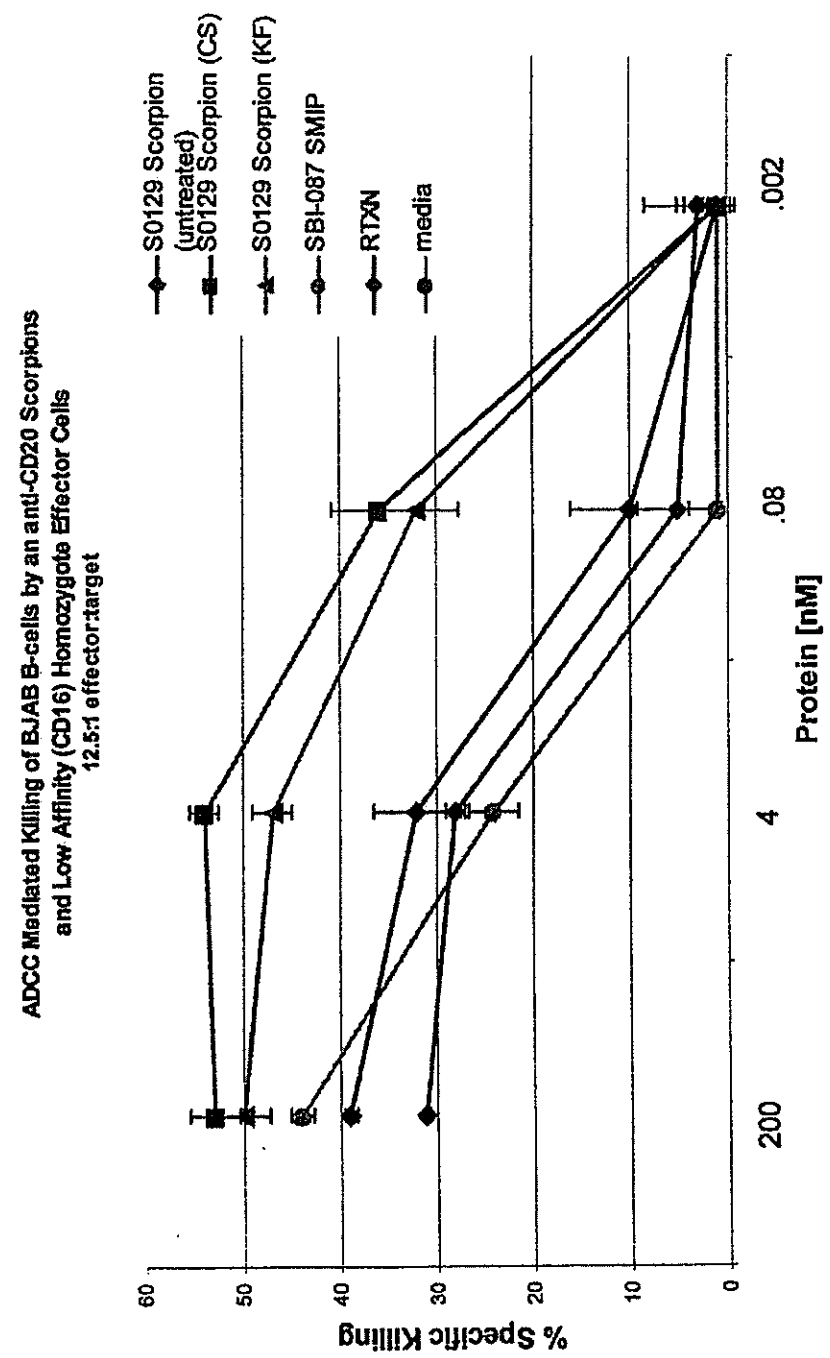

ADCC Mediated Killing of BJAB B-cells by an anti-CD20 Scorpions and Low Affinity (CD16) Homozygote Effector Cells
12.5:1 effector:target
S0129 Scorpion (untreated)
S0129 Scorpion (CS)
S0129 Scorpion (KF)
SBI-087 SMIP
RTXN
media
% Specific Killing
0
10
20
30
40
50
60
200
4
.08
.002
Protein [nM]

