FUJIFILM
Value from Innovation

TECHNICAL BULLETIN

FUJIFILM liposomes with immune checkpoint inhibitors

Making strides in R&D to combat cancer

Our scientists take a comprehensive approach to understanding cancer and drug discovery. Treating cancer requires a deep understanding of the challenges faced within the industry and the complexity of the disease. We have made great strides in our understanding of cancer, but more needs to be done.

Cancer incidence and mortality are rapidly growing worldwide. It is estimated that in 2018, 9.6 million people died from cancer[1] and the number of global cancer deaths is projected to reach over 13 million in 2030[2].

Our goal is to expand current clinical trials for cancer drugs, develop new drug delivery systems and produce liposomes designed by harnessing nanotechnologies to be an efficient delivery vehicle for highly-advanced drug candidates to help combat the disease.

As our understanding of cancer and drug delivery systems expands, so does our hope for developing treatments that may change people's lives.

FUJIFILM Pharmaceuticals U.S.A., Inc. is determined to help combat cancer by continuing to invest in research, by collaborating closely with partners, and by developing innovative healthcare solutions.

Adapting liposomes for drug delivery

Developed by harnessing nanotechnologies, liposomes are artificially constructed vesicles made from organic phospholipids similar to those that make up cell and bio membranes. These small particles provide a type of drug delivery system (DDS) technology that can deliver the required amount of a drug to the specific area of the body on a predetermined schedule.

New DDS technologies are urgently needed to support delivery of the anti-cancer therapeutic to the cancerous cells. Oftentimes anti-cancer agents can act on healthy tissues and cells instead of the cancerous tumor, leading to adverse side effects. By encapsulating a drug in a liposome, it is expected that the drug will be preferentially delivered to the tumor, suppressing side effects, and enhancing the pharmacological efficacy of the drug.

Fujifilm is developing new drug delivery systems — including liposomal formulations — to advance therapeutic progress to meet unmet medical needs, such as cancer.

The advantage of Fujifilm's liposomal particles is evident in its physical characteristics. Unique emulsification methods were optimized to yield liposomes that are uniform in size and shape, which enables a controllable release rate, and makes the technology applicable to various lipid formulations. The uniform shape in nano size allows the encapsulated drug to be delivered to the targeted area of concern.

[1] "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." *A Cancer Journal for Clinicians*. (2018). Retrieved from https://onlinelibrary.wiley.com/doi/full/10.3322/caac.21492

[2] Cancer. *World Health Organization*. (2019). Retrieved from https://www.who.int/cancer/resources/keyfacts/en/

Combination Therapy with Immune Checkpoint Inhibitors

Cancer cells' ability to escape the immune system renders traditional immunotherapeutics, such as vaccines and immunostimulants, inefficient in eliminating tumors. Progressing through the activation of immune checkpoint pathways that suppresses the body's anti-tumor responses, cancer cells are able to bypass immunosurveillance, and consequently, stimulating the immune system becomes a fruitless effort by the time the tumor is clinically apparent.

This scenario has changed thanks to the introduction of immune checkpoint inhibitors. This new therapeutic approach redirects immunosuppression functions towards anti-tumor immunity by inhibiting immune checkpoint molecules used by cancer cells to evade an immune system attack.

Immune checkpoint inhibitors currently approved by the FDA target molecules CTLA-4, PD-1, and PD-L1. Although antibody-mediated blockade of these molecules has shown promising results against melanomas and long-term responses in the treatment of renal cell and small-cell cancers, in most cases, monotherapy with checkpoint inhibitors results in treatment failure. This issue has been addressed by combining immune checkpoint inhibitors with conventional cancer therapies. This article introduces Fujifilm's approach to cancer treatment by combining immuno-oncology and liposomal drugs.

Pre-clinical research findings for FF-10832

FF-10832 is a liposomal-based agent encapsulating gemcitabine (Gemzar®), an anti-cancer agent developed by Eli Lilly and Company, indicated for pancreatic cancer, ovarian cancer, metastatic breast cancer, among others. Preclinical studies demonstrated that FF-10832 increases plasma stability of gemcitabine, resulting in high dose delivery of the drug to tumor tissues.

