# BSA01, a bispecific antibody-drug conjugate targeting EGFR and membrane-bound MUC1-C, exhibits anti-tumor efficacy *in vivo*

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## ABSTRACT

**Background:** EGFR and the surface glycoprotein MUC1 are commonly co-expressed in several malignancies, including esophageal squamous cell car cinomas, non-small cell lung cancers (NSCLC), and triple-negative breast cancers. To overcome limitations of current EGFR- and MUC1-targeting thera pies, we generated a novel bispecific antibody-drug conjugate conjugated with monomethyl auristatin E, BSA01, which targets both antigens. EGFR and MUC1 antibodies were identified using RenLite® fully human common light chain mice and further evaluated for efficacy and specificity *in vitro* and *in vivo*.

**Methods:** Parental MUC1 antibody was tested in a binding competition assay alongside a benchmark identifying cleaved MUC1 [1]. Surface plasmon resonance and flow cytometry was employed to determine the affinity and species cross-reactivity of anti-MUC1. Binding avidity was assessed by flow cytometry. Internalization and cytotoxicity were assessed by Incucyte® live cell imaging. The specificity of BSA01 was assessed by comparing the cyto toxicity of tumor cells and neonatal Human Epidermal Keratinocytes (HEKn). The efficacy of BSA01 in preventing growth of cell line-derived (CDX) and patient-derived xenograft (PDX) tumors *in vivo* was subsequently evaluated.

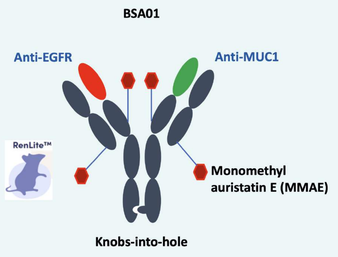
**Results:** Binding competition assays indicate that the parental MUC1 antibody of BSA01 binds to MUC1-C*, which remains membrane-bound after cleavage. The affinity of the anti-MUC1 antibody was similar to human and cynomolgus monkey antigens. BSA01 BsAbs bound EGFR$^+$MUC1$^+$ cell lines (HCC827 and HCC70) with stronger avidity than a single-positive cell line (A431). The internalization activity of BSA01 BsAbs was superior to its mon ovalent parental antibodies. BSA01 was able to effectively induce cytotoxicity in vitro, while only marginally affecting human normal cells that express low levels of MUC1 and EGFR. Notably, BSA01 showed superior anti-tumor efficacy when compared with benchmark ADCs in CDX and pancreatic PDX models *in vivo*. In NSCLC PDX tumors, BSA01 performed similar to MUC1 benchmark.

**Conclusions:** We generated a novel bispecific ADC targeting EGFR and MUC1. The MUC1 arm of BSA01 binds to the cleaved MUC1-C*protein, which remains membrane bound on tumor cells. BSA01 exhibits strong affinity and internalization activity in vitro, while also demonstrating a good safety pro file. Moreover, BSA01 shows superior anti-tumor efficacy to benchmarks in certain in vivo PDX models evaluated.

References

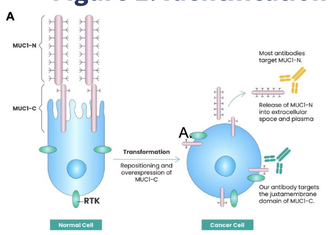
1. Wu, G., Kim, D., Kim, J. N., Park, S., Maharjan, S., Koh, H., Moon, K., Lee, Y., & Kwon, H. J. (2018). A Mucin1 C-terminal Subunit-directed Monoclonal Antibody Targets Overexpressed Mucin1 in Breast Cancer. Theranostics, 8(1), 78–91. https://doi.org/10.7150/thno.21278

## Introduction

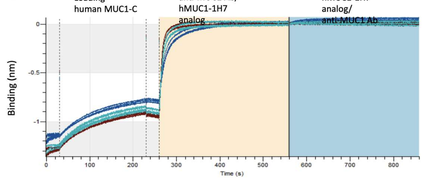



- EGFR and MUC1 antibodies were generated from fully human common light chain RenLite® mice.
- MUC1 antibody binds to MUC1-C*, which remains membrane-bound after cleavage.
- BSA01 BsADC has best-in-class potential.