FIG. 44

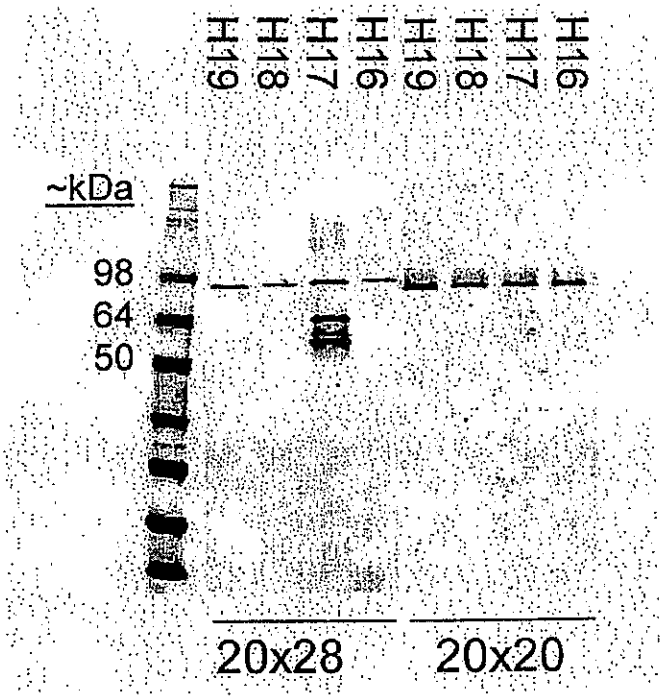


FIG. 45

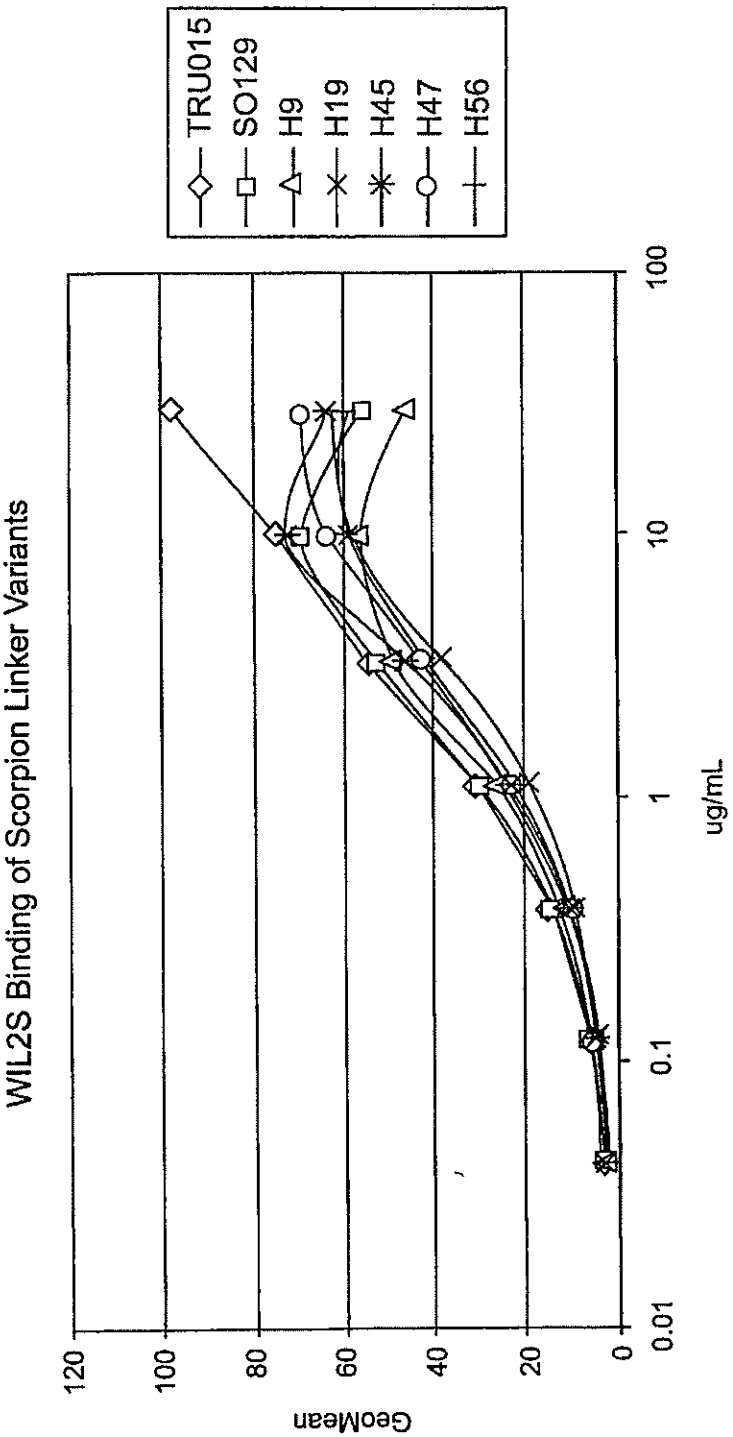

WIL2S Binding of Scorpion Linker Variants
TRU015
SO129
H9
H19
H45
H47
H56
GeoMean
120
100
80
60
40
20
0
0.01
0.1
1
10
100
ug/mL

FIG. 46

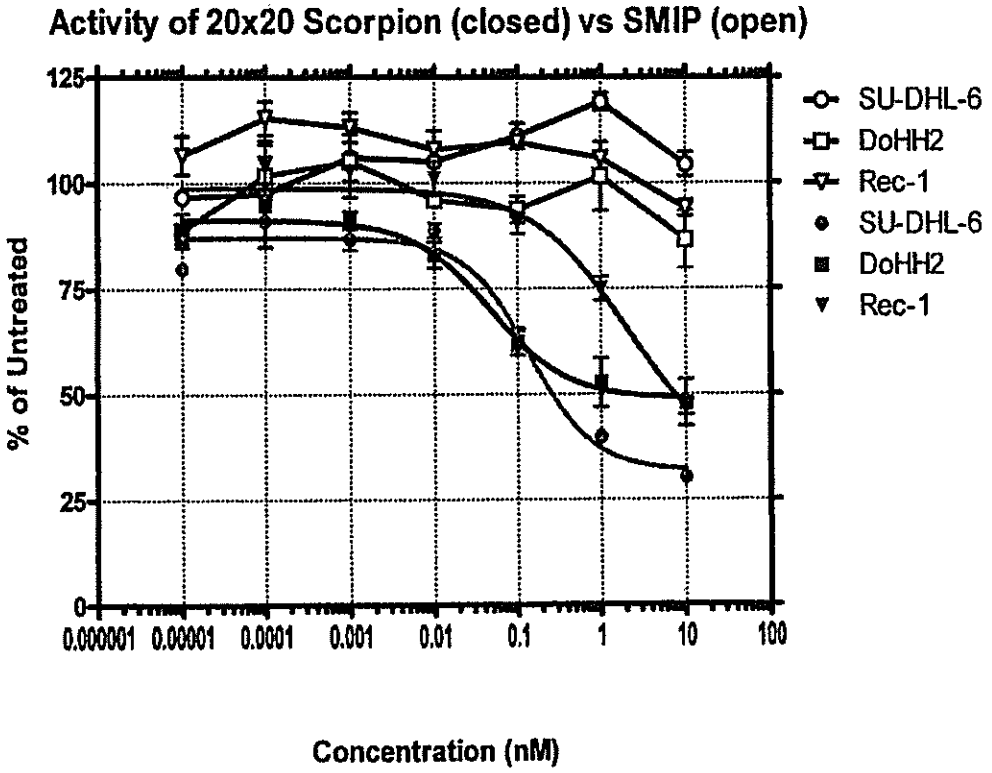

Activity of 20x20 Scorpion (closed) vs SMIP (open)
% of Untreated
125
100
75
50
25
0
0.000001
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
SU-DHL-6
DoHH2
Rec-1
SU-DHL-6
DoHH2
Rec-1

