FUJIFILM
Value from Innovation

TECHNICAL BULLETIN

Liposomes improving efficacy of anti-cancer agent — FF-10832

Scientists are on the bleeding edge of innovation in drug development. In the past few years, major breakthroughs, such as CRISPR, CAR-T, immuno-oncology and gene therapies have ushered in a new era of therapeutic options and renewed hope for patients and their families.

Drug delivery is particularly formidable in addressing cancer, which is a group of many diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. The World Health Organization (WHO) listed cancer as one of its top ten threats to global health in 2019 — and for good reason — cancer claimed the lives of an estimated 9.6 million people around the globe in 2018.

There are multiple and an ever-changing array of cancer treatment options available today. These range from traditional, still effective chemotherapy and radiation treatment to new immunotherapies that must selectively target the cancerous cells, while minimizing toxicity from the therapeutic to healthy cells.

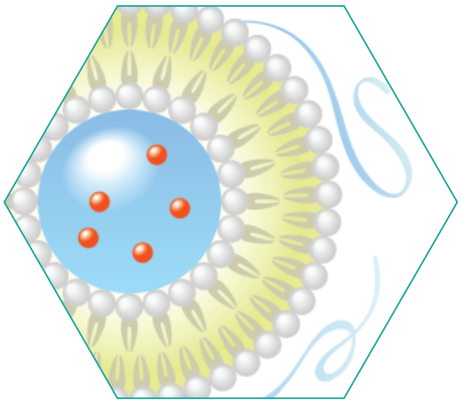
Liposomes coupled with anti-cancer agents for increased pharmacological efficacy

Developed by harnessing nano-dispersion technologies, 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 the delivery of anti-cancer agents 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 an anti-cancer agent in a liposome, we can expect the agent to be preferentially delivered to the tumor, therefore 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. Moreover, the uniform shape in nano size allows the encapsulated drug to be delivered to the targeted area of concern.



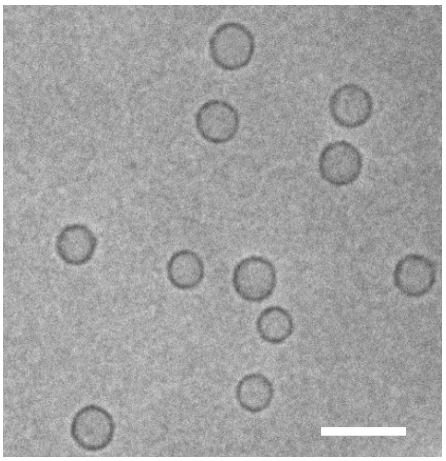
FF-10832

FF-10832 is a liposome-based agent encapsulating gemcitabine (Gemzar®), an anti-cancer agent developed by Eli Lilly and Company indicated for pancreatic cancer, among others. As gemcitabine has a short half-life, the goal is for FF-10832 to stabilize the therapy in the blood stream, accumulating and releasing the therapy at tumors with an enhanced permeability and retention (EPR) effect[1] , where the agent accumulates within the tumor and is retained for an extended period of time before it is released. FF-10832 is currently in Phase 1 clinical trials in the U.S.

FF-10832 is a liquid injection containing liposomes in suspension. FF-10832 has unilamellar vesicles encapsulating gemcitabine in solution (**Figure 1A**), and is stable for at least 18 months when stored between 4-10°C (**Figure 1B**). Initial pharmacokinetics studies demonstrated that liposome encapsulation greatly increases the half-life of gemcitabine. FF-10832 can be detected for at least 48 hours, twice longer than gemcitabine, in the plasma of mice that had received a single intravenous dose of the drug (**Figure 2**).

The pharmacological benefits of FF-10832 were studied in mice, using different pancreatic cancer models as described below.

