Received: 18 April 2019 | Revised: 17 June 2019 | Accepted: 4 July 2019

DOI: 10.1111/cas.14123

ORIGINAL ARTICLE

Cancer Science WILEY

FF-10832 enables long survival via effective gemcitabine accumulation in a lethal murine peritoneal dissemination model

Tamami Higuchi[1,2] | Takehiko Yokobori[3,4] | Ryo Takahashi[5] | Tomoharu Naito[2] | Hiromu Kitahara[2] | Takeshi Matsumoto[2] | Chihaya Kakinuma[2] | Shinji Hagiwara[2] | Hiroyuki Kuwano[5] | Ken Shirabe[5] | Takayuki Asao[6]

[1]Department of Oncology Clinical Development, Gunma University, Graduate School of Medicine, Maebashi, Japan

[2]Pharmaceutical and Healthcare Research Laboratories Research & Development Management Head Quarters, Fujifilm Corporation, Ashigarakami-gun, Japan

[3]Division of Integrated Oncology Research, Gunma University Initiative for Advanced Research (GIAR), Maebashi, Japan

[4]Department of Innovative Cancer Immunotherapy, Gunma University, Maebashi, Japan

[5]Department of General Surgical Science, Gunma University, Graduate School of Medicine, Maebashi, Japan

[6]Big Data Center for Integrative Analysis, Gunma University Initiative for Advanced Research (GIAR), Maebashi, Japan

Correspondence
Takayuki Asao, Big Data Center for Integrative Analysis, Gunma University Initiative for Advanced Research (GIAR), 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan.
Email: asao@gunma-u.ac.jp

Takehiko Yokobori, Division of Integrated Oncology Research, Gunma University Initiative for Advanced Research (GIAR), 3-39-22 Showamachi, Maebashi 371-8511, Japan.
Email: bori45@gunma-u.ac.jp

Funding information
Fujifilm Corporation

Abstract

Chemotherapy has been the treatment of choice for unresectable peritoneal dissemination; however, it is difficult to eradicate such tumors because of poor drug delivery. To solve this issue, we developed FF-10832 as liposome-encapsulated gemcitabine to maintain a high concentration of gemcitabine in peritoneal tumors from the circulation and ascites. A syngeneic mouse model of peritoneal dissemination using murine Colon26 cell line was selected to compare the drug efficacy and pharmacokinetics of FF-10832 with those of gemcitabine. Despite the single intravenous administration, FF-10832 treatment enabled long-term survival of the lethal model mice as compared with those treated with gemcitabine. Pharmacokinetic analysis clarified that FF-10832 could achieve a more effective gemcitabine delivery to peritoneal tumors owing to better stability in the circulation and ascites. The novel liposome-encapsulated gemcitabine FF-10832 may be a curative therapeutic tool for cancer patients with unresectable peritoneal dissemination via the effective delivery of gemcitabine to target tumors.

KEYWORDS
gemcitabine, liposomes, nanotechnology, peritoneal neoplasms, survival analysis

1 | INTRODUCTION

Peritoneal dissemination is a poor prognostic factor in several gastrointestinal and gynecological cancers.[1,2] It is difficult to completely remove the peritoneal dissemination surgically; therefore, systemic chemotherapy has typically been performed for peritoneal dissemination. However, its therapeutic effect is insufficient at present owing to drug resistance as well as poor drug delivery to disseminated tumors from the circulation and ascites.[3,4]

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine; dFdCyd) is a deoxycytidine analog that is incorporated into DNA, resulting in an anticancer effect via chain termination.[5] This drug is currently used as the standard chemotherapeutic agent for patients with malignant abdominal tumors that may cause unresectable peritoneal dissemination.[6-9] However, it has been reported that gemcitabine can induce an interesting pharmacological effect not only on gemcitabine-treated cancer cells but also on the surrounding untreated cells. This unique transmission of therapeutic effect has been identified as the "bystander effect" of nucleoside analogs such as gemcitabine and ganciclovir.[10] From these observations, it has been suggested that maintaining a high gemcitabine concentration in the circulation and ascites that are in contact with disseminated tumors is important in curing peritoneal dissemination because effective gemcitabine delivery to a portion of the target tumors is expected to transmit the therapeutic effect to the entire population of disseminated tumor cells. However, gemcitabine has a rapid clearance from the circulation (half-life ($t_{1/2}$) = 0.3 hour), which may be a critical disadvantage in the eradication of peritoneal tumors because gemcitabine is known to be a time-dependent anti–tumor drug.[11] Therefore, improvement of the in vivo delivery and stability of gemcitabine is imperative for cancer patients with peritoneal dissemination.

Researchers have attempted several drug modifications to strengthen the enhanced permeability and retention (EPR) effect for effective drug delivery. For instance, various types of liposome-encapsulated anticancer drugs have shown significantly better effects against peritoneal dissemination via drug stability in the circulation due to an improvement in the EPR effect.[12-14] To solve the problem of the rapid clearance of gemcitabine, we developed FF-10832, as a liposome-encapsulated gemcitabine, to maintain a high drug concentration in the circulation and ascites that are in contact with the disseminated tumors.

The purpose of the present study was to evaluate the therapeutic efficacy of newly developed FF-10832, a liposome-encapsulated gemcitabine (Lipo-GEM), and to perform pharmacokinetic analysis to evaluate the EPR effect using a peritoneal dissemination mouse model. In the present study, we show a significant tumor reduction and survival extension by FF-10832 administration through the effective accumulation of gemcitabine not only in the circulation and ascites but also in target disseminated tumors.