FIG. 47

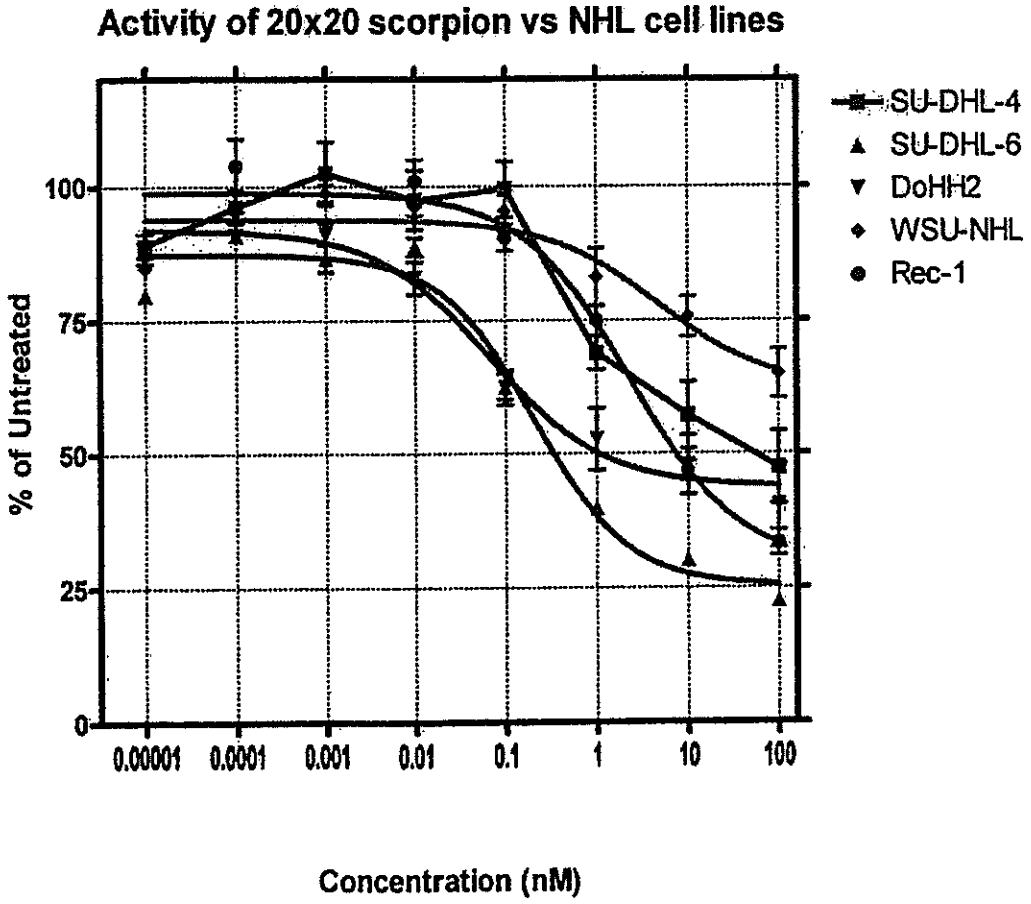

Activity of 20x20 scorpion vs NHL cell lines
% of Untreated
100
75
50
25
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
SU-DHL-4
SU-DHL-6
DoHH2
WSU-NHL
Rec-1

FIG. 48

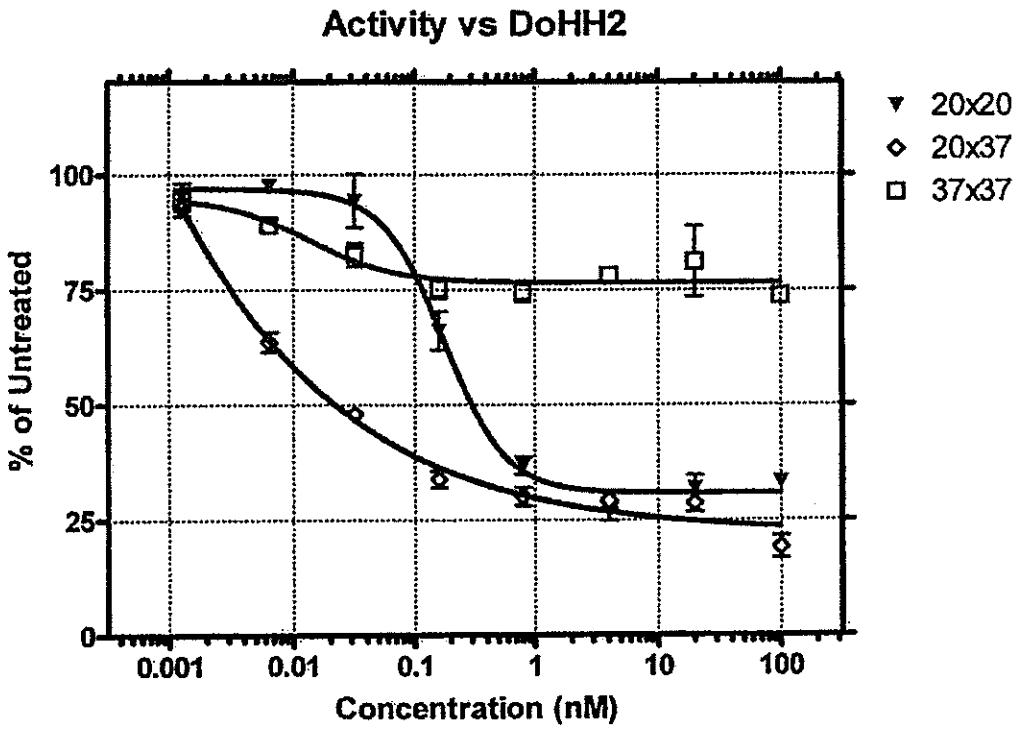

Activity vs DoHH2
% of Untreated
100
75
50
25
0
0.001
0.01
0.1
1
10
100
Concentration (nM)
20x20
20x37
37x37

