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Supplementary Materials for

Deglycosylated bleomycin has the antitumor activity of bleomycin without pulmonary toxicity

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/8/326/326ra20/DC1)

Figure #S1

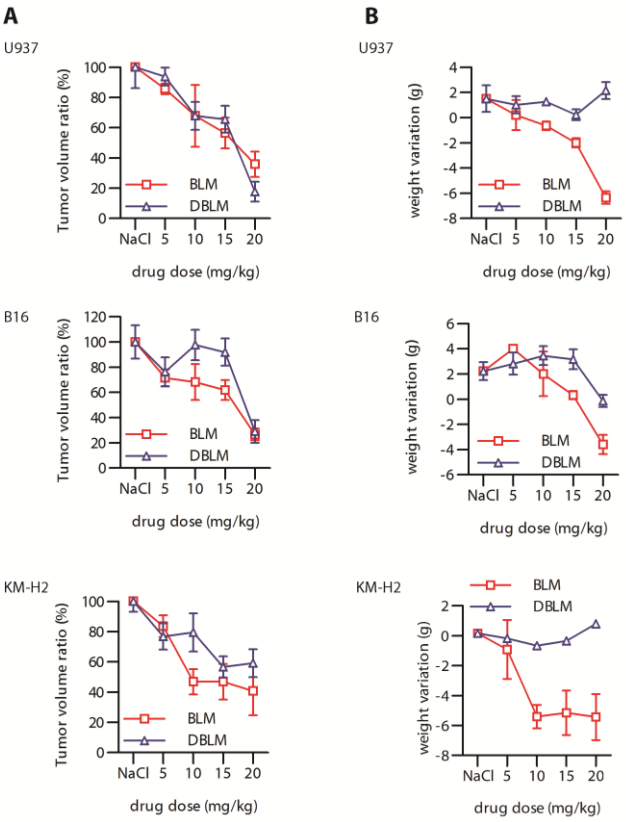

Figure S1. *Deglyco-BLM has the same antitumoral profile as BLM but lacks toxic side effects.* U937, B16, or KM-H2 cells were subcutaneously injected into nude, C57BL/6, or NOG mice, respectively. When the tumor volume reached 62.5 mm^3, mice received i.p. injections of NaCl, BLM, or deglyco-BLM (DBLM) every other day for 8 (U937, B16) or 10 (KM-H2) days. A) Dose-dependent effect of BLM and DBLM (5, 10, 15, and 20 mg/kg) after 8 or 10 days. Data presented as the ratio of tumor size in the BLM- and DBLM-treated normalized to the NaCl-treated animals. B) Difference in weight between mice treated with BLM or DBLM at the indicated doses compared to NaCl-treated mice.

