THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives

RESEARCH ARTICLE

# LIPID PRODRUG NANOPARTICLES CARRYING A MIMETIC PEPTIDE OF APOLIPOPROTEIN A-II TARGET PANCREATIC ADENOCARCINOMA

**Aiqun Xue[1], Sarah F. Smith[1], Anthony Gill[2], Ross C. Smith[3]***

[1]Cancer Surgery Research Laboratory, Kolling Institute, St Leonards NSW 2065 Australia.
[2]Professor, Department of Surgical Pathology, University of Sydney, Sydney NSW 2006 Australia.
[3]Professor Emeritus, The University of Sydney, Sydney NSW 2006, Australia.

*ross.smith@sydney.edu.au

OPEN ACCESS

PUBLISHED
31 July 2024

CITATION
Xue A., et al., 2024. Lipid Prodrug Nanopartlcles Carrying a Mimetic Peptide of Apolipoprotein A-II Target Pancreatic Adenocarcinoma. Medical Research Archives, [online] 12(7). https://doi.org/10.18103/mra.v12i7.5442



DOI
https://doi.org/10.18103/mra.v12i7.5442

ISSN
2375-1924

## ABSTRACT

The easy production, improved efficacy, and low toxicity of lipid prodrug nanoparticles (LPNP) represent a promising new drug delivery technology for pancreatic ductal adenocarcinoma (PDAC), when carrying gemcitabine (Gem-LPNP).

This paper follows on from our previous study where we showed that lipid-based nanoparticles carrying a gemcitabine prodrug inhibit growth of human and cell-line PDAC xenografted onto mice. Using only 4.5mg/Kg of the clinical dose of gemcitabine in the prodrug, Gem-LPNP inhibited tumour growth as much as the significantly greater clinical dose of free gemcitabine (75-100mg/Kg). When apolipoprotein A-II (ApoA-II) was added to Gem-LPNP, growth was inhibited further. We determined that ApoA-II was actively targeting PDAC cells via the scavenger receptor-B1.

To improve the safety and cost of our targeting nanoparticles, we have now designed a short peptide of ApoA-II (SQ31) to be attached to Gem-LPNP (Gem-LPNP-SQ31) and aimed to compare the effects of Gem-LPNP with Gem-LPNP-SQ31 in a murine xenograft model.

Cell-line PDAC xenografts were implanted in one loin of twenty five immunodeficient mice. When the xenografts reached a measurable size, the mice were randomly assigned into five groups. They were given 200µL twice weekly of either 1) IV saline, 2) IP free gemcitabine 75mg/kg, 3) IV free gemcitabine 4.5mg/kg (equivalent dose to the nanoparticles), 4) IV Gem-LPNP or 5) IV Gem-LPNP-SQ31.

Transdermal xenograft measures showed that over four weeks, Gem-LPNP-SQ31 inhibited PDAC growth as much as high-dose free gemcitabine, but using only a fraction of the high free gemcitabine dose. Although xenograft sizes after Gem-LPNP-SQ31 were significantly smaller than those after Gem-LPNP treatment, this was only a small difference. Both Gem-LPNP and Gem-LPNP-SQ31 xenografts were significantly smaller than xenografts after the equivalent low-dose free gemcitabine. There was no histological evidence of complications in the mice. It is concluded that the addition of SQ31 to Gem-LPNP increased the inhibition of PDAC growth and this nanoparticle construct should be developed for clinical evaluation in humans.

## Introduction

Treatment of PDAC has not progressed over the last several years as well as for other cancers. Prodrugs of established chemotherapy agents like gemcitabine promise to reduce complications while maintaining effectiveness. In a previous paper we describe progress in inhibiting the growth of PDAC using innovative nanoparticles which carry a prodrug of gemcitabine and a targeting apolipoprotein[1].

The economic burden of advanced PDAC is high for non-active treatment[2] and for all stages combined, 5-year survival is only 13%. Addition of antiangiogenic drugs and the co-delivery of different agents are potential advantages for prodrugs incorporated in nanoparticle treatments, but further work is required to develop these technologies. Gemcitabine has been widely used to treat PDAC[1] but its efficacy is reduced because of the need to penetrate the extracellular fibrous[1] tumour capsule, the frequent occurrence of complications leading to treatment failure and its rapid clearance through the kidney, reducing the time of exposure to cancer tissue.

Gemcitabine prodrugs are formed when a lipid chain is attached to the gemcitabine molecule. We investigated different naturally-occurring lipids to determine the most functional chain: oleyl, linoleyl and phytanyl. When the prodrug was combined with phospholipids and cholesterol, biomimetic Gem-lipid prodrug nanoparticles (Gem-LPNP), are created and their ability to form liposomes was verified by SSAXS and cryo-TEM[11]. Testing of Gem-LPNP in several PDAC in cell lines and in mice showed lower toxicity and greater tumour suppression with Gem-LPNP than with free gemcitabine[1].

