Identification of novel senolytic targets and development of Conditionally Active Biologic-based-drug conjugates for targeted senescence-associated secretory phenotype elimination *in vivo*

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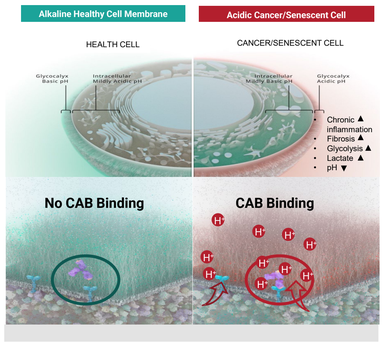
Abstract No. 7376

INTRODUCTION

- Elimination of senescent cells has been reported to improve normal and pathological changes associated with aging and cancer in mice. However, most senolytic agents inhibit anti-apoptotic pathways that do not differentiate between hyper-inflamed senescence-associated secretory phenotype (SASP) cells from relatively non-inflamed, valuable functional senescent cells, leading to off-target effects in normal tissues [1,2].
- Conditionally Active Biologics (CAB) technology is a proprietary platform unique in its ability to be selectively active in the context of diseased, acidic tissues (e.g. inflamed tissue and tumor microenvironment), but not in alkaline normal tissues [3,4]. The aberrant accumulation of senescent cells in aged and cancerous tissue triggers increasing inflammatory signaling through SASP, promoting aging and tumor progression [5,6]. Since these cells have an acidic surface environment, similar to cancer cells, we explored whether CAB technology allows selective removal of senescent cells in SASP-associated microenvironments. In addition, to complement this selectivity, we identified more specific senolytic targets to further increase selectivity and the efficiency of senescence cell removal and SASP reduction [7].
- In our previous report, CAB antibodies exhibit preferential elimination of senescent cells by targeting novel senescence markers in the glycolytic/acidic SASP microenvironment (low pH 6.5-7.0), while displaying low activities in alkaline physiological conditions (pH ≥7.4). In the unilateral ureteral obstruction (UUO) model, mice treated with CAB antibodies experienced a significant reduction in senescent and inflammatory cells infiltrating in the renal cortex compared to those treated with benchmark and isotype antibodies [7].
- In this study we developed several fibrosis models in mice, including in kidney UUO model, bleomycin-induced pulmonary fibrosis in lung, high-fat diet (HFD)+CCl_4-induced non-alcoholic steatohepatitis (NASH) in liver, and isoproterenol-induced cardiac fibrosis. We confirmed that the novel senescence-specific antigens we identified in our *in vitro* system are highly expressed in fibrotic tissues with newly induced senescent cells and associated with inflammation in *in vivo* models. Furthermore, we developed duocarmycin-based CAB-antibody drug conjugates (CAB-ADC) to study whether CAB-ADC reduces senescent cell occurrence and inflammation *in vivo*.

- BioAtla discovered that acidic pH at the disease cell surface unveils binding sites that are shielded at normal pH of healthy cells
- BioAtla invented CAB technology, creating antibodies that bind ***only*** to these unveiled sites on cancer/aged cells [3]
- CAB binding region is not masked or caged and thus different from prodrugs that require irreversible enzymatic cleavage to become activated
- CAB antibodies have the potential for increased efficacy with improved safety relative to traditional antibodies



Selective and targeted Conditionally Active Biologics (CAB) technology widens therapeutic window [3]

RESULTS

1. Elevated expression of a novel senescence target (ST-001) in Palbociclib-induced senescent cells

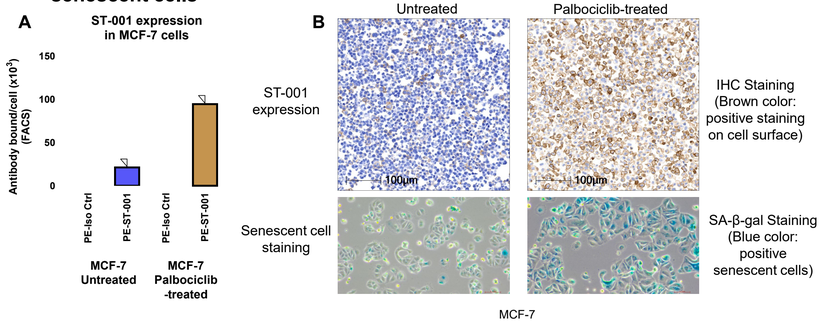


Figure 1. Elevated ST-001 expression in senescent cells. Senescent MCF-7 cells were induced by treatment of Palbociclib (CDK4/6 inhibitor, 3uM) for 1 week. (A) The levels of ST-001 expression were detected by FACS analysis using phycoerythrin (PE)-conjugated antibodies. (B) The cell surface expression of ST-001 was detected by ST-001 IHC staining in Pal-induced senescent MCF cells. The newly formed senescent cells were detected by using the Senescence β-Galactosidase Staining Kit (CST, #9860).