Figure #S2

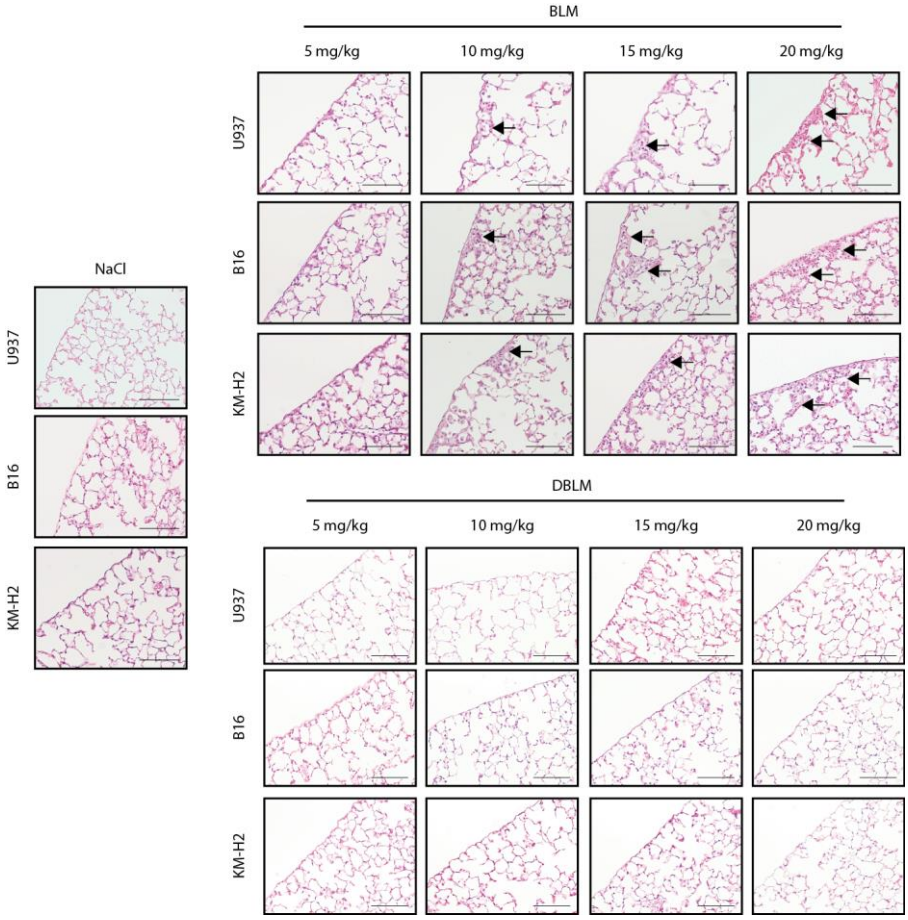

Figure S2. *BLM, but not deglyco-BLM, induces lung toxicity.*

U937, B16, or KM-H2 cells were subcutaneously injected into nude, C57BL/6, or NOG mice, respectively. When the tumor volume reached 62.5 mm^3, mice received i.p. injections of NaCl, BLM, or DBLM every other day. Representative histology after 8 (U937, B16) or 10 (KM-H2) days at indicated doses (H&E staining, scale bars = 100 μm, arrows highlight the injured areas). n=6 mice/group.

Figure #S3

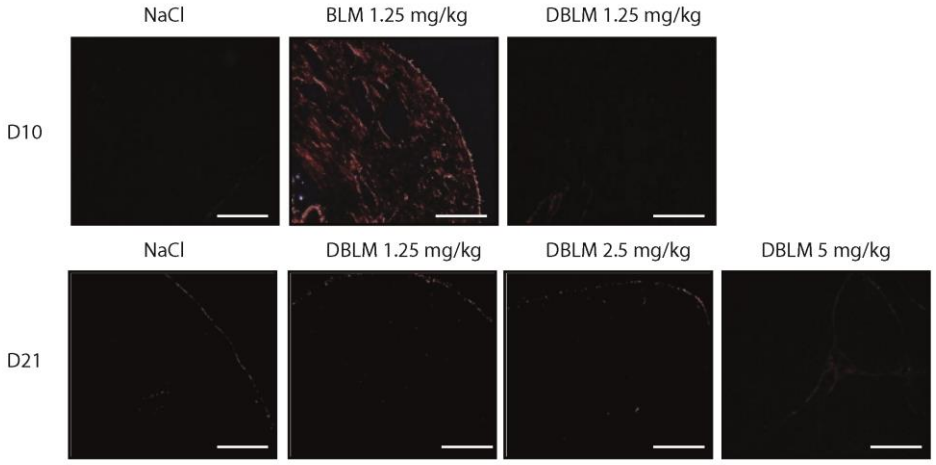

Figure S3. *BLM, but not deglyco-BLM, induces collagen accumulation in the lung.*

Collagen accumulation in C57BL/6 mouse lung sections, 10 and 21 days after i.t. administration of BLM, DBLM, or NaCl (Picrosirius red staining, scale bars = 100 µm, n=9 mice in BLM or DBLM groups, n=6 mice in NaCl group).

Figure #S4

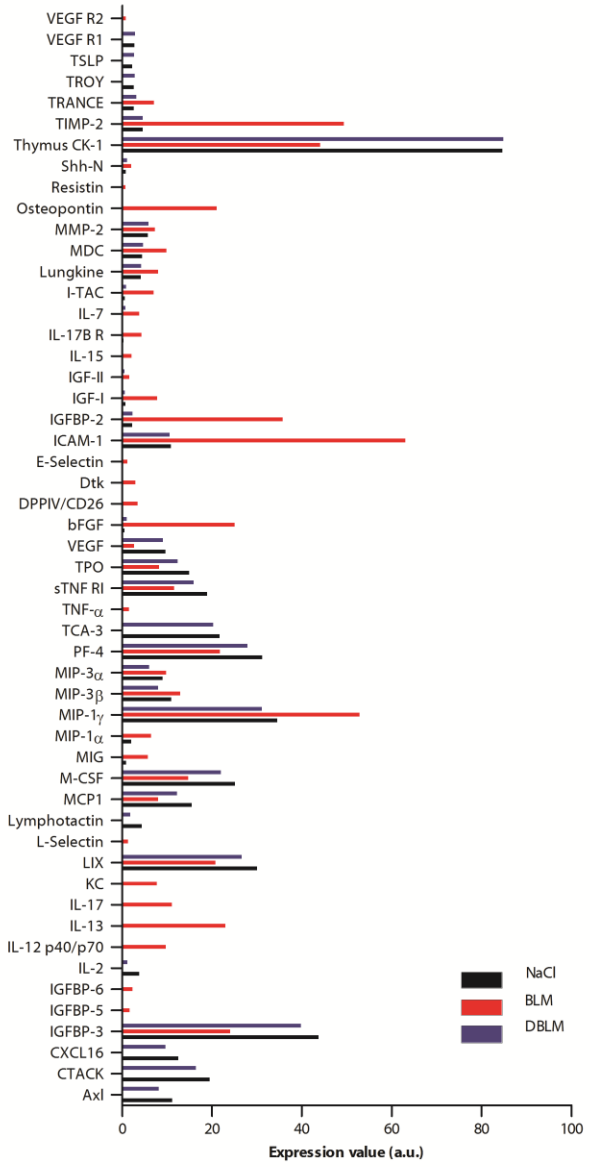

Figure S4. *Deglyco-BLM does not induce inflammation and/or fibrosis-related cytokines.*

Cytokine concentrations were measured in the lungs of mice treated with NaCl, BLM, or DBLM (5 mice per group) at day 4. Expression value is presented as arbitrary units (a.u.).