We reported that ApoA-II is preferentially taken up by PDAC[1]. We hypothesised that ApoA-II attached to a gemcitabine prodrug Gem-LPNP would target PDAC to deliver gemcitabine into the tumour.

Subsequent work with our Gem-LPNP carrying ApoA-II (Gem-LPNP-A2) as a potential targeting agent (Gem-LPNP-A2) further slowed growth in semi-resistant PDAC cells and in resistant human PDAC xenografts without complications. We demonstrated that our Gem-LPNP-A2 targeted PDAC better than apolipoprotein A-I, which had been used unsuccessfully for a long time[8]. In an additional study, SR-B1 was seen to be over-expressed in PDAC cells and xenografts[1]. Targeting by ApoA-II was seen to be suppressed by anti-SR-B1[4], supporting our concept that SR-B1 is the receptor enabling the uptake of ApoA-II into PDAC cells.

Because the full length of ApoA-II is derived from human plasma, its use in humans could carry the risk of disease[9;10]. Studies to evaluate its efficacy would be prohibitively expensive. We therefore aimed to determine in a pilot study, the effectiveness of a mimetic peptide of ApoA-II (SQ31) in targeting PDAC in a murine xenograft model.

## Methods

### Design of synthetic peptide to mimic the targeting action of ApoA-II

We hypothesised that a mimic peptide[1] would have similar targeting properties to the full length ApoA-II. The design of our SQ31 peptide utilises the triple helix structure of ApoA-II, and that Helix 1 most strongly binds and clarifies emulsions[5]. Using this knowledge, a 31-amino acid peptide starting from the "N" terminal of natural ApoA-II, which included the first helix, was manufactured to make the synthetic apolipoprotein (SQ31). This peptide has the benefit of avoiding the usual manufacture of ApoA-II from human blood which carries the risk of cross-infection. SQ31 was manufactured by Peptide2.0, 4410 Brookfield Corporate Dr #223983, Chantilly, VA 20151, United States.

Its amino acid sequence is N-QAKEPCVESLVSQ YFQTVTDYGKDLMEKVKS-C. The sequence and the 98% purity of SQ31 were confirmed at the Kolling Institute, University of Sydney, Australia by MALDI-TOF and HPLC analysis.

## Manufacture of nanoparticles

The prodrug concept used in the manufacture of our nanoparticles was developed by Dr M.J. Moghaddam at the Commonwealth Scientific and Industrial Research Organisation in Sydney, Australia[11].

Gem-LPNP prodrug nanoparticles were manufactured and stabilized with 30% PEG as previously published[1]. ApoA-II was added to Gem-LPNP nanoparticles to create Gem-LPNP-A2 prodrug nanoparticles. SQ31 was added to Gem-LPNP to create Gem-LPNP-SQ31 prodrug nanoparticles. ApoA-II and SQ31 replaced the PEG in Gem-LPNP and they were seen to be stable over 12 months[13]. Nanoparticles were manufactured and tested at NanoMed Pty Ltd, Lane Cove West, NSW Australia using light scattering technology.

The remaining work was performed at the Kolling Institute of Medical Research, University of Sydney, St Leonards NSW 2065, Australia.

## PDAC cell lines and cell culture

CFPAC-1 and PANC-1 cell lines were provided by Prof. Barry Allen (St. George Hospital, Kogarah, NSW, Australia). The cells were cultured and their identities confirmed as previously described[13].

## Agarose gel studies

The sizes and composition of LPNP +/- free ApoA-II and free SQ31 were compared using electrophoresis. A sheet of agarose gel was prepared and exposed to an electric field. The different components were placed in chambers in the gel to test their relative movement along the gel. A fluorescent image of the gel was then taken by a Kodak small animal imaging station.

## Confocal microscopy

To compare the targeting action on individual cells of SQ31 and ApoA-II, confocal micrograph studies were undertaken on two cell lines, CFPAC-1 and PANC-1. DiD red dye was added to visualise lipid and Alexa 488 fluoro green was added to stain for peptides. DAPI blue fluorescent dye was added to identify the cell nucleus.

## Xenograft preparation

Six-week old male NSG mice were obtained from the Animal Research Centre, Perth, WA, Australia and acclimatised for one week in the Kearns Facility, Kolling Institute. Animal care and housing was undertaken following the institutional guidelines of Northern Sydney Local Health District.

Subcutaneous cell line-derived xenografts were established by implanting 2-3 x$10^6$ cells from CFPAC-1 cultures as previously described[1], following the Northern Sydney District Animal Care & Ethics Committee Protocol (Number 1011-015A).

## *In-vivo* studies comparing the effect of Gem-LPNP with Gem-LPNP-SQ31 on xenograft growth

After 10 days' growth, when xenografts had reached ~80-100$mm^3$, mice were randomised into 5 groups of 5 mice. They were given 200µL twice weekly of either 1) IV saline, 2) IP free gemcitabine 75mg/kg (GemH), 3) IV free gemcitabine 4.5mg/kg (the same dose as in the nanoparticles, GemL), 4) IV gemcitabine prodrug Gem-LPNP, giving 4.5mg/kg or 5) IV gemcitabine prodrug Gem-LPNP-SQ31, also giving 4.5mg/kg gemcitabine.