2 | MATERIALS AND METHODS

2.1 | Preparation of liposome-encapsulated gemcitabine, FF-10832

Cholesterol, hydrogenated soy phosphatidylcholine (HSPC) and N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (N-MPEG-DSPE) were dissolved in organic solvent containing ethanol and ethyl acetate, in reference to WO2015166985A1. FF-10832 consists of 0.5 mg/mL gemcitabine, cholesterol, HSPC and N-MPEGDSPE. Liposome was prepared using cholesterol, HSPC and N-MPEG-DSPE in a 4:15:1 molar ratio. The optimized lipid composition allowed for effective drug encapsulation over 97.5% with respect to the total amount (0.5 mg/mL) of gemcitabine. Electron microscopy data clearly revealed that FF-10832 had a homogeneous appearance and unilamellar vesicles and consisted of liposomes with a mean particle size of 80 nm.

2.2 | Cell culture and animals

The luminescent mouse colorectal carcinoma cell line Colon26-luc (Colon26) was cultured in RPMI-1640 medium (Sigma Aldrich) containing 10% FBS, 50 U/mL penicillin, 50 mg/mL streptomycin (Pen-strep; Gibco, Thermo Fisher Scientific) and 10 μg/mL puromycin (Sigma Aldrich) at 37°C in a humidified atmosphere with 5% CO_2. Inbred female BALB/cCrSlc mice (Japan SLC) were obtained at 5 weeks of age and maintained under specific pathogen-free conditions. These mice were used for experiments at 6 weeks of age. All experiments and procedures for care and treatment of the animals used in the present study were carried out in accordance with the requirements of the Gunma University Animal Care and Experimentation Committee (Experimental Protocol: No. 14-026; Gunma University, Maebashi, Japan).

2.3 | Murine peritoneal dissemination model

Thirty mice were intraperitoneally inoculated with Colon26 cells (1×10^6 cells in 0.5 mL PBS) (day 0). Mice were randomly divided into 3 groups on day 3 according to the intensity of luminescence, as described previously [15]: vehicle-treated group (n = 10), GEM-treated group (240 mg/kg Eli Lilly, n = 10) and FF-10832-treated group (3 mg/kg, Fujifilm, n = 10). In our lethal model mice, we had already validated the establishment of tumor inoculation on peritoneal cavity pathologically on day 5 after intraperitoneal injection (data not shown).These drugs were administered to the mice on day 5 by intravenous injection. Peritoneal dissemination was examined using an in vivo luciferase assay on days 3, 7, 12, 17, 22 and 29 following transplantation. We plotted body weight until day 22 following transplantation and Kaplan-Meier survival curves until day 56 following transplantation in each group. On day 56 following transplantation, surviving mice were killed to evaluate the peritoneal tumor conditions using an ex vivo luciferase assay, as previously described.[15] The

peritoneal tumors in each group were observed using H&E staining. The tumor samples were collected from moribund mice in the vehicle-treated and GEM-treated groups.

2.4 | Pharmacokinetic analysis

The time course of gemcitabine concentration was evaluated in the plasma and ascites of each group. The gemcitabine concentrations were measured in plasma and ascites following a single intravenous administration of 240 mg/kg GEM or 3 mg/kg FF-10832 to Colon26-bearing mice 7 days after intraperitoneal inoculation. Both doses were decided as the maximum tolerated dose from our preliminary study.[16] For the plasma pharmacokinetics of gemcitabine, blood samples were collected in tubes containing 100 μg/mL tissues of tetrahydrouridine (THU) at 1, 4, 24, 48 and 72 hours following FF-10832 treatment, and at 1, 4 and 24 hours following GEM treatment (n = 3 or 4). For the ascites pharmacokinetics, the inside of the ascites was washed with 500 μL physiological saline, and the washing solution was collected into tubes containing 100 μg/g tissues of THU at 1, 4, 24 and 72 hours following FF-10832 treatment, and at 1, 4 and 24 hours following GEM treatment (n = 3 or 4). For tumor tissue distribution of gemcitabine, tumor tissue samples were collected into tubes containing 100 μg/g tissues of THU at 24 hours following FF-10832 or GEM treatment (n = 3). Tumor tissues were harvested, weighed and flash frozen in liquid nitrogen. The plasma, ascites and tumor samples were analyzed by LC/MS/MS (Applied Biosystems; gemcitabine Q1/Q3 = 264.08/112.1.) for gemcitabine content. The pharmacokinetic parameters of gemcitabine were determined by non-compartmental analysis using WinNonlin (version 6.4; Pharsight Corporation).

2.5 | H&E staining

Tissues were fixed in 10% neutral-buffered formalin (Wako), embedded in paraffin (Sakura Finetek Japan), and 2-μm sections were subsequently prepared. The sections were stained with H&E (Hematoxylin 3G, Sakura Finetek, Japan; Eosin, Wako, Japan) using standard procedures (hematoxylin, 1 minute; eosin, 1 minute). Snapshots of histology were taken using an Olympus BX51 microscope.

2.6 | Statistical analysis

Overall survival was measured from the day of Colon26 injection and plotted according to the Kaplan-Meier method; the log-rank test was used for comparisons. Concentrates of gemcitabine in tumor tissue were assessed using the Wilcoxon test. A *P*-value of ≤0.05 was defined as statistically significant. Statistical analyses were performed using the JMP software (SAS Institute).