A



B

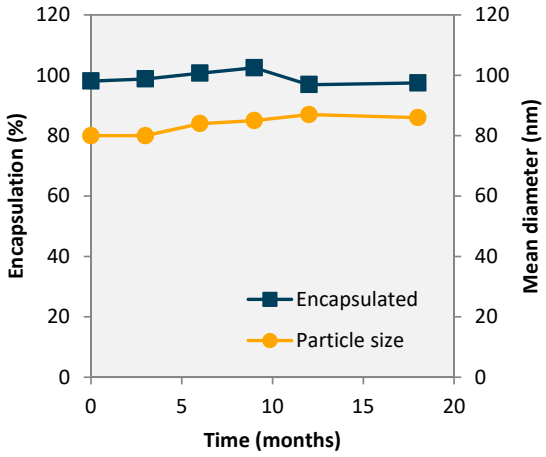
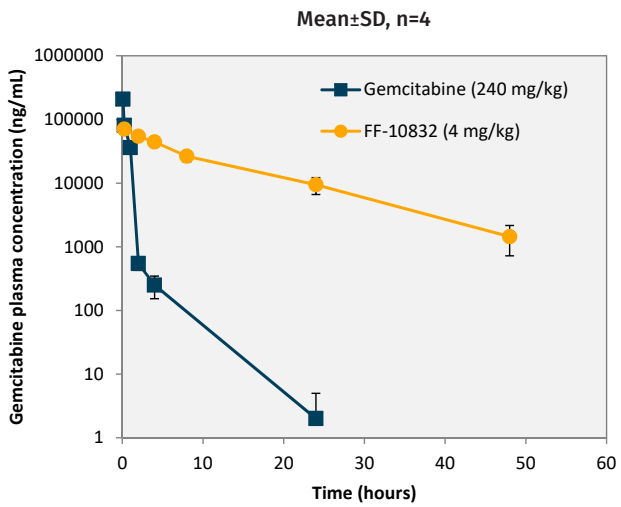


Figure 1. FF-10832 features. A) Transmission electron microscopy of FF-10832. Scale bar: 100 nm. B) Gemcitabine encapsulation rate and FF-10832 particle size upon refrigerated storage (4-10 °C).

[1]As they grow, tumors promote angiogenesis, generating blood vessels that are not fully developed and have larger gaps that are not found in normal blood vessels. When liposomes and polymers are retained within the blood, they do not permeate the walls of normal blood vessels, which have small gaps, permeating only the vascular walls around the tumor. In addition, as lymphatic vessels are not fully developed in tumors, the liposomes and polymers that have permeated cannot be easily eliminated, resulting in the accumulation of these structures and molecules in the tumor. This is called the enhanced permeability and retention (EPR) effect.

A



B

	Gemcitabine	FF-10832
CL (ml/min/kg)	54.8	0.0812
Vd_{SS} (ml/kg)	1400	59.2
$T_{1/2}$ (h)	2.90	8.18

Figure 2. Phamacokinetics in mice. A) Plasma phamacokinetics of gemcitabine following a single IV bolus injection. B) Phamacokinetics comparison of gemcitabine and FF-10832.

Research results for FF-10832 — drug release mechanism

FF-10832 was labeled with a fluorescent dye and administered to mice transplanted with human-derived pancreatic cancer cells, Capan-1. Image analysis and histology revealed that FF-10832 had accumulated in the vicinity of pancreatic cancer cells (**Figure 3**), particularly within tumor-associated macrophages, as evaluated by flow cytometry (**Figure 4**). As such, it is observed that FF-10832 accumulates in tumor tissue through the EPR effect, and after being incorporated into macrophages, gradually releases gemcitabine in the vicinity of tumor cells.

The release of gemcitabine was studied in two different experiments. First, gemcitabine and DiI incorporated in FF-10832 were quantified in the plasma and tumor of Capan-1 xenograft mouse models that had received an intravenous dose of the drug. The percent release of gemcitabine was calculated as follows:

$$GEM_{released} = \frac{GEM_{enc}(0) - GEM_{enc}(t)}{GEM_{enc}(0)} \times 100(\%)$$

Where $GEM_{enc}(0)$ is the encapsulated ratio of gemcitabine in injection solution, and $GEM_{enc}(t)$ is the encapsulated ratio of gemcitabine at t (hour) post-injection.

