# Tumor priming sequences combined with novel nanoparticle drug carriers for enhanced therapeutic efficacy in pancreatic cancer:

# a tripartite USA/Northern Ireland/Republic of Ireland consortium

**SHORT REPORT**

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**Evidence Brief**

Why did we start? (The need)

Pancreatic cancer is one of the most highly intractable and difficult-to-treat cancers, where survival rates have remained unacceptably low without significant improvement over several decades. Although of relative infrequent occurrence, being only the 10th most common cancer in the UK, it is the 6th cause of cancer-related deaths, showing that it is a disease of urgent clinical unmet need. Clearly, current strategies for the development of therapies that have should activity in other cancers have not shown similar efficacy in pancreatic cancer – new approaches and new strategies at a discovery level are needed.

What did we do? (The methods)

Our research was focused on the development of new therapeutics for the treatment of this disease. Specifically, we focused on the development of antibody and nanotechnology-based strategies for the treatment of this disease. Working with colleagues in both Dublin (DCU and St Vincent's) and University at Buffalo (USA) in a US-Ireland tripartite consortium, we aimed to develop new therapies that can be tested in cutting edge preclinical models of pancreatic cancer.

What answer did we find? (The findings)

We successfully developed a range of nanoparticle- and antibody-based therapies against several clinically relevant targets in pancreatic cancer. We worked with our colleagues in DCU to develop new models and ways of analysing our experimental agents and then worked with UB to assess efficacy in human pancreatic cancer models. All platforms underwent extensive in vitro and in vivo analysis which demonstrated their ability to potently reduce tumour volume in multiple pancreatic cancer models. In the case of some they were able to completely eradicate the tumour until treatment ceased.

What should be done now?

We believe the treatments developed warrant further investigation in more advanced in vivo models. This would derisk the technologies and strategies we have developed to successfully engage large pharma downline to take this technology forward to clinical trials. Indeed interest in our work has now led to the Scott group being a partner in an International NCI/CRUK Cancer Grand Challenge to enhance targeting and delivery of macromolecule drugs – the MAGIC Consortium (shortlisted grant currently being prepared)

## Background

The survival rates for many cancers have improved significantly over the last forty years. This has largely been accredited to an increase in early diagnosis and an improvement in treatment. Unfortunately, pancreatic cancer has not enjoyed this trend, having one of the lowest 10-year survival rates among the 20 most common cancers in the UK of 1.1%. Surgery remains the only opportunity for a complete cure but only around 20% of patients are deemed eligible for this surgery at diagnosis.

When surgery is not an option a patient is likely to be treated with gemcitabine or a combination of 4 drugs called FOLFIRINOX, however the highest median survival reported from these treatments is less than one year. Clearly, there is a desperate need for an improvement in treatment options.

Antibody conjugated nanoparticles (ACNPs) represent and exciting opportunity for the development of new pancreatic cancer treatments. As with antibody drug conjugates (ADCs), antibodies are used to target tumour specific antigens to aid delivery and internalisation of a cytotoxic payload. ACNPs have the added advantage of presenting the antibody in a multivalent format which can aid efficacy in some cases. They also have a much higher drug to antibody ratio allowing for less toxic payloads to be used.

In this work we developed numerous treatments capable of reducing tumour volume in mice In QUB we developed an EGFR targeted polymeric nanoparticle, a DR5 targeted polymeric nanoparticle and an EGFR targeted ADC (in collaboration with UCL). In Buffalo a DR5 targeted liposome was developed. These formulations were all tested in vivo in Buffalo.

## Aims and Objectives

- Develop and characterise various nanoparticle formulations and antibody-drug conjugates against pancreatic cancer targets including EGFR, DR5 and DLL4
- Undertake comprehensive in vitro analysis of the various platforms to examine readouts such as target binding, cellular uptake, cytotoxicity and mechanism of action
- Progress lead formulations to in vivo efficacy testing in murine models of pancreatic cancer including cell line xenografts and clinically relevant patient-derived xenografts

## Methods

DR5 – in QUB numerous formulations with different payloads were assessed based on drug entrapment, efficacy and reproducibility. Ultimately a camptothecin entrapped polymeric nanoparticle was chosen for further development. The anti-DR5 antibody AMG 655 (conatumumab) was provided as a kind gift from Amgen. This antibody was conjugated to the surface of the nanoparticle via carbodiimide chemistry and efficacy was tested against numerous pancreatic cancer models in vitro and in vivo. siRNA and CRISPR technologies were used to investigate the mechanism behind the synergistic effect between the two therapeutics employed on the nanoparticle formulation.

