FF-10832 combined with immune checkpoint blockade shows favorable antitumor activity

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Introduction

Although gemcitabine (GEM)-based therapies have been used for the treatment of several advanced solid cancers, it doesn't satisfy the medical needs due to its limited efficacy. It appears that the rapid clearance of GEM is one of the reasons for poor clinical outcomes and drug resistance; therefore, we designed FF-10832, a liposomal gemcitabine, to produce long-term exposure of tumor cells to GEM. We have previously reported that FF-10832 is stable in blood, accumulates preferentially in tumor tissues, is efficiently released GEM in tumors, and exhibits superior antitumor effects in xenograft models (Fig.1).

Cancer immunotherapy, especially the immune checkpoint inhibitor (ICI), is an important area of research in cancer treatment. Some patients obtain long-term tumor free survival, but response rates are not always high. In order to improve response rates, many combination studies are underway. GEM is one of the combination partners because some research has shown the immune-modulating effects of GEM in clinical and nonclinical studies which indicates GEM-induced immunogenic cell death or decrease of immune suppressive cells, such as MDSC, Treg or M2 macrophages in tumor microenvironment. We reported that FF-10832 released GEM after being phagocytized by macrophages in tumor microenvironments (Fig. 2); thus, FF-10832 may also modify the tumor microenvironment with respect to the antitumor situation in addition to the direct effect on tumor cells.

In the present study, we verified the combined effects of FF-10832 and immune checkpoint inhibitors using GEM sensitive (MBT-2) and GEM partial sensitive (EMT6) syngeneic mouse models (Table 1).

Schematic view of EPR effect and GEM release in tumor tissues

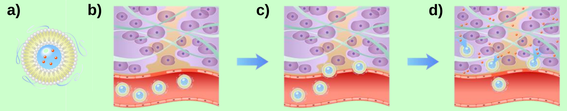


Fig. 1 a) FF-10832 consists of 0.5 mg/mL GEM, cholesterol, HSPC, and N-MPEG-DSPE. FF-10832 is considered to be b) retained for a long time in blood with good stability, c) accumulating in tumor, and d) releasing GEM in tumor tissues.

Phagocytization and GEM release in tumor tissues

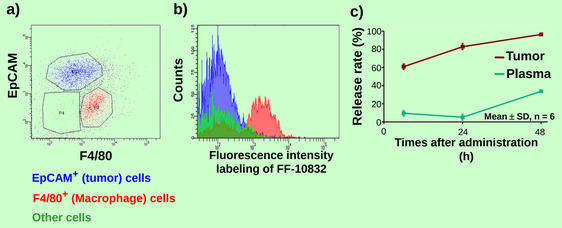


Fig. 2 a) Seventy-two hours after the administration of DiI-labeled FF-10832, tumor tissue was harvested and dissociated into single-cell suspensions, then labeled with mAb of EpCAM and F4/80. Cells were divided into three types of cells: EpCAM positive, F4/80 positive, and double-negative cells, b) DiI intensity was measured in these cells. c) GEM and DiI incorporated into FF-10832 were measured and release rate was calculated in plasma and tumor tissues.

Results

1. Response to single agent

Table 1 Tumor growth inhibition rate of single agent in each syngeneic model

TGI rate (%)	Cell lines				
Intervention	MBT2	CT26	EMT6	4T1	LLC1
αPD-1 mAb	22	22	40	3	-4
αPD-L1 mAb	12	15	31	-2	-5
αCTLA-4 mAb	78	76	36	-6	-23
GEM	79	84	50	56	21

Mice were subjected to subcutaneous implantation of each cell. Seven days after implantation, ICI antibodies (10 mg/kg) were intraperitoneally administered twice a week or GEM (240 mg/kg) was intravenously administered once a week. Tumor volume was measured at 7–10 days after treatment initiation and calculated tumor growth inhibition (TGI) rate using the formula TGI rate (%) = (1 – TVt/TVc) x 100, where TVt and TVc are the mean tumor volume of treated and control groups, respectively.

3. Immune cell analysis in tumor

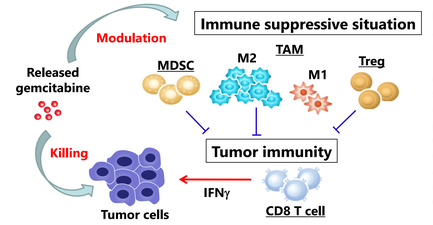


Fig. 4 Schematic illustration of the assumed mode of action of the tumor microenvironment

Some studies have reported that gemcitabine decreases immune suppressive MDSC, Treg or M2 macrophages in clinical and preclinical conditions. Released gemcitabine from FF-10832 may not only attacks tumor cells but also modulates the pro-tumor microenvironment to the antitumor condition.

