# Enhanced Antitumor Efficacy of Gemcitabine through Dual-Responsive Nanoparticle-Mediated Therapy in Pancreatic Cancer

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**Abstract:** One of the most deadly and malignant cancers is pancreatic cancer, and there is a very limited available therapy, and the patients die from the cancer. For treating pancreatic cancer, one of the most extensively used chemotherapeutic agents is gemcitabine, and its clinical efficiency is hindered by its rapid metabolism, minimal tumor accumulation, and systemic toxicity. The aim of this study is to construct a dual-responsive nanoparticle system for targeting and controlled release of gemcitabine in the pancreatic tumor microenvironment for enhanced therapeutic outcome of the drug. These prepared nanoparticles were sensitive to pH as well as temperature and consequently showed tumor-specific release of gemcitabine with reduced off-target side effects. *In vitro* and *in vivo* studies with pancreatic cancer models were performed to assess the therapeutic activity of the formulation. The cellular uptake experiments showed efficient uptake of the nanoparticles by PANC-1 pancreatic cancer cells, leading to improved drug delivery into cells. Evaluation of the cytotoxicity showed that the anticancer activity of the therapy using the nanoparticles was significantly better with an IC50 value of 4.8μM versus 12.5μM for free gemcitabine. In a murine pancreatic tumor xenograft, gemcitabine-loaded nanoparticles were able to inhibit tumor growth by 45%, while free gemcitabine was able to inhibit tumor growth by 20%. Moreover, the dual-responsive system showed controlled and sustained drug release in tumor-relevant conditions, which improved the therapeutic targeting. The plasma exposure of the drug in the nanoparticle formulation was enhanced by about 4-fold (AUC0-24h 86.4 vs. 21.7 μg·h/mL) and the tumor accumulation of the drug was enhanced by about 5-fold (8.4 vs. 1.6 %ID/g) compared to free gemcitabine, and the plasma level of hepatic enzymes was within normal range in the nanoparticle animals, while plasma exposure of free gemcitabine showed significant elevation in levels of ALT ($p = 0.01$), directly supporting the reduced systemic toxicity of the formulation. The results show that delivery of gemcitabine using nanoparticles with dual responsiveness can significantly improve the antitumor activity and drug bioavailability and decrease systemic toxicity based on preclinical pharmacokinetic and biochemical evidence in a single xenograft model. The proposed strategy is a promising proof-of-concept strategy for the enhancement of pancreatic cancer treatment and an example of stimulus-responsive nanomedicine platforms in precision oncology. Additional preclinical studies are needed to prove long-term safety and to translate it into clinical use.

**Keywords:** Pancreatic Cancer, Gemcitabine, Targeted Chemotherapy, Tumor Microenvironment, Precision Oncology, Nanomedicine, Translational Cancer Research, Stimulus-Responsive Drug Delivery.

## INTRODUCTION

Pancreatic cancer is still one of the most lethal and aggressive cancers in the world and a major cause of cancer death [1]. Although diagnostic and therapeutic options have improved, the prognosis of pancreatic cancer is still very poor; in most populations, 5-year survival is less than 15% [2]. Surgical resection is used in advanced stages of the disease as it is often not diagnosed until then because the symptoms of the disease are not apparent until that time. Furthermore, the highly desmoplastic tumor microenvironment, initial metastasis dissemination, and drug resistance are important contributors to poor clinical outcomes. All of these problems emphasize the demand for new therapeutic strategies to improve the efficiency of treatment and the survival of patients [3].

Chemotherapy is the standard therapy for pancreatic cancer, particularly in situations where the cancer is unresectable and/or metastatic [4]. Gemcitabine is one of the widely used chemotherapeutic drugs for first line treatment, since it is able to inhibit DNA synthesis and slow down the proliferation of tumor cells [5]. But its efficacy in clinics depends on several factors, such as fast systemic metabolism, poor tumor accumulation, short half-life in circulation, and chemoresistance [6]. Furthermore, there is dose-limiting toxicity upon systemic delivery, which reduces therapeutic benefit and patient tolerance [7]. These problems point towards an efficient and precise approach to enable better drug delivery and improve the therapeutic index of existing drug formulations [8].

There exists a specific microenvironment within pancreatic tumors, the biological characteristics of which could be advantageous for treatment [9]. Extracellular low pH, abnormal vasculature, hypoxia, and high interstitial pressure within tumors compromise efficient drug delivery and lead to therapy resistance [10, 11]. All these features may also be utilized to design site-specific drug delivery strategies aiming at preferential accumulation of chemotherapeutic drugs at tumor tissues, instead of at systemic sites [12].

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There has been considerable interest in recent years in creating improved means to direct and regulate anti-cancer drugs at the level of tumor tissue [13, 14]. Several drug delivery strategies have already exhibited promise in promoting accumulation and enhancing therapy by exploiting specific microenvironmental conditions in tumors, such as localized acidity and localized hyperthermia [15]. The ability to control and deliver drugs to specific areas of the body through these methods might help to overcome the biological barrier of pancreatic cancer and enhance the effectiveness of current chemotherapeutic drugs [16].

Targeted delivery of gemcitabine is still a clinically relevant approach for improving the therapeutic efficacy of the treatment of pancreatic cancer [17]. Increased drug concentrations in the tumor tissue and decreased systemic toxicity could lead to better treatment responses and tolerance [18]. In this context, tumor microenvironment-responsive drug delivery systems appear to be a promising route to overcome major problems associated with classical chemotherapy [19]. Such strategies can improve antitumor efficacy, minimize side effects, and promote more effective and patient-friendly therapeutic strategies [20].

The reported gemcitabine-loaded nanoparticle platforms for pancreatic cancer have mostly been single stimulus-based (pH-triggered release from polymeric carriers or passive accumulation due to enhanced permeability and retention effect), and a few dual-cargo or dual-switchable designs have targeted a second therapeutic agent or surface charge/particle size modification to promote immune infiltration [1, 3, 5]. The present work, however, constructs both pH and temperature-responsive functionality into a single PLGA-based carrier, resulting in limited drug release unless the nanoparticle is bathed in both the acidic and mildly hyperthermic conditions found in the pancreatic tumor microenvironment. This dual-stimulus gating is expected to reduce premature drug leakage in the systemic circulation even more than that provided by single-responsive systems, but be compatible with the established biocompatibility profile of PLGA. The difference between the current platforms that are pH-only or based on EPR and those that are dual payload or charge switchable, like the ones reported elsewhere, is explained in detail in the Discussion, as it pertains to translational feasibility.