FIG. 49

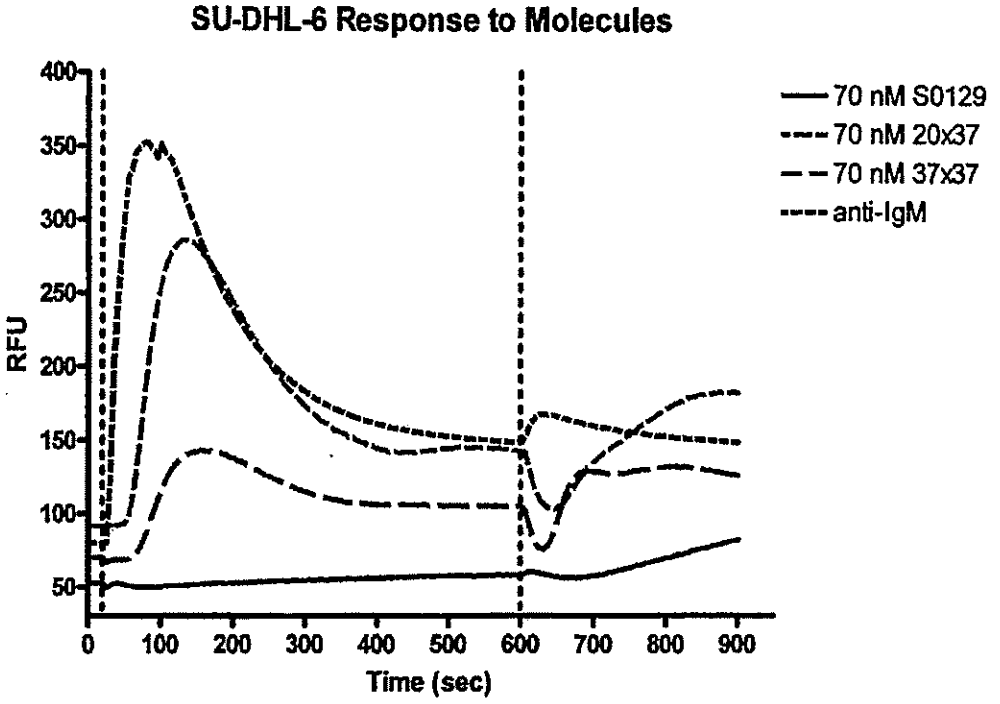

SU-DHL-6 Response to Molecules
RFU
400
350
300
250
200
150
100
50
0
100
200
300
400
500
600
700
800
900
Time (sec)
70 nM S0129
70 nM 20x37
70 nM 37x37
anti-IgM

FIG. 50

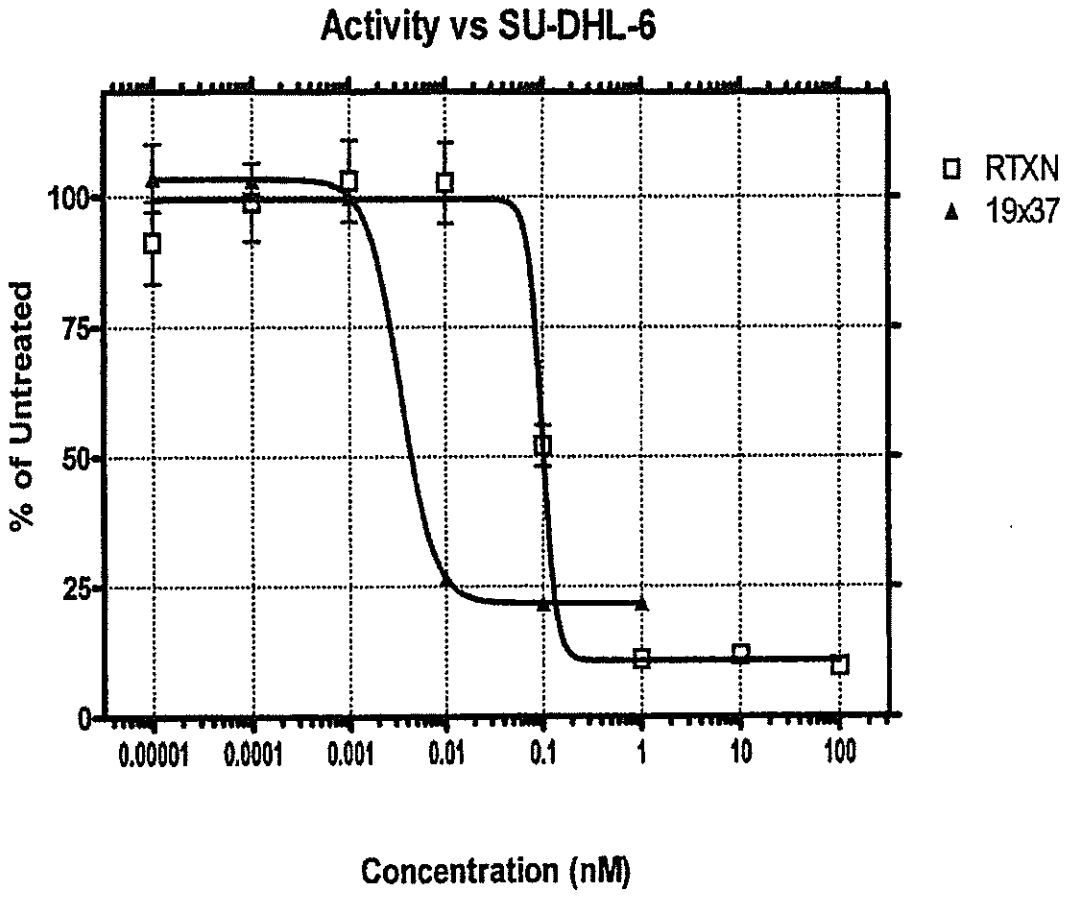

Activity vs SU-DHL-6
% of Untreated
100
75
50
25
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration (nM)
RTXN
19x37