EGFR – Three EGFR-targeted therapies were developed throughout the course of this project. These included:

(1) a camptothecin-loaded polymeric nanoparticle functionalised with cetuximab (full IgG) via conventional carbodiimide chemistry.

(2) a refined iteration of (1), consisting of a camptothecin-loaded polymeric nanoparticle functionalised with cetuximab (Fab fragment) via a site-specific chemistry. This superior conjugation approach ensured that antibody paratopes were presented in an optimal conformation on the surface of the nanoparticles for maximal target binding.

(3) an antibody-drug conjugate consisting of cetuximab coupled to the cytotoxic warhead monomethylauristatin-E.

All platforms underwent comprehensive in vitro analysis including assessment of their EGFR binding potential and cytotoxicity in a panel of pancreatic cancer cell lines. This was followed by in vivo evaluation in murine models of pancreatic cancer, employing either pancreatic cell lines or patient-derived material as xenografts.

DLL4 – Work on DLL4 involved the development of a camptothecin-loaded polymeric nanoparticle functionalised with DLL4 VNAR via maleimide chemistry. In vitro analysis of the DLL4 binding potential and cytotoxicity of this formulation was performed in a panel of pancreatic cancer and endothelial cell lines. In a separate body of work, we explored an alternative approach for the site-selective coupling of the DLL4 VNAR to our nanoparticle. Targeting of this formulation was assessed through binding assays with recombinant DLL4.

Full details on the methods employed for construction and validation of all of the above platforms have now been published [1–6].

Personal and Public Involvement (PPI)

*Benefits/impacts of PPI to the study*

There has been continued application of PPI. One of the most important developments that the grant has allowed us to do is to establish a network of pancreatic cancer at the Patrick G Johnston Centre for Cancer Research (formally the Centre for Cancer Research and Cell Biology), including the funding of an additional PhD student as part of the programme – the John Martin Scholarship. These efforts have been fundamental in growing pancreatic cancer as a key area for the future in the Centre, leading to developments such as Cancer Focus NI now supporting Dr Richard Turkington and his translational and clinical work in this area

*Challenges/barriers to PPI*

We had intended to run a PPI event publicising the research in pancreatic cancer that we do in QUB and with the US-Ireland network in March 2020 but this has had to be postponed for now due to COVID-19. We will revisit and reorganise this when appropriate to do so.

*Role of PPI in dissemination of the research*

Through the new networks established with our PPI, we have been able to reach out to community groups such as the Black Box science club, Rotary clubs, Ulster Farmers Union, The Perennials Rugby club and other groups with our research. This would not have been possible without that engagement and indeed leadership offered by our PPI.

Findings

EGFR - Epithelial growth factor receptor has been adopted as a therapeutic target in other cancers such as colorectal cancers. A lead antibody cetuximab has been on the market for several years and has shown much efficacy. EGFR is also up-regulated in pancreatic cancers, but unfortunately almost 90% of these tumours over-expressing the receptor are actually resistant to cetuximab due to mutations in a key oncogene, K-Ras, which over-rides any inhibition of EGFR. Nonetheless, we hypothesised that as these tumours are still EGFR positive, cetuximab could be repositioned as a targeting moiety to deliver chemotherapy-loaded nanoparticles to these tumours. We developed an EGFR targeted nanomedicine and have now demonstrated its efficacy in pancreatic tumour models, in collaboration with colleagues in Buffalo. This work was published in the top-tier RSC journal Nanoscale in late 2019 [1]. In brief, we found that whilst a drug-loaded nanoparticle could elicit specific therapeutic effects itself in pancreatic tumour models, the efficacy of the nanoparticle was further enhanced through EGFR targeting (using cetuximab antibody). Leading on from this work, we wished to further refine the formulation of these nanoparticles, to progress them to a standard that would be needed to satisfy regulatory requirements. Through a collaborative multi- disciplinary effort with UCL, we developed an approach for the highly controlled and homogeneous coupling of cetuximab F(ab) to the nanoparticles. In comparison to conventional methods for nanoparticle functionalisation, we showed that our bioconjugation approach resulted in superior EGFR binding activity and enhanced delivery of an entrapped payload to pancreatic cancer cells. This work was also published in Nanoscale in 2020 [3]. In addition to our EGFR targeted nanomedicine approach, we also developed a novel EGFR targeted antibody-drug conjugate, again through collaboration with our colleagues in UCL. This is additional work to that which was originally proposed in the project itself. After performing the in vitro characterisation of this compound here at QUB, the material was evaluated in xenograft models in Buffalo. We are delighted to report that the work is now completed and using animal models at Buffalo we have been able to show excellent activity of the antibody drug conjugate in EGFR positive pancreatic tumour models. The importance of this work for patients is that although many pancreatic cancer patients are insensitive to the antibody drug targeting EGFR called Cetuximab, the receptor is still of clinical relevance for drug targeting approaches. This work was accepted for publication in the British Journal of Cancer in 2020 [4].

