**ABSTRACT**

APS001F is a live recombinant bifidobacterium longum expressing cytosine deaminase (CD), which is an enzyme to catalyze the hydrolytic deamination of cytosine to uracil. *Bifidobacterium* is a non-pathogenic anaerobic bacterium derived from normal human intestinal flora. It can easily survive and grow at low oxygen environment such as solid tumor. Intra-tumoral administration of APS001F to patients leads to colonization of B. longum and production of CD enzyme only inside the solid tumor. Locally expressed CD enzyme converts orally taken prodrug 5-FU, which increases 5-FU concentration specifically inside tumor and results in alleviation of side effects caused by attacking normal organs. The phase 1 clinical trial for APS001F is conducted in the US sponsored by us. It is reported that 5-FU upregulating PD-L1 expression on the surface of tumor cells, which drive to combine anti-PD-1 antibody with the APS001F therapy. At this present study, systemic administration of APS001F and 5-FU significantly inhibited tumor growth in CT26 bearing syngeneic mice model which is our preclinical study. Additionally, anti-murine PD-1 antibody combined with APS001F and 5-FU further suppressed tumor progression and some of individual samples achieved complete regression of CT26 tumors. Experiments revealed that PD-L1 level was enhanced. Early administration of APS001F followed by mPD-1 mAb showed treatment showed superior anti-tumor effects. Moreover, FACs analysis suggested activation of immune reaction in tumor by the combination treatment. More efforts need to be fulfilled in elucidating the mechanism of such combination attempt, nevertheless, the notable combination effect of anti-murine PD-1 antibody and APS001F with 5-FU revealed a new application approach for APS001F.

**RESULTS**

1. APS001F combined with anti-mPD-1 mAb increases PD-L1 expression level in syngeneic CT26 tumor model

   - Non-treated
   - Anti-mPD-1 mAb
   - Combination: APS001F + anti-mPD-1 mAb

   FACs analysis on day 14 as same schedule of anti-tumor effect study in result 2

   - Total: Tumor cells + CD8+ cells
   - APS001F treatment or combined with anti-mPD-1 antibody
   - Combination: APS001F + anti-mPD-1 mAb

   • Order: CR, CR/0, CR/0, CR/0
   • ##: P<0.01
   • ###: P<0.001
   • S-way: ANOVA, Tukey multiple comparison

2. Synergistic anti-tumor effect of APS001F combined with anti-mPD-1 antibody against CT26 in Balb/c mice

3. Anti-tumor immune reaction induced by APS001F treatment or combined with anti-mPD-1 antibody

   - Combination of APS001F and anti-mPD-1 antibody enhanced CD8+/tumor ratio and decreased Treg in CD4+ cells

4. Early administration of APS001F followed by mPD-1 mAb treatment showed superior anti-tumor effects

**BACKGROUND**

Features of APS001F, CD Expressing B. longum

- B. longum, is derived from flora in human intestine
- Obligate anaerobe
- Selectively colonized only in hypoxic solid tumors
- Produce CD enzyme inside the tumor to convert 5-FU to 5-FC

**Tumor-Selective Localization of APS001F**

- APS001F was clearly observed localized into tumor, and grow completely from all organs within 3 days of APS001F injection. (Bile, heart, spleen, lung, kidney, brain, small intestine, large intestine, stomach, liver, skin, lung, intestine)

**Conclusion & Perspectives**

- Combination treatment of APS001F with anti-mPD-1 antibody showed more significant anti-tumor efficacy than that of either single treatment.
- A decrease of Treg in the tumor and an increase of CD8+/Tumor ratio were observed under the above combination therapy.
- Early administration of APS001F followed by mPD-1 mAb showed superior anti-tumor efficacy.
- More investigations are needed to find an optimal dosing schedule of the combination for the effective treatment in clinical setting.