FIG. 51

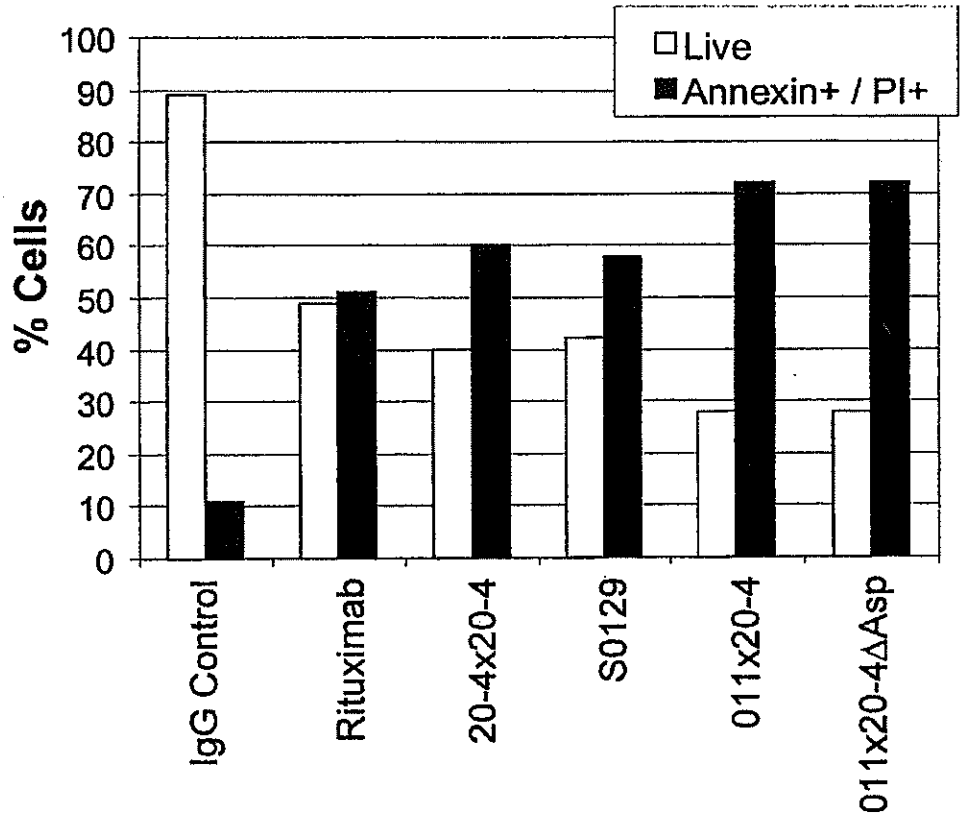

Live
Annexin+ / PI+
% Cells
100
90
80
70
60
50
40
30
20
10
0
IgG Control
Rituximab
20-4x20-4
S0129
011x20-4
011x20-4ΔAsp

FIG. 52

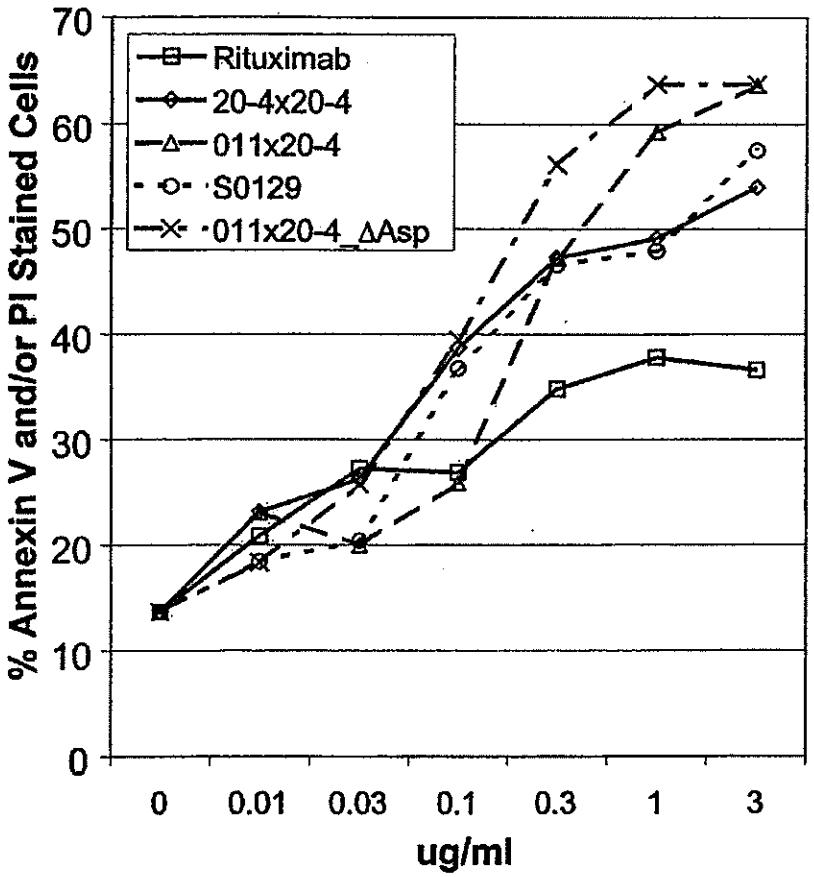

% Annexin V and/or PI Stained Cells
70
60
50
40
30
20
10
0
Rituximab
20-4x20-4
011x20-4
S0129
011x20-4_ΔAsp
0
0.01
0.03
0.1
0.3
1
3
ug/ml

FIG. 53

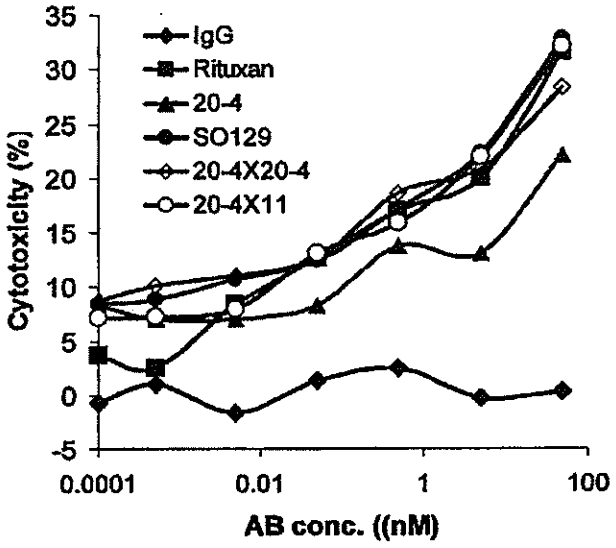

35
30
25
20
15
10
5
0
-5
Cytotoxicity (%)
IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X11
0.0001
0.01
1
100
AB conc. ((nM)

FIG. 54

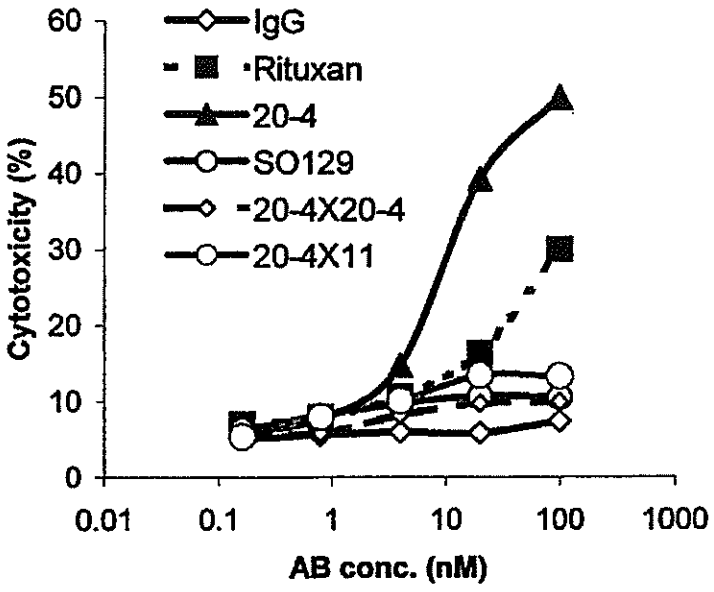

IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X11
Cytotoxicity (%)
60
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
AB conc. (nM)

