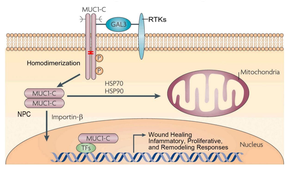
MUC1-C expression is associated with an immunosuppressive TME and represents a promising target for ADC therapy in NSCLC

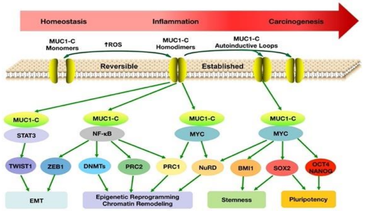
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Background

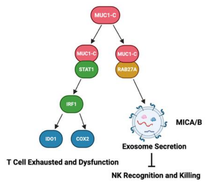
Lung cancer remains the leading cause of cancer-related mortality worldwide, and the prognosis of patients with advanced or unresectable non-small cell lung cancer (NSCLC) remains unsatisfactory. Immune checkpoint inhibitors (ICIs) have transformed the treatment landscape of NSCLC and have recently been incorporated into perioperative treatment strategies for resectable disease. Although perioperative ICIs improve clinical outcomes, a substantial proportion of patients still experience recurrence, highlighting the need to identify mechanisms of immune resistance and develop therapeutic strategies capable of enhancing antitumor immunity.

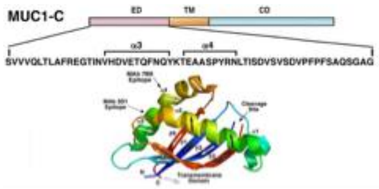
Activation of the MUC1-C Subunit
Wound Healing → Cancer Progression





An immunologically “cold” tumor microenvironment (TME), characterized by limited infiltration and impaired function of tumor-reactive immune cells, is strongly associated with ICI resistance. Cancer cells can also acquire an epigenetically maintained memory of prior immune pressure, enabling rapid reactivation of immune-resistance programs after subsequent immune attack. This immune-evasion memory is mediated, at least in part, by chronic interferon signaling and persistent activation of STAT1-regulated interferon-stimulated genes (ISGs), including OAS1 [Qiu et al., 2023].
MUC1-C is the oncogenic transmembrane subunit of MUC1, a protein that evolved to protect epithelial barrier tissues from loss of homeostasis. In cancer, MUC1-C promotes proliferation, epithelial–mesenchymal transition, cancer stem-like properties, and resistance to anticancer therapies [Kufe, Nat Rev Cancer 2009; Kufe, Carcinogenesis 2020]. MUC1-C also contributes to immune evasion by inducing PD-L1 expression and suppressing antitumor immunity [Bouillez et al., 2017]. Furthermore, MUC1-C silencing suppresses OAS1 and other ICI resistance-associated ISGs in molecularly targeted therapy-resistant NSCLC cells. Similar findings in breast cancer models support a reproducible role for MUC1-C in regulating interferon-responsive immune-resistance programs [J Immunother Cancer. 2021].



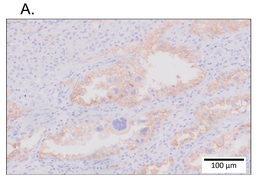


There are currently no approved agents targeting MUC1-N or MUC1-C. Previous approaches targeting MUC1-N have shown limited efficacy, partly because MUC1-N is shed from the cancer-cell surface, generating a circulating antigen pool that can interfere with antibody delivery. In contrast, MUC1-C remains on the cell surface and contains activation-exposed extracellular α-helices that are accessible to antibody targeting [Pancham et al., 2018; Kufe, 2020]. MAb3D1 binds the α3 helix of the MUC1-C extracellular domain with low-nanomolar affinity and selectively recognizes MUC1-C-expressing EGFR-mutant NSCLC cell lines and primary NSCLC cells. MAb3D1 is internalized following cell-surface binding, enabling intracellular delivery of a cytotoxic payload. It was therefore conjugated through a cleavable linker to monomethyl auristatin E (MMAE), a tubulin-disrupting agent used in clinically approved antibody–drug conjugates (ADCs). The resulting MAb3D1–MMAE ADC reduced NSCLC-cell viability and demonstrated antitumor activity in syngeneic tumors, human tumor xenografts, and patient-derived xenograft models without apparent toxicity in MUC1-transgenic mice [Pancham et al., 2018].
Based on these findings, we investigated the association between tumor-cell MUC1-C expression and the immune TME in clinical NSCLC specimens and evaluated MUC1-C as a potential therapeutic target for ADC therapy.