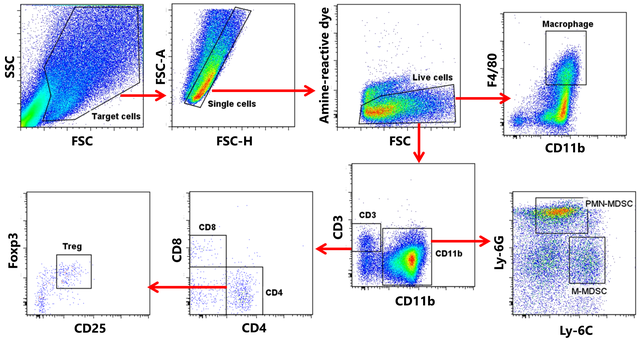
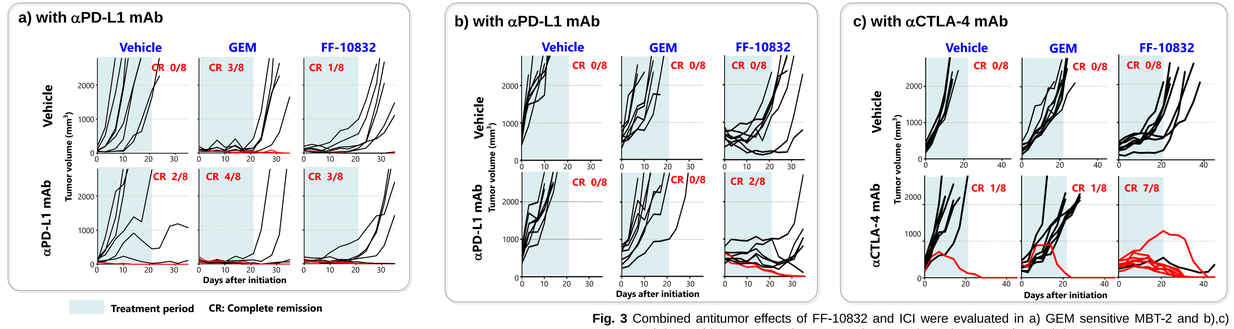


Fig. 5 In EMT6 model, tumor was harvested and dissociated at 16 or 17 days after treatment initiation. Dissociated cells were sorted by CD45 microbeads and stained with fluorescence-conjugated antibodies then analyzed by flow cytometry. After they were gated on live cells, macrophage ($CD11b^+$, $F4/80^+$), PMN-MDSC ($CD11b^+$, $Ly6G^+$, $Ly6C^{low}$), M-MDSC ($CD11b^+$, $Ly6C^+$, $Ly6G^-$), CD8T cell ($CD3^+$, $CD8^+$), and Treg ($CD3^+$, $CD4^+$, $CD25^+$, $Foxp3^+$) were identified using cell-specific markers.

2. Anti-tumor effects of FF-10832 in combination with ICI



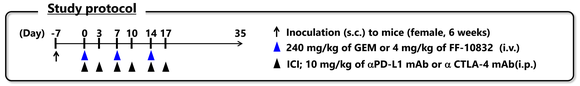


Fig. 3 Combined antitumor effects of FF-10832 and ICI were evaluated in a) GEM sensitive MBT-2 and b),c) GEM partial sensitive EMT6 subcutaneously transplanted syngeneic models. FF-10832 or GEM was intravenously administered once a week and ICI antibody was intraperitoneally administered twice a week for three weeks. After the treatment period, treatment was withdrawn and tumor volume was continually observed. Each line indicates the tumor volume of individual mice. The red line indicates individuals that achieved complete tumor reduction (CR; complete remission). In the MBT-2 model, FF-10832 showed comparable combination effect to GEM. In the EMT6 model, FF-10832 exerted synergistic effects that were superior to GEM.

4. Immune cell modulation in tumor

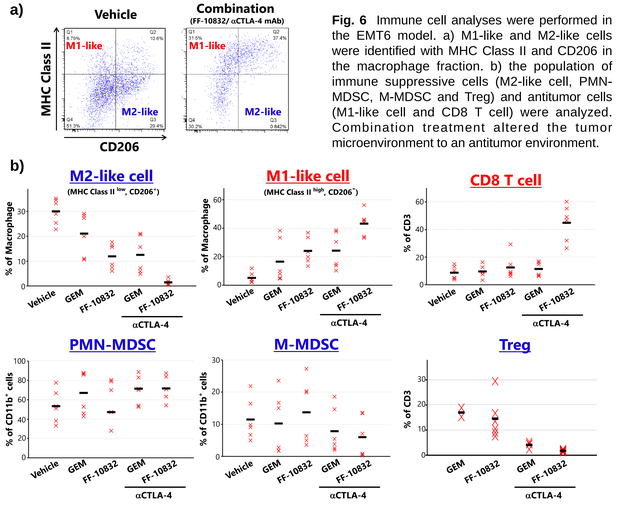


Fig. 6 Immune cell analyses were performed in the EMT6 model. a) M1-like and M2-like cells were identified with MHC Class II and CD206 in the macrophage fraction. b) the population of immune suppressive cells (M2-like cell, PMN-MDSC, M-MDSC and Treg) and antitumor cells (M1-like cell and CD8 T cell) were analyzed. Combination treatment altered the tumor microenvironment to an antitumor environment.

5. Effects of CD8 T cells

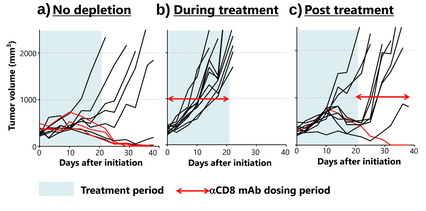


Fig. 7 αCD8 mAb (10mg/kg, twice a week) was used to deplete CD8 T cells in EMT6 model. a) combination treatment using FF-10832 (4mg/kg, once a week) and αCTLA-4 mAb (10 mg/kg, twice a week) for 3 weeks was performed. αCD8 mAb was treated b) during combination treatment or c) post combination treatment. The red line indicates the individual that became tumor free. αCD8 mAb abolished the combination effect.

Conclusion

1. FF-10832 showed a strong synergistic effect with ICI even when GEM was not effective
2. The combination of FF-10832 and αCTLA-4 mAb altered the M1/M2 ratio to the antitumor condition and induced CD8 T cell infiltration
3. CD8 depletion abolished the combination effect

These data indicate that combination with FF-10832 is a promising approach for ICI to augment antitumor effects.

Conflict of Interest Disclosure :
This study was designed and funded by FUJIFILM Corporation.
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