FIG. 55

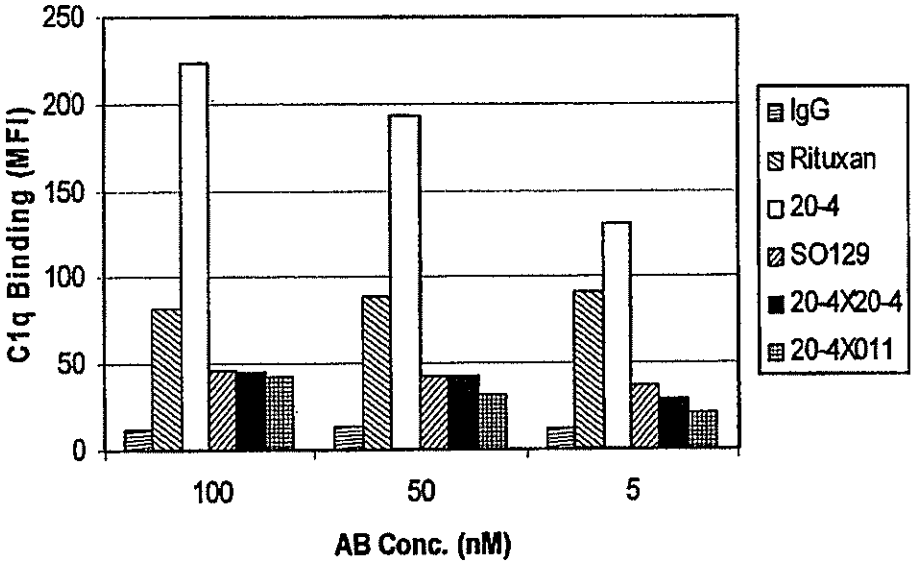

250
200
150
100
50
0
C1q Binding (MFI)
100
50
5
AB Conc. (nM)
IgG
Rituxan
20-4
SO129
20-4X20-4
20-4X011

FIG. 56

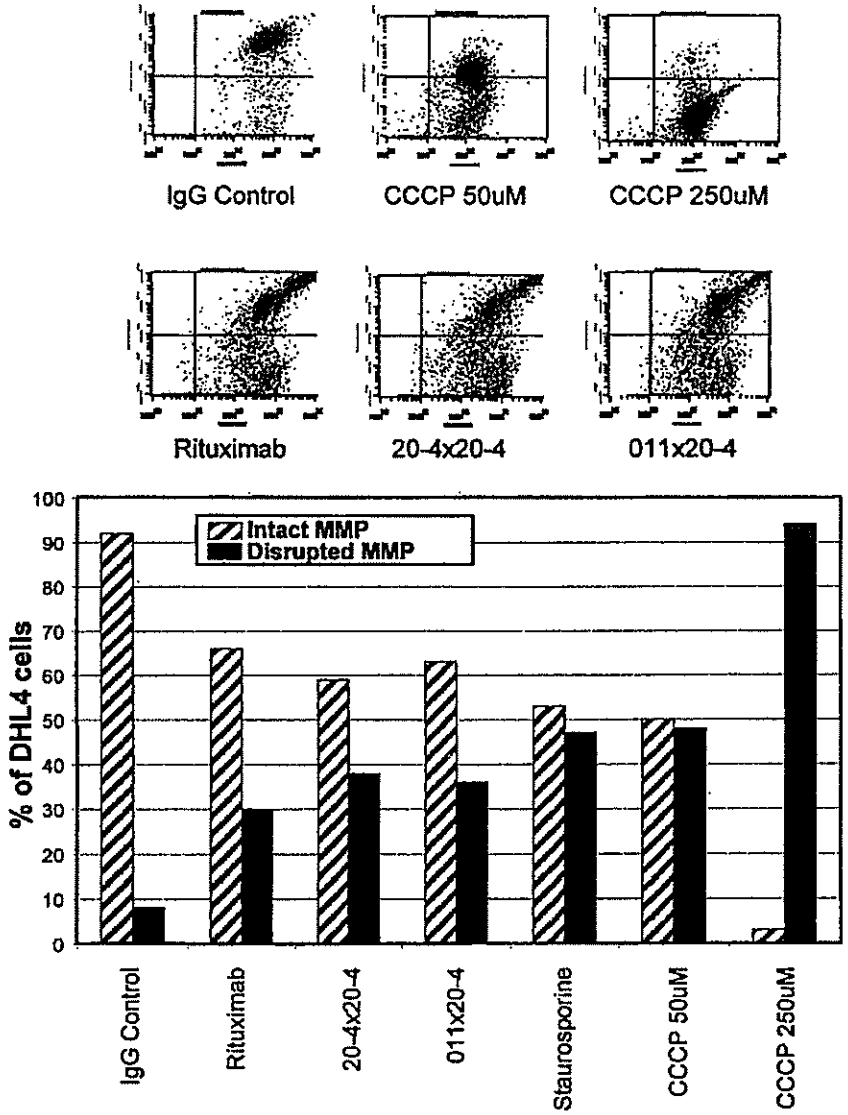

IgG Control
CCCP 50uM
CCCP 250uM
Rituximab
20-4x20-4
011x20-4
Intact MMP
Disrupted MMP
% of DHL4 cells
100
90
80
70
60
50
40
30
20
10
0
IgG Control
Rituximab
20-4x20-4
011x20-4
Staurosporine
CCCP 50uM
CCCP 250uM