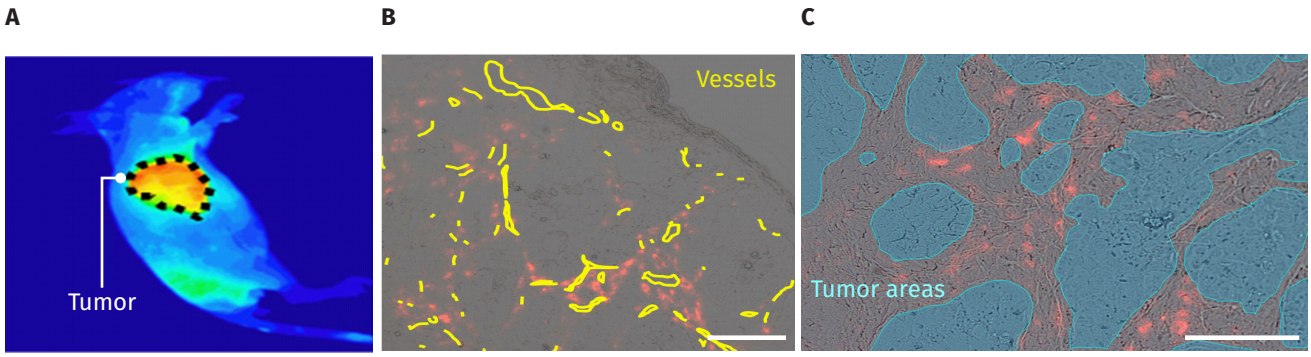


Figure 3. Accumulation of FF-10832 in tumors. A) Imaging of DiI-labeled FF-10832 administered to Capan-1 xenograft model. Photo taken 72 h after administration. B) Tumor cryosection stained for CD31 (tumor vessels, yellow) and merged with DiI-fluorescence imaging (red). C) Tissue section indicating tumor areas (aqua), as observed by hematoxylin and eosin staining, merged with DiI-fluorescence imaging (red). Scale bars: 100 µm.