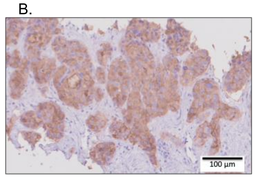
Result

1. MUC1-C Expression in Resected Lung Adenocarcinoma

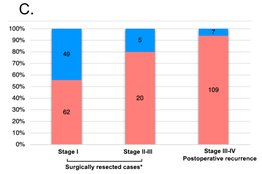
A.



B.



C.



MUC1-C expression was assessed in completely resected pathologic stage I–III lung adenocarcinoma specimens by immunohistochemistry using the MUC1-C rabbit monoclonal antibody D5K9I (Cell Signaling Technology; 1:100) following antigen retrieval with Target Retrieval Solution (pH 6) in an autoclave. Cytoplasmic and membranous staining was evaluated using the Allred score, and tumors were classified as MUC1-C-low or MUC1-C-high using the median score of 6 as the cutoff.
Representative images of MUC1-C-low (A) and MUC1-C-high tumors (B) are shown. Scale bars, 100 μm.
(C) Proportion of tumors with membranous MUC1-C expression according to disease stage. The prevalence of MUC1-C expression increased with advancing stage, reaching 94.0% in stage III–IV disease.

2. High MUC1-C Expression Is Associated with an Immunosuppressive Tumor Microenvironment and Poor Prognosis

A.

Factors		MUC1-C		p
		Low (n=228)	High (n=149)	
Age	< 70 years	122 (53.5%)	78 (52.3%)	0.8254
	≥ 70 years	106 (46.5%)	71 (47.7%)	
Sex	Female	132 (57.9%)	59 (39.6%)	0.0005
	Male	96 (42.1%)	90 (60.4%)	
Smoking	never	126 (55.3%)	60 (40.3%)	0.0044
	ever	102 (44.7%)	89 (59.7%)	
Stage	I	197 (86.4%)	103 (69.1%)	<0.0001
	II-III	31 (13.6%)	46 (30.9%)	
Ly +	Negative	215 (94.3%)	121 (81.2%)	<0.0001
	Positive	13 (5.7%)	28 (18.8%)	
V +	Negative	192 (84.2%)	97 (65.1%)	<0.0001
	Positive	36 (15.8%)	52 (34.9%)	

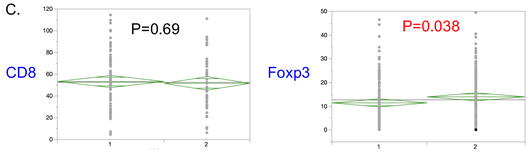
A. Among 377 patients, 228 and 149 tumors were classified as MUC1-C-low and MUC1-C-high, respectively. High MUC1-C expression was significantly associated with male sex (60.4% vs 42.1%, P = 0.0005), a history of smoking (59.7% vs 44.7%, P = 0.0044), pathologic stage II–III disease (30.9% vs 13.6%, P < 0.0001), lymphatic invasion (18.8% vs 5.7%, P < 0.0001), and vascular invasion (34.9% vs 15.8%, P < 0.0001), but not with age (P = 0.8254).

B.

PD-L1 TPS	MUC1-C low expression	MUC1-C high expression	p
Negative (TPS <1%)	127 (76.5%)	107 (64.5%)	0.0161
Positive (TPS ≥1%)	39 (23.5%)	59 (35.5%)	

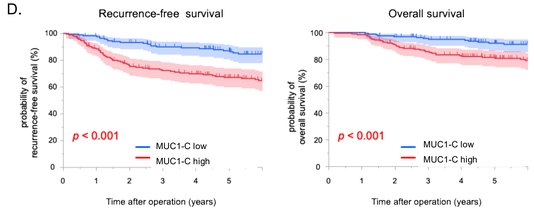
B. Among tumors evaluable for PD-L1 expression, PD-L1 positivity, defined as a tumor proportion score ≥1%, was more frequent in the MUC1-C-high group than in the MUC1-C-low group (35.5% vs 23.5%, P = 0.0161).