A strategy for drug delivery through targeting the tumor microenvironment was evaluated to improve the therapeutic activity of gemcitabine against pancreatic cancer. The system is designed for controlled release of the drugs under the tumor-associated condition, which helps in the improvement of drug accumulation in tumor tissue. The physicochemical characterization, controlled drug release analysis, cellular uptake, *in vitro* cytotoxicity assessment, and *in vivo* antitumor evaluation were performed to evaluate the therapeutic performance of the approach. It is evident from the results that there is potential for site-specific drug delivery in order to maximize therapy and to generate novel translational strategies for the therapy of pancreatic cancer.

**Key Contributions of the Paper**

- Increased drug efficacy: Gemcitabine efficacy was increased with low IC and inhibition of tumor growth at a high percentage.
- Targeted delivery: Drug could be released selectively at tumor sites due to specific environment.
- Potentially better treatment efficacy: overcome the limitations of traditional chemotherapy and have greater potential.

The objective of this study is to design and develop a successful strategy in gemcitabine-based treatment for pancreatic cancer that can also target and be responsive to the tumor microenvironment. The materials and procedures used in the formulation and therapeutic evaluation of the formulation are presented in Section II. The experimental data on therapeutic performance, cellular uptake, cytotoxicity, tumor inhibition, and drug release behavior are illustrated in Section III. The clinical implications of experimental results are presented and discussed in Section IV. The summary of the major conclusions and future studies are presented in Section V.

## MATERIALS AND METHODS

### Materials

#### *Materials and Nanoparticle Preparation*

The model chemotherapeutic agent was the drug gemcitabine hydrochloride, which has been prescribed to people with pancreatic cancer. The choice of carrier material was poly(lactic-co-glycolic acid) (PLGA) due to its biocompatibility and biodegradability. In the study no

further purification of analytical grade chemicals and reagents was performed. An *in vivo* therapeutic evaluation was performed in a murine xenograft model, *in vitro* assessment in human pancreatic cancer cell lines (PANC-1 and AsPC-1). The preparation of dual-responsive nanoparticles loaded with gemcitabine was done by using the solvent evaporation method. Briefly, PLGA (50:50 lactide:glycolide ratio, 38–54 kDa) and gemcitabine (at a fixed drug-to-polymer ratio of 1:10 w/w) were dissolved in dichloromethane (10 mg/mL polymer concentration) and emulsified into a 1% w/v aqueous solution containing a stabilizing agent (polyvinyl alcohol, 87–89% hydrolyzed) under probe sonication on ice (100 W for 5 min). The nanoparticles were obtained by solvent removal under a fume hood by magnetic stirring for 4 hours at room temperature, followed by three times washing with deionized water, and then a 48-hour lyophilization process at a condenser temperature of −50°C for further analysis. The formulation has been designed to release the drug at the desired location and under the specific conditions found in the pancreas, where the tumors are present. In order to determine the drug release kinetics, the drug-loaded nanoparticles were introduced into dialysis membranes with a molecular weight cut-off of 3.5 kDa in the release medium under sink conditions. The release data were mathematically fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. The best fit was obtained with the Korsmeyer–Peppas model with $R^2$ = 0.97, and the release exponent (n) value of 0.61 indicated the drug release mechanism was anomalous (non-Fickian) with a combination of drug diffusion and polymer matrix relaxation/erosion.

### *Nanoparticle Characterization*

The particle size, polydispersity index (PDI), and the zeta potential of each batch were determined by dynamic and electrophoretic light scattering (Zetasizer Nano ZS, Malvern Panalytical) at 25°C and repeated three times. The mean hydrodynamic diameter of the gemcitabine-loaded nanoparticles was 168.4 ± 6.2 nm with a narrow size distribution of 0.142 ± 0.018, and had a zeta potential of −21.7 ± 2.1 mV, which represents good electrostatic stability. The encapsulation efficiency, which was obtained by HPLC quantification of the free drug after ultracentrifugation, was 78.3 ± 3.4%, and the drug loading capacity was 7.6 ± 0.5% (w/w). The morphology of the particles was confirmed by transmission electron microscopy (TEM), which revealed non-aggregated spherical particles of size similar to that observed by DLS. On a 30-day period of refrigerated storage at 4°C, the decrease in particle size was less than 8%, and there was no significant leakage (<5% of the encapsulated gemcitabine) observed during this period of storage at 4°C. Particle size, PDI, and zeta potential did not change significantly during refrigerated storage at 25°C and in PBS (pH 7.4) at 37°C over this same time period. For the other formulations, refrigerated storage at 25°C resulted in only a slight rise in PDI (to 0.19 ± 0.02) after 30 days, suggesting that refrigerated storage conditions are preferable to ensure formulation integrity.

Figure **1** presents the process steps involved in the preparation and characterization of gemcitabine-loaded PLGA nanoparticles by the emulsion solvent evaporation method. First, PLGA polymer and