3 | RESULTS

3.1 | Characteristics of FF-10832 as a novel liposome-encapsulated gemcitabine

In the present study, we developed FF-10832, a liposome-encapsulated gemcitabine in the dissolved state using Fujifilm nanotechnology (Figure 1A). The formulation of FF-10832 was a liquid injection containing a liposome suspension; unilamellar vesicles encapsulating gemcitabine in solution. Transmission electron microscopy analysis clarified that our nanotechnology for liposome encapsulation enabled production of FF-10832 with a homogeneous appearance and

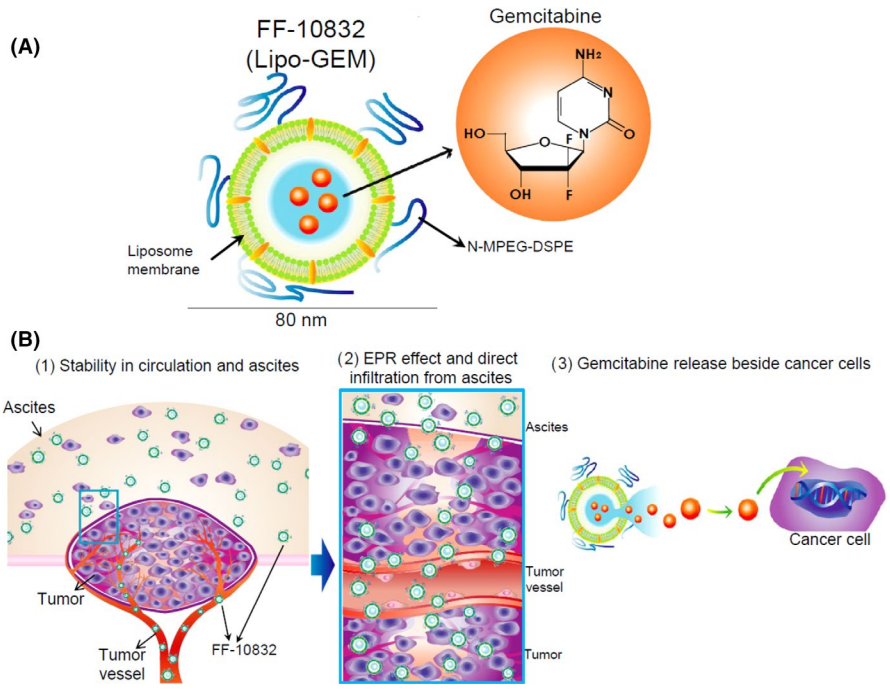
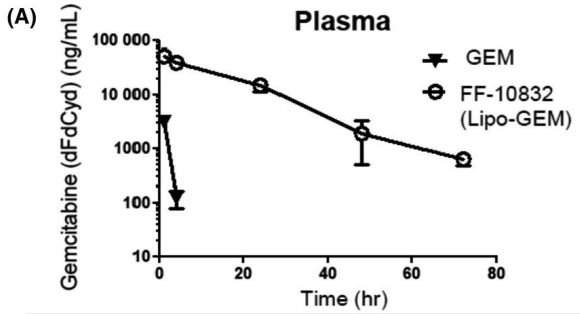
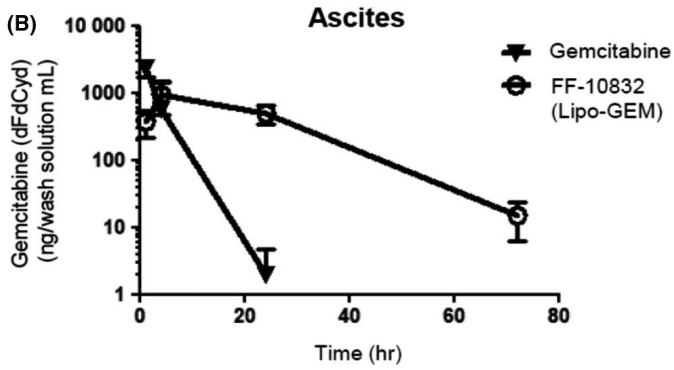


FIGURE 1 Structure and characteristics of novel liposome-encapsulated gemcitabine, FF-10832. A, Characteristic scheme of FF-10832 (Lipo-GEM) as liposome-encapsulated gemcitabine. PEG, polyethylene glycol. B, Advantages of FF-10832 as compared with non-encapsulated gemcitabine. (1) Stability in the circulation and ascites, (2) enhanced permeability and retention effect from tumor vessels and direct infiltration from the ascites and (3) gemcitabine release in close proximity to the cancer cells



		GEM	FF-10832 (Lipo-GEM)
Dose	mg/kg	240	3
$t_{1/2}$	hr	0.641	10.9
CL	mL/min/kg	467	0.0570
Vdss	mL/kg	25900	54.3
$AUC_{0\text{-last}}$	hr・ng/mL	8460	867000
$AUC_{0\text{-last}}$ / Dose	-	35.3	289000