Xenograft growth was measured twice weekly for four weeks using vernier calipers to measure length (L) and breadth (B). Xenograft volume was derived by the formula ($L*B^2$)/2. Thirty days after first treatment animals were culled. Organs and xenograft were harvested from two mice in each group for histopathology. Xenograft morphology was confirmed to remain the same as the original PDAC tumour[14].

## Histology

To examine tissues for any toxic effects, formalin-fixed, paraffin-embedded 4-µm liver, spleen, kidney and xenograft sections were cut and stained with haematoxylin and eosin (H & E).

### Statistical analysis

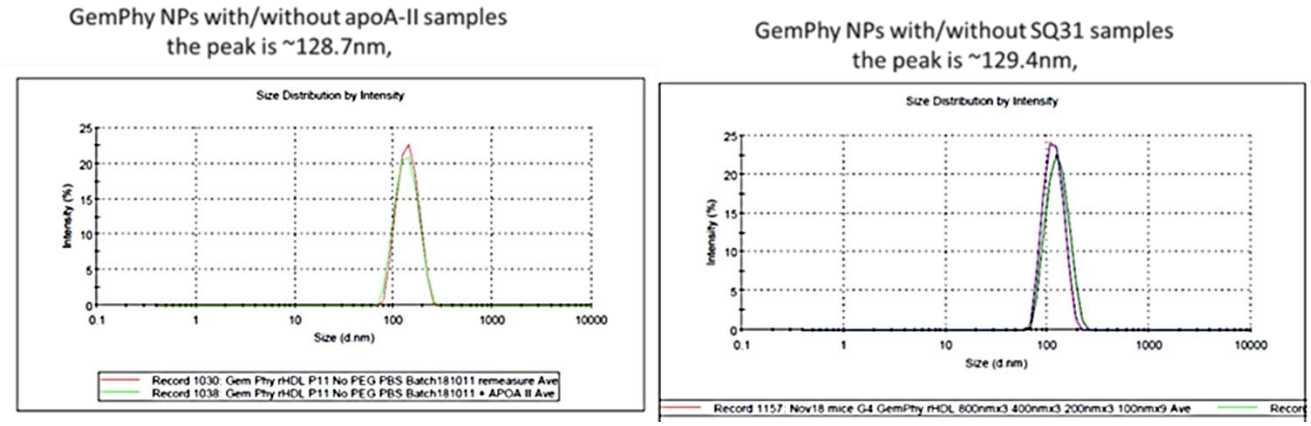
To determine if the mean rank xenograft volumes of any one group were different to any other group, final volumes of the xenografts were compared using the Kruskal-Wallis test and post-hoc the groups were compared by the Conover test. MedCalc® Statistical Software version 22.021 was used (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024). A P value of 0.05 was regarded as indicating a significant difference.

## Results

### Physical properties of LPNP with SQ31 or ApoA-II

Results of dynamic light scattering tests are shown in Figure 1. The size distribution of the nanoparticles was almost the same whether the full length ApoA-II or the peptide SQ31 was attached to the Gem-LPNP nanoparticles. The median size of Gem-LPNP-A2 nanoparticles was 128.7nm and of the Gem-LPNP-SQ31 was 129.4nm.

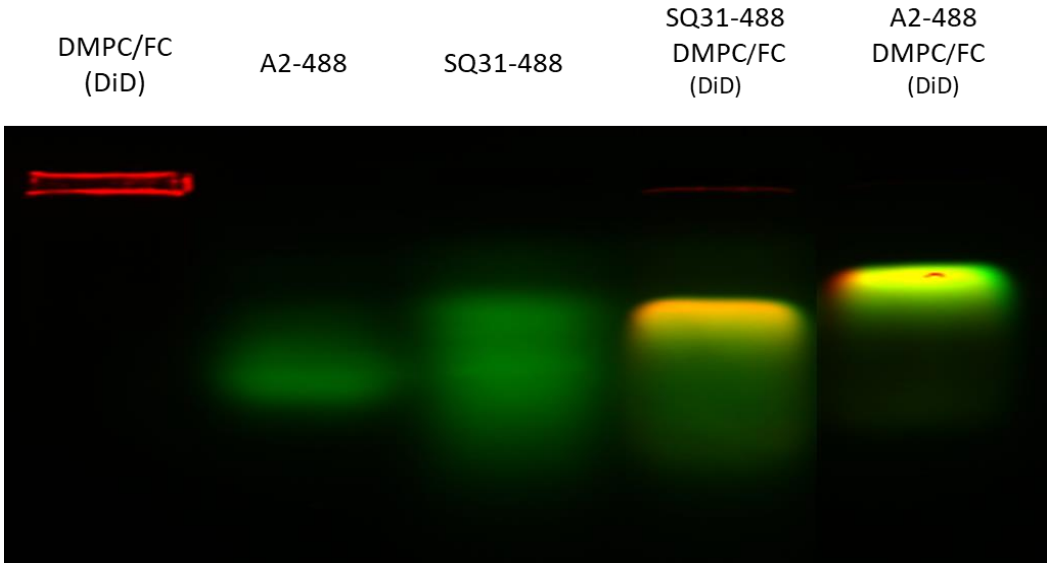
**Figure 1.** Dynamic light scattering measurements comparing sizes of (L) Gem-LPNP nanoparticles with and without ApoA-II, and (R) Gem-LPNP nanoparticles with and without SQ31. There are minimal differences between sizes.




