Opportunity-Led Research Award COMM/5335/19

Experimental Cancer Medicine Centre (ECMC): Support for clinical trial development – CDC7 and IAP inhibition.

Final report

A major aim of Belfast ECMC is the translation of discovery science undertaken by academic scientific researchers in NI into Belfast-led, biologically informed clinical trials. This award has supported the development of context-specific pre-clinical models to accelerate the development of potential clinical trials and provide underpinning data for funding applications to evaluate novel therapeutic approaches. It has also enabled leveraged funding from industry to support both the pre-clinical and clinical research programmes and supported training and progression of early career researchers here in NI, building future capacity.

CDC7 inhibition:
The former CRUK Combinations Alliance was an industry partnership that made available novel therapeutics for evaluation in non-commercial areas of interest. This included the First-In-Class CDC7 inhibitor from Lilly. CDC7 is a protein with key roles in regulating cell cycle progression that is often overexpressed in malignant cells, in particular those with TP53 mutations.

Basic scientific research in QUB (McDade, Longley) demonstrated activity in pre-clinical colorectal cancer (CRC) models. This data informed a successful funding application led by Wilson (Belfast ECMC lead, now Glasgow ECMC) to CRUK for a FIC Phase I clinical trial to evaluate the safety and tolerability of this agent in patients with advanced solid tumours with planned expansion cohorts in CRC and other tumour types.

Preliminary findings from this trial were presented at the American Society of Clinical Oncology Annual Meeting (Gallagher PF, Journal of Clinical Oncology, 2022), (https://doi.org/10.1200/JCO.2022.40.16_suppl.3103). Monotherapy treatment was safe and tolerated however, significant clinical activity was not observed (including in the CRC expansion cohort). Future evaluation would therefore be needed within combination strategies. Pre-clinical modelling supported by a CRUK Clinical Doctoral Fellowship and this award showed combination therapy with standard of care cytotoxic chemotherapy agents could be an effective treatment strategy in CRC. Unfortunately, there was no further commercial support for development of therapeutic approaches using this agent and no other agents in class available hence; any potential clinical trials will not be realized. However, this work has led to improved understanding of the associated tumour biology and formal publication of the data obtained is in progress.

IAP inhibition:
This award also supported further development of preclinical models to evaluate Inhibitors of Apoptosis Proteins (IAPs) in combination with standard care chemotherapy in CRC. IAPs are frequently deregulated in cancer, and are a major

contributor to chemotherapy resistance through inhibition of apoptosis ad pro-survival NF-kappaB signaling. Restoration of apoptotic function through inhibition of IAPs is an attractive therapeutic strategy with an additional potential for enhanced cell death via immunomodulation of the tumour microenvironment. Initial data to support this therapeutic approach in cell line and xenograft models was obtained via an academic programme of discovery science research (Longley) with another agent in class, birinapant (Crawford N, Cell Death Differ, 2018 25(11): 1952-1956).

ASTX660 (tolinapant) is a novel small molecule potent IAP antagonist, inhibiting both XIAP and cIAP1. A pre-clinical collaboration with Astex Pharmaceuticals (Longley) supported evaluation of this agent in combination with chemotherapy in pre-clinical CRC models showing cell death *in vitro* and tumour growth inhibition in xenograft models. At the same time, ASTX660 was undergoing clinical evaluation as monotherapy in patients with advanced cancer and from this trial; a recommended Phase 2 dose and schedule were identified.

While our pre-clinical data broadly supported potential clinical evaluation of ASTX660 in combination with standard care chemotherapy in patients with advanced CRC, we felt evaluation in an immunocompetent model would be more compelling as the immunomodulatory effects of treatment could be demonstrated in addition to the cell death effects. We included some preliminary mouse organoid data in our outline clinical trial application, and in recognition of the potential dual treatment effect were asked to further develop this by the funder (CRUK).

This award supported the development of the pre-clinical models that underpinned the subsequent successful clinical trial application (CRUK Clinical Research Committee). Briefly, we completed evaluation of ASTX660 in combination with oxaliplatin/fluorouracil chemotherapy (modelling the FOLFOX regimen administered to patients) in mouse colon organoids with relevant genetic contexts (APC/KRAS/P53) showing a significant reduction in organoid viability *in vitro.* These organoid models were used to enable evaluation in immunocompetent mouse models and we went on to demonstrate that the combination of FOLFOX and ASTX660 resulted in significant tumour growth inhibition *in vivo* compared to chemotherapy or ASTX660 alone. This data has been published as part of the overall programme of work (Crawford N, Mol Cancer Ther, 2021 20(9): 1627-1639.

The work supported by this award has contributed to the successful funding of the ASTFOX clinical trial. ASTFOX is a Phase I study of the IAP antagonist, ASTX660, in combination with standard of care chemotherapy in metastatic colorectal cancer (CRCPJT\100021; £472k, CI Coyle, QUB). While the trial has encountered delays due to the pandemic, obtaining regulatory approvals and site set-up pressures) it has opened to recruitment in Glasgow ECMC in July 2024. Belfast and Leicester ECMC remain at the site set up stage. Unfortunately, due to lack of clinical research infrastructure support (in particular statistical support for a Bayesian design) QUB is not the host institution for the funding (Glasgow is host institution and trial sponsor). However, the trial is translationally rich and tissue-based translational

research endpoints will be analysed in QUB (Precision Medicine Centre). The trial will build local research capacity via PPI and co-investigator involvement: it has achieved recognition from the funder and REC for its exemplary PPI input in trial design and development (via Kerr and Irvine, PPI applicants from NICRCF) and has attracted a Clinical Fellow to Belfast to further develop the associated pre-clinical research via the Wellcome HRB ICAT Programme (O'Brien).

This award will be acknowledged in any output arising from the clinical trial.

Additional information:
This award and the associated work has taken longer than anticipated with a downturn in research activity due to the pandemic hence the previous requests for no cost extensions.