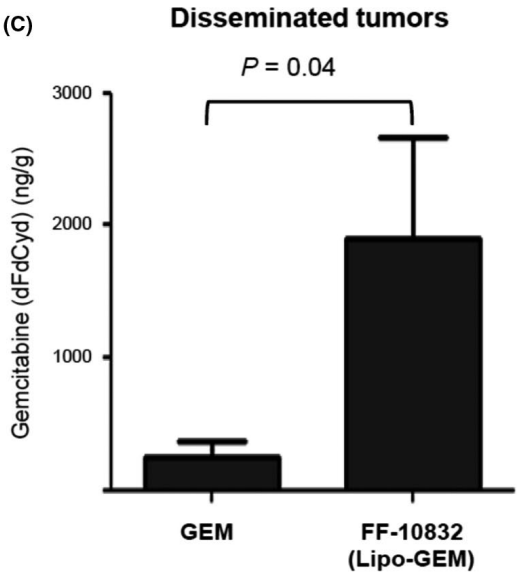


FIGURE 2 Pharmacokinetic analysis of gemcitabine in Colon26 peritoneal dissemination model mice following a single administration of gemcitabine (GEM) or FF-10832. A, Gemcitabine (dFdCyd) concentration-time curves in plasma and pharmacokinetic parameters. Plasma samples were collected at various intervals. Each data represents the mean ± standard deviation (n = 3 or 4). B, Gemcitabine concentration-time curves in ascites. Ascites samples were collected at various intervals. Each data represents the mean ± standard deviation (n = 3 or 4). C, Gemcitabine concentrations in the tumor 24 h following administration. Each data represents the mean ± standard deviation (n = 3). AUC0-last, area under the curve; AUC0-last/dose, dose-normalized area under the curve; CL, total clearance; $t_{1/2}$, half-life; Vdss, volume of distribution at steady state

size (80 nm). FF-10832 stock solution has been shown to be stable for at least 18 months.[16] Newly developed FF-10832 was constructed to acquire several advantages, such as (1) stability in circulation and ascites; (2) EPR effect from tumor vessels and direct infiltration from ascites; and (3) gemcitabine release from liposomes in close proximity to the cancer cells (Figure 1B). In particular, our nanotechnology improved drug delivery from the ascites and stability in the ascites as compared with ordinary liposomal drugs.

3.2 | Liposome encapsulation improved gemcitabine delivery to peritoneal tumors via increased stability of FF-10832 in the plasma and ascites

Using the peritoneal dissemination mouse model, the pharmacokinetics of gemcitabine in plasma and ascites were analyzed: non-liposomal gemcitabine (GEM) vs FF-10832. GEM and FF-10832 were administered intravenously. Figure 2A shows the gemcitabine (dFdCyd) concentration-time curves and pharmacokinetic parameters in plasma. The gemcitabine concentration in plasma 24 hours following GEM administration was below the limit of quantitation (lower limit of quantitation = 2 ng/mL). The area under the curve (AUC_{0-last}) value in plasma was 8460 hours*ng/mL following 240 mg/kg GEM administration. In contrast, the AUC value was 867 000 hours*ng/mL following 3 mg/kg FF-10832 administration. The dose-normalized plasma AUC_{0-last} (AUC0-last/dose) was 8190-fold higher in FF-10832-treated mice than in GEM-treated mice. An extended plasma $t_{1/2}$, lower total clearance (CL) rate and smaller volume of distribution at the steady state (Vdss) were observed following FF10832 administration as compared with GEM administration (GEM: $t_{1/2}$ = 0.641 hour, CL = 467 mL/min/kg, Vdss = 25 900 mL/kg; FF-10832: $t_{1/2}$ = 10.9 hours, CL = 0.057 mL/min/kg, Vdss = 54.3 mL/kg). Figure 2B shows the gemcitabine concentration-time curves in ascites. In addition to an improvement in the $t_{1/2}$ in plasma, the $t_{1/2}$ value of FF-10832 in ascites was longer than that of GEM (GEM:

