Post programme report

Cancer is a major global health burden associated with millions of deaths annually. Whilst current chemotherapeutic treatments for cancer are effective, they possess substantial off-target effects (Hepatotoxicity, Neurotoxicity and Cardiotoxicity) which all have a substantial impact on long-term survival. These off-target effects of chemotherapeutics are significantly more severe in children when used to treat childhood cancers such as medulloblastoma. It is therefore imperative to evaluate new therapeutics for their efficacy and potential off-target effects.

A potential rich and diverse source of these new therapeutics is those derived from plants termed phytopharmaceuticals. Phytopharmaceuticals have been used for millennia to treat various disorders during traditional medicinal practices worldwide, however several traditional compounds are now routinely used clinically. Numerous lead compounds have been highlighted for the development of clinically utilised agents. One of these being Celastrol, a pentacyclic triterpene isolated from *Tripterygium wilfordii*, which is shown to possess anti-inflammatory and antioxidant properties in vitro. Investigations also reveal that Celastrol possesses anti-cancer properties against various cancer types.

Following a recent collaboration with Kidscan Children's Cancer Research, a numerous phytochemicals have been identified as producing potent cytotoxicity against medulloblastoma cells, with the most potent of these being celastrol. Celastrol was shown to produce EC_{50} values of less than 500nM against the group 3 medulloblastoma cell line HD-MB03 through the induction of apoptotic cell death highlighting its potential as a therapeutic agent for medulloblastoma.

The objectives of the project revolved around the evaluation of celastrol on medulloblastoma subtypes, something that has never been characterised, and explore its cardiotoxic potential. My project focussed to see if previously described activity was also the case in other medulloblastoma subtypes including the sonic hedgehog subtype cell line, DAOY. I preliminarily investigated the potential cardiac implications of celastrol utilising an in vitro model of cardiotoxicity (H9c2 cells). To evaluate these effects, technical research methodologies including flow cytometry, live cell microscopy and fluorescent microscopy were utilised to determine the mechanism of action observed in group 3 medulloblastoma cells is mimicked in DAOY cells.

The results of this studentship showed celastrol to be effective as a chemotherapeutic agent against both HD-MB03 and DAOY cell lines in a time and concentration dependent manner (Figure 1A&B), with EC_{50} values ranging between 0.24–0.51µM and 0.21–0.64µM respectively. It was also found that cytotoxicity of celastrol was irreversible after treatment for 24 hours when treated with concentration of >500nM before removal and washout of the celastrol (Figure 1C&D). Using time-lapse live-cell microscopy we identified that celastrol produced no effect on cellular migration (Figure 2). Fluorescent microscopy revealed that celastrol significantly increased the activation of Caspase 3/7 (Figure 3), providing further evidence of apoptotic cell death and providing preliminary information on the mechanism of cell death induced by celastrol.

Treatment of terminally differentiated cardiac myoblasts (H9c2 cells) with celastrol, to screen for cardiotoxicity, revealed that celastrol induced cardiotoxicity at concentrations >0.5µM following treatment for 72 hours (Figure 4) with no toxicity observed at lower treatment timepoints (Data not shown).

These data highlight celastrol as a relatively safe and effective therapeutic lead for the treatment of medulloblastoma. The research group will now seek to continue this study by exploring changes in key apoptosis and cell cycle regulatory gene and protein expression by qRT-PCR and Western Blotting in medulloblastoma cells following treatment with celastrol. The group is also seeking to develop an internal collaboration with nano-medicine specialists, to evaluate if celastrol can be nano-encapsulated to increase its potency and minimise off-target effects.

This project may evoke other scientists to think about exploring traditional medicine-derived compounds for their wider therapeutic potential and develop an understanding of their molecular mechanisms of action. In traditional medicine, thousands of compounds/extracts are used to treat numerous ailments, however, there remains limited exploration of the molecules within these extracts and their mechanisms of action. The development of an understanding of the mechanisms may make them more desired for translation to in-vivo study or clinical trials, as well as for scientists to have a basis for the generation of modified derivatives which possess more potent effects or less off target toxicity.

Throughout this project we continued to develop our in vitro model of cardiotoxicity to screen novel compounds as cardiotoxicity is a common side effect of chemotherapies. As phytomedicines traditionally have fewer off-target effects than synthetic compounds, by simultaneously screening compounds for their therapeutic and cardiotoxic potential, will highlight promising lead compounds to take forward for molecular evaluation. This could be of interest to the wider bioscience community as a tool for developing novel compounds of therapeutic interest.