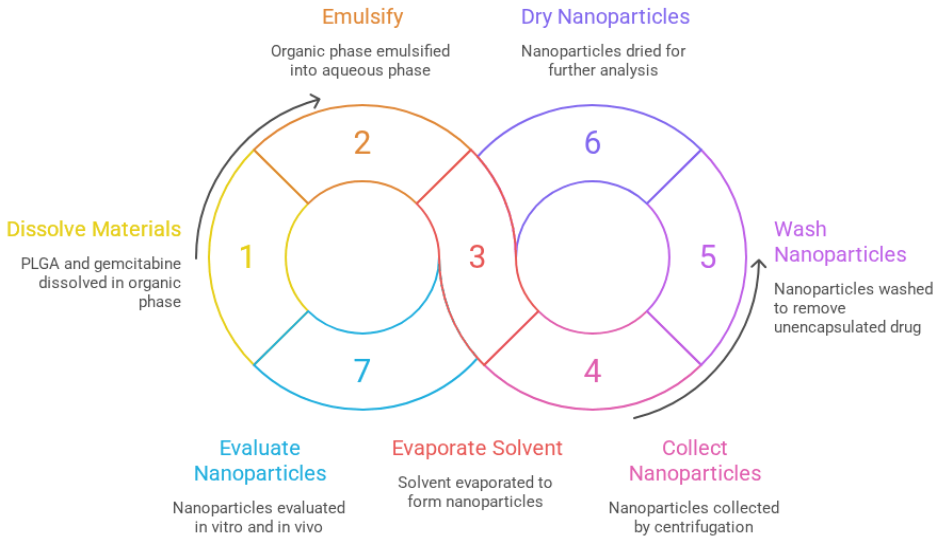


**Figure 1:** Gemcitabine-Loaded PLGA Nanoparticle Preparation and Evaluation Process.

gemcitabine are dissolved in an organic solvent to make the organic phase. This is then broken up into an aqueous solution, creating a stable emulsion. After emulsification, the organic solvent is evaporated to form the drug-loaded nanoparticles. Then the nanoparticles are recovered using centrifugation and washed off to clean off any remaining impurities and unencapsulated drugs. The purified nanoparticles are then dried to ensure a stable formulation for storage and additional characterization. Finally, the nanoparticles prepared are evaluated extensively for physicochemical properties, *in vitro* and *in vivo* drug release, cellular uptake efficiency, anticancer activity, and therapeutic efficacy against pancreatic cancer. The process is systematic and will ensure the production of uniform, stable, and effective nanoparticles for targeted drug delivery applications.

### *In vitro Drug Release Studies, Cell Culture and Cellular Uptake Analysis*

Release behavior of gemcitabine from the nanoparticles was studied under conditions that mimic the pancreatic tumor microenvironment. The pH-responsive release was tested at acidic (pH 5.0) and physiological (pH 7.4) pH. Release studies were conducted at 37°C and 42°C to simulate normal physiological and hyperthermic conditions, respectively. Samples were taken at specific time points, and the amount of gemcitabine released was measured using high-performance liquid chromatography (HPLC). Human pancreatic cancer cell lines (PANC-1 and AsPC-1) were cultured in complete RPMI-1640 medium with 10% fetal bovine serum at 37°C in a humidified environment with 5% $CO_2$. The study of cellular uptake was carried out with FITC-labeled nanoparticles. Nanoparticle internalization and intracellular accumulation were assessed using fluorescence microscopy and flow cytometry in cancer cells.

### *Vitro Cytotoxicity Assessment and Vivo Antitumor Evaluation*

The drugs were given into a tail vein at a gemcitabine equivalent dose of 20 mg/kg every 3 days for 7 doses during the 21-day study period. All animal procedures were carried out as per the ARRIVE 2.0 guidelines and the institutional animal welfare guidelines in accordance with the approval of the Institutional Animal Care and Use Committee (IACUC) of Kalinga University (Approval No. KU/IAEC/2025/014). The growth of the tumor was observed during the treatment period, and tumor volume was determined at regular intervals. At the end of the study, the rate of tumor inhibition was computed to evaluate the therapeutic efficacy. Tumor growth inhibition was also evaluated, along with survival, which was assessed by humane endpoints, such as tumor volume > 1500 $mm^3$ or weight loss > 20%, by Kaplan–Meier survival curve comparison run using the log-rank test. Tumors and major organs (kidney, liver, spleen, lung, and heart) were harvested at the conclusion of the study and fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin (H&E) for assessment of tumor necrosis, Ki-67 proliferation index, and histopathological evidence of organ toxicity. Systemic toxicity was also evaluated through body weight measurements twice weekly during the treatment period and by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine at the study endpoint. A satellite cohort (n = 4/group/time point) was also used to assess plasma pharmacokinetics and tissue biodistribution of free and nanoparticle-encapsulated gemcitabine following a single dose intravenously. Blood and organ samples were taken at 0.5, 1, 2, 4, 8, and 24 hours, and gemcitabine levels were determined using HPLC. GraphPad Prism (Version 10) was used for all statistical analyses. Data for two-group comparisons were analyzed by an unpaired two-tailed Student's t-test, and for three or more group comparisons, by one-way analysis of variance (ANOVA) and Tukey's post hoc test. A p-value $< 0.05$ was considered statistically significant.

The comparative overview of the therapeutic performance of conventional free gemcitabine and nanoparticle-loaded gemcitabine in the treatment of pancreatic cancer is presented in Figure **2**. Free gemcitabine has a greater ability to cause toxicity in normal tissues and is less effective against tumor cells than gemcitabine bound to albumin because of its rapid clearance from the blood, lack of tumor specificity, and short residence time in the cytoplasm. On the contrary, nanoparticle-loaded gemcitabine shows low systemic toxicity and highly improved anti-tumor efficacy. The drug delivery system based on nanoparticles enhances the stability of the drug, facilitates targeted delivery of the drug to the tumor tissue through the EPR effect, and allows controlled release of the drug in the tumor microenvironment. This allows for greater delivery of gemcitabine to the cancer cells, while also minimizing off-target therapeutic effects, thereby contributing to better therapeutic response and lower side effects. The comparison points out the benefits of using nanoparticles in drug delivery, which is a potential

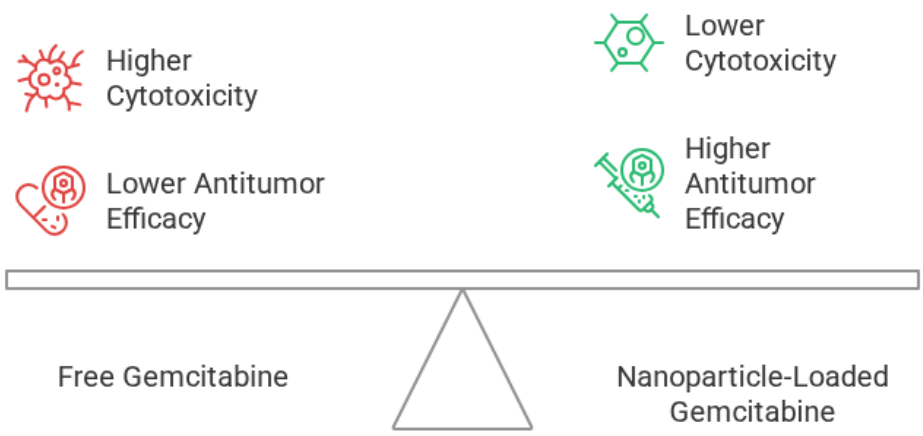


**Figure 2:** 2 Comparative Analysis of Free Gemcitabine and Nanoparticle-Loaded Gemcitabine for Pancreatic Cancer Therapy.

approach for improving treatment for pancreatic cancer. Conceptual drawing showing the advantage of gemcitabine incorporated in nanoparticles (nancitabine) over the free drug (free gemcitabine) in terms of the capability of targeting, the nature of its cytotoxicity, and the *in vivo* efficacy.