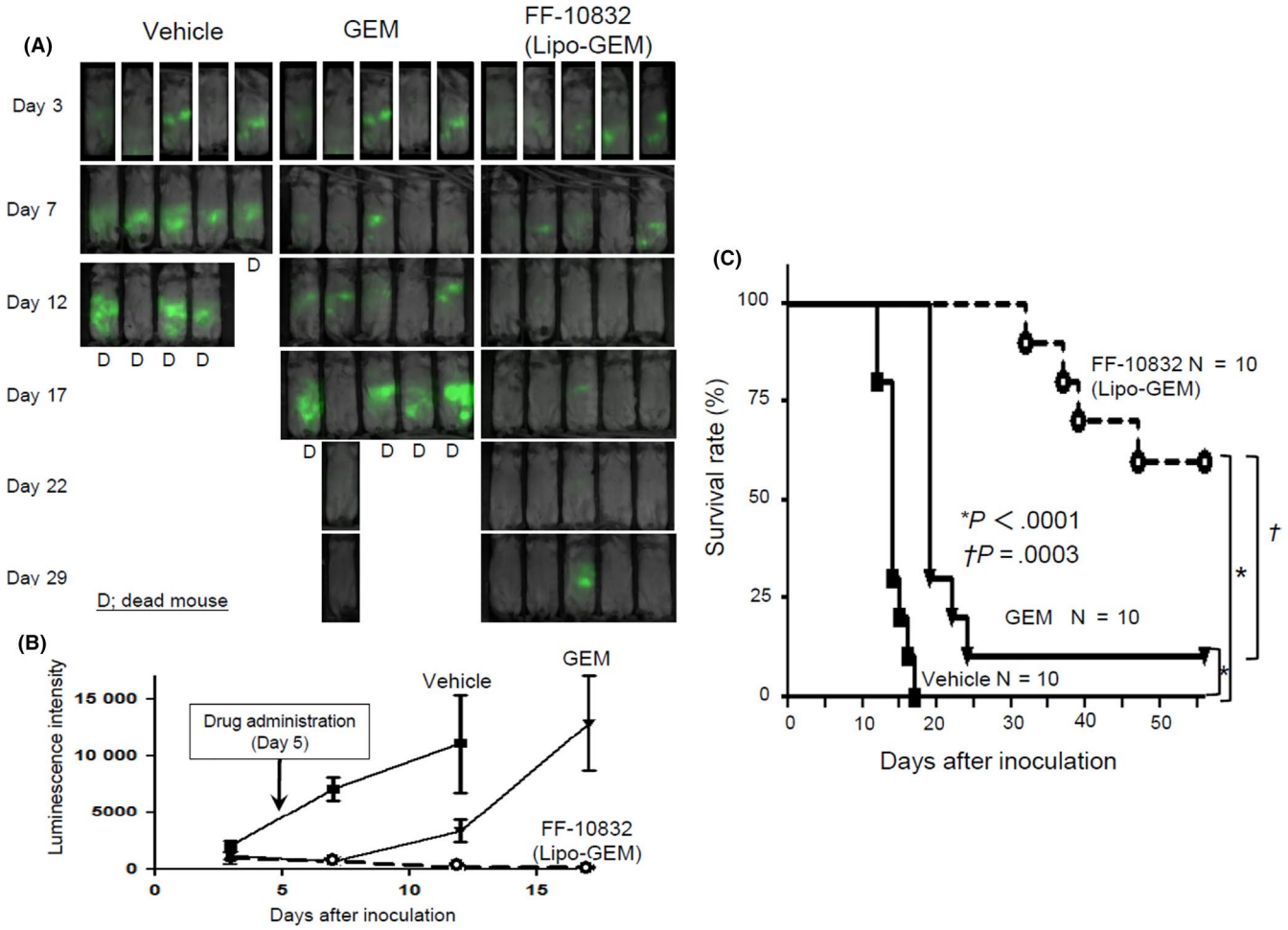


FIGURE 3 Luminescence intensity in the in vivo luciferase assay and Kaplan-Meier survival plots in Colon26 peritoneal dissemination model mice according to each drug administration. A, In vivo luciferase assay from day 3 to day 29 following transplantation in the 3 treatment groups; vehicle, gemcitabine (GEM) and FF-10832. B, Luminescence intensity was calculated using the Image J software. Luminescence intensity was significantly weaker in the FF-10832-treated group than that in vehicle- and GEM-treated groups. C, FF-10832-treated mice (n = 10) had a significantly better overall survival than mice in the vehicle-treated (n = 10) and GEM-treated groups (n = 10). Vehicle vs GEM ($P < 0.0001$); vehicle vs FF-10832 ($P < 0.0001$); GEM vs FF-10832 ($P = 0.0003$). Drug administration was performed on day 5

$t_{1/2}$ = 2.32 hours; FF-10832: $t_{1/2}$ = 10.9 hours). Moreover, Figure 2C shows the gemcitabine concentration in peritoneal tumors 24 hours following administration of GEM or FF-10832. As a result, the intratumoral gemcitabine concentration in the FF-10832 group was significantly higher than that in the GEM group ($P = 0.04$). Figure 2A-C suggests that FF-10832 achieved a long circulatory time in plasma and ascites, which allowed successful delivery of gemcitabine to the disseminated tumors via liposome encapsulation.

3.3 | FF-10832 administration prolonged survival in the peritoneal dissemination mouse model

In the in vivo luciferase assay, luminescence was observed on day 3 through the intact abdominal wall in 30/34 mice (88%; data not shown). The 30 mice with viable peritoneal tumors were randomly divided into 3 groups according to the luminescence intensity: vehicle (n = 10), GEM (n = 10) and FF-10832 (n = 10).

To quantitatively evaluate disseminated tumor growth, we performed an in vivo luciferase assay in 15 representative mice (vehicle, n = 5; GEM, n = 5; FF-10832, n = 5) on days 7, 12, 17, 22 and 29 following drug administration on day 5 (Figure 3A). All mice in the FF-10832-treated group survived to day 29. In contrast, all mice in the vehicle-treated group died by day 17, and almost all mice in the GEM-treated group died by day 22 (Figure 3A). The luminescence intensity in the vehicle-treated and GEM-treated groups gradually increased; however, that in the FF-10832-treated group was dramatically decreased (Figure 3B).