Figure #S5

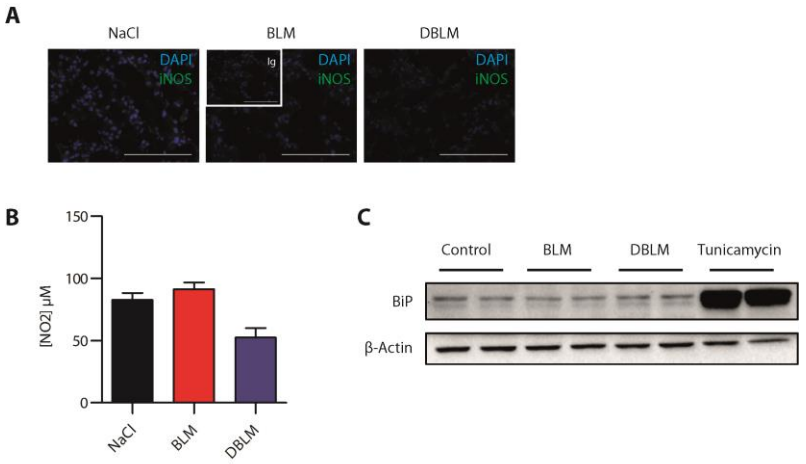

Figure S5. *Deglyco-BLM and BLM do not induce nitrogen species or ER stress in A549 cells.*

A) NO amount was determined in lung sections from mice i.t. injected with BLM, DBLM (2.5 mg/kg), or NaCl (n=5/group) using iNOS immunostaining (green). Nuclei are stained with DAPI (blue). Insert Ig, negative IgG control. Scale bars = 50 µm. B) Nitrite concentration in lung lysates from the mice described in A. Data shown as mean ± SEM. C) Western blot analysis for the ER stress marker BiP in cells treated for 24 hours with NaCl (control), BLM, DBLM (100 µM), or tunicamycin (ER stressor, positive control, 0.1 µg/ml).

Figure #S6

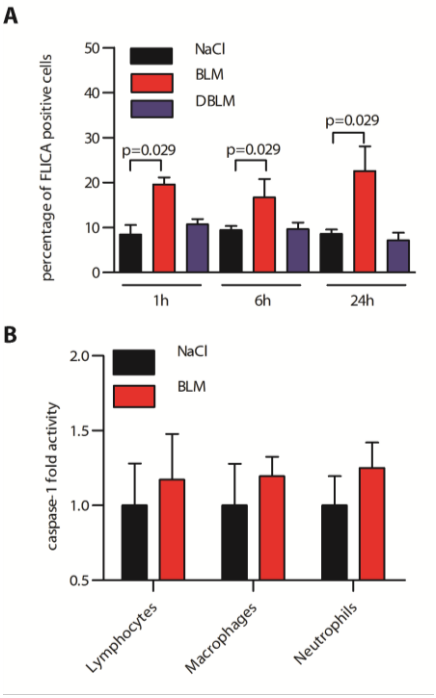

Figure S6. *BLM induces caspase-1 activation in alveolar epithelial cells but not in immune cells.* A) FLICA-measured caspase-1 activity in A549 cells either left untreated or treated with BLM or DBLM (200 µM). Representative data (3 independent experiments). B) Lungs from C57BL/6J mice intra-tracheally injected with NaCl or BLM (5 mg/kg) were collected at D3, and lung homogenates were stained for caspase-1 with FLICA probe as well as for lymphocyte (CD3), macrophage (F4/80), and neutrophil (Ly6G) markers. Staining was followed by flow cytometry. Data are presented as mean ± SEM of caspase-1 activation normalized to the control.

Figure #S7

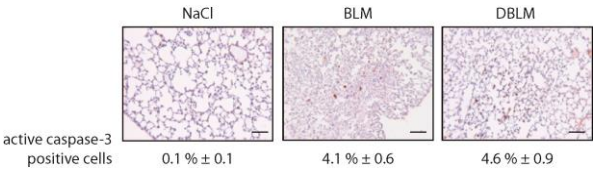

Figure S7. *Deglyco-BLM and BLM induce similar caspase-3 cleavage.*

Cleaved caspase-3 was detected in the parenchyma of the lungs in nude mice after NaCl, 20 mg/kg BLM, or DBLM i.p. administration every other day for 8 days. Representative images are shown. 5 mice/group. Scale bars = 100 μm.