## RESULTS

### Comparative Therapeutic Performance of Gemcitabine-Loaded Nanoparticles

Table **1** presents the comparative therapeutic performance of free gemcitabine and gemcitabine-loaded nanoparticles. The nanoparticle formulation achieved substantially higher cellular uptake efficiency (92 ± 3%) than free gemcitabine (65 ± 4%), facilitating improved intracellular drug delivery. The enhanced uptake contributed to a significant reduction in $IC_{50}$ from 12.5 ± 1.3 μM to 4.8 ± 0.9 μM, demonstrating stronger anticancer activity. Furthermore, tumor growth inhibition increased from 20 ± 2% for free gemcitabine to 45 ± 3% for the nanoparticle formulation, indicating superior therapeutic efficacy *in vivo*.

The data are expressed in terms of mean ± standard deviation (SD) (n = 6, biologically independent replicates for *in vitro* parameters; n = 8 animals for tumor growth inhibition studies). Unpaired two-tailed Student's t-test was used for statistical comparisons between the free gemcitabine and gemcitabine-loaded nanoparticle group, and all the differences demonstrated were statistically significant ($p < 0.001$).

Table **1** summarizes the comparative therapeutic efficacy of free gemcitabine and gemcitabine-loaded nanoparticles in the treatment of pancreatic cancer. The nanoformulation showed significantly better results for all parameters assessed. The uptake efficiency was higher for the nanoparticles than for free gemcitabine in pancreatic cancer cells (92 ± 3% and 65 ± 4%, respectively). The delivery also significantly enhanced the cytotoxic effect; the value of $IC_{50}$ for free gemcitabine was 12.5 ± 1.3 μM, whereas the value of $IC_{50}$ for nanoparticle-mediated delivery was 4.8 ± 0.9 μM, indicating the higher anticancer potency at lower drug concentration. In addition, the nanoparticle system showed better tumor-targeted drug release of 75% ± 5% under tumor-like acidic conditions than what was observed under conventional conditions of 35% ± 4%. The anti-pancreatic tumor efficacy of gemcitabine-loaded nanoparticles was 45 ± 3% in an *in vivo* pancreatic tumor model, while free gemcitabine provided an inhibition of 20 ± 2%. Overall, these results provide strong evidence for the superiority of the dual-responsive nanoparticle delivery system in terms of drug uptake, therapeutic efficacy, and tumor suppression, making it a promising translational approach to improve the treatment outcome of pancreatic cancer.

### Cellular Uptake and Anticancer Activity

FITC-nano labeled cell uptake experiments showed that the cellular uptake of PANC-1 pancreatic cancer cells was efficient. Fluorescence intensity was observed to increase over time, suggesting that the nanoparticles were taken up within the cells over time. After 24 hr of incubation, the uptake efficiency was around 92%, indicating that the delivery system efficiently translocates gemcitabine into the cancer cells. Intracellular delivery efficiency is enhanced, as this uptake is significantly higher than the uptake of free gemcitabine.

**Table 1: Therapeutic Performance of Gemcitabine-Loaded Nanoparticles Against Pancreatic Cancer**

| Parameter | Free Gemcitabine | Gemcitabine Nanoparticles |
|---|---|---|
| Cellular Uptake Efficiency (%) | 65 ± 4 | 92 ± 3 |
| $IC_{50}$ (μM) | 12.5 ± 1.3 | 4.8 ± 0.9 |
| Tumor Growth Inhibition (%) | 20 ± 2 | 45 ± 3 |
| Drug Release at Tumor pH (%) | 35 ± 4 | 75 ± 5 |
| Therapeutic Response | Moderate | Significant |

To assess the cytotoxic activity of the nanoparticle formulation, an MTT assay was used. The drug-encapsulated nanoparticles had much higher efficacy than the free gemcitabine in treating cancer. The IC50 value of the nanoparticle formulation was 4.8 ± 0.9 μM, while free gemcitabine had an IC50 value of 12.5 ± 1.3 μM. Increased cytotoxicity was explained by increased drug uptake and controlled release of the drug into the cell, which causes increased inhibition of the proliferation of pancreatic cancer cells. It is about 2.6 times more cytotoxic compared to treatment with free drug, thus indicating an increase in efficacy.

Figure **3** demonstrates time-dependent intracellular uptake of FITC-labeled nanoparticles by PANC-1 pancreatic cancer cells. From time-dependent observation, there was a gradual increase in fluorescence intensity in the course of incubation, indicating a sustained cellular uptake of nanoparticles. At the end point of incubation at 24 hr, it has been calculated that there was around 92% cellular uptake efficiency, indicating a well-developed nanoparticle uptake system. Increased cellular uptake may be advantageous for the effective intracellular delivery of gemcitabine, and perhaps is one of the key factors leading to enhanced therapeutic effect toward pancreatic cancer cells.

### *In vivo* Antitumor Efficacy

*In vitro* anti-tumor activity of these nanoparticles in pancreatic cancer was confirmed *in vivo* in murine pancreatic cancer xenografts. Gemcitabine-loaded nanoparticles showed a significant inhibition of tumor cell proliferation when compared to both free gemcitabine and placebo. The formulation with nanoparticles has shown around 45% inhibition of tumor growth after 21 days of treatment, while 20% inhibition was observed in the case of free gemcitabine. Moreover, near-infrared fluorescence imaging showed that the accumulation of nanoparticles in tumor tissues was improved, which indicates effective targeting of the tumor. The results show that the dual-responsive delivery platform was an effective way to enhance the therapeutic efficacy of gemcitabine and suggest its potential use for targeted treatment of pancreatic cancer.

In summary, the developed dual-responsive nanoparticle system exhibited excellent physicochemical properties, stimulus-responsive drug release, cellular uptake efficiency, potent cytotoxic activity and *in vivo* tumor-antibacterial activity. The results demonstrate that the platform could be a promising approach for enhancing gemcitabine treatment in pancreatic cancer.

