Conditionally Active Biologics targeting novel senescence markers effectively eliminate the senescence-associated secretory phenotype in both *in vitro* and *in vivo* models

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INTRODUCTION

- Clearing senescent cells has demonstrated promising effects against cancer and age-related pathologies in preclinical models as well as in early human clinical trials. However, current senescent cell elimination strategies lack specificity, targeting both hyper-inflamed or SASP senescent cells and non-inflamed senescent cells. This indiscriminate approach limits the clinical utility of senolytics due to their cytotoxicity toward normal cells or potentially beneficial senescent cells. There is a pressing need for a more effective and selective approach to remove or reduce senescent cells while minimizing side effects. (1,2).

- Conditionally Active Biologics (CAB) technology is a proprietary platform unique in its ability to be selectively active in the context of diseased tissues, but not normal tissues (3,4). The aberrant accumulation of senescent cells in aged and cancerous tissue triggers inflammatory signaling through a senescence-associated secretory phenotype (SASP), promoting aging and tumor progression (5, 6). Leveraging our CAB technology, currently being evaluated in cancer therapy targeting cell surface markers such as AXL, ROR2, and CTLA4 in clinical studies, we investigated whether CAB technology allows for selective removal of senescent cells in SASP-associated microenvironments.

- In our previous report, we identified several novel senescence-specific surface antigens upregulated in senescent cells. CAB antibodies targeting these senescence markers exhibit minimal binding to the target antigen on senescence cells under physiological, alkaline conditions, but demonstrate strong binding in glycolytic, acidic SASP conditions in *in vitro* binding assays (7). Furthermore, our studies demonstrated that CAB antibodies were more potent against senescent cells compared to proliferating cells and displayed high selectivity for the glycolytic, acidic microenvironment condition in antibody-dependent cell-mediated cytotoxicity (ADCC) *in vitro* assays.

- Here, we developed a unilateral ureteral obstruction (UUO) mouse model to induce senescent cells, fibrosis, and inflammation in the mouse kidney. We investigated whether treatment with CAB antibodies could reduce senescent cell occurrence in this *in vivo* senescence model.

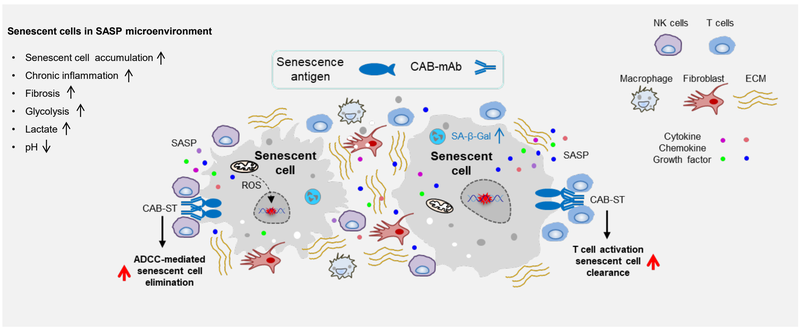
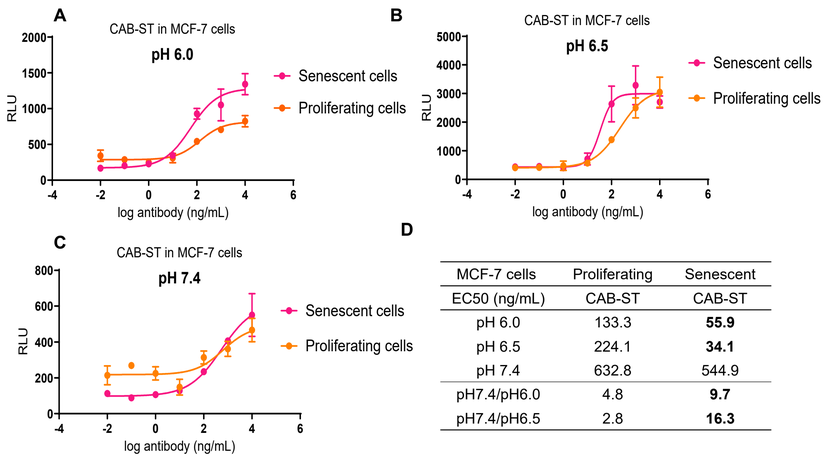


Figure 1. CAB antibodies selectively eliminate senescence cells in glycolytic/acidic SASP microenvironments. The accumulation of senescent cell in SASP microenvironment leads to increased tissue inflammation and fibrosis. CAB therapeutics are designed to preferentially target senescent cells in SASP, glycolytic/acidic microenvironment, promoting their clearance, while sparing non-senescence cells and non-SASP senescence cells from elimination.

