Targeting the MUC1-C oncoprotein is associated with downregulation of TIGAR and ROS- mediated CTCL cell death

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OBJECTIVES

Evaluate the role of the Mucin-1 oncoprotein in the pathogenesis cutaneous T cell lymphoma.

METHODS AND RESULTS

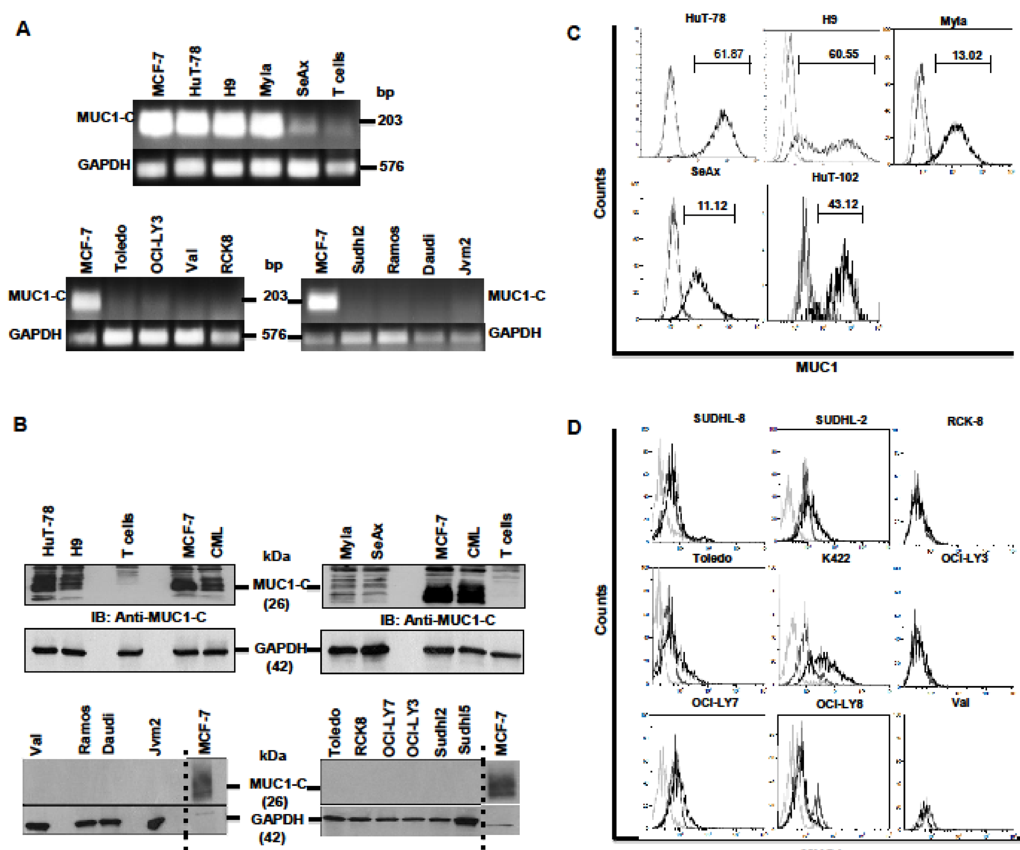

Figure 1. Expression of MUC1 by CTCL cell lines. Sezary Syndrome (HuT-78, H9, SeAx) and Mycosis Fungoides (Myla, HuT-102) cell lines were analyzed for MUC1 mRNA expression by reverse transcription (RT)-PCR. The results demonstrated prominent expression of MUC1 in the CTCL cells and minimal levels in normal T cells from healthy donors and in B-cell lymphoma cells (Figure. 1A). These findings were confirmed by immunoblot analysis which showed high levels of the MUC1-C cytoplasmic protein in CTCL cell lines in comparison to T cells from normal healthy donors and B-cell lymphoma cell lines including diffuse large B-cell lymphoma (Val, Toledo, RCK-8, OCI-LY7, OCI-LY3, SUDHL2, SUDHL5), Burkitt's (Ramos and Daudi) and Mantle cell lymphoma (JVM-2) (Figure. 1B). Flow cytometric analysis of the cells with another antibody, DF3 (anti-MUC1-N) targeting the extracellular N-terminal subunit further demonstrated that MUC1 is expressed in 11-62 % of the sezary cells and 13-43 % of the mycosis cells as opposed to 0-2 % of the B-cell lymphoma cells (Figures. 1C and 1D).