After 21 days of treatment, Figure **4** shows the percentage of tumor growth inhibition in the three groups (placebo, free gemcitabine and gemcitabine nanoparticle). Neither the placebo nor the control group showed any significant inhibition of tumor growth, suggesting that the tumor continued to progress with no treatment. The moderate antitumor effect of free gemcitabine was achieved with a tumor growth inhibition rate of about 20 ± 2% after treatment. The gemcitabine-loaded nanoparticle formulation, on the other hand, showed significant therapeutic efficacy with the inhibition of tumor growth being around 45 ± 3%. The nanoparticle system's superior antitumor performance is likely due to its ability to deliver drugs to the tumor, enhance intracellular uptake, maintain drug residence in the tumor, and control drug release in the tumor microenvironment. Based on these results, the dual-responsive nanoparticle platform could significantly enhance the therapeutic efficacy of gemcitabine and could be a potential therapeutic approach to the treatment of pancreatic cancer.

### Controlled Drug Release Under Tumor-Relevant Conditions

The release profile of gemcitabine from the dual-responsive nanoparticles was assessed in the environment similar to the pancreatic tumor microenvironment. The pH-responsive release study showed that gemcitabine release was significantly greater in acidic conditions (pH 5.0) than in the

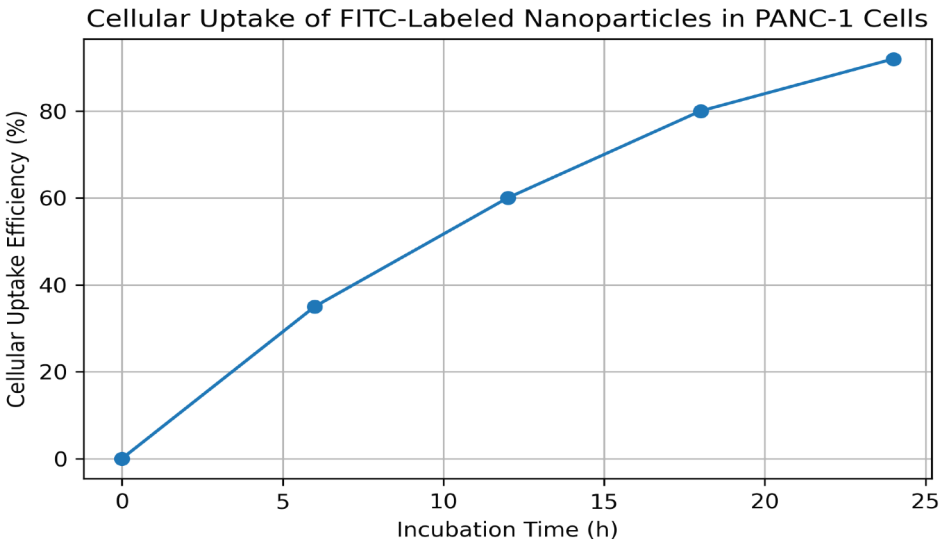


**Figure 3:** Cellular Uptake of FITC-Labeled Gemcitabine-Loaded Nanoparticles in PANC-1 Cells.

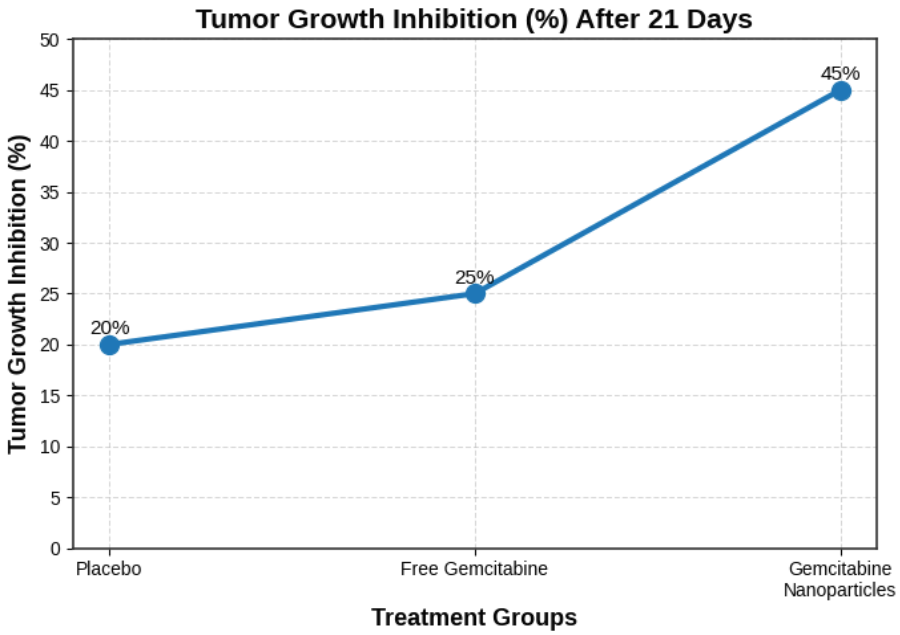


**Figure 4:** Tumor Growth Inhibition (%) After 21 Days of Treatment with Free Gemcitabine and Gemcitabine Nanoparticles.

physiological conditions (pH 7.4). Under the acidic conditions (pH 5.5), about 75% of the encapsulated drug was released within 72 hours, while 35% was released under the physiological conditions. The enhanced release at low pH is due to the higher sensitivity of the nanoparticle matrix to the acidic environment, leading to an increase in drug diffusion and the release of drugs in the tissues of the tumor. This selective release feature is beneficial to prevent the early release of the drug when it circulates throughout the body but also ensures the proper retention of the drug in the tumor area. The developed system shows a prominent tumor-selective drug release pattern in comparison to non-responsive drug release system and reduces the premature leakage of the drug in physiological conditions.