This project has provided me with a diverse set of highly sought skills, both practical and transferable, in the research field. This studentship has enabled me to build a comprehensive portfolio of laboratory skills, enhancing my post-graduation employability and preparing me for potential postgraduate research pursuits such as MRes, or a PhD programmes. These skills range from fundamental laboratory techniques like cell culture and cell viability assays to more advanced methodologies such as time-lapse live-cell microscopy, fluorescence microscopy, and flow cytometry.

During instances when my experiments encountered setbacks I showed resilience, honed my problem-solving abilities to pinpoint sources of experimental error and gained proficiency in troubleshooting technical issues by collaborating with fellow research students, academics, and technical staff. Furthermore, I had the opportunity to participate in workshops, which significantly contributed to the development of my analytical and communication skills. This encompassed areas like academic writing, data presentation, and statistical analysis of biological data. Consequently, I was able to deliver an oral presentation summarising the findings and conclusions of my studentship to fellow members of the research laboratory and a broader audience of academic staff. The results of my research are promising and may lead

to the creation of a conference abstract for potential presentation at scientific research conferences in the future. Most notably, this experience afforded me a deep sight into the day-to-day responsibilities of a research scientist engaged in interdisciplinary research, something I could not experience without this studentship.

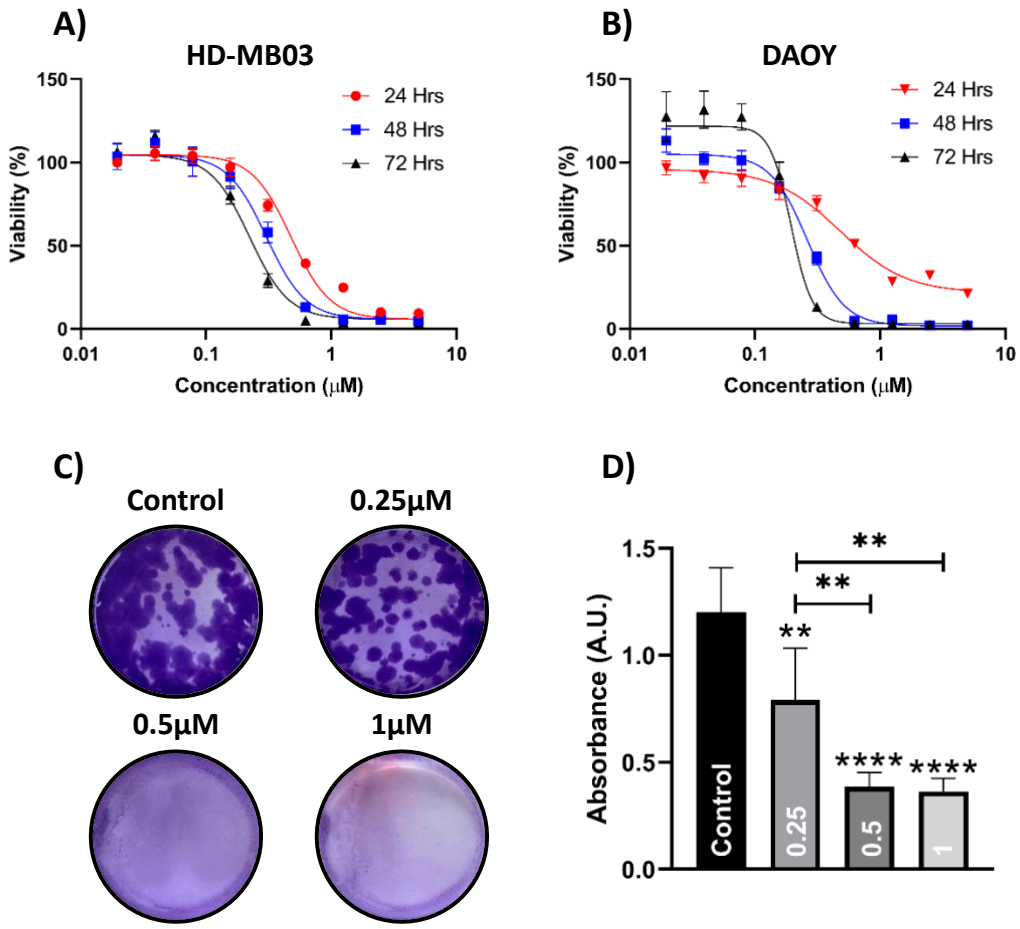

Figure 1 - The cytotoxic effects of celastrol against medulloblastoma subtypes. (A & B) Dose response curves comparing the cytotoxicity of celastrol and against HD-MB03 (Panel A) and DAOY (Panel B) cell lines following treatment for 24 (Red), 48 (Blue) and 72 hours (Black). (C) Representative crystal violet staining images showing HD-MB03 colonies arising after celastrol wash-out over 10 days recovery. Pre-treatments with celastrol were carried out over 48 hours before wash-out. (D) Corresponding Mean ± SD crystal violet absorbance derived from 6 independent repeats. All data compared to vehicle alone (0.5 % v/v DMSO). Statistical significance ($P < 0.05$) was determined following a one-way ANOVA with a Tukey's post-hoc test **$P < 0.01$; ***$P < 0.001$; **** $P < 0.0001$. n = 6 independent repeats for all experiments.

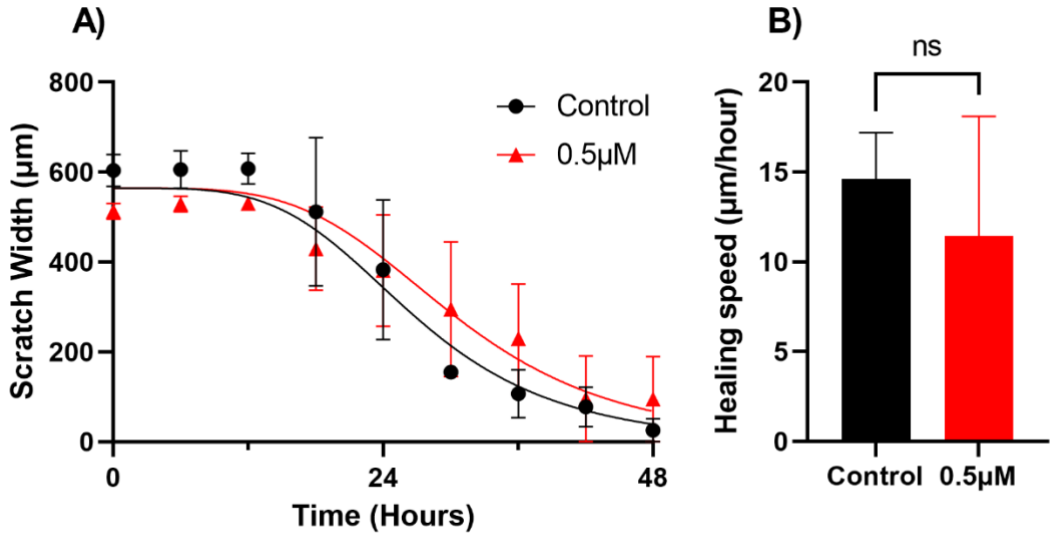

Figure 2 – The anti-migratory effects of celastrol in Sonic Hedgehog (DAOY) medulloblastoma cells. (A) The time-width profile of scratch assays following treatment with control vehicle (0.5% *v/v* DMSO) (Black) and 0.5uM celastrol (Red). (B) The effect of control vehicle and celastrol on cellular migration speed. Statistical significance ($P < 0.05$) was determined using a student's t-test. ns = non-significant. Data shown generated from 3 independent repeats for all experiments.