A preclinical study was conducted to assess the efficacy of FF-10832 in combination with immune checkpoint inhibitors on a syngeneic mice model. The mice were inoculated with the breast mammary carcinoma line EMT6, a cell line resistant to gemcitabine and anti-PD-1/L1 antibodies (**Figure 1A**), and partially sensitive to anti-CTLA-4 antibodies. After that, three doses of FF-10832 (4 mg/kg) or gemcitabine (240 mg/kg), and six doses of anti-PD-L1 or anti-CTLA-4 antibodies (10 mg/kg for both) were administered in a period of 17 days (**Figure 1B**).

The FF-10832 therapy resulted in tumor growth delay, which was not observed with gemcitabine therapy. Anti-PD-L1 antibody monotherapy did not show any efficacy, much like the vehicle treatment (control). The gemcitabine and anti-PD-L1 antibody combination therapy resulted in slight tumor growth delay, but without complete tumor regression. On the other hand, combination therapy with FF-10832 and anti-PD-L1 antibody showed tumor growth delay and complete tumor remission in two out of eight mice (**Figure 1C**). These results show that combination of FF-10832 and anti-PD-L1 antibody is a more effective treatment compared to gemcitabine and anti-PD-L1 antibody combined.

The combination with the anti-CTLA-4 antibody, to which EMT6 is partially sensitive to, resulted in complete tumor remission in seven out of eight animals, whereas anti-CTLA-4 antibody alone, or in combination with gemcitabine, resulted in only one complete remission each (**Figure 2**).

The results of combination therapy with FF-10832 and checkpoint inhibitors show superior efficacy compared to non-liposomal gemcitabine and checkpoint inhibitors.

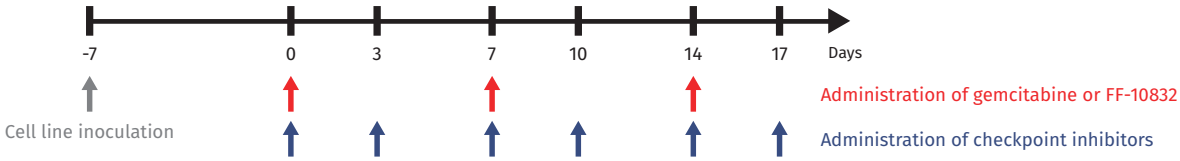
A

Model information

	Gemcitabine	Anti-PD-1/L1 mAB	Anti-CTLA-4 mAb
EMT6	Resistant	Resistant	Partially sensitive