### Composition and sizes of study nanoparticles

Agarose gel electrophoresis results are shown in Figure 2. DMPC/FC represents base lipid nanoparticles LPNP without attachments. It is used as a control and appears red. The colour appears yellow when ApoA-II combines with LPNP to form LPNP-A2 and SQ31 combines with LPNP to form LPNP-SQ31, showing that ApoA-II and SQ31 are attached to the lipid nanoparticles. The combined band is more tightly seen than the free peptide SQ31 or free ApoA-II alone.

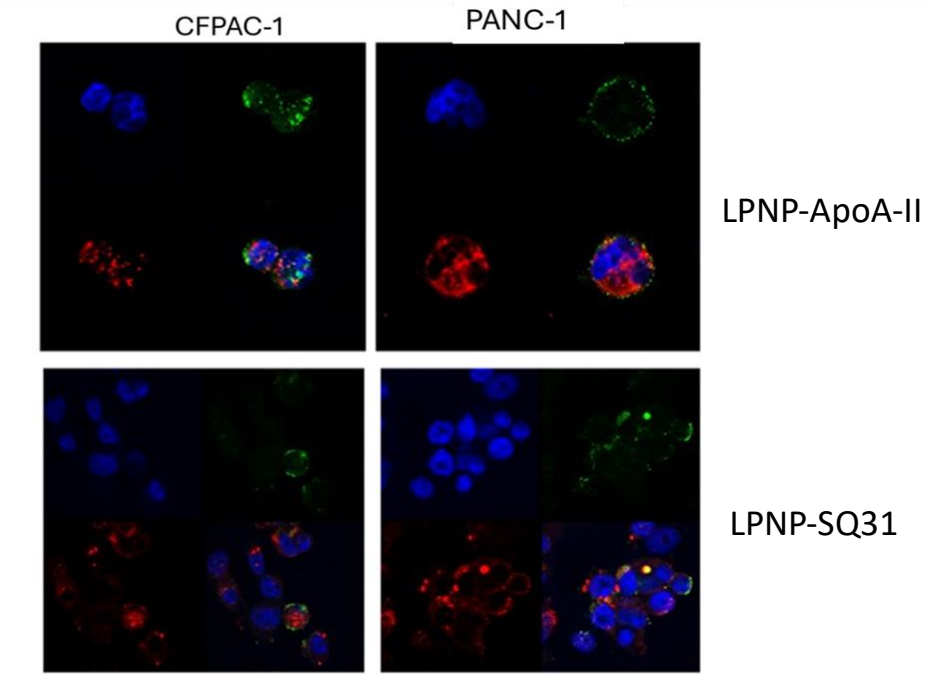
**Figure 2.** Results of electrophoresis showing that SQ31 combines with LPNP similarly to ApoA-II. LPNP is stained red with DiD, ApoA-II is stained green with Atto 488, SQ31 is stained green with 488), LPNP-SQ31 and LPNP-A2 are yellow.



## Confocal microscopy

Results of confocal microscopy are shown in Figure 3. ApoA-II is seen on the surface of the cells, more strongly on CFPAC-1 than on the PANC-1 cells. SQ31 also attaches to the surface of both cell lines, although in a patchier manner. The images show that SQ31 targets these cells and delivers lipid nanoparticles into them.