Gemcitabine (GEM) or FF-10832 was administered at 5 day following intraperitoneal injection of Colon26 cells. The median survival in the vehicle-treated and GEM-treated groups was 14 and 19 days, respectively. The vehicle-treated group had a poorer prognosis than the GEM-treated group ($P < 0.0001$) (Figure 3C). In the FF-10832-treated group, 60% (6/10) of the mice survived for the entire evaluation period of 56 days; in other words, a single

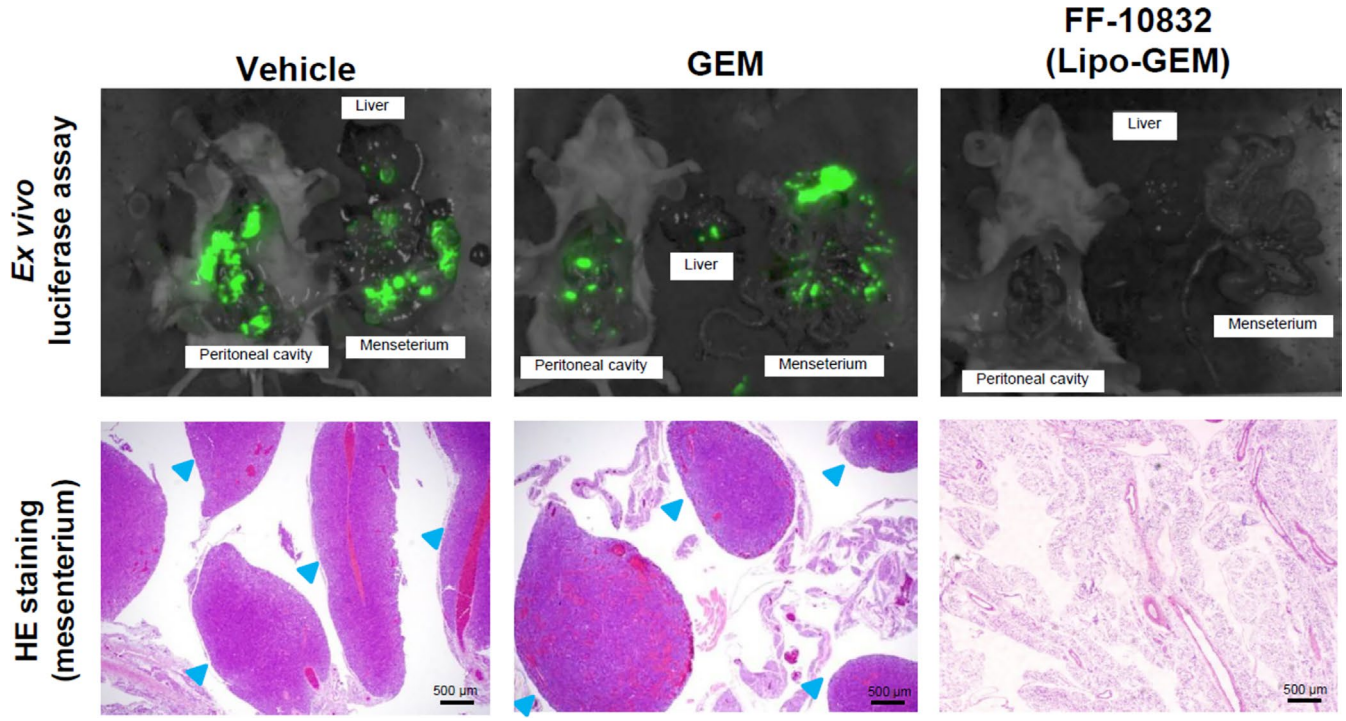


FIGURE 4 Representative ex vivo luciferase assay and microscopic images of peritoneal disseminated Colon26 tumors in dissemination model mice. Upper panel: Ex vivo luciferase assay to observe the abdominal wall, retroperitoneum, liver, gastrointestinal tract and mesenterium of vehicle-treated, gemcitabine (GEM)-treated and FF-10832-treated mice. Lower panel: Microscopic findings in moribund mice treated with vehicle or GEM-treated mice were evaluated on days 14 and 23, respectively. Surviving mice in the FF-10832 treatment group were evaluated on day 56, the final day of the observation interval (magnification: 20×). Blue arrows show the microscopically detectable tumors

administration of FF-10832 enabled the long-term survival of lethal model mice with peritoneal dissemination. The FF-10832-treated group had a significantly better prognosis than either the vehicle-treated ($P < 0.0001$) or GEM-treated ($P = 0.0003$) group (Figure 3C).

In the present study, we measured the body weight in each group (Figure S1). After administration, the body weight in the GEM group was decreased by 5.2%, while that in FF-10832 was decreased by 2.5%. No other obvious side effects were observed in both groups. Body weight loss in the vehicle-treated group was observed on day 12 followed by initiation of death. In the GEM-treated group, a significant body weight loss was observed from day 12 due to progression of peritoneal disseminated tumors. In contrast, continuous body weight gain was observed in the FF-10832-treated group until day 22. All mice in the FF-10832-treated group were alive during the measurement period of 22 days.

3.4 | FF-10832 administration strongly suppressed tumor growth in the peritoneal dissemination mouse model

The luminescence spots of disseminated tumors were strongly detected in the vehicle-treated group as compared with the GEM-treated group. In contrast, disseminated tumors in the FF-10832-treated group were undetectable as luminescence spots using a highly sensitive ex vivo luciferase assay that can diagnose the existence of invisible tumors (Figure 4; upper panel). Interestingly, microscopy analysis clarified that viable disseminated tumor mass in the FF-10832-treated group was almost completely absent; however, evident tumor masses existed in the vehicle-treated and GEM-treated groups (Figure 4; bottom panel).

4 | DISCUSSION

In the present study, we developed a novel liposome-encapsulated gemcitabine, FF-10832, with a significantly improved EPR effect and stability in the circulation and ascites. Moreover, a single administration of FF-10832 to the peritoneal dissemination mouse model showed a tumor reduction effect and significantly prolonged the survival interval. This was the result of effective gemcitabine delivery to peritoneal tumors due to increased stability in the circulation and ascites as compared with GEM treatment.