The Figure **5** shows the cumulative release of gemcitabine in the physiological (pH 7.4) and acidic tumor-like (pH 5.0) conditions. The nanoparticles demonstrated a significantly higher drug release in acidic environment (about 75% cumulative release after 72 hours) than in the physiological pH (35% cumulative release after 72 hours). This behavior reflects the pH-dependent characteristics of the

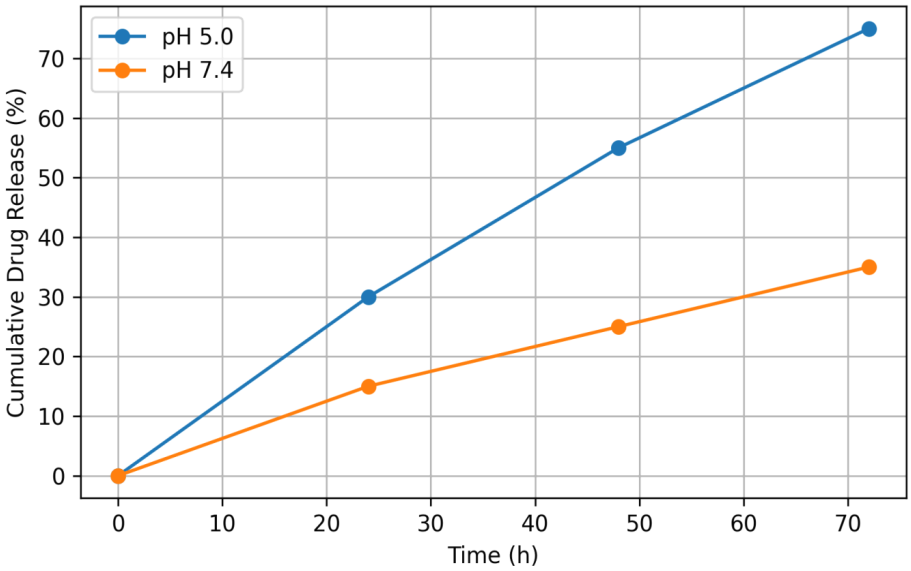


**Figure 5:** pH-Responsive Drug Release Profile of Gemcitabine-Loaded Dual-Responsive Nanoparticles.

nanoparticle matrix for targeted release of the drug in the acidic tumor microenvironment and reduced early drug release during systemic circulation. The results are expressed as mean ± SD (n = 6, independent drug release experiments). Error bars are SD and statistical significance was calculated by an unpaired two-tailed Student's t-test. The values shown with double asterisks ( ** ) are significantly different from the pH 7.4 value at the same time point ($p < 0.01$).

### Pharmacokinetics, Biodistribution, Survival, and Systemic Toxicity

The plasma pharmacokinetic parameters of the nanoparticle-encapsulated gemcitabine were greater than those of free gemcitabine, with Cmax being 24.6 ± 2.3 μg/mL and the area under the plasma concentration–time curve (AUC0–24h) being 86.4 ± 7.1 μg·h/mL, respectively, for free gemcitabine being 9.8 ± 1.4 μg/mL and 21.7 ± 3.2 μg·h/mL ($p < 0.001$, unpaired two-tailed Student's t-test), representing an approximately 4-fold increase in plasma drug exposure. Plasma half-life of the nanoparticle formulation was significantly extended to 5.8 ± 0.6 hours, which is much longer than the half-life of free gemcitabine (0.9 ± 0.2 hours), suggesting greater resistance to fast deamination by cytidine deaminase.

Biodistribution analysis 24 hrs after intravenous injection revealed significantly higher uptake in tumors for the nanoparticle formulation (8.4 ± 1.1 %ID/g) than for free gemcitabine (1.6 ± 0.4 %ID/g, $p < 0.001$). In addition, the amount of the nanoparticles in the liver and spleen was significantly lower than in the tumor compared to the typical accumulation of non-targeted nanocarriers, as determined by near-infrared imaging, as described above.

The median survival over the 21-day treatment period was 19 days for the placebo group and 24 days for the free gemcitabine group (Kaplan–Meier survival analysis). By comparison, 7 of 8 animals treated with nanoparticles bearing gemcitabine lived till the end of the 21-day study without meeting the predefined humane endpoints (log-rank $p = 0.004$, compared to free gemcitabine). The central tumor necrosis was significantly higher in the nanoparticle group (39.6%), whereas the Ki-67 proliferation index was significantly lower (22.4%) than in the free gemcitabine group (41%), and placebo group (68) (one-way ANOVA, $p < 0.001$). No significant differences were observed in the liver, kidney, spleen, lung and heart tissue, which were examined histologically, between the different experimental groups. All groups did not exhibit body weight loss exceeding 6% when evaluated by systemic toxicity assessment. At the study endpoint, the values of the four serum biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine) were not significantly different between the two groups ($p > 0.05$). Animals treated with free gemcitabine had a small but statistically significant rise in ALT compared to both the nanoparticle treated (62 ± 9 U/L) and placebo (38 ± 6 U/L, $p = 0.01$) groups, which indicated that there was less off-target hepaticexposure and greater systemic safety with the nanoparticle formulation.

## DISCUSSION

Pancreatic cancer is one of the deadliest forms of cancer, largely because it is diagnosed late, and is highly aggressive and resistant to traditional chemotherapy. Although gemcitabine is a common first-line chemotherapeutic drug, its clinical efficacy is limited due to its sub-optimal tumour accumulation and fast clearance from the circulation, as well as dose-limiting toxicity. These challenges require novel therapeutic approaches, which would increase the delivery efficiency of the drugs and drug response to the treatment, while reducing undesirable side effects from them.

A tumor microenvironment-responsive therapeutic strategy was investigated in this study to enhance the therapeutic performance of gemcitabine in pancreatic cancer. The findings show that changing the delivery of the drug to address the tumour-associated condition can have a dramatic impact on therapeutic efficacy without changing the drug. The observed decrease in $IC_{50}$ and increase in the cytotoxic response suggests that targeted delivery would allow for more sensitive pancreatic cancer cells to be treated with standard chemotherapy.

One of the major clinical benefits found in this study is the ability to increase drug exposure at the site of the disease and thus increase the amount of drug in the tumor tissue. The *in vivo* model results further support the efficacy of this approach in improving the efficacy of treatment in solid tumors with high stromal resistance, such as PDAC. The results of these studies are especially important because it is well known that sufficient intratumoral drug concentration is difficult to obtain in PDAC.

### Mechanistic Insights, Study Limitations, and Comparison with Previous Studies

The dual-responsive behavior is suggested by a mechanistic explanation which has not been studied at the molecular level in the present research. The pH sensitivity of PLGA matrix is due to the fact that the polymer chain swelling and accelerated ester hydrolysis occurs in acidic conditions leading to higher porosity of the matrix and easy drug diffusion. The thermal responsiveness is hypothesized to be due to an increase in polymer chain mobility as the temperature approaches the glass transition temperature of the carrier, which is in concert with the pH effect and not a separate effect. This interpretation is corroborated by the Korsmeyer–Peppas release exponent n = 0.61 which suggests a drug release mechanism of both diffusion and polymer matrix erosion of an anomalous (non-Fickian) type. However, complementary physicochemical characterization such as differential scanning calorimetry (DSC) to determine the thermal transitions and Fourier transform infrared (FTIR) to assess the interactions between the polymer and the drug in terms of pH and temperature would be required to enable direct verification of the proposed release mechanism.