## Figure 1. Identification of an antibody targeting cleaved, membrane-bound MUC1-C*

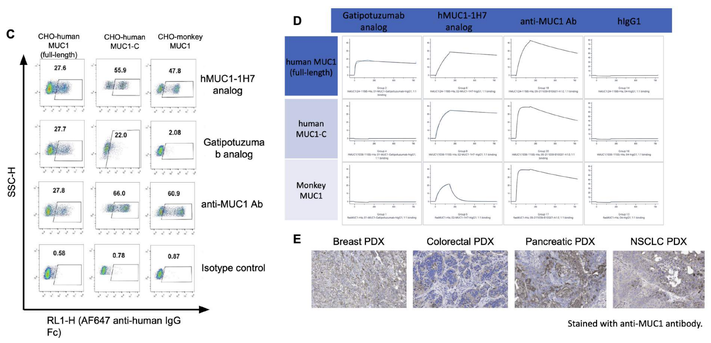



**A.** Diagram of MUC1 processing during cell transformation (adapted from Kufe [1]). MUC1-N is shed from the surface of cancer cells. As a result, the free MUC1-N domain may sequester anti-MUC1 antibodies in circulation and reduce the fraction of antibodies that bind MUC1 proteins on the cell surface after cleavage. The targeting of the juxtamembrane ectodomain of MUC1 may be a better strategy to improve efficacy.



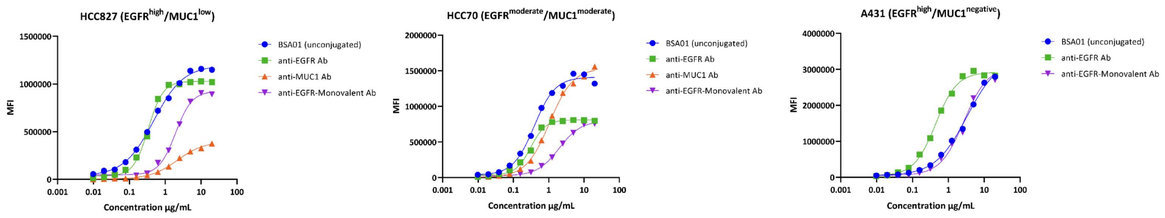

**B.** Anti-MUC1 Ab shares epitope on MUC1-C* with a reference anti-MUC1 antibody clone 1H7. 1H7 clone recog nizes MUC1-C*, which is the membrane-bound MUC1 after cleavage [2]. In this binding competition assay, sequential injection of anti-MUC1 Ab followed by 1H7 induced no additional binding signal (ForteBio Octet), and vise versa, indicating anti-MUC1 Ab and 1H7 share an overlapping epitope on MUC1-C*.

1. Kufe, D. MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. *Oncogene* 32, 1073–1081 (2013). https://doi.org/10.1038/onc.2012.158
2. Wu, G., Kim, D., Kim, J. N., Park, S., Maharjan, S., Koh, H., Moon, K., Lee, Y., & Kwon, H. J. (2018). A Mucin1 C-terminal Subunit-directed Monoclonal Antibody Targets Overexpressed Mucin1 in Breast Cancer. *Theranostics*, 8(1), 78–91. https://doi.org/10.7150/thno.21278



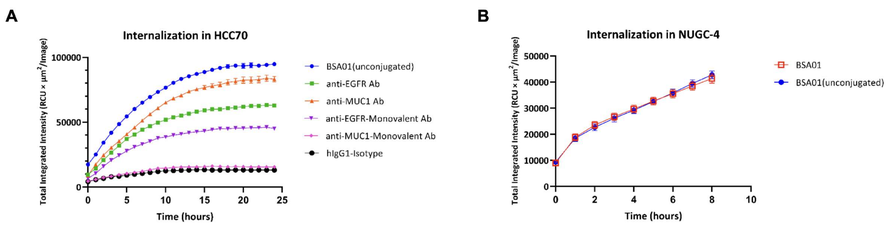

**C.** Species cross-reactivity of MUC1 antibodies (flow cytometry). Anti-MUC1 Ab binds both human and cynomolgus monkeys exogenously expressed MUC1. **D.** Affinity measurement (Biacore). Anti-MUC1 Ab showed similar affinity to human and cynomolgus monkey antigens. **E.** Immunohistochemistry (IHC) staining of PDXs showed cell membrane expression of MUC1.