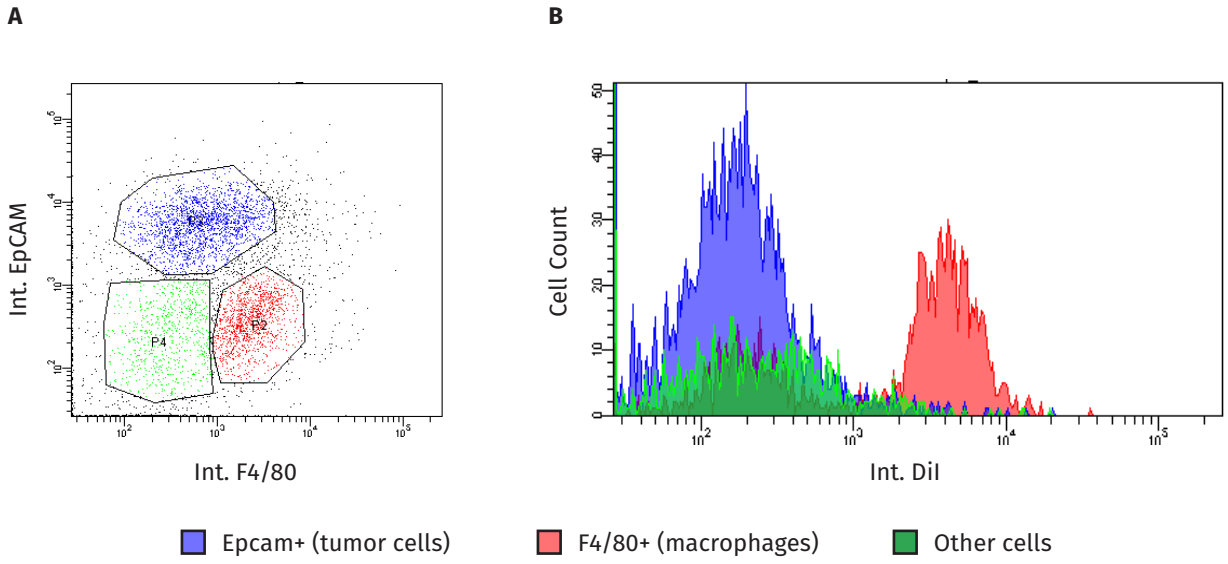


Figure 4. Accumulation of FF-10832 in tumor-associated macrophages. A) Identification of tumor cells (EpCAM-positive) and macrophages (F4/80-positive) from tumor-dissociated cells. B) FF-10832 uptake (DiI fluorescence) in EpCAM-positive tumor cells and F4/80-positive macrophages.

Through this experiment, we confirmed that gemcitabine is successfully released from liposomes, and the release rate is higher in tumors when compared to plasma (**Figure 5A**). In the second study, FF-10832 was intraperitoneally injected in mice. Three hours later, peritoneal macrophages were harvested and seeded in culture dishes so the supernatant could be collected for gemcitabine quantification. The release rate was calculated as the ratio between the drug concentration in the supernatant and the drug concentration at seeding. The results demonstrated that gemcitabine is completely released from the macrophages within 24 hours (**Figure 5B**).

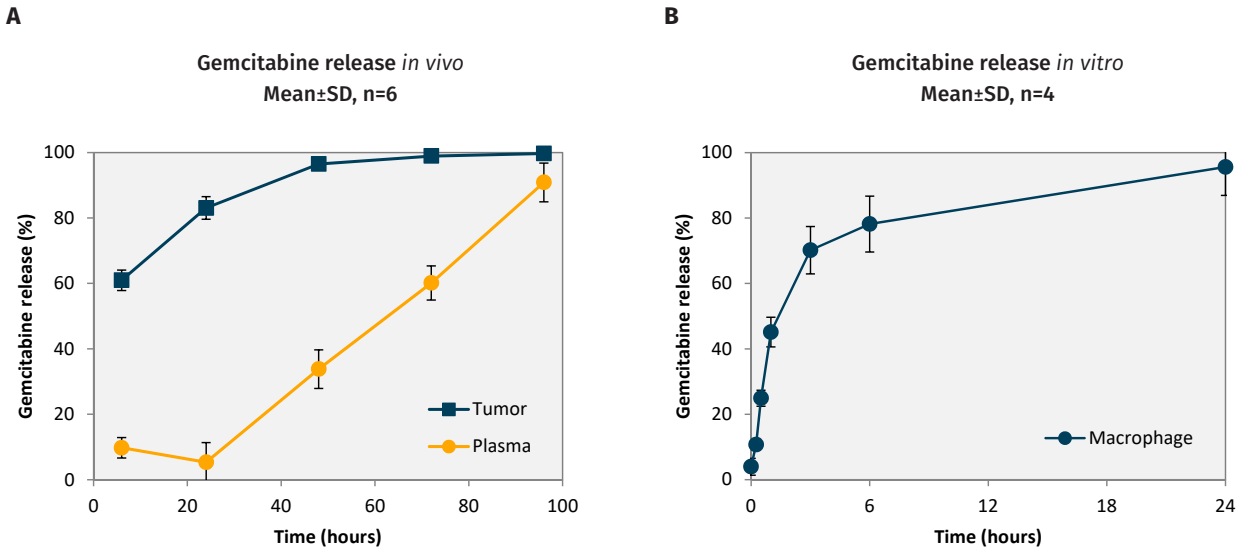


Figure 5. Gemcitabine release rate from FF-10832. A) Gemcitabine release from FF-10832 *in vivo*. B) Gemcitabine release from FF-10832 by peritoneal macrophages *in vitro*

Following administration, gemcitabine is intracellularly converted into difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate (dFdCTP). dFdCTP acts as an inhibitor of DNA polymerase, and therefore DNA synthesis, conferring gemcitabine with antineoplastic activity. Because of that, one of the side effects of the drug is bone marrow depression, as manifested by leukopenia, thrombocytopenia, and anemia. Using the Capan-1 xenograft model, we measured the concentration of dFdCTP in tumor and bone marrow of mice that received either gemcitabine or FF-10832 intravenously. We observed that the dFdCTP tumor/bone marrow AUC ratio was significantly higher in FF-10832 treated mice (AUC ratio = 7, **Figure 6A**) compared to gemcitabine alone (AUC ratio = 0.8, **Figure 6B**). These results suggest that liposome encapsulation has the potential to reduce gemcitabine side effects in the bone marrow, as the drug is more efficiently distributed in tumors.