B

Experiment design



C

Tumor growth curve

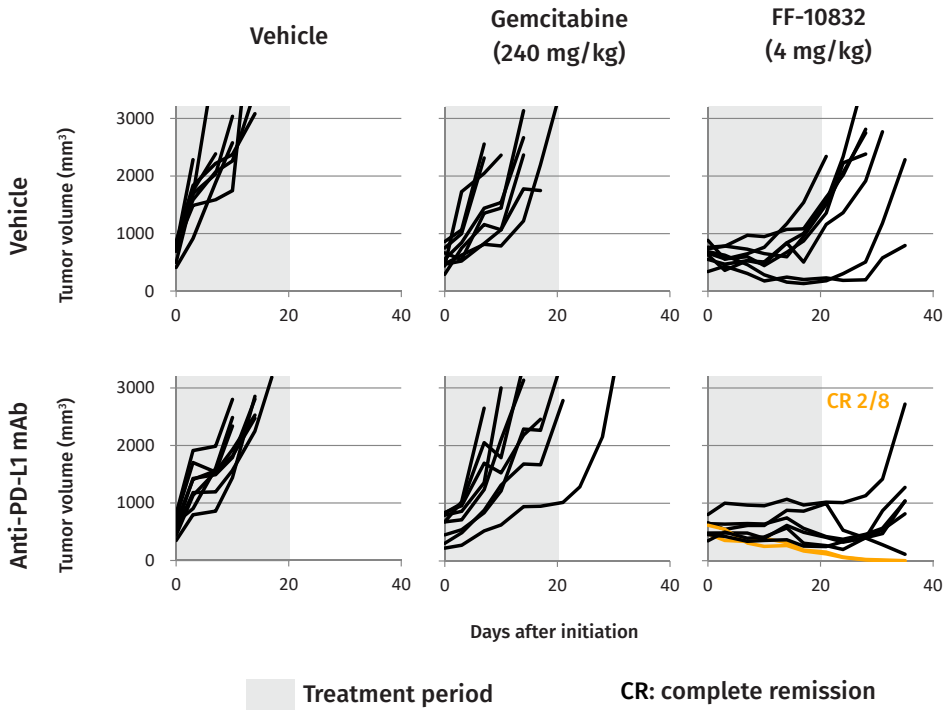


Figure 1. Anti-tumor effects of PD-L1 combination therapy on gemcitabine-resistant EMT6 breast mammary carcinoma model. A) EMT6 model information. B) Experiment design and treatment timeline. C) Tumor growth curves demonstrating the combined anti-tumor effects of FF-10832 and the immune checkpoint inhibitor PD-L1.

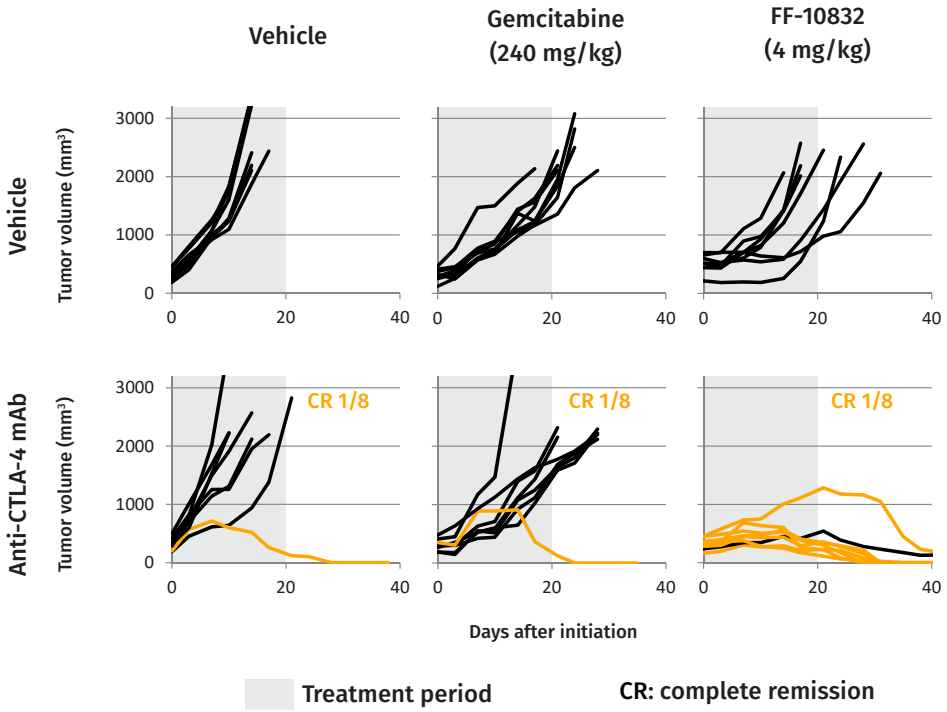


Figure 2. Anti-tumor effects of CTLA-4 combination therapy on gemcitabine-resistant EMT6 breast mammary carcinoma model. Tumor growth curves demonstrating the combined anti-tumor effects of FF-10832 and the immune checkpoint inhibitor CTLA-4.

Immune cell analysis in tumors of a syngeneic mice model revealed that the FF-10832 and CTLA-4 combination therapy altered the tumor microenvironment to an anti-tumor environment. M1- and M2-like cells were identified with MHC Class II and CD206, in the macrophage fraction (**Figure 3A**). The combination therapy decreased the number of immune suppressive M2 macrophages and increased that of immune active M1 macrophages. The combination also increased the number of CD8 T cells, which has immunity against cancer cells in the tumor tissue. These results indicated that the combination therapy drastically changed the immune suppressive microenvironment to an immune active microenvironment (**Figure 3B**).