C.



C. Immunohistochemical evaluation of tumor-infiltrating lymphocytes showed no significant association between MUC1-C expression and CD8+ cell infiltration (P = 0.69), whereas MUC1-C-high tumors exhibited significantly greater FOXP3+ cell infiltration (P = 0.038), suggesting an immunosuppressive tumor microenvironment.

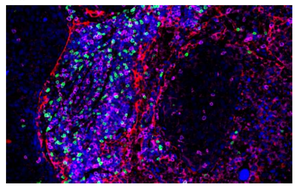
D.



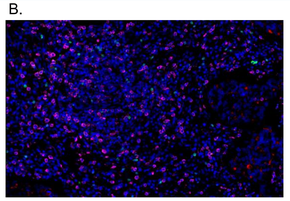
D. Kaplan–Meier analyses demonstrated that high MUC1-C expression was significantly associated with shorter recurrence-free survival and overall survival (both log-rank P < 0.001).
In multivariable Cox regression analysis, high MUC1-C expression remained an independent adverse prognostic factor for recurrence-free survival (HR, 1.96; P = 0.012).

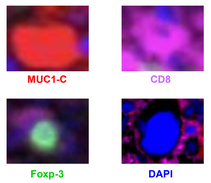
3. Multiplex immunofluorescence analysis of MUC1-C expression and immune-cell infiltration in representative NSCLC tumors according to clinical benefit from perioperative ICI therapy.

A.



B.

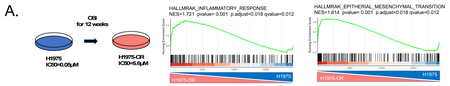




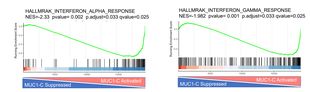
A. A representative ICI-resistant case with stage IIIA NSCLC and a PD-L1 tumor proportion score of 90% showed high MUC1-C expression. Although CD8-positive T cells were present within the tumor microenvironment, prominent infiltration of FOXP3-positive regulatory T cells was observed around MUC1-C-expressing tumor cells. The patient did not achieve a pathologic complete response or major pathologic response after neoadjuvant ICI therapy, with 90% residual viable tumor.
B. In contrast, a representative case with durable clinical benefit from ICI therapy, with stage IIIB NSCLC and a PD-L1 tumor proportion score of 50%, showed low MUC1-C expression, limited FOXP3-positive regulatory T-cell infiltration, and prominent CD8-positive T-cell infiltration. The patient remained recurrence-free for 70.6 months after surgery and postoperative ICI therapy. Enlarged representative images of individual markers are shown on the right. These findings suggest that high MUC1-C expression may be associated with a FOXP3-rich immunosuppressive tumor microenvironment and reduced sensitivity to perioperative ICI therapy.

4. MUC1-C Sustains ICI Resistance–Associated Interferon Programs in Osimertinib-Resistant NSCLC Cells

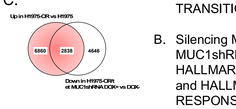
A.



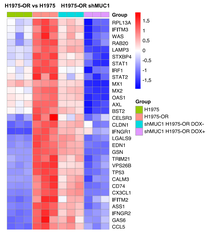
B. H1975-OR/tet-MUC1shRNA DOX+ vs DOX-



C.



D.



A. H1975 cells were treated with increasing osimertinib concentrations for 12 weeks to select for osimertinib resistant H1975-OR cells[1]. H1975-OR vs H1975 cells and identified upregulation of the HALLMARK INFLAMMATORY RESPONSE and HALLMARK EPITHELIAL MESENCHYMAL TRANSITION gene signatures.