## Figure 2. BSA01 (unconjugated) showed strong binding avidity to EGFR$^+$ MUC1$^+$ cells but not EGFR$^+$ MUC1$^-$ cells



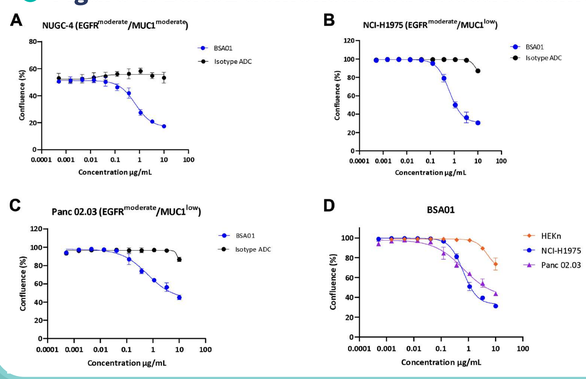

Cell surface binding of anti-EGFR and anti-MUC1 antibodies (bispecific, monoclonal, and monovalent) was determined by flow cytometry. Notably, BSA01 (unconjugated) bound double positive HCC827 and HCC70 cells more readily than the single positive A431 cells with ~8-fold difference in EC50 values (0.5/0.4 vs 3.3).

## Figure 3. BSA01 (unconjugated) demonstrated enhanced internalization compared with monovalent parental antibodies




Internalization of BSA01 and parental antibodies as measured by Incucyte® live-cell assays. **A.** BSA01 (unconjugated) demonstrated enhanced internalization compared with monovalent parental antibodies in HCC70 (EGFR$^{moderate}$/MUC1$^{moderate}$) cells. **B.** BSA01 and BSA01 (unconjugated) exhibited nearly identical internalization activity in NUGC-4 (EGFR$^{moderate}$/MUC1$^{moderate}$) cells.

## Figure 4. BSA01 demonstrated selective tumor cytotoxicity *in vitro*

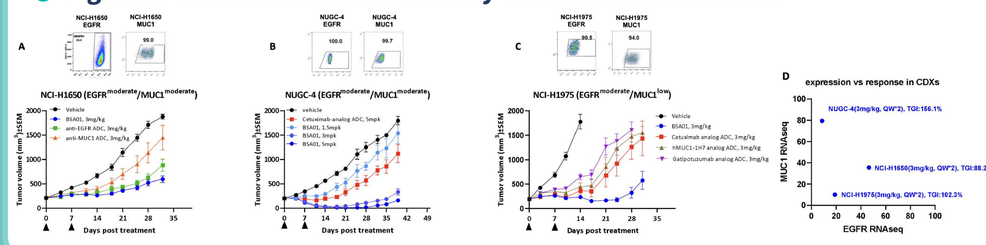



| | Tumor cells | | | |
|---|---|---|---|---|
| IC50 (µg/mL) | NUGC-4 (Gastric cancer ) | NCI-H1975 (NSCLC) | Panc 02.03 (pancreatic cancer) | HEKn * |
| BSA01 | 0.68 | 0.63 | 0.63 | ~4.9 |

* Primary Epidermal Keratinocytes; Normal, Human, Neonatal Foreskin (HEKn) (ATCC, PCS-200-010™)