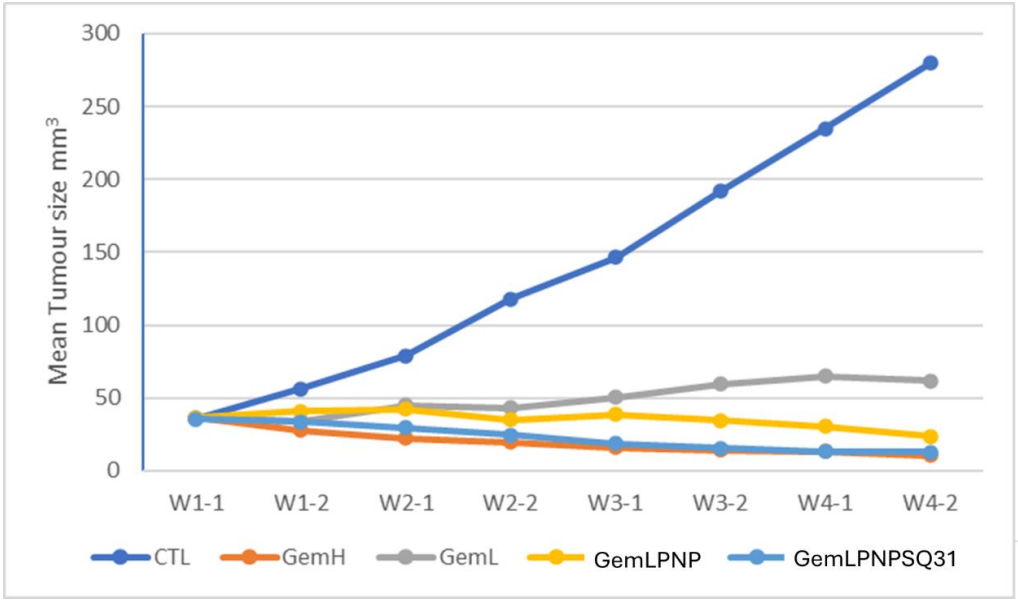
**Figure 3.** Confocal micrographs comparing CFPAC-1 and PANC-1 cells treated with nanoparticles carrying full-length ApoA-II or peptide SQ31. Red: lipid LPNP, green: peptides in ApoA-II and SQ31, blue: nucleus.




## *In-vivo* studies

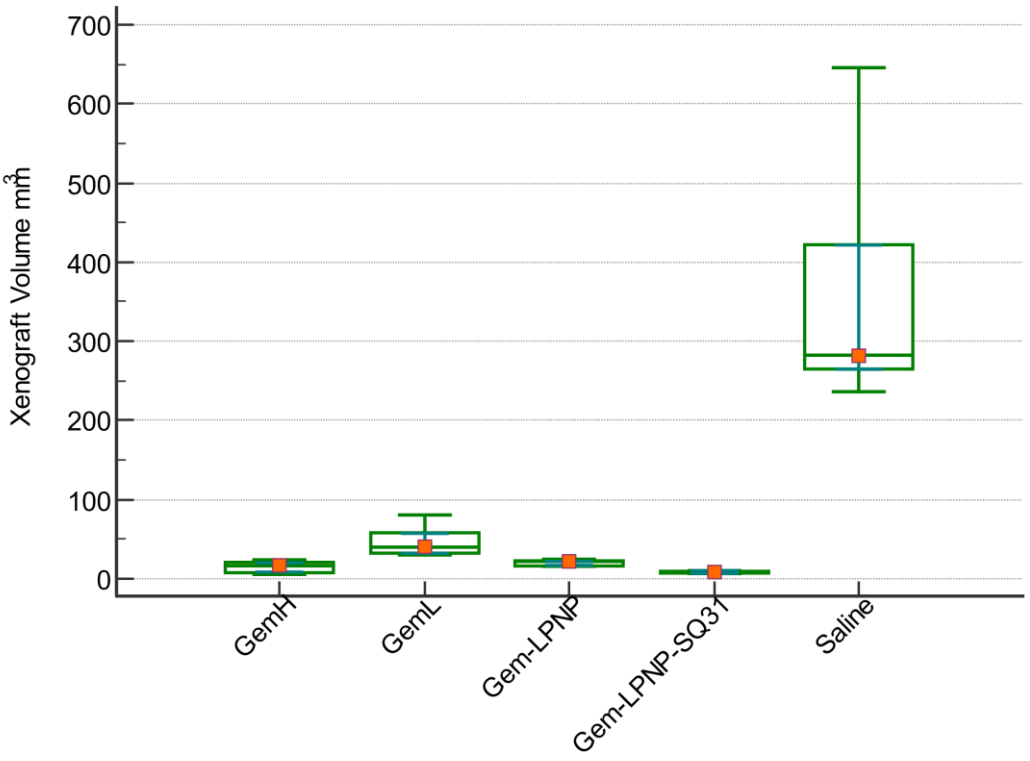
Results of *in vivo* studies are shown in Figure 4a. All active agents suppressed xenograft growth more than the saline control. Growth was suppressed most strongly by high-dose free gemcitabine and LPNP-SQ31. Although Gem-LPNP with 30% PEG has a significant effect on xenograft growth (greater than control and than free gem 4.5 mg), Gem-LPNP-SQ31 has a stronger suppression effect and more closely follows that of high-dose free gemcitabine.

**Figure 4a.** Xenograft growth over 4 weeks after twice-weekly doses of saline control (dark blue), free gemcitabine 75mg/kg (red), free gemcitabine 4.5mg/kg (grey), gem-LPNP (yellow), gem-LPNP-SQ31 (pale blue). Sizes normalised to time of first injection.



Final xenograft volumes are displayed as boxplots in Figure 4b. Volumes in the active treatment groups are obviously different to volumes in the saline control group. The GemL group received the same dose of gemcitabine as groups receiving the nanoparticles, but growth was more suppressed by the nanoparticles. Growth in the SQ31 group appears suppressed to a similar degree to the GemH and GemLPNP groups, although the difference appears to be marginal.