2. ST-001 is highly expressed in fibrotic tissues with newly induced senescent cells and co-expressed with senescence makers in *in vivo* models

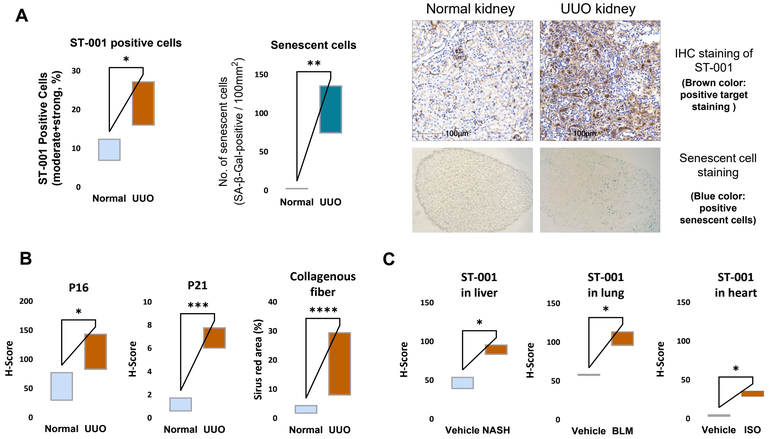
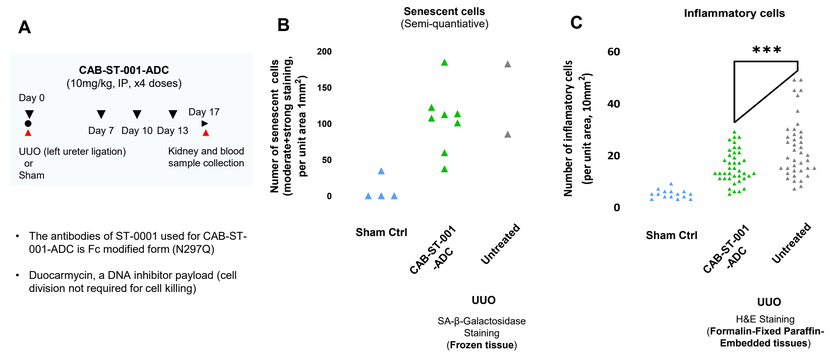


Figure 2. Elevated expression of ST-001 in UUO and other fibrosis *in vivo* models. A UUO model were established in 8-10 weeks old female C57BL/6 mice by ligating the left ureter for 2 weeks (n=3). Normal: no ureter ligation mice (n=4). (A) The levels of ST-001 expression were detected by ST-001 IHC staining. The newly formed senescent cells were detected by using the SA-β-Gal Staining Kit in frozen tissues. (B) Senescent and fibrosis (Sirus red staining) markers were detected in UUO and normal mice kidneys. (C) ST-001 is highly expressed in other fibrosis models. NASH: high-fat diet (HFD)+CCl4-induced non-alcoholic steatohepatitis in liver; BLM: Bleomycin-induced pulmonary fibrosis in lung; ISO: Isoproterenol induced cardiac fibrosis in heart. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, unpaired t-test.

3. New approach of CAB-ADC targeting senescence by treatment of CAB-ST-001-ADC in UUO model



- The antibodies of ST-0001 used for CAB-ST-001-ADC is Fc modified form (N297Q)
- Duocarmycin, a DNA inhibitor payload (cell division not required for cell killing)

Figure 3. Evaluation of CAB-ST-001-ADC efficacy in UUO model. (A) UUO mice were treated by CAB-ST-001-ADC (n=8) as indicated. Sham Ctrl: no ureter ligation mice (n=4). Untreated: untreated UUO mice. (B) Senescent cells in UUO and Sham mice kidneys were identified and measured using SA-β-Galactosidase staining kit. **(C)** Inflammatory cells infiltrated in the renal cortex were evaluated by H&E staining. Inflammatory cells (neutrophils and other inflammatory cells) were counted per unit area (five random fields in each animal's cortex). *** $p < 0.001$, unpaired t-test.

CONCLUSIONS

- We identified several novel senescence-specific surface antigens including a senescence-specific antigen ST-001 (Fig.1) as senolytic targets both *in vitro* and *in vivo* [7]. ST-001 is also highly expressed in fibrotic tissues with newly induced senescent cells and associated with inflammation in multiple *in vivo* fibrosis models (Fig. 2).
- In the UUO model, mice treated with CAB-ADC targeting ST-001-expressed senescent cells reduced inflammatory cells infiltrating in the renal cortex (Fig.3) suggesting that the senescent cells can be targeted using a CAB anti-senescence-specific receptor drug conjugate strategy.
- CAB antibodies exhibit preferential elimination of senescent cells by targeting novel senescence markers in the glycolytic/acidic SASP microenvironment (low pH 6.5-7.0), while displaying low activities in alkaline physiological conditions (pH ≥7.4). The CAB technology represents a new generation of biologics with an enhanced safety and therapeutic index for targeting SASP senescence cells in both cancer and age-related diseases.

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