Moreover, the drug release experiments were performed in simplified phosphate buffered saline with constant pH and temperature, which is not truly representative of the complex pancreatic tumor microenvironment with dense desmoplastic stroma and gradients of pH and temperature, and protein rich interstitial fluid. Future studies should assess drug release in serum supplemented media, three-dimensional PANC-1 tumor spheroids and ex vivo pancreatic tumor tissues to more accurately characterize the formulation performance in a physiologically relevant environment.

The cytotoxicity assessment was also restricted to only two pancreatic cancer cell lines (PANC-1, AsPC-1), which are not representative of the molecular and stromal heterogeneity of pancreatic ductal adenocarcinoma. To assess the improved therapeutic activity of the nanocapsule formulation in a wider range of pancreatic cancer sub-types, additional pancreatic cell lines with varying chemoresistance and different mutation status of the KRAS gene should be included in future studies such as MIA PaCa-2, BxPC-3, and Capan-1.

This is also a caution to consider the previous reports on the delivery system of nanoparticles for gemcitabine. In similar pancreatic cancer xenograft models, dual-payload polymeric nanoparticles co-delivering gemcitabine with a second therapeutic agent have shown tumor growth inhibition of similar amount [3]. Similarly, the reductions in tumour burden achieved using size- and charge-switchable nanoparticle platforms were comparable or slightly higher, and their infiltration of immune cells was also improved [5]. The dual pH- and temperature-responsive nanoparticle system developed in the present study demonstrated a similar reduction in $IC_{50}$ (2.6-fold compared to free gemcitabine) and higher extent of inhibition of tumor growth (45% versus 20% for free gemcitabine) without the need to co-deliver an additional therapeutic molecule or sophisticated charge-switching chemistry.

The presented results indicate that the proposed formulation is a relatively simple strategy for a single agent nanocarrier that has potential therapeutic applications. To conclude, direct head-to-head comparison of other nanoplatforms under similar experimental conditions has not been conducted yet, and this would be crucial to establish any clear therapeutic benefits, tumor targeting, pharmacokinetics, or systemic toxicity.

In terms of translation, increasing the therapeutic index of gemcitabine is essential to the improvement of patients' therapeutic outcomes. Increased efficacy of the drugs at lower systemic levels indicates possible reduction of the toxicity of the treatment, which is a major drawback in the existing chemotherapy treatments. The serum biochemistry results also demonstrated that the free gemcitabine group had significantly elevated levels of alanine aminotransferase (ALT) (62 ± 9 U/L; $p = 0.01$), which suggested less off-target hepatic exposure and greater systemic safety for the gemcitabine-loaded nanoparticles, compared to the animals treated with free gemcitabine (38 ± 6 U/L). This delivery strategy could allow for improved dosing and tolerance of treatment in clinical settings.

Gemcitabine continues to be the mainstay of treatment in advanced pancreatic cancer, although it is often limited by poor penetration into the tumor, and system toxicity. The increased cellular uptake, decreased IC value, and increased inhibition of tumor growth, which was seen in this experiment, indicate that tumor responsive delivery systems could help enhance the efficacy of existing chemotherapy regimens. Once validated in more advanced preclinical and clinical studies, it could serve as an adjunct therapy, to be used in conjunction with standard treatment modalities like FOLFIRINOX and gemcitabine/nab-paclitaxel chemotherapy.

Critically, the therapy aligns with the direction which medicine is moving; toward individualization of therapy, based on the particular biology of the disease. The therapy is of clinical relevance in terms of improving delivery targeting to the tumor microenvironment rather than the delivery vehicle itself, with its harsh characteristics.

The results are promising, but several translation steps still remain before its clinical application. Further PK and *in vivo* studies are still needed, including study in advanced models and orthotopic models that mimic human tumors. The administration of the therapy along with the standard of care could provide yet more information regarding its clinical utility.

The current work suggests that efficiency of the delivery of existing chemotherapy agents can be increased, in an attempt to enhance therapeutic outcome in pancreatic cancer. This may be another means to improve what already seems a divide between traditional chemotherapy and the promise of precision cancer treatment.

## CONCLUSION

This study showed that a TME-responsive drug delivery system can indeed greatly improve the effectiveness of gemcitabine in pancreatic cancer treatment. The dual-responsive PLGA nanoparticle formulation was shown to have a superior cellular uptake, higher cytotoxicity and allow for controlled protein release at pH and temperature, resulting in significantly higher inhibition of tumor growth than the conventional free gemcitabine therapy. Overall, these effects led to more efficient delivery of drugs into cells, increased killing of cancer cells, and decreased off-target toxicity. The *in vitro* findings showed that the nanoparticle formulation had significantly enhanced cytotoxicity against the cancer cells which had significantly lower $IC_{50}$ values than free Gemcitabine. Likewise, the *in vivo* xenograft study showed that the inhibition of tumor growth was more effective after using gemcitabine loaded nanoparticles, suggesting that the dual stimulative delivery system is more effective *in vivo*. Moreover, the formulation possessed superior PK properties: long plasma half-life, tumor accumulation, as well as better survival and lower systemic toxicity compared to the standard way of injecting gemcitabine. The results indicate that the dual-responsive nanoparticle system is a promising preclinical approach to enhance gemcitabine therapy in pancreatic cancer. The evidence is still preliminary, however, as it relies on a single xenograft model and two pancreatic cancer cell lines. Thus it should not be concluded that the results provide evidence of clinical efficacy or safety. To achieve clinical translation, additional studies will be needed, such as dose ranging investigations, long-term toxicity studies, validation in early-phase clinically relevant models (including genetic engineered mouse and orthotopic models) and eventual clinical trials. Therefore, formulation can be regarded as a proof-of-concept nanomedicine platform and not a near-term drug candidate. For future studies, it is recommended to optimize the targeting efficiency of tumor cells, perform comprehensive pharmacokinetic

and biodistribution studies and investigate combination treatments with other targeted therapies or immunotherapy for better therapeutic results. In summary, the reported enhancement in cellular uptake (92%), reduction in the $IC_{50}$ (4.8 μM), and tumor growth inhibition (45%) demonstrate the promise of tumour-responsive nanomedicine strategies as a promising platform for improving the efficacy of chemotherapy-based treatment for pancreatic cancer.