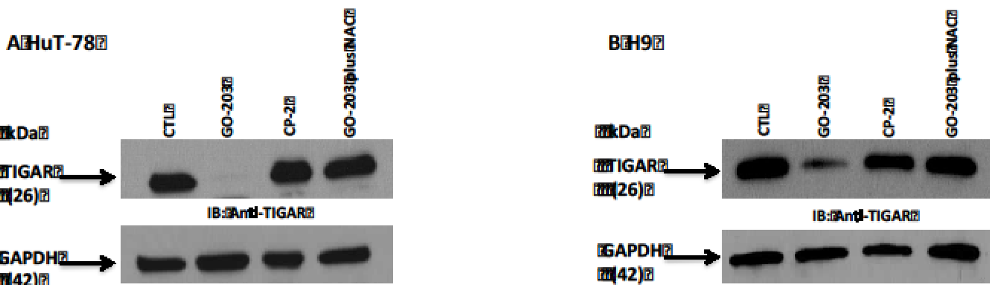

Figure 4. MUC1 inhibition is associated with decreased levels of TIGAR. To investigate the mechanism by which MUC1 inhibition results in increased ROS levels in CTCL, we examined the impact of GO-203 on TIGAR expression, a p53-inducible protein that regulates glycolysis and protects against oxidative stress. We demonstrated that exposure of CTCL cells to GO-203 was associated with the marked reduction of levels of TIGAR in HuT-78 and H9 cells (Figures. 4A and 4B).

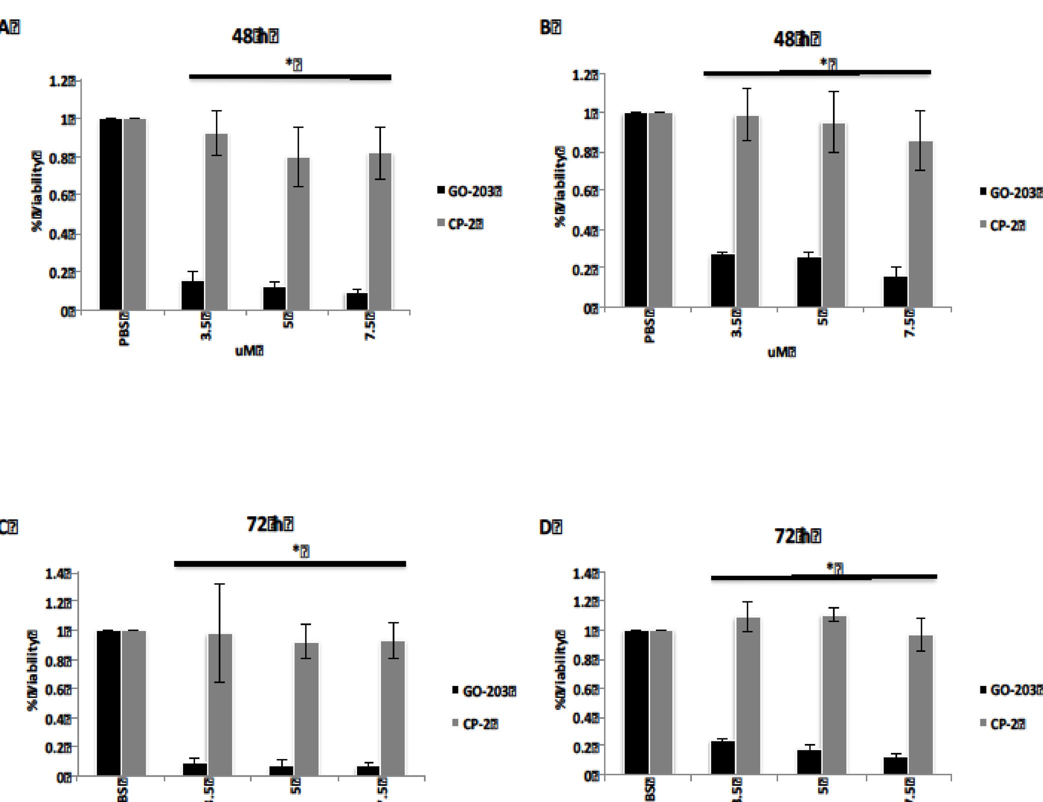

Figure 7. Targeting of MUC1-C in primary CTCL cells with GO-203. Consistent with these findings, exposure to GO-203 induced cell death in primary CTCL cells. The malignant clonal population was isolated from 3 primary CTCL samples by flow cytometric sorting for the TCR Vβ of interest (Figure. 2D). Culture of these cells with different concentrations of GO-203 (3.5-7.5 uM) resulted in decreased viability at 48 hours and 72 hours (10-30%) as opposed to CP-2 treated and untreated cells (80-100 %) (Figures. 7A-7D