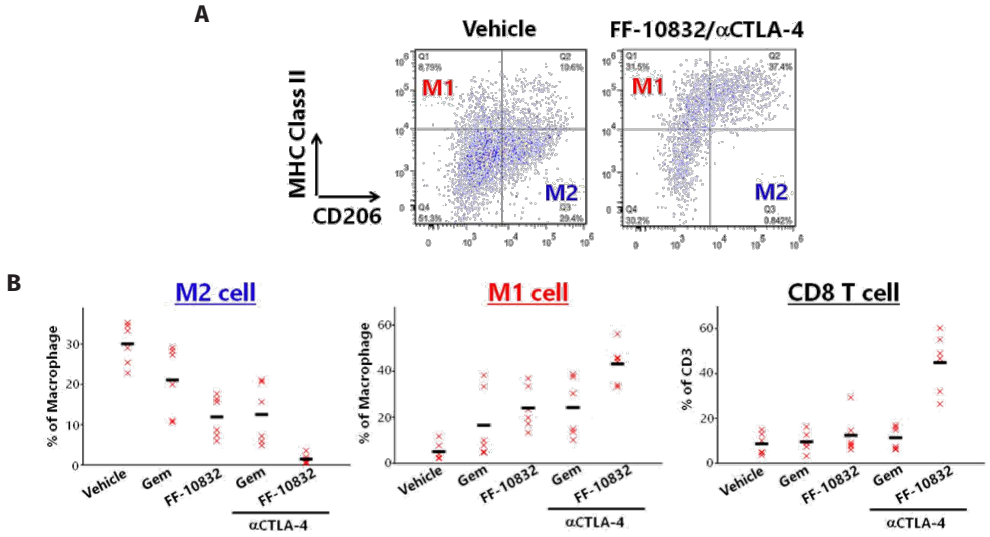


Figure 4. Immune cell modulation in tumors. Immune cell analysis of EMT6 gemcitabine-resistant models treated with FF-10832 in combination with the immune checkpoint inhibitor CTLA-4. A) M1- and M2-like cells were identified with MHC Class II and CD206 in the macrophage fraction. B) Analysis of immune suppressive cell (M2-like cell) and anti-tumor cell (M1-like cell) populations. Gem: gemcitabine.

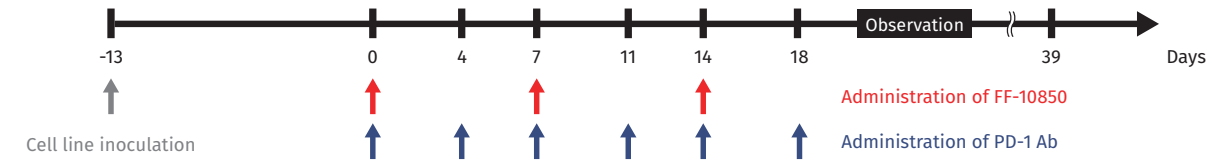
Pre-Clinical Research Findings for FF-10850

FF-10850 is a liposomal-based agent encapsulating topotecan (Hycamtin®), an anti-cancer agent developed by GlaxoSmithKline plc., and distributed by Novartis as a treatment option for ovarian cancer, small-cell lung cancer, and cervical cancer. Preclinical studies demonstrated that FF-10850 enables stable encapsulation and prolonged half-life of topotecan. Furthermore, FF-10850 modulates the pharmacokinetic profile of the drug, dramatically improving anti-tumor effects, while reducing the toxicity of topotecan.

A combination therapy study using FF-10850 and an anti-PD-1 antibody to treat colon cancer in a syngenic mouse model revealed the synergistic effects of FF-10850 in combination with an immune checkpoint inhibitor. Mice inoculated with the murine CT26 colon carcinoma line received three doses of FF-10850 (2 mg/kg), and six doses of anti-PD-1 antibody (10 mg/kg) within a period of 18 days. Tumor volume was monitored for up to 39 days, or until the tumor reached 2000 mm^3, at which point the animals were euthanized (**Figure 4A**). Treatment effects were observed in both FF-10850 and anti-PD-1 antibody treated mice; FF-10850 delayed tumor growth in all animals beyond the observation period of 39 days, whereas PD-1 treatment resulted in significant tumor growth delay in two mice. The combination therapy, however, resulted in complete tumor remission in two out of eight mice, suggesting that the FF-10850 and anti-PD-1 antibody combination is more effective than its parts alone (**Figure 4B**).