The well-known cytotoxic agent gemcitabine was selected as a liposome-encapsulated medicinal ingredient in the present study for the following reasons. The initial reason was that gemcitabine can induce the "bystander effect," which can transmit therapeutic efficacy from drug-delivered cells to surrounding cells without drug exposure via intracellular gap junctions.[10] Exploiting this unique characteristic of gemcitabine, we hypothesized that improved gemcitabine

delivery by liposome encapsulation may strengthen the therapeutic effect as compared with other cytotoxic drugs that lack this phenomenon. The second reason for choosing gemcitabine for encapsulation is that it is already recognized as a standard and traditional cytotoxic drug; therefore, we knew the majority of important and fundamental information prior to beginning the study, such as drug efficacy mechanism and representative side effects.[17-19] In recent years, several drugs have been developed against novel molecular targets; however, it is difficult to completely predict unexpected adverse effects of such drugs in the clinic. For instance, the humanized anti–VEGF monoclonal antibody bevacizumab causes hypertension and hemorrhage as specific adverse effects,[20] and the EGFR inhibitor gefitinib results in pulmonary toxicity.[21] These findings suggested that the use of novel medications in human trials is challenging for both patients and clinicians. Accordingly, we hypothesized that FF-10832 would be less likely to result in unknown side effects because a plethora of usage experiences with gemcitabine have already accumulated in the clinic; therefore, it is not as difficult for clinicians to deal with the traditional adverse effects of cytotoxic agents, such as nausea and vomiting, blood disorders and organ dysfunction. At this point, FF-10832 will have certain advantages in future clinical trials as compared with newly developed drugs. In fact, a phase I clinical trial to clarify the safety and efficacy of FF-10832 is already ongoing (ClinicalTrials.gov Identifier: NCT03440450). In the future, a large-scale trial will be conducted depending on the results of this phase I trial.

In the present study, we focused on the liposome encapsulation of gemcitabine to improve anticancer drug delivery against peritoneal disseminated tumor cells, which cause death in patients with gastrointestinal and gynecological cancer. Intraperitoneal injection can achieve continuation of a high drug concentration in the peritoneal cavity as compared with systemic injection.[22] Armstrong et al,[23] report that intraperitoneal administration of cisplatin and paclitaxel improves prognosis in ovarian cancer patients with peritoneal dissemination as compared with intravenous systemic chemotherapy. However, it is known that intraperitoneal injection has certain disadvantages, such as injury of abdominal organs and catheter-related complications, including obstruction, infection and catheter migration into the abdominal cavity.[24]

In the present study, an extended plasma $t_{1/2}$, lower clearance rate (CL) and smaller volume of distribution (Vdss) were observed following FF-10832 administration as compared with GEM administration. These results are indicative of the drug remaining primarily in the blood when administered in liposomes. In addition, an extended ascites $t_{1/2}$ was observed, indicating that a high stability in plasma owing to liposome encapsulation allowed longer exposure in the ascites due to permeation of liposomes from plasma. Interestingly, the levels of Vdss, a factor for drug distribution into non-target sites, in FF-10832 was lower than that in liposomal gemcitabine established by other researchers,[25] suggesting the effective accumulation of FF-10832 into target sites, including peritoneal tumors and ascites in our lethal model mice. Therefore, we assume that FF-10832, with low Vdss, has an important characteristic to deliver gemcitabine into peritoneal disseminated tumors from both of the circulations by increased-EPR effects and the ascites. This report provides the first confirmation of the effective delivery of intravenously injected liposomal gemcitabine into ascites and peritoneal disseminations as target sites.

Our animal experiments show that a single intravenous administration of FF-10832 enabled the long-term survival of lethal model mice without drug administration-related complications. Interestingly, despite the use of the ex vivo luciferase assay with a high cancer detection sensitivity, we could not detect any viable cancer cells on day 56 in the peritoneal cavity of model mice treated with FF-10832 (Figure 4). Our data suggest that intravenous FF-10832 administration in the clinic is a curative therapeutic tool for cancer patients with unresectable peritoneal dissemination through the effective delivery of gemcitabine from not only the circulation but also from the ascites.

In conclusion, we developed a novel liposome-encapsulated gemcitabine, FF-10832, to improve drug delivery to disseminated tumors. Despite the employment of a single administration, FF-10832 treatment enabled long-term survival of lethal model mice via improvement of the EPR effect and gemcitabine stability in the circulation and ascites as compared with non-encapsulated gemcitabine. The ongoing phase I clinical trial will clarify the safety and efficacy of FF-10832 (NCT03440450); nevertheless, a large-scale study will be needed in the future to evaluate the curative effect of FF-10832 in cancer patients with peritoneal dissemination.

ACKNOWLEDGMENTS

The authors would like to thank Shigetomo Tsujihata, Mariko Nakamura, Sayaka Okada, Sawa Nagayama and Hiroko Nemoto for their advice and technical assistance. This work was carried out in part at the Bioresource Center, Gunma University Graduate School of Medicine.

DISCLOSURE

This research was supported by the FUJIFILM Corporation. Tamami Higuchi, Tomoharu Naito, Hiromu Kitahara, Takeshi Matsumoto, Chihaya Kakinuma, Shinji Hagiwara are employees of the FUJIFILM Corporation. Takehiko Yokobori received a donation from Ono Pharmaceutical, Chugai Pharmaceutical and Memolead.

