

**SUPPLEMENTARY METHODS:*****In vivo* efficacy studies**

Female NSG-NOD scid mice (Experimental Radiation Oncology Breeding Core from MD Anderson) (6 weeks old, five mice per group) were injected with  $5 \times 10^6$  cells of MDA-MB-231 at the mammary fat pad (1:1 v/v with matrigel). Once tumor volumes reached 40-60 mm<sup>3</sup>, animals were treated every other day for 5 total injections with PBS (IV, tail vein) or and GrB(C210A)-Fc-IT4 (100 mg/kg) (IV or IP). Animals were monitored and tumor growth was measured every 3-4 days for an additional 40 days. Data are presented as mean tumor volume (mm<sup>3</sup>)  $\pm$  SD.

**SUPPLEMENTARY TABLES****Table 1S.** Effect of time/exposure on GrB(C210A)-Fc-IT4 cytotoxicity on MDA-MB-231 cells.

<b>Time of exposure (h)</b>	<b>IC<sub>50</sub> (nmol/L)<sup>1</sup></b>
1	245 $\pm$ 46
2	184 $\pm$ 25
4	261 $\pm$ 46
8	136 $\pm$ 15
12	81 $\pm$ 21
24	46 $\pm$ 12
48	36 $\pm$ 8
72	11 $\pm$ 5

<sup>1</sup> Mean  $\pm$ SD of at least 3 independent experiments.

**Table 2S.** Macroscopic and microscopic findings in the different organs and tissues extracted from mice in acute and recovery groups.

Organ Morphologic Diagnosis	Acute		Recovery	
	Vehicle	GrB(C210A)-Fc-IT4	Vehicle	GrB(C210A)-Fc-IT4
<b>Liver/Gall Bladder</b>				
Inflammation, chronic, multifocal, interstitium		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Lung</b>				
Ectopic tissue, focal, bone, bronchiole				minimal, rare <10%
Adenoma/adenocarcinoma, alveolar-bronchiolar				minimal, rare <10%
<b>Spleen</b>				
Hyperplasia, lymphoid, diffuse, white pulp		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Heart</b>				
Inflammation, chronic, focal, interstitium, myocardium				minimal, rare <10%
<b>Adrenal Glands</b>				
Degeneration, X-zone, multifocal, corticomedullary junction		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Brain</b>				
Cyst, epidermal inclusion				cyst minimal, rare <10%
Gliosis, multifocal, neuropil with apoptotic cells				minimal, rare <10%
<b>Nose</b>				
Malformation, focal, septum & turbinates				minimal, rare <10%
<b>Eyes, Ears</b>				
Inflammation, acute, diffuse, bilateral, middle-ear				minimal, rare <10%
<b>Stomach</b>				
Depletion, Parietal cells, multifocal, mucosa		minimal, rare <10%	minimal, rare <10%	Mild, infrequent 10-20%
Metaplasia, intestinal, multifocal, mucosa		minimal, rare <10%		minimal, rare <10%
Hyperplasia, epithelial, multifocal, mucosa		minimal, rare <10%		
Inflammation, chronic, multifocal, interstitium		minimal, rare <10%		
<b>Jejunum</b>				
Inflammation, acute, multifocal, submucosal		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Colon and Rectum</b>				
Inflammation, acute, multifocal, submucosal		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Mesenteric Lymph Node</b>				
Hyperplasia, lymphoid, multifocal, cortex		minimal, rare <10%	minimal, rare <10%	minimal, rare <10%
<b>Salivary Gland</b>				
Regeneration, acinar cells, focal, periductular				minimal, rare <10%
<b>Mammary Gland</b>				
Inflammation, chronic, focal, periductular				
Hyperplasia, epithelial, focal, subcutaneous		minimal, rare <10%		
<b>Urinary Bladder</b>				
Inflammation, acute, multifocal, submucosal				
Inflammation, chronic-active, multifocal, submucosal		minimal, rare <10%		minimal, rare <10%
<b>Femur/Knee Joint</b>				
Inflammation, acute, multifocal, periosteal		minimal, rare <10%		
Degeneration, focal, myocytes, skeletal muscle, periosteal		minimal, rare <10%		
Regeneration, focal, myocytes, skeletal muscle, periosteal		minimal, rare <10%		
Proliferation, focal, synovial membrane		minimal, rare <10%		

**SUPPLEMENTARY FIGURE LEGEND:**

**Figure 1S.** *In vivo* activity of the GrB(C210A)-Fc-IT4 fusion construct on an orthotopic MDA-MB-231 tumor model. A, MDA-MB-231 cells were implanted under the mammary fat pad of NSG-NOD scid mice and groups of mice (n=5) were treated with vehicle (PBS 10% Maltose ●), GrB(C210A)-Fc-IT4 (100 mg/kg IV □) or GrB(C210A)-Fc-IT4 (100 mg/kg IP ▲). Treatment was every other day when the tumors reached approximately 75 mm<sup>3</sup>. Arrows represent IV treatment via tail vein and diamonds represent IP treatment. Tumor size was assessed by direct caliper measurement. Efficacy data was plotted as mean tumor volume (in mm<sup>3</sup>) ± SD. B, Fold change of tumor size on day 48 after tumor implantation normalized to initial tumor size before treatment (day 7). Data are plotted as fold change of individual tumors ± SD. *P*<0.0021, GrB(C210A)-Fc-IT4 IP group compared with vehicle; *P*<0.0013, GrB(C210A)-Fc-IT4 IV group compared with vehicle.