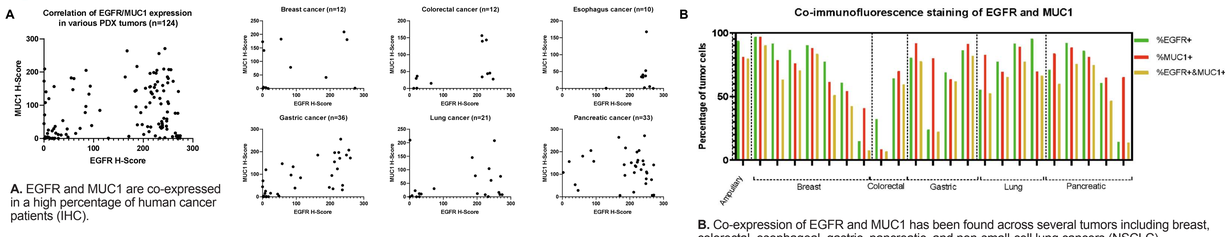
**A-C.** BSA01 showed effective killing of NUGC-4 (EGFR$^{moderate}$/MUC1$^{moderate}$), NCI-H1975 (EGFR$^{moderate}$/MUC1$^{low}$), and Panc 02.03 (EGFR$^{moderate}$/MUC1$^{low}$) cells in vitro. **D.** BSA01 inhibited tumor cell viability, while only marginally affecting normal human epidermal keratinocytes, neonatal (HKEn).

## Figure 5. Robust anti-tumor activity of BSA01 in CDX models




**A.** Increased efficacy of BSA01 in an NCI-H1650 xenograft model compared with parental anti-EGFR and anti-MUC1 ADCs. **B.** BSA01 dose-dependently inhibited NUGC-4 xenograft growth. **C.** BSA01 demon strated stronger antitumor efficacy compared with benchmark ADCs with the same payload/DAR in NCI-H1975 (EGFR L858R & T790M) models. **D.** In the CDX models tested, there was a response to EGFR/MUC1 double positivity regardless of its level of expression.

## Figure 6. Co-expression of EGFR and MUC1 in PDX models (in-house analysis)

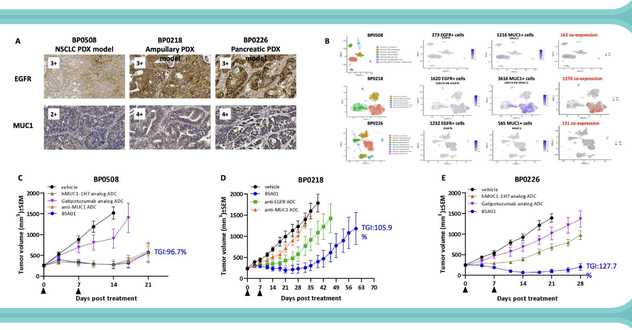



**A.** EGFR and MUC1 are co-expressed in a high percentage of human cancer patients (IHC).

**B.** Co-expression of EGFR and MUC1 has been found across several tumors including breast, colorectal, esophageal, gastric, pancreatic, and non-small-cell lung cancers (NSCLC).

## Figure 7. Potent anti-tumor efficacy of BSA01 in PDX models

BSA01 exhibits potent anti-tumor efficacy in PDX models. **A.** Expression of EGFR and MUC1 in PDX tumors by IHC. Note that two different scoring systems were used for the two tumor antigens. **B.** Single-cell RNA sequencing of PDX models indicates co-expression of EGFR and MUC1 in a subset of cancer cells. **C-E.** Efficacy studies of BSA01. C. ADCs targeting MUC1-C* (BSA01 and 1H7-ADC) were more potent in tumor growth inhibition than gatipotuzumab-ADC, which binds to MUC1-N. D. BSA01 in the BP0218 model showed superior anti-tumor activity compared with the parental mAb ADCs. E. BSA01 was more potent in the BP0226 model than the benchmark control ADCs. All ADCs carried the same payload with the same DAR. All models received the indicated treatments at 3 mg/kg twice weekly, i.v.




## SUMMARY

- Co-expression of EGFR and MUC1 in multiple solid tumors suggests that simultaneous targeting of EGFR and MUC1 with bsADC has the potential to enhance efficacy and improve safety.
- BSA01 is an EGFR- and MUC1-targeting bsADC derived from the proprietary, RenLite® common light chain, fully human antibody technology.
- BSA01 binding to MUC1-C* remains membrane-bound after cleavage and exhibits excellent affinity and internalization activity.
- The EGFR arm of BSA01 was selected to have reduced binding and internalization capability, designed to reduce the known skin toxicity of EGFR targeting.
- BSA01 demonstrated potent anti-tumor activity in multiple CDX and PDX models, with improved efficacy over parental mAb ADCs and benchmark ADCs in certain models.

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