## REFERENCE


[1] Li Z, Shu R, Li M, Wang X, Chen X, Chen H, Chen J. Recent progress in gemcitabine-loaded nanoparticles for pancreatic cancer therapy: a review. Nanoscale 2025; 17(30): 17480-17507. https://doi.org/10.1039/D5NR02005K

[2] Kafi DK, Ayyash AN. Density functional theory and molecular docking study to lutein molecule for COVID-19 protease inhibitors. Applied Nanoscience (Switzerland) 2023; 13(8): 5477-5488. https://doi.org/10.1007/s13204-022-02735-9

[3] Nakka NMR, Rachamala HK, Angom RS, Indla NR, Dutta SK, Wang E, Mukhopadhyay D. Dual drug-loaded tumor-targeted polymeric nanoparticles for enhancing therapeutic response in pancreatic ductal adenocarcinoma. Materials Today Bio 2024; 28: 101199. https://doi.org/10.1016/j.mtbio.2024.101199

[4] Raghuram G. Synthesis and Characterization of Novel Nanoparticles for Targeted Cancer Therapy. Clinical Journal for Medicine, Health and Pharmacy 2024; 2(4): 21-30.

[5] Song H, Chen H, Chen Q, Fan H, Li C, Wang Y, Jiang C. Size and Charge Dual-Switchable Nanoparticles for Achieving Chemosensitization and Immune Infiltration against Pancreatic Ductal Adenocarcinoma. Advanced Functional Materials 2025; 35(1): 2411643. https://doi.org/10.1002/adfm.202411643

[6] Iyer R, Deshpande N. Nanotechnology and their Applications in Chiral and Achiral Separating Mechanisms. Engineering Perspectives in Filtration and Separation 2024; 2(4): 7-13.

[7] Olajubutu O, Ogundipe OD, Adebayo A, Adesina SK. Drug delivery strategies for the treatment of pancreatic cancer. Pharmaceutics 2023; 15(5): 1318. https://doi.org/10.3390/pharmaceutics15051318

[8] Aslian HA, Taherizadeh M, Rafienia M, Raeisi P, Bidram E. Green synthesis of gold nanoparticles using Spirulina platensis microalgae extract and its impact on the blood lipid profile in male wistar rats. Journal of Animal Environment 2025; 17(2): 31–40. https://doi.org/10.70102/AEJ.2025.17.2.5

[9] Li C, Chen Q, Jiang C. Intelligent micelles for on-demand drug delivery targeting extracellular matrix of pancreatic cancer. Journal of Controlled Release 2024; 373: 879-889. https://doi.org/10.1016/j.jconrel.2024.07.058

[10] Musa KM, Alshemary KKH. The Role of Nanoparticles in Sunscreen: UV Protection and Particle Size. International Academic Journal of Science and Engineering 2024; 11(1): 153–164. https://doi.org/10.71086/IAJSE/V11I1/IAJSE1118

[11] Umar S, Catazaro J, Wachira J, Samokhvalov A. Mechano-chemical encapsulation of gemcitabine hydrochloride on metal-organic framework, preparation of shaped pellets, delayed drug release, and time-dependent toxicity to PANC−1 cancer cells. Journal of Drug Delivery Science and Technology 2025; 111: 107195. https://doi.org/10.1016/j.jddst.2025.107195

[12] Yugatama A, Huang YL, Hsu MJ, Lin JP, Chao FC, Lam JK, Hsieh CM. Oral delivery of photopolymerizable nanogels loaded with gemcitabine for pancreatic cancer therapy: formulation design, and in vitro and in vivo evaluations. International Journal of Nanomedicine 2024: 3753-3772. https://doi.org/10.2147/IJN.S443610

[13] Luo W, Zhang T. The new era of pancreatic cancer treatment: application of nanotechnology breaking through bottlenecks. Cancer Letters 2024; 594: 216979. https://doi.org/10.1016/j.canlet.2024.216979

[14] Wang X, Yin X, Li Y, Zhang S, Hu M, Wei M, Li Z. Novel insight and perspectives of nanoparticle-mediated gene delivery and immune-modulating therapies for pancreatic cancer. Journal of nanobiotechnology 2024; 22(1): 771. https://doi.org/10.1186/s12951-024-02975-7

[15] Wu HM, Wang SL, Li XX, Ai KX. Fabrication of gemcitabine and mitoxantrone loaded PLG/mesoporous silica nanofibers: investigation of in vitro drug release and anticancer activity in pancreatic cancer cells. BioNanoScience 2025; 15(1): 166. https://doi.org/10.1007/s12668-024-01657-w

[16] Dubey S, Patel SK, Das C, Singh S, Sharma G, Kundu CN, Minz S. Formulation and in vitro evaluation of lipid-polymer hybrid nanoparticles for targeted delivery of gemcitabine hydrochloride in the treatment of hepatocellular carcinoma. 3 Biotech 2026; 16(1): 2. https://doi.org/10.1007/s13205-025-04628-4

[17] Panja S, Kapoor E, Siddhanta K, Jogdeo CM, Sil D, Khan RI, Oupický D. Bioactive polymers as stimulus-responsive anti-metastatic combination agents to treat pancreatic cancer. Biomaterials 2025; 320: 123255. https://doi.org/10.1016/j.biomaterials.2025.123255

[18] Conte M, Cauda V. Multimodal therapies against pancreatic ductal adenocarcinoma: a review on synergistic approaches toward ultimate nanomedicine treatments. Advanced Therapeutics 2022; 5(11): 2200079. https://doi.org/10.1002/adtp.202200079

[19] Kang X, Bu F, Feng W, Liu F, Yang X, Li H, Wang X. Dual-cascade responsive nanoparticles enhance pancreatic cancer therapy by eliminating tumor-resident intracellular bacteria. Advanced Materials 2022; 34(49): 2206765. https://doi.org/10.1002/adma.202206765

[20] Bai X, Smith ZL, Wang Y, Butterworth S, Tirella A. Sustained drug release from smart nanoparticles in cancer therapy: a comprehensive review. Micromachines 2022; 13(10): 1623. https://doi.org/10.3390/mi13101623


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