RESULTS

1. The CAB-senescence target (ST) antibody exhibits high selectivity and potency against senescent cells in the glycolytic/acidic (pH 6.0-6.5) microenvironment condition



D

MCF-7 cells	Proliferating	Senescent
EC50 (ng/mL)	CAB-ST	CAB-ST
pH 6.0	133.3	**55.9**
pH 6.5	224.1	**34.1**
pH 7.4	632.8	544.9
pH7.4/pH6.0	4.8	**9.7**
pH7.4/pH6.5	2.8	**16.3**

Figure 1. CAB-ST antibody mediated ADCC assays. Palbociclib-induced senescent cells or proliferating cells were treated with CAB-ST antibody for 6 hours in pH 6.0 (A), pH 6.5 (B) or pH 7.4 (C) culture conditions. A summary table of EC50 is shown in D. ADCC assay was performed using ADCC Reporter Bioassay, Core Kit (Promega Cat. G7018).

2. Treatment with CAB-ST antibody resulted in a reduction of senescent cells and inflammatory cell infiltration in *in vivo* UUO kidneys

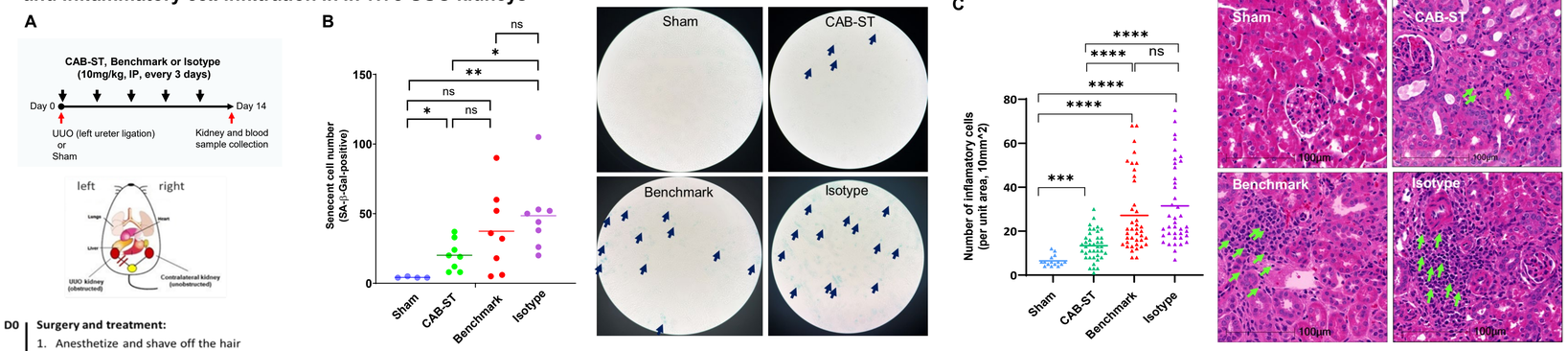


Figure 2. Evaluation of CAB-ST efficacy in UUO model. A: A UUO model were established in 8-10 weeks old female C57BL/6 mice by ligating the left ureter for 2 weeks. The mice were treated by CAB-ST antibody (n=8), the benchmark (non-CAB antibody) (n=8), or an isotype control (n=8) as indicated. Sham: non treatment control (n=4). B: Senescent cells in UUO and Sham mice kidneys were identified and measured using SA-β-Galactosidase staining kit (Cell Staining Technology #9860). Black arrows indicate SA-β-Gal staining positive senescent cells in representative images. 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). Green arrows indicate infiltrated inflammatory cells in representative images. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significantly (unpaired t-test).

CONCLUSIONS

- CAB antibodies exhibit preferential elimination of senescent cells by targeting novel senescence markers in the glycolytic/acidic SASP microenvironment (low pH 6.0-6.5), 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.
- Moreover, alongside on-going human clinical trials in cancer, CABs are currently under evaluation as senolytic therapies in disease models associated with aging.
- The CAB technology represents a new generation of biologics with an enhanced safety margin and therapeutic index for targeting SASP senescence cells in both cancer and age-related diseases.

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