**Figure 4B**. Boxplots of xenograft volumes at the end of treatment



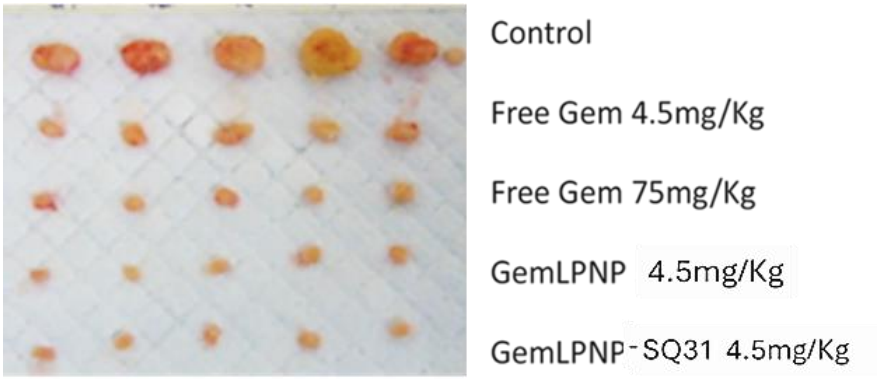

Kruskal-Wallis testing showed a significant difference between the groups (P=0.00035). Conover testing confirmed that suppression by SQ31 and GemH were similar and both suppressed more than GemLPNP to the 5% significance level. See Table 1.

**Table 1**. Post-hoc analysis comparing tumour sizes at the endpoint (Conover test for pair-wise comparisons after significance seen on Kruskal-Wallis test). nr: non-parametric risk.

| Factor | n | Average Rank | Different (P<0.05) from factor nr |
|---|---|---|---|
| (1) CONTROL | 5 | 23.00 | (2)(3)(4)(5) |
| (2) GemH (IP) | 5 | 8.20 | (1)(3) |
| (3) GemL (IV) | 5 | 18.00 | (1)(2)(4)(5) |
| (4) Gem-LPNP 4.5mg/Kg | 5 | 11.40 | (1)(3)(5) |
| (5) Gem-LPNP-SQ31 4.5mg/Kg | 5 | 4.40 | (1)(3)(4) |

The macroscopic appearance of explanted xenografts is seen in Figure 4c and confirms the discrepancy in size between active treatment groups and control. Xenografts in the GemL group appear larger than the other active treatment groups.

**Figure 4c.** Explanted tumours from mice in five groups receiving 1) saline as control, 2) GemL, 3) GemH, 4) GemLPNP or 5) GemLPNP-SQ31.




An image of explanted spleens from all treated mice is shown in Figure 5.

**Figure 5.** Explanted spleens from the treated mice were examined for evidence of toxicity.


The spleens from mice receiving gem-LPNP appear bulkier than those in he other groups, possibly indicating a greater turnover of red cells in that group. Apart from this observation, no toxic effects were seen on macroscopic and histological examination by author AG.

## Discussion

This study confirms that lipophile nanoparticles containing a prodrug of gemcitabine, Gem-LPNP, slowed the growth of PDAC xenografts in mice, and when carrying a mimic peptide of ApoA-II (Gem-LPNP-SQ31), growth of xenografts slowed further ($P<0.05$). Importantly, nanoparticle size was no different whether it was carrying the mimic peptide or the complete ApoA-II. Note that the dose of gemcitabine in both gem-LPNP and Gem-LPNP-SQ31 was 4.5mg/Kg which was approximately 6 percent of the usual clinical dose 75mg/Kg. Results show that Gem-LPNP SQ31 has a similar suppressive effect to high-dose free gemcitabine, but its use will be associated with fewer toxic effects.

Two cell lines were used in this study to provide diversity of biology. CFPAC-1 is derived from a patient with cystic fibrosis and PDAC. It is a less aggressive tumour than PANC-1.

The green stain seen in confocal microscopy here is on the surface of the cells where the SR-B1 receptors would lie, consistent with ApoA-II and SQ31 utilising

the SR-B1 receptors on the surface of the cells. More green stain is seen on the less-aggressive CFPAC-1cells.[1]

We chose NSG mice as our hosts for the xenografts because our lab had experience and we were comfortable with their ability to carry xenografts. They were unlikely to have secondary immune reactions which could have confused our interpretation of results. Also, that we had a 100 percent take with this model which meant that the xenograft sizes were similar at the commencement of the study, and which supports the choice of NSG mice. This also allowed us to study small groups of 5 mice, as requested by our animal ethics committee, and to have sufficiently tight data to allow for statistical interpretation.