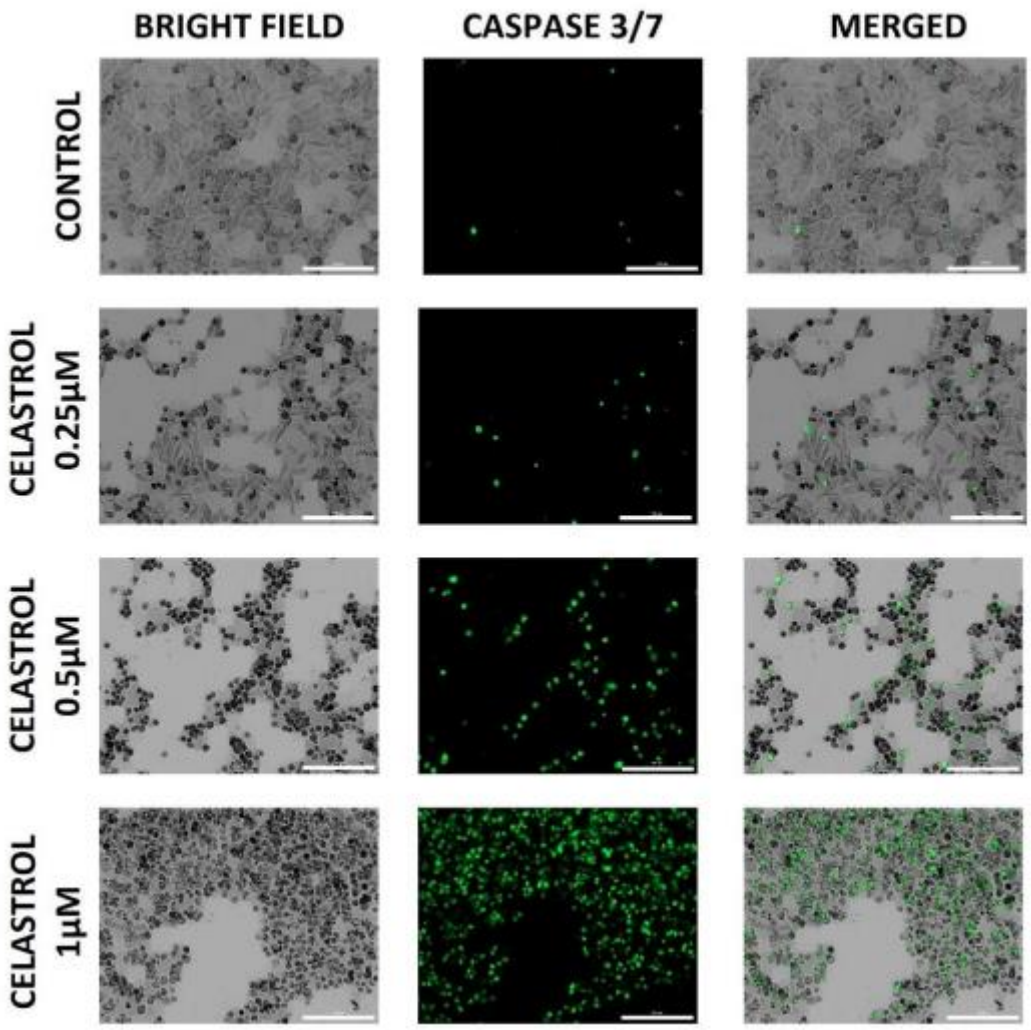

Figure 3 – The effects of celastrol on caspase mediated apoptosis. Specimen light and fluorescent micrographs (100x total magnification) showing the activation of Caspase 3/7 following staining with Invitrogen CellEvent Caspase-3/7 detection agent following treatment with Celastrol (0.25, 0.5 and 1 µM) for 24 hours. Scale bars = 200 µm.

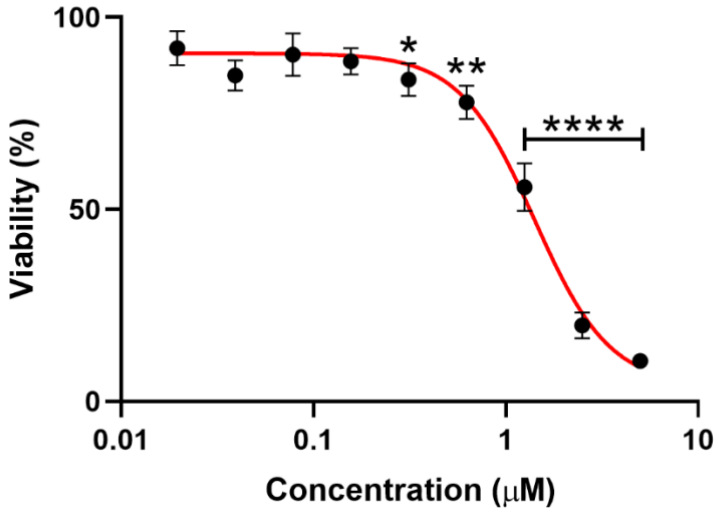

Figure 4 – The cardiotoxic effects of celastrol. Dose response curves comparing the cytotoxicity of celastrol and against H9c2 terminally differentiated cardiac myocytes following treatment for 72 hours. Statistical significance ($P < 0.05$) was determined following a one-way ANOVA with a Dunnett's post-hoc test. * $P < 0.05$, **$P < 0.01$; **** $P < 0.0001$. n = 6 independent repeats for all experiments.