A

Model information



B

Tumor growth curve

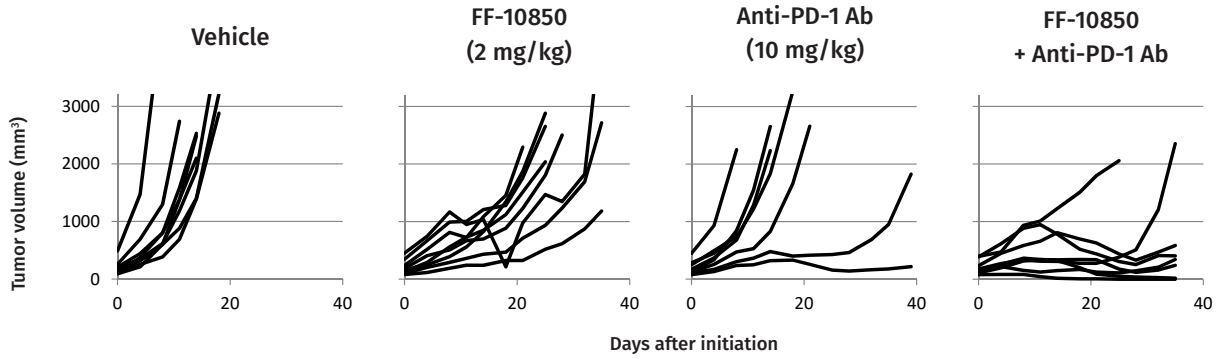


Figure 3. Anti-tumor effects of PD-1 combination therapy on CT-26 colon carcinoma model. A) Experiment design and treatment timeline. B) Tumor growth curves demonstrating the combined anti-tumor effects of FF-10850 and the immune checkpoint inhibitor PD-1.

Advancing Fujifilm's Pipeline – Phase I Clinical Trials Underway

With sights set on helping patients along the entire care pathway, Fujifilm has made the significant investment required in research and clinical development for success in the pharmaceutical industry. Research across Fujifilm's clinical and development programs demonstrates the company's commitment.

Preclinical findings are promising, however, there is still more to come from next phase clinical studies. Developments within trials are often marred with questions regarding the safety, tolerability and efficacy of new approaches. Going forward, Fujifilm will study the safety, tolerability, pharmacokinetics, and initial efficacy of FF-10832 and FF-10850 on advanced solid tumors in Phase I clinical trials in the United States.

Fujifilm stands at the forefront of the industry by offering new solutions and advancing DDS technologies. By taking on some of the most challenging diseases, Fujifilm is dedicated to developing treatments for unmet needs and improving the quality of life for patients around the globe.

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About Fujifilm

Established in 2010, FUJIFILM Pharmaceuticals U.S.A., Inc. is based in Boston, Massachusetts, and specializes in clinical research and development of pharmaceutical products. FUJIFILM Pharmaceuticals U.S.A., Inc. strives to contribute to the further development of global health care through new drug development of unique therapeutic compounds, to combat illnesses such as influenza, Alzheimer's and cancer.

FUJIFILM Holdings Corporation, Tokyo, Japan, brings cutting edge solutions to a broad range of global industries by leveraging its depth of knowledge and fundamental technologies developed in its relentless pursuit of innovation. Its proprietary core technologies contribute to the various fields including healthcare, graphic systems, highly functional materials, optical devices, digital imaging and document products. These products and services are based on its extensive portfolio of chemical, mechanical, optical, electronic and imaging technologies. For the year ended March 31, 2019, the company had global revenues of $22 billion, at an exchange rate of 111 yen to the dollar. Fujifilm is committed to responsible environmental stewardship and good corporate citizenship.

For more information, please visit: www.fujifilmpharma.com