FIG. 57

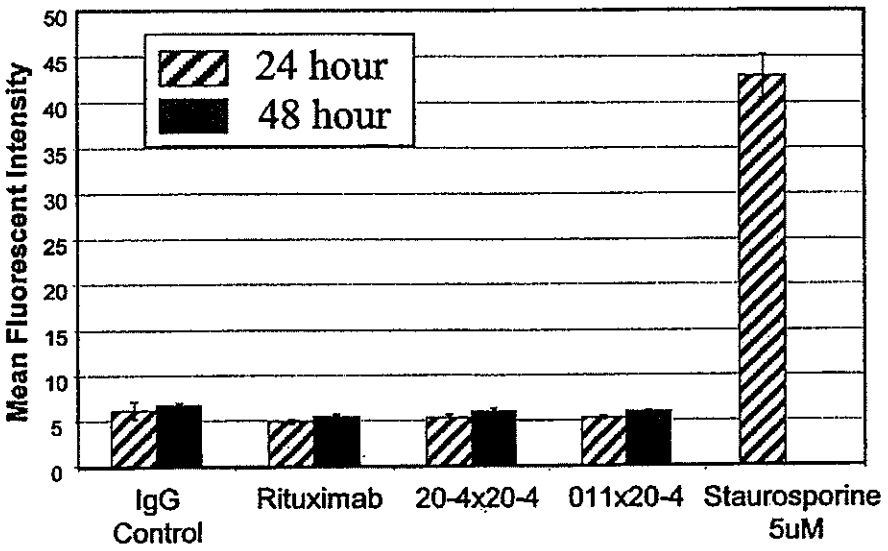
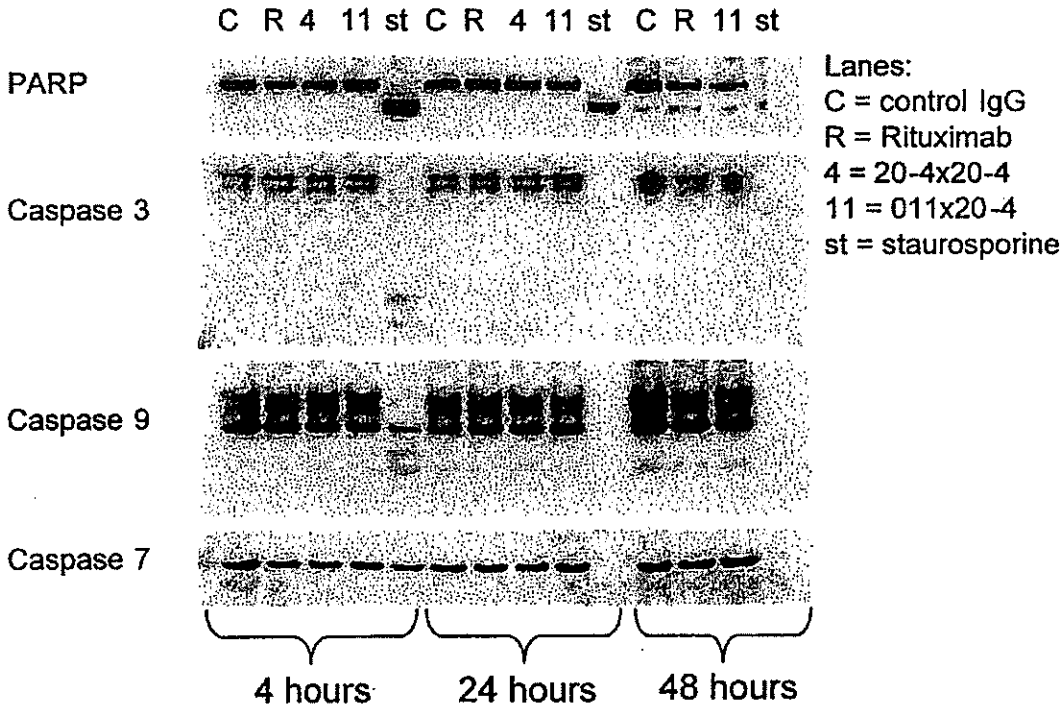

Mean Fluorescent Intensity
50
45
40
35
30
25
20
15
10
5
0
24 hour
48 hour
IgG Control
Rituximab
20-4x20-4
011x20-4
Staurosporine 5uM

FIG. 58


C R 4 11 st C R 4 11 st C R 11 st
PARP
Caspase 3
Caspase 9
Caspase 7
Lanes:
C = control IgG
R = Rituximab
4 = 20-4x20-4
11 = 011x20-4
st = staurosporine
4 hours
24 hours
48 hours

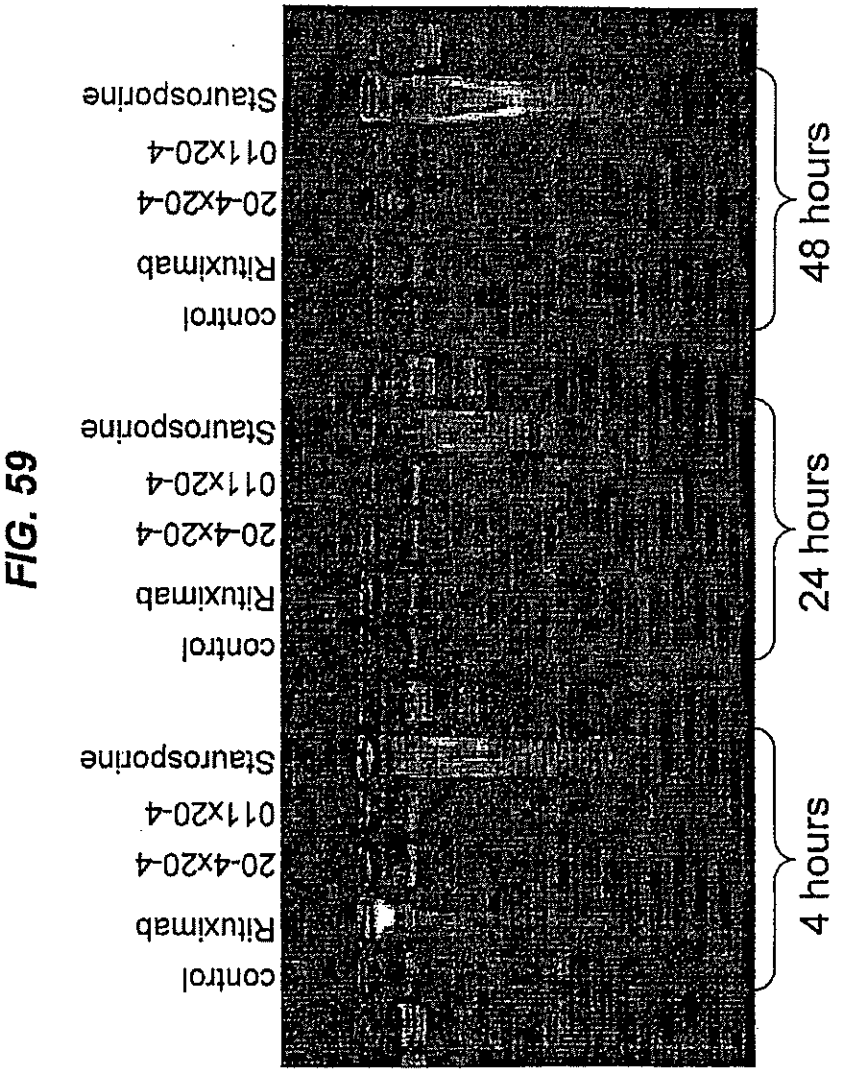
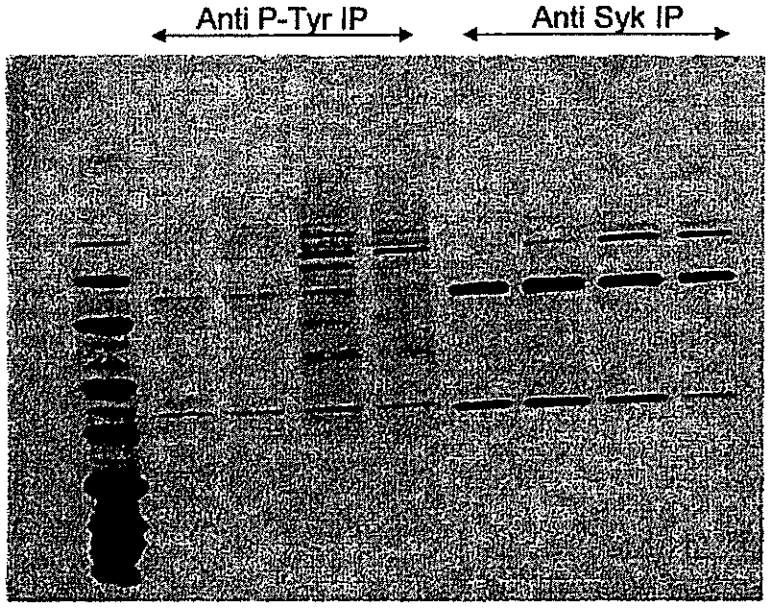