PDACcontinues to be a serious unsolved problem and the nanoparticle construct described here offers potential benefit. Gemcitabine is frequently used for PDAC, either as a monotherapy or in addition to other agents. Unfortunately, its use is associated with the risk of many complications. Furthermore, gemcitabine continues to be widely used in many different advanced cancers. Complications are indicated by nausea, renal and liver blood indices and marrow suppression and therefore must be carefully managed, frequently by dose reduction[9]. Although dose reduction reduces the effectiveness of the treatment, it is widely used even in gemcitabine and nab-paclitaxel protocols with some benefit[15]. These side issues could be avoided by targeting with Gem-LPNP-SQ31 treatment. Although these patients are highly motivated, they frequently become unhappy when approached with the next course of treatment. We hypothesise that because these side effects are dose-dependent, the targeting nanoparticleconstruct will result in a greatly reduced negative experience compared to the normal gemcitabine protocols.

In future, the nanoparticle concept may also permit oral administration of gemcitabine, although the half-life exposure to tumours has not been seen to increase by use of a prodrug after oral treatment[16].

We found a more prolonged half-life of nanoparticles compared to free gemcitabine[4] IV and IP. Future possible improvement in treatment may be achieved by combining nanoparticles with agents for photodynamic therapy[17], extracellular vesicles[18], free-radical targeting[19], different gold nanoparticles[20], and small-sized hollow mesoporous silica nanoparticles[21]. Also, liposomal derivatives may improve anticancer activity[22]. Prodrugs may avoid the need to use the hENT1 pathway for absorption into cancer tissue[23].

It should be noted that the prodrug nanoparticle used in this study can be manufactured without the use of complex and risky manufacturing processes and the mimic peptide can also be constructed from sterile amino acids and therefore could be the basis of human therapy. Further, some publications discuss reactons to PEG[24] ,which can be avoided by the use of our mimic peptide. We have not seen any complications from the use of these nanoparticles with or without ApoA-II or SQ31.

We appreciate that there is only a small, although significant, benefit of Gem-LPNP-SQ31 over Gem-LPNP and the choice depends on whether it is worth the cost of developing the SQ31 peptide further. Human trials will be necessary to determine this. Also, this study has a similar result to that when the complete ApoA-II was used in a xenograft model when the more immunogenic mouse, NOD-SCID, was the host. There seems to be little reason not to move to a Phase I/II study in humans.

## Author Contributions Statement:

Dr Aiqun Xue conceptualised and designed the study. She undertook xenograft induction then IV and IP treatments, and managed the caliper measurement of xenografts, euthanasia of mice at the endpoint along with measurement of explanted xenografts and spleens and preparation for histological review. She cut the fixed tissue and prepared the slides for histological examination; she collected and undertook preliminary assessment of results.

Dr Sarah F. Smith helped with the preparation of the manuscript and edited and reviewed the final version.

Professor Anthony Gill reviewed the xenograft histology.

E/Professor Ross C. Smith conceptualised the project, overviewed the laboratory protocols, undertook the statistical assessment, wrote the early draft of the manuscript and approved the final draft of the manuscript. Ross C. Smith is a Co -CEO of NanoMed Pty Ltd.

## Conflict of Interest Statement:

None

## Acknowledgements Statement:

Dr Minoo Moghaddam conceptualised and manufactured the lipophile nanoparticles used in this study. Dr. Minoo Moghaddam is a Co-CEO of NanoMed Pty Ltd.

## References:

(1) Smith RC, Bulanadi JC, Gill AJ, Rye KA, Hugh T, Proschogo N et al. Pancreatic adenocarcinoma preferentially takes up and is suppressed by synthetic nanoparticles carrying apolipoprotein A-II and a lipid gemcitabine prodrug in mice. *Cancer Lett* 2020; **495**:112-122.

(2) Winer A, Handorf E, Dotan E. Dosing Schedules of Gemcitabine and nab-Paclitaxel for Older Adults With Metastatic Pancreatic Cancer. *JNCI Cancer Spectr* 2021; **5**(5).

(3) Cheng J, Fuller J, Feldman R, Tap W, Owa T, Fuks Z et al. Enhancement of Soft Tissue Sarcoma Response to Gemcitabine through Timed Administration of a Short-Acting Anti-Angiogenic Agent. *Cell Physiol Biochem* 2020; **54**(4):707-718.

(4) Julovi SM, Xue A, Thanh LE TN, Gill AJ, Bulanadi JC, Patel M et al. Apolipoprotein A-II Plus Lipid Emulsion Enhance Cell Growth via SR-B1 and Target Pancreatic Cancer In Vitro and In Vivo. *PLoS One* 2016; **11**(3):e0151475.

(5) Smith LE, Segrest JP, Davidson WS. Helical domains that mediate lipid solubilization and ABCA1-specific cholesterol efflux in apolipoproteins C-I and A-II. *J Lipid Res* 2013; **54**(7):1939-1948.

(6) Wu Q, Ma X, Zhou W, Yu R, Rosenholm JM, Tian W et al. Co-Delivery of Paclitaxel Prodrug, Gemcitabine and Porphine by Micelles for Pancreatic Cancer Treatment via Chemo-Photodynamic Combination Therapy. *Pharmaceutics* 2022; **14**(11).