ORCID

Tamami Higuchi https://orcid.org/0000-0002-6049-3666

REFERENCES

1. Kato A, Kataoka H, Yano S, et al. Maltotriose conjugation to a chlorin derivative enhances the antitumor effects of photodynamic therapy in peritoneal dissemination of pancreatic cancer. *Mol Cancer Ther*. 2017;16:1124-1132.

2. Abiko K, Mandai M, Hamanishi J, et al. PD-L1 on tumor cells is induced in ascites and promotes peritoneal dissemination of ovarian cancer through CTL dysfunction. *Clin Cancer Res*. 2013;19:1363-1374.
3. Gomez-Roman N, McGregor F, Wheate NJ, Plumb JA. Cucurbit [7] uril encapsulated cisplatin overcomes resistance to cisplatin induced by Rab25 overexpression in an intraperitoneal ovarian cancer model. *J Ovarian Res*. 2015;8:62.
4. Emoto S, Yamaguchi H, Kamei T, et al. Intraperitoneal administration of cisplatin via an in situ cross-linkable hyaluronic acid-based hydrogel for peritoneal dissemination of gastric cancer. *Surg Today*. 2014;44:919-926.
5. Mini E, Nobili S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. *Ann Oncol*. 2006;17:v7-v12.
6. Pignata S, Cecere SC, Du Bois A, Harter P, Heitz F. Treatment of recurrent ovarian cancer. *Ann Oncol*. 2017;28:viii51-viii56.
7. Krantz BA, O'Reilly EM. Biomarker-based therapy in pancreatic ductal adenocarcinoma: an emerging reality? *Clin Cancer Res*. 2018;24:2241-2250.
8. Jimenez-Fonseca P, Solis MP, Garrido M, et al. Gemcitabine plus capecitabine (Gem-Cape) biweekly in chemorefractory metastatic colorectal cancer. *Clin Transl Oncol*. 2015;17:384-392.
9. Vogl TJ, Gruber-Rouh T, Eichler K, et al. Repetitive transarterial chemoembolization (TACE) of liver metastases from gastric cancer: local control and survival results. *Eur J Radiol*. 2013;82:258-263.
10. Garcia-Rodriguez L, Perez-Torras S, Carrio M, et al. Connexin-26 is a key factor mediating gemcitabine bystander effect. *Mol Cancer Ther*. 2011;10:505-517.
11. Kornmann M, Butzer U, Blatter J, Beger HG, Link KH. Pre-clinical evaluation of the activity of gemcitabine as a basis for regional chemotherapy of pancreatic and colorectal cancer. *Eur J Surg Oncol*. 2000;26:583-587.
12. Graeser R, Bornmann C, Esser N, et al. Antimetastatic effects of liposomal gemcitabine and empty liposomes in an orthotopic mouse model of pancreatic cancer. *Pancreas*. 2009;38:330-337.
13. Iinuma H, Maruyama K, Okinaga K, et al. Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. *Int J Cancer*. 2002;99:130-137.
14. Sadzuka Y, Hirota S, Sonobe T. Intraperitoneal administration of doxorubicin encapsulating liposomes against peritoneal dissemination. *Toxicol Lett*. 2000;116:51-59.
15. Takahashi R, Yokobori T, Osone K, et al. Establishment of a novel method to evaluate peritoneal microdissemination and therapeutic effect using luciferase assay. *Cancer Sci*. 2016;107:341-346.
16. Matsumoto T, Kitahashi T, Komori T, et al. Abstract 5148: liposomal gemcitabine, FF-10832, improves gemcitabine (GEM) pharmacokinetics (PK) and increases anti-tumor efficacy. *Cancer Res*. 2017;77:5148.
17. Saung MT, Zheng L. Current standards of chemotherapy for pancreatic cancer. *Clin Ther*. 2017;39:2125-2134.
18. Burris HA 3rd, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol*. 1997;15:2403-2413.
19. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med*. 2013;369:1691-1703.
20. Keating GM. Bevacizumab: a review of its use in advanced cancer. *Drugs*. 2014;74:1891-1925.
21. Dawson NA, Guo C, Zak R, et al. A phase II trial of gefitinib (Iressa, ZD1839) in stage IV and recurrent renal cell carcinoma. *Clin Cancer Res*. 2004;10:7812-7819.
22. Kodera Y, Imano M, Yoshikawa T, et al. A randomized phase II trial to test the efficacy of intra-peritoneal paclitaxel for gastric cancer with high risk for the peritoneal metastasis (INPACT trial). *Jpn J Clin Oncol*. 2011;41:283-286.
23. Armstrong DK, Bundy B, Wenzel L, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med*. 2006;354:34-43.
24. Al-Quteimat OM, Al-Badaineh MA. Intraperitoneal chemotherapy: rationale, applications, and limitations. *J Oncol Pharm Pract*. 2014;20:369-380.
25. Cosco D, Bulotta A, Ventura M, et al. In vivo activity of gemcitabine-loaded PEGylated small unilamellar liposomes against pancreatic cancer. *Cancer Chemother Pharmacol*. 2009;64:1009-1020.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Higuchi T, Yokobori T, Takahashi R, et al. FF-10832 enables long survival via effective gemcitabine accumulation in a lethal murine peritoneal dissemination model. *Cancer Sci*. 2019;110:2933–2940. https://doi.org/10.1111/cas.14123