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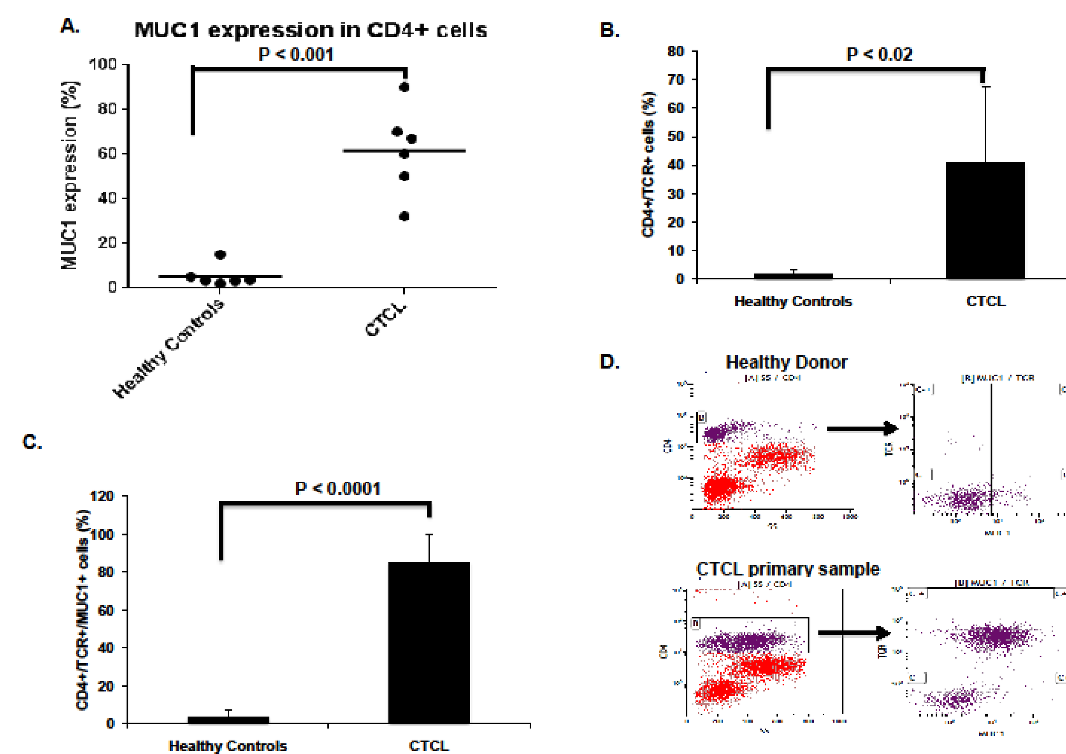

Figure 2. MUC1 expression in primary CTCL cells as compared to T cells derived from normal healthy donors. Based on the findings with CTCL cell lines, PBMCs from six L-CTCL patients with previously identified CD4 dominant TCR Vβ malignant clones were analyzed for MUC1 expression by flow cytometry and compared to PBMCs from 6 healthy donors as controls[20]. Analysis of the unselected CD4+ population demonstrated high levels of MUC1 expression in L-CTCL samples (avg. 61.5 %) as compared to normal healthy donor controls (avg. 5.34 %) that was statistically significant (Figure. 2A). The dominant malignant clonal population was further isolated using the previously identified specific TCR Vβ which comprised a mean of 40.65 % as compared to 1.7 % of the CD4+ cells derived from the CTCL patients and normal controls, respectively (Figure. 2B). Consistent with the prior findings, a majority of the cells in the CTCL patients that expressed the TCR Vβ malignant clone also exhibited MUC1 expression (avg. 85.43 %) in comparison with the normal healthy donor controls (avg. 3.66 %) (Figure. 2C). A representative FACS plot of a CTCL patient (in which TCR Vβ1 is re-arranged in the malignant cells) and corresponding healthy donor are shown in Figure. 2D).