Death Receptor 5 - We have previously shown that DR5 can be effectively targeted with nanomedicines bearing antibodies towards this cell surface receptor. A key attribute to this approach is that the density of the antibodies on the surface of the nanoparticles induce not only receptor binding, but also the engagement of DR5 signalling, which results in cell death. This sets this particle system apart from the previously described EGFR system in that it has a dual role of (i) targeting the DR5 positive tumour cells and (ii) inducing a cell death signal. We completed a range of pancreatic tumour models and shown that this approach to activation of apoptosis has application in this disease. With the addition of chemotherapy to the formulation we see synergistic effects over simple delivery of a non-targeted nanomedicine and furthermore we have found that this mechanism is consistently observed in different models regardless of initial levels of drug resistance.

We were able to map these sensitivities back to apoptotic pathways and reveal the molecular basis for activities observed; revealing further mechanistic insight into the development of pancreatic cancer and validating our approach to treat. Our PhD student was able to travel to Buffalo to train a PhD student there in the conjugation of antibodies to polymeric particles. He not only gained a wealth of experience, including shadowing the production of liposomes, he was also able to learn in vivo experiment design and the ability to manage large in vivo experiments remotely. A paper showing this DR5 work was published in the pharmaceutical journal Journal of Controlled Release [6].

DLL4 – The success of our work on EGFR and DR5 attracted the interest of Scottish biotechnology company Elasmogen Ltd., who we worked alongside to develop new DLL4 targeted nanoparticle systems (particularly for pancreatic cancer) using soloMER (shark antibody) technology. We showed that nanoparticle targeting to pancreatic tumour cells could be markedly enhanced through surface functionalisation with DLL4 VNAR. Importantly, these effects also translated to endothelial cell cultures, allowing for enhanced delivery of the entrapped payload and highlighting the potential for simultaneous targeting of both tumour cells and neovasculature. Further work alongside our UCL colleagues focused on the development of a novel conjugation chemistry for the highly controlled and oriented coupling of DLL4 VNAR to our nanoparticles. Nanoparticles formulated via this approach showed significant improvements in DLL4 binding that outperformed those constructed using conventional conjugation approaches. Collectively, this body of work resulted in two publications [5,7].

## Conclusion

This US Ireland partnership programme has enabled an incredibly fruitful collaboration between QUB, SUNY at Buffalo and DCU. Over the course of the project, numerous formulations were tested as potential treatments for pancreatic cancer. These treatments were highly effective in the majority of models tested. The capabilities of each partner synergistically enabled work not possible in one place alone. Numerous articles and reviews have been published as a result which greatly benefits the partners and the career development of the postdocs and PhD students involved.

This early translational work has led to many new opportunities (pathways to impact)

1. Development of new industrial collaborations – companies such as Elasmogen have been excited by our work and sufficiently convinced of its potential and derisking to engage further development projects to target pancreatic and other cancers

2. Track record in macromolecule drug delivery established by the Scott group has enabled our involvement in one of the most prestigious cancer grant applications worldwide – a Cancer Grand Challenge Consortium – we have been one of 11 teams shortlisted from almost 90 applicants and are currently developing the project application – lead applicant Terry Rabbitts, Institute of Cancer Research.

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