Recombinant Immunotoxin with T Cell Epitope Mutations that Greatly Reduce Immunogenicity for Treatment of Mesothelin Expressing Tumors

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SUPPLEMENTARY INFORMATION

Table S1. Cytotoxic activity in vitro in five cell lines

Disease model **Cell line** IC₅₀ (pMolar) SS1-LR-Relative Relative SS1P LMB-T20 **GGS** activity activity* Mesothelioma HAY 9 ± 2 2 ± 0 450% 3 ± 1 300% 335% Pancreatic cancer KLM1 47 ± 17 7 ± 1 671% 14 ± 0 L55 41 ± 13 205% 24 ± 6 178% Lung cancer 20 ± 2 Stomach cancer MKN74 33 ± 10 7 ± 2 471% 13 ± 1 253% Epidermoid 25% A431/H9 0.4 ± 0 1.3 ± 0 31% 1.6 ± 0 carcinoma

± SEM

 IC_{50} values were determined by averaging IC_{50} of three independent assays

^{*}Relative activity compared to SS1P

Table S2. In vivo toxicity of RITs

	Dose (mg/kg)	Response	Average change in weight	Number of doses	Frequency	Number of mice
SS1-LR-	5	1/8 Died	(-5%)	2	QOD	8
GGS	10	2/2 Died	*	1	-	2
LMB-T20	5	Healthy	(-0.1%)	6	QD	8
	10	Healthy	(-1%)	2	72 hours	3
	15	Healthy	(-9%)	2	72 hours	3
	20	1/2 Died	(-12%)	2	72 hours	2

^{*}No data-mice died prior to weight measurement

QOD, every other day

QD, every day

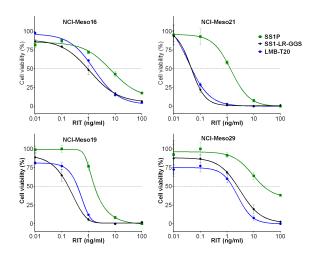


Figure S1. Cytotoxic activity in patients cells curves.

Cells cultured from the pleural fluid or ascites of four mesothelioma patients (NCI-Meso16, NCI-Meso19, NCI-Meso21, and NCI-Meso29) were treated with increasing concentrations of RITs. After 72 hours, cells were evaluated for viability using a WST-8 assay and IC $_{50}$ were calculated. The IC $_{50}$ are plotted in Figure 5. Cells were treated in three replicas. Line represents mean, error bar show SEM

IC₅₀ (SS1P: 111pM. 51pM. 22pM and 146pM, for SS1-LR-GGS: 22pM, 4pM, 1pM and 57pM and for LMB-T20 33pM, 8pM, 1pM and 51pM in patients NCI-Meso16, NCI-Meso19, NCI-Meso21 and NCI-Meso29, respectively).