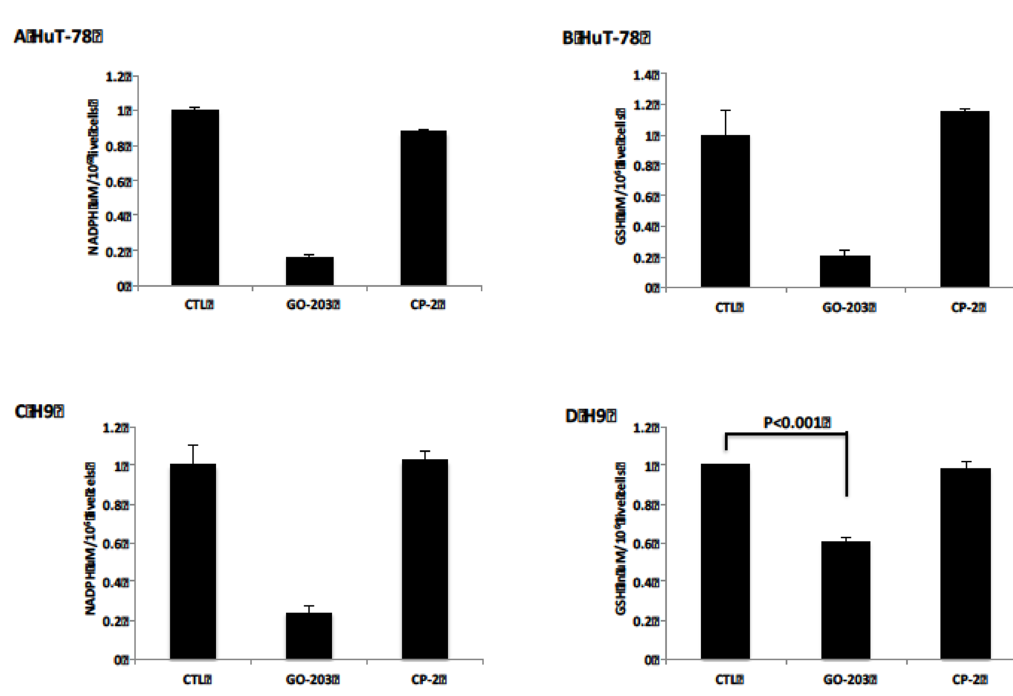

Figure 5. TIGAR redirects glycolytic intermediates to the pentose phosphate pathway, increasing NADPH production. Accordingly, inhibition of MUC1-C and down-regulation of TIGAR in HuT-78 cells was associated with a marked decrease in NADPH levels (Figure. 5A). NADPH is necessary for the production of GSH and the scavenging of ROS. In this regard, GO-203 treatment resulted in a reduction in GSH levels in HuT-78 cells (Figure. 5B). Similar findings were observed in H9 cells (Figures. 5C and 5D). In summary, inhibition of MUC1-C results in increased levels of ROS in the context of reduction in TIGAR and thereby NADPH and GSH

Conclusions

1. MUC1-C expression is upregulated in CTCL cells and patient samples.
2. MUC1-C inhibition initiates a cascade of (a) down-regulation of TIGAR (b) decreases in NADPH and GSH (c) ROS production; and (d) ROS-mediated late apoptosis/necrosis.
3. The first-in-man MUC1-C inhibitor has entered phase 1 evaluation in patients with AML to establish a maximum tolerated dose that could be used for treatment of patients with relapsed/refractory CTCL.
4. Our finding of MUC1 inhibition leading to increased sensitivity of the CTCL cells to stress induced apoptosis has led us to explore synergistic combination strategies of GO-203 with other drugs with marked anti T-cell lymphoma activity.