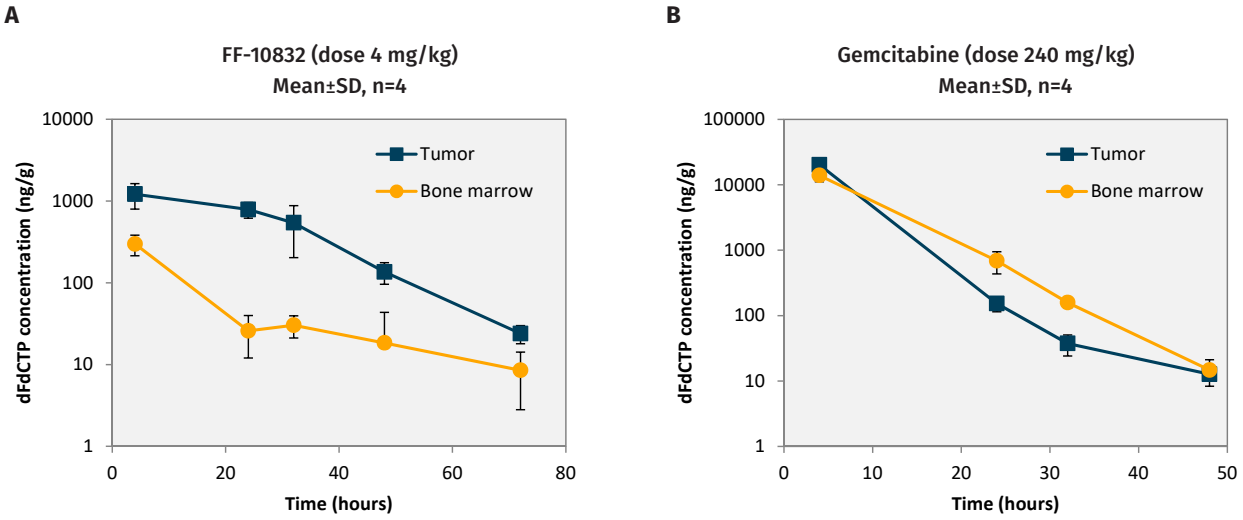


Figure 6. Distribution of gemcitabine in Capan-1 xenograft model. Concentration of gemcitabine active form, gemcitabine triphosphate (dFdCTP), following a single IV dose in Capan-1 xenograft models. A) dFdCTP tumor/bone marrow AUC ratio was 7 for FF-10832. B) Tumor/bone marrow AUC ratio was 0.8 for gemcitabine.

Anti-tumor effects

To study the anti-tumor effects of FF-10832, gemcitabine and FF-10832 were administered intravenously, once a week for three weeks, to Capan-1 (on days 9, 16 and 23), a gemcitabine sensitive model, and BxPC-3 (on days 35, 42 and 49), a gemcitabine resistant xenograft model for pancreatic cancer. In both models, despite the difference in sensitivity to gemcitabine, FF-10832 demonstrated superior anti-tumor effects at much lower doses than gemcitabine alone (**Figure 7**).