control
Rituximab
20-4x20-4
011x20-4
Staurosporine
control
Rituximab
20-4x20-4
011x20-4
Staurosporine
control
Rituximab
20-4x20-4
011x20-4
Staurosporine
4 hours
24 hours
48 hours

FIG. 59

FIG. 60

DHL6 Cells Treated with 20x37 Scorpion



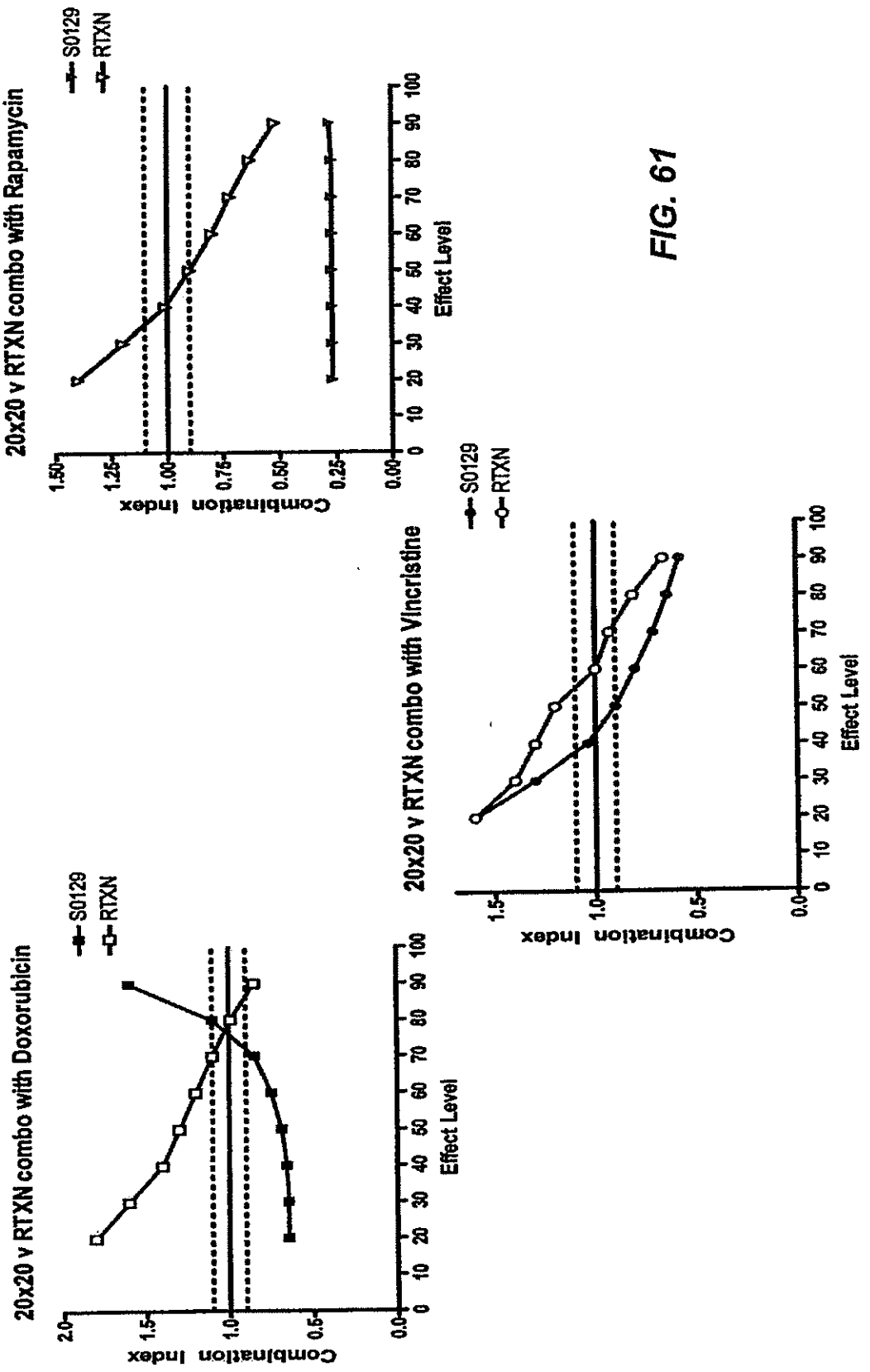

20x20 v RTXN combo with Rapamycin
S0129
RTXN
Combination Index
Effect Level
20x20 v RTXN combo with Vincristine
S0129
RTXN
Combination Index
Effect Level
20x20 v RTXN combo with Doxorubicin
S0129
RTXN
Combination Index
Effect Level

FIG. 61