(7) Otowa Y, Kishimoto S, Saida Y, Yamashita K, Yamamoto K, Chandramouli GVR et al. Evofosfamide and Gemcitabine Act Synergistically in Pancreatic Cancer Xenografts by Dual Action on Tumor Vasculature and Inhibition of Homologous Recombination DNA Repair. *Antioxid Redox Signal* 2023; **39**(7-9):432-444.

(8) Oberle R, Kuhrer K, Osterreicher T, Weber F, Steinbauer S, Udonta F et al. The HDL particle composition determines its antitumor activity in pancreatic cancer. *Life Sci Alliance* 2022; **5**(9).

(9) Rye KA, Wee K, Curtiss LK, Bonnet DJ, Barter PJ. Apolipoprotein A-II inhibits high density lipoprotein remodeling and lipid-poor apolipoprotein A-I formation. *J Biol Chem* 2003; **278**(25):22530-22536.

(10) Clay MA, Cehic DA, Pyle DH, Rye KA, Barter PJ. Formation of apolipoprotein-specific high-density lipoprotein particles from lipid-free apolipoproteins A-I and A-II. *Biochem J* 1999; **337 (Pt 3)**(Pt 3):445-451.

(11) Bulanadi JC, Xue A, Gong X, Bean PA, Julovi SM, de CL et al. Biomimetic Gemcitabine-Lipid Prodrug Nanoparticles for Pancreatic Cancer. *Chempluschem* 2020; **85**(6):1283-1291.

(12) Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Adv Drug Deliv Rev* 2021; **176**:113851.

(13) Julovi SM, Xue A, Thanh LE TN, Gill AJ, Bulanadi JC, Patel M et al. Apolipoprotein A-II Plus Lipid Emulsion Enhance Cell Growth via SR-B1 and Target Pancreatic Cancer In Vitro and In Vivo. *PLoS One* 2016; **11**(3):e0151475.

(14) Smith RC, Bulanadi JC, Gill AJ, Rye KA, Hugh T, Proschogo N et al. Pancreatic adenocarcinoma preferentially takes up and is suppressed by synthetic nanoparticles carrying apolipoprotein A-II and a lipid gemcitabine prodrug in mice. *Cancer Lett* 2020; **495**:112-122.

(15) Jung K, Choi S, Song H, Kwak K, Anh S, Jung JH et al. Real-world dose reduction of standard and modified FOLFIRINOX in metastatic pancreatic cancer: a systematic review, evidence-mapping, and meta-analysis. *Ther Adv Med Oncol* 2023; **15**:17588359231175441.

(16) Thompson BR, Shi J, Zhu HJ, Smith DE. Pharmacokinetics of gemcitabine and its amino acid ester prodrug following intravenous and oral administrations in mice. *Biochem Pharmacol* 2020; **180**:114127.

(17) Xue A, Scarlett CJ, Jackson CJ, Allen BJ, Smith RC. Prognostic significance of growth factors and the urokinase-type plasminogen activator system in pancreatic ductal adenocarcinoma. *Pancreas* 2008; **36**(2):160-167.

(18) Lee H, Hong HJ, Ahn S, Kim D, Kang SH, Cho K et al. One-Pot Synthesis of Double-Network PEG/Collagen Hydrogel for Enhanced Adipogenic Differentiation and Retrieval of Adipose-Derived Stem Cells. *Polymers (Basel)* 2023; **15**(7).

(19) DiMartini ET, Lowe CJ, Shreiber DI. Alternative Chemistries for Free Radical-Initiated Targeting and Immobilization. *J Funct Biomater* 2023; **14**(3).

(20) Kesharwani P, Ma R, Sang L, Fatima M, Sheikh A, Abourehab MAS et al. Gold nanoparticles and gold nanorods in the landscape of cancer therapy. *Mol Cancer* 2023; **22**(1):98.

(21) Slapak EJ, El MM, Ten Brink MS, Kros A, Bijlsma MF, Spek CA. CAPN2-responsive mesoporous silica nanoparticles: A promising nanocarrier for targeted therapy of pancreatic cancer. *Cancer Lett* 2024; **590**:216845.

(22) Milano G, Innocenti F, Minami H. Liposomal irinotecan (Onivyde): Exemplifying the benefits of nanotherapeutic drugs. *Cancer Sci* 2022; **113**(7):2224-2231.

(23) Wang R, Li Y, Gao J, Luan Y. WRQ-2, a gemcitabine prodrug, reverses gemcitabine resistance caused by hENT1 inhibition. *Drug Discov Ther* 2022; **16**(6):286-292.

(24) Desai AK, Hodovan J, Belcik JT, Lindner JR. Hypersensitivity Cross-Reactivity for Ultrasound-Enhancing Agents and COVID-19 Vaccines 3. *J Am Soc Echocardiogr* 2022; **35**(5):523-525.