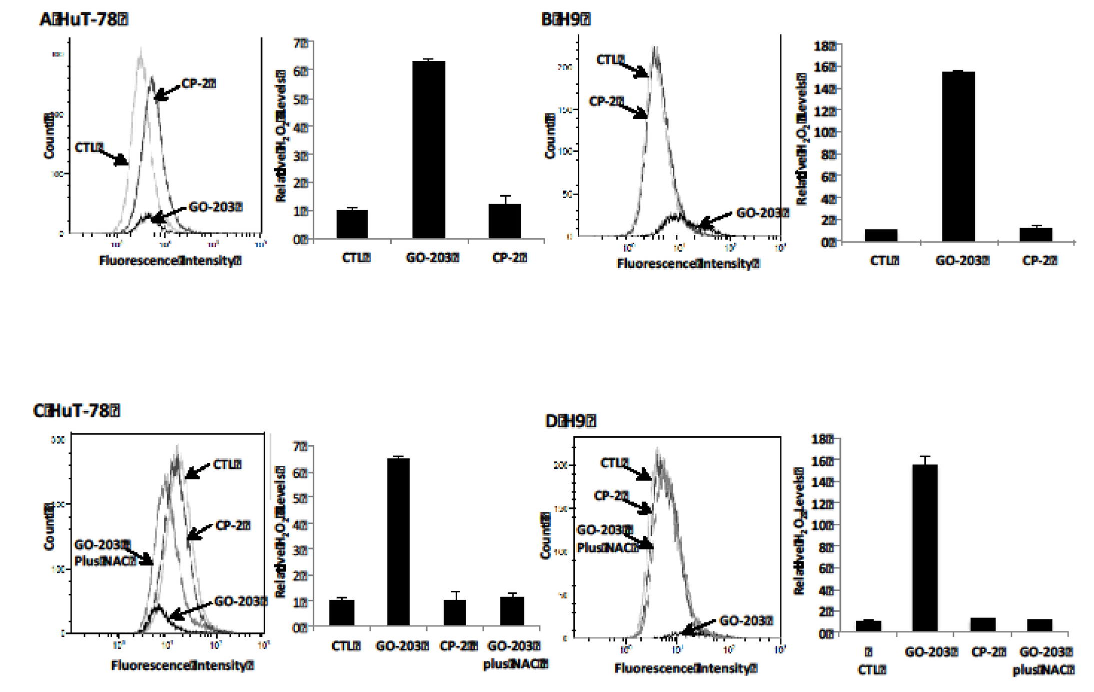

Figure 3. Inhibition of MUC1-C increases ROS in CTCL cells. Treatment of HuT-78 sezary cells with the MUC1-C inhibitor, GO-203, was associated with increases in hydrogen peroxide levels in contrast to that obtained with, CP-2 (Figure. 3A). Similar results were obtained in GO-203–treated H9 sezary cells (Figure. 3B). The induction of ROS by GO-203 was reversed by concurrent exposure of HuT-78 and H9 cells to the antioxidant NAC that promotes the scavenging of ROS (Figures. 3C-3D).