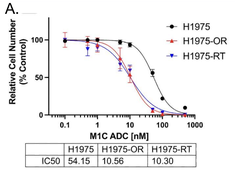
B. Silencing MUC1-C in DOX-treated H1975-OR/tet-MUC1shRNA cells downregulated the HALLMARK INTERFERON ALPHA RESPONSE and HALLMARK INTERFERON GAMMA RESPONSE gene signatures[2]

C. Venn diagram of 2838 shared genes upregulated in H1975-OR vs H1975 cells and downregulated in H1975-OR cells with MUC1-C silencing.

D. Heatmaps of ISGs in (i) H1975-OR vs. H1975, (ii) H1975-OR/tet-MUC1shRNA treated with DOX vs. DMSO. The shared genes identified IFNGR1/2, STAT1/2 and IFN stimulated genes (ISGs), including (i) MX1 that contributes to the IFN-related DNA damage resistance gene signature (IRDS) [Weichselbaum, Proc Natl Acad Sci USA, 2008], and (ii) OAS1, which has been linked to inflammatory memory and immune evasion [Qiu, Nat Cancer, 2023]

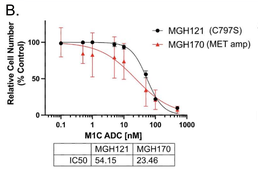
5. Antitumor Activity of a MUC1-C–Targeted ADC in Lung Cancer ModelsC

A.



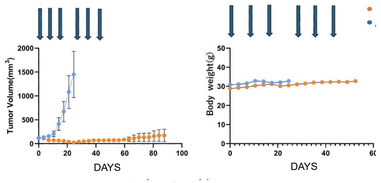
	H1975	H1975-OR	H1975-RT
IC50	54.15	10.56	10.30

B.

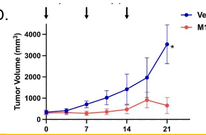


	MGH121	MGH170
IC50	54.15	23.46

C.



D.



A. H1975 parental and H1975-OR, cells were treated with increasing concentrations of the MUC1-C ADC, and relative cell numbers were determined as percentages of untreated controls. The IC_{50} values were 54.15, and 10.56, respectively.

B. Osimertinib-resistant MGH121 cells harboring EGFR C797S and MGH170 cells with MET amplification were treated with the MUC1-C ADC. The IC_{50} values were 54.15 and 23.46 nM, respectively, demonstrating activity against distinct mechanisms of osimertinib resistance.

C. Mice bearing H69 small-cell lung cancer xenografts were treated with vehicle control or the MUC1-C ADC at the time points indicated by arrows. Treatment with the MUC1-C ADC markedly inhibited tumor growth without appreciable loss of body weight.

D. Mice bearing MGH170 MET-amplified patient-derived xenografts were treated with vehicle or the MUC1-C ADC at the indicated time points. The MUC1-C ADC significantly suppressed tumor growth compared with vehicle. P < 0.05 versus vehicle[3]

Conclusion

High MUC1-C expression in resected lung adenocarcinoma was associated with aggressive clinicopathologic features, increased PD-L1 expression, enhanced FOXP3+ regulatory T-cell infiltration, and poorer clinical outcomes, supporting its role in an immunosuppressive tumor microenvironment. In osimertinib-resistant NSCLC cells, MUC1-C sustained interferon-response programs associated with ICI resistance, which were suppressed by MUC1-C silencing. Furthermore, the MUC1-C–targeted ADC demonstrated antitumor activity in vitro and in vivo, including in osimertinib-resistant models. These findings identify MUC1-C as a potential biomarker of immune resistance and a promising therapeutic target for ADC therapy in NSCLC.

1) Haratake N, et al. MUC1-C is a common driver of acquired osimertinib resistance in NSCLC. *J Thorac Oncol.* 2024;19:434–450.
2) Haratake N, et al. MUCIN 1 confers inflammatory memory of tyrosine kinase inhibitor resistance in non-small cell lung cancer. *Signal Transduct Target Ther.* 2025;10:389.
3) Haratake N, et al. Activation of APOBEC3 cytidine deaminases and endogenous retroviruses is integrated by MUC1-C in NSCLC cells. *Cell Death Discov.* 2025;11:372.