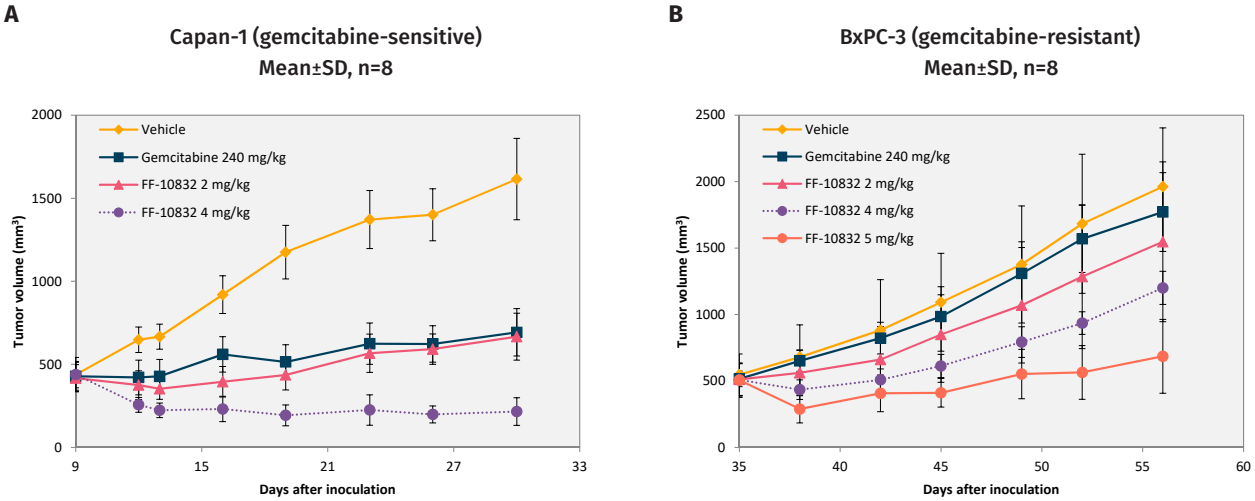


Figure 7. Anti-tumor effects in subcutaneous xenograft model. Tumor volume changes after gemcitabine and FF-10832 IV administration. A) Tumor growth curve of gemcitabine-sensitive model Capan-1. B) Tumor growth curve of gemcitabine-resistant model BxPC-3.

Likewise, the survival rate of mice treated with FF-10832 was higher than that of gemcitabine-treated mice, as observed in experiments using SUIT-2 orthotopic xenograft models. In this experiment, gemcitabine and FF-10832 were administered intravenously once a week for 11 weeks, and the survival rate of mice that received FF-10832 treatment (4 mg/kg) was 40% higher than that of mice that received gemcitabine alone (240 mg/kg) (**Figure 8**).

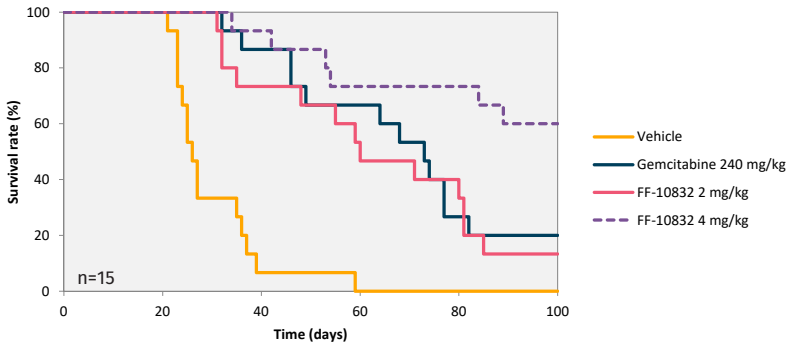


Figure 8. Effect on survive rate. Effect of gemcitabine and FF-10832 on survival rate of SUIT-2 orthotopic xenograft model.

The promise of liposomal technologies

Fujifilm's biggest strength is its wide range of advanced technologies, such as chemical synthesis, analysis, nano-technologies and manufacturing engineering.

Currently, the company is also undertaking initiatives with the aim of applying the technologies not only to existing drugs but expanding to next-generation therapeutics, such as nucleic acid therapy and gene therapy. Working towards meeting the global supply of liposome drugs, Fujifilm has built a new facility to manufacture liposome formulations at the pharmaceutical production site of the Fujifilm group company Toyama Chemical Co., Ltd. Operations at the plant started in February 2020.

By offering new therapies and advancing DDS technologies, 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.

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