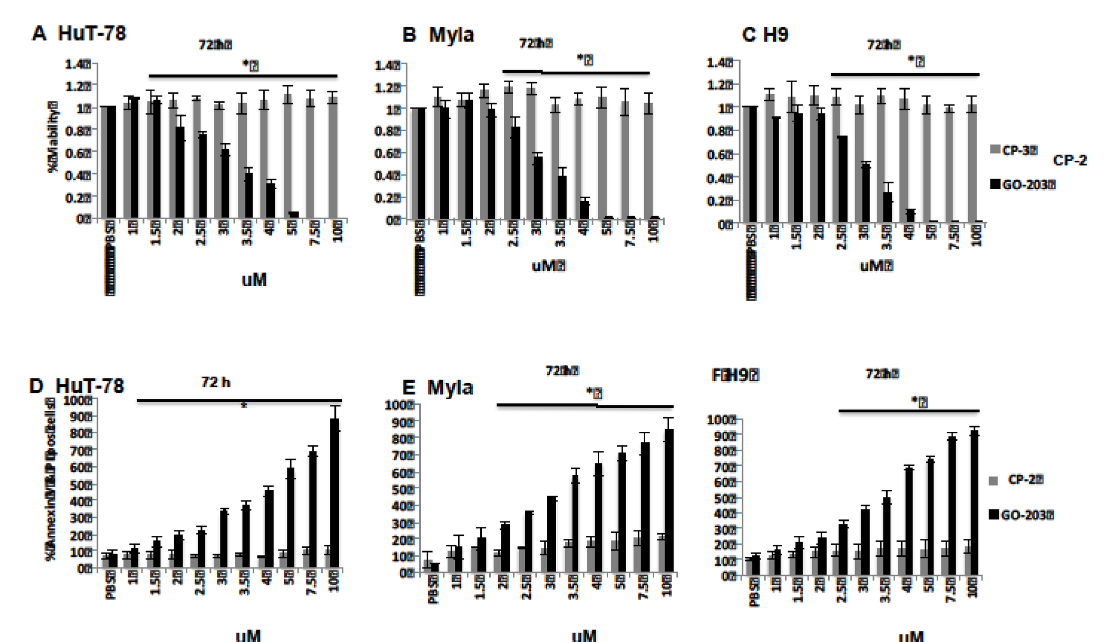

Figure 6. GO-203 induces apoptosis and cell death in CTCL. CTCL cells were co-cultured with escalating concentrations of the MUC1-C inhibitor GO-203 (1 to 10 uM/L) and levels of apoptosis and cell death was quantified at 48, 72 and 96 hours of exposure. The Sezary cell line HuT-78, H9 and MF cell line Myla demonstrated dose and time dependent sensitivity to GO-203 relative to CP-2 starting as low as 3 uM at 48 hours. Cell viability was reduced to 40-60% at 72 hours in all cell lines when incubated at 3.5 uM GO-203; by contrast, CP-2 had little effect (Figures. 6A, 6B and 6C). Flow cytometric analysis of propidium iodide (PI)/annexin V–FITC staining in the treated CTCL cells (Figures. 6D, 6E and 6F) was consistent with late apoptosis/necrosis in 65 %, 62 % and 74 % of HuT-78, Myla and H9 cells, respectively. In contrast, exposure to the control peptide, CP-2 alone, resulted in apoptosis/necrosis in 8 %, 12 % and 11 % of the HuT-78, Myla, and H9 cells, respectively).

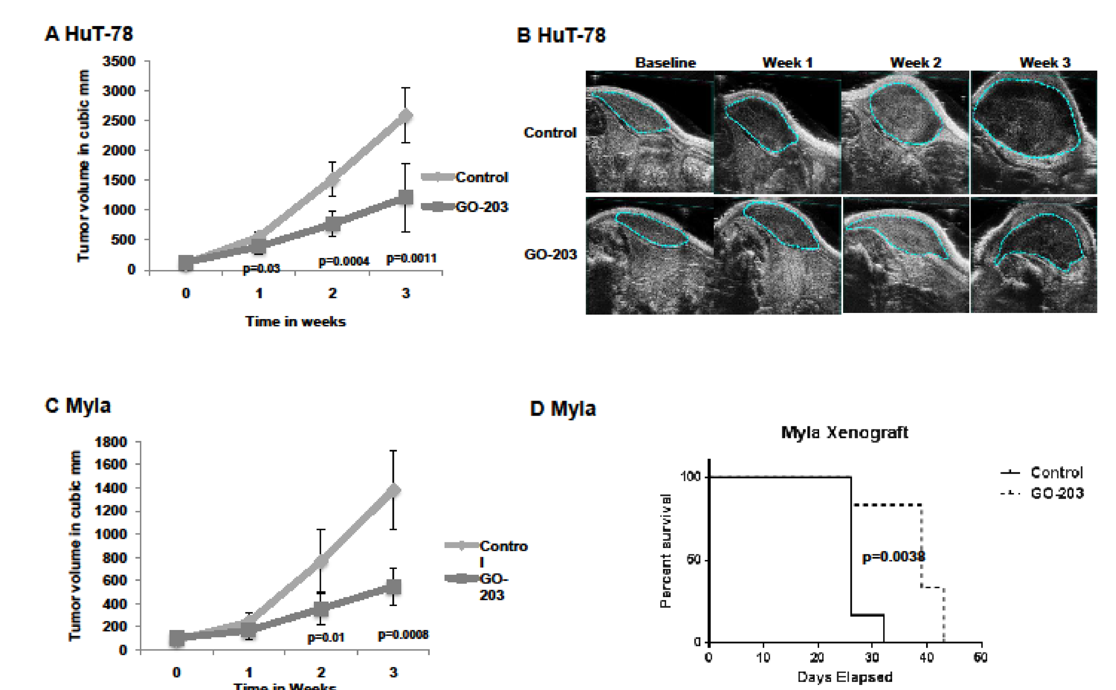

Figure 8. Inhibition of MUC1-C induces tumor reduction in xenograft murine models of CTCL. To assess *in vivo* antitumor activity of GO-203, HuT-78 cells were inoculated s.c in the flank of NSG mice. After the xenograft tumors reached a volume of approximately 100 mm3 as determined by 3D ultrasonography, they were randomized to control (treated with PBS) versus GO-203 treatment daily via id injection for 21 days. At least a 2.5 fold reduction of tumor volume was seen with the administration of GO-203 compared with that obtained with the vehicle (PBS)

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