

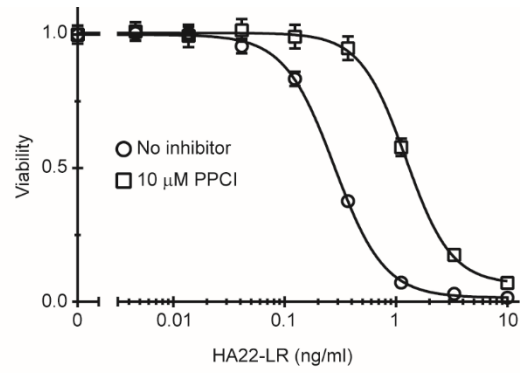
Table S1. Residue preferences at furin cleavage sites

Position	P6	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'
Most Common Residues at Furin Sites	R 14.5%	R 22.3%	R 85.6%	K 16.9%	K 45.8%	R 100%	S 33.1%	L/V 18.4%	S 13.6%	A 11.9%	A 12.6%
	K/S 10.5%	S 13.2%	I 5.9%	R 13.6%	R 36.4%	-	A 15.7%	A 17.6%	D/G 11.0%	Q/S 10.2%	G 10.9%
	P/T 9.7%	K 10.7%	V 4.2%	S 11.9%	A 4.2%	-	F 10.2%	S 10.4%	P 10.2%	T 9.3%	E 10.1%
PE Furin Site	R 14.5%	H 5.0%	R 85.6%	Q 9.3%	P 3.4%	R 100%	G 7.1%	W 0.8%	E 5.9%	Q 10.2%	L 7.6%

Table S2. HA22-LR variant proteins

RIT	Antibody	Linker	Furin Site	Catalytic Domain
1) HA22-LR			R H R Q P R G W E Q L	
2) HA22-LR R274H			H H R Q P R G W E Q L	
3) HA22-LR H275R			R R R Q P R G W E Q L	
4) HA22-LR Q277R			R H R R P R G W E Q L	
5) HA22-LR P278K	RFB4		R H R Q K R G W E Q L	
6) HA22-LR R279G	[GTHW]		R H R Q P G G W E Q L	PTGAE...REDLK
7) HA22-LR G280S	anti-CD22	KASGG	R H R Q P R S W E Q L	[Native PE 395-613]
8) HA22-LR W281L	disulfide- stabilized (ds)Fv*		R H R Q P R G L E Q L	
9) HA22-LR E282D			R H R Q P R G W D Q L	
10) HA22-LR Q283T			R H R Q P R G W E T L	
11) HA22-LR L284S			R H R Q P R G W E Q S	
12) HA22-LR/FUR			H R R R K R G L D T S	
13) HA22			Native PE 251-364	Native PE 381-613

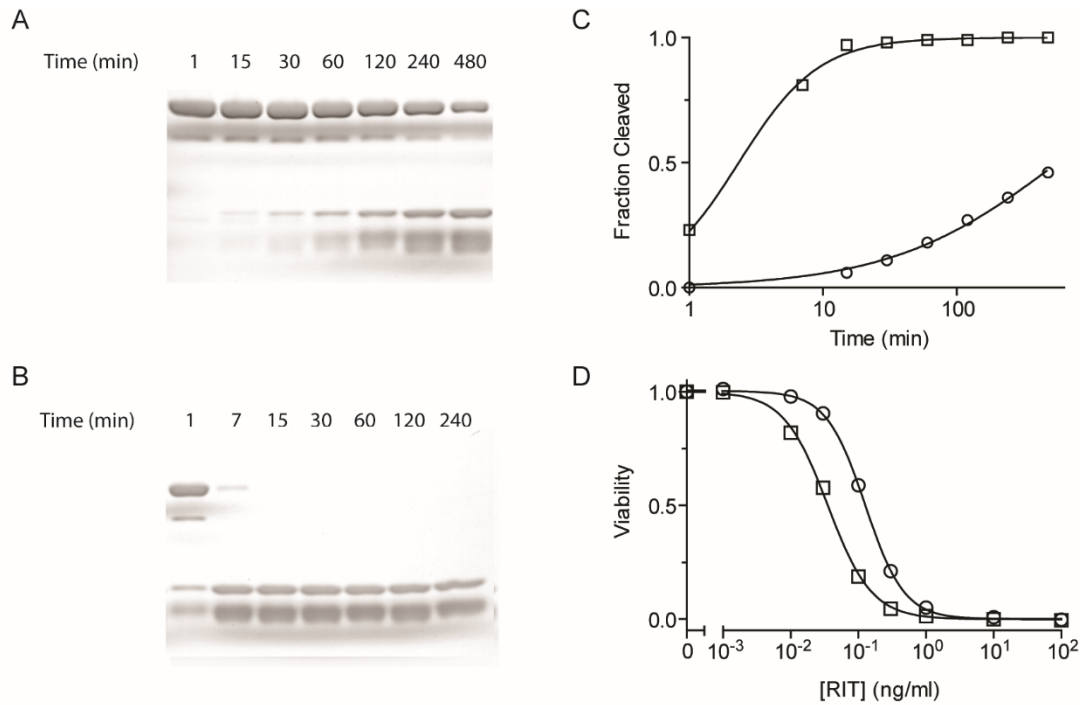
*Salvatore, G., Beers, R., Margulies, I., Kreitman, R. J., and Pastan, I. (2002) Improved cytotoxic activity toward cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display. *Clin. Cancer Res.* 8, 995-1002.



Supplementary Figure 1

Figure S1. HA22-LR cytotoxicity in the presence of a furin inhibitor.

The cytotoxicity of HA22-LR was evaluated against CA46 cells in the presence (open squares) and absence (open circles) of proprotein convertase inhibitor (PPCI).



Supplementary Figure 2

Figure S2. Representative analyses of furin site mutants. An *in vitro* assay was used to evaluate the efficiency of furin cleavage (A-C). Example SDS-PAGE gels from assays evaluating (A) HA22-LR and (B) HA22-LR/FUR are shown. Band intensity was quantified by densitometry, plotted against time, and fit to a four-parameter sigmoid model (C). The cytotoxicity of each mutant was also evaluated on the CA46 cell line (D). HA22-LR (open circles; $EC_{50} = 0.18$ ng/ml) and HA22-LR/FUR (open squares; $EC_{50} = 0.04$ ng/ml) are shown. A comparison of the relative cytotoxicity and relative cleavage efficiency for all variants is presented in Figure 2.