Cancer research update

Report for Breast Cancer Research Aid from the European Cancer Stem Cell Research Institute
August 2023

How has Breast Cancer Research Aid support helped? We are pleased to report that your gifts have continued to accelerate our research and have leveraged further funding for this vital work. Thank you once again for your ongoing support.

Professor Richard Clarkson

Director of European Cancer Stem Cell Research Institute

This year has seen a significant step towards the delivery of our leading novel cancer therapeutic to clinical trials. Thanks to your donations, we were able to provide consumables support for three Master's student projects in our laboratory.

The projects have verified our earlier studies indicating a role for Bcl3 in influencing immune surveillance of breast tumours and confirming the recently published role of Bcl3 in repairing damaged DNA during and after chemotherapy.

These observations were used as supporting evidence in a recent submission by ourselves and our industrial partner, TNA Therapeutics, to UK Government for Innovate UK funding to support our Bcl3-inhibitory drug development pipeline (see appendix). This application was successful thanks specifically to this supporting scientific evidence, which was highlighted by the reviewers as a particular strength of the proposal:

"The application has a very strong scientific base. Applicant used their prior data very effectively to demonstrate the candidate drug is biologically active and has a novel mechanism of action. The appendix contains a particularly strong representation of the science underpinning this application."

The award of £1.5M now allows us to complete the final pre-clinical data and submit to the FDA and EMA for approval for clinical trials. These future trials will be coordinated out of Cardiff by our collaborator Prof Rob Jones.

Furthermore, we are pleased to announce that the findings of the previous work of Professor Matt Smalley, supported for many years by Breast Cancer Research Aid (BCRA), have also been submitted to a scientific journal, in which BCRA are acknowledged.

The study has made new discoveries about the role of the gene LYN kinase in the breast, but Professor Smalley has concluded that this gene would not be a good therapeutic target in breast cancer. This is a crucial contribution to the wider field as researchers continue to seek an effective treatment for breast cancer.

Supporting the next generation of breast cancer researchers

BCRA funding was used to support two incredibly talented breast cancer research students this year. This includes Eulalia Noguera-Delgado (MA 2022), studying at Cardiff University as an Erasmus student, who helped us to pilot our BCRA-funded cancer stem cell platform. Eulalia used the platform to monitor the activity of cancer stem cells within breast tumour cell populations in real time by fluorescence microscopy.

Eulalia's work was particularly important as it demonstrated proof of principle for our cFLIP-related work looking at the effect of our cFLIP inhibitors on chemotherapy mediated stimulation of cancer stem cells (which we described in our previous report). This proof of principle was included in our successful application for 3-years funding from Breast Cancer Now, and has subsequently employed my senior post-doc Gillian Seaton for a further 3 years. Gillian's role will be to develop the stem cell platform.

Off the back of her work in my lab, Eulalia was successful in being offered a very prestigious PhD studentship at the National Cancer Institute in Amsterdam with Professor Jacco Van Rheenen, studying the initiation and progression of cancer.

The second student supported by your donations this year was my PhD student, Hannah Smith (PhD 2022-), who is completing a study of the role of Bcl3 in senescence.

Senescence is a process in which a cell ages and permanently stops dividing but does not die. Over time, large numbers of senescent cells can build up in tissues throughout the body. These cells remain active and can release harmful substances that may cause inflammation and damage to nearby healthy cells, and it is believed that this may contribute to the development of cancer.

The final data Hannah generated has forced us to reassess our model for how Bcl3 influences cell viability in p53-wild-type breast cancers. Our initial hypothesis was that cells became senescent when Bcl3 was inhibited, when in fact we now believe this is more likely a cell-cycle arrest rather than entry into a senescent state. This is important for the implications of using Bcl3 inhibitors in breast cancer patients with wild-type p53 (approximately half of all patients) as it means that tumour cells are less likely to lie dormant (senesce) for long periods after treatment and may consequently be more prone to targeted therapy.

Produced by Development and Alumni Relations for Breast Cancer Research Aid. For more information contact Bethany Morrissey on MorrisseyB@cardiff.ac.uk or 029 208 70326.

We plan to use your generous £21,000 funding to further Hannah's work, elucidating the mechanism by which Bcl3 inhibitors influence the inner-workings of breast cancer cells, and to follow up previous observations on the immune response of breast cancers to Bcl3-inhibition.

The funds will be used to run two key platform assays. Firstly, a PAMGene Kinase assay will be performed to identify, at the protein level within cancer cells, which signalling pathways are activated and repressed when we treat breast cancer cells with our Bcl3 inhibitor. There is mounting evidence that Bcl3 controls several key cancer related pathways, and identifying which of these are 'dominant' in breast cancers will be critical to choosing which patients we treat and what other drugs these patients should receive.

We also intend to collaborate with researchers at Cardiff University to gain access to a multi-fluorescence platform assay for immune cells recruited to the tumour microenvironment following Bcl3 inhibitor treatment. This will follow up the exciting data we reported above showing that the immune system may be enhanced with Bcl3 inhibition. This 'multiplex' platform will identify which of the many sub-types of immune cells are recruited to the tumours after treatment and will be used to inform on whether we should include existing immune-therapy agents in clinical trials with our Bcl3 inhibitor.

Funding state-of-the-art equipment

Your donations have enabled us to purchase vital research equipment this year, including the cancer stem cell platform mentioned previously in this report. Alongside the platform, we allocated your £10,000 equipment grant towards acquiring a 'low oxygen' cell culture incubator. This has been used by our laboratory mainly, but also by other cancer research groups within the Institute to look at cancer cell behaviour in a more relevant low-oxygen or 'hypoxic' environment which mimics the conditions inside tumours, and which promotes metastatic disease.

With this incubator, we have been able to show that our Bcl3 inhibitor is more effective at suppressing some cancer related signals, such as Wnt signalling, when the cells are in a low oxygen environment. We are continuing to use this equipment on a weekly basis to explore these areas.

Appendix

Figures used in our Innovate UK application to support the potential for Bcl3 inhibitors to target immune cells and DNA damage in breast tumours. All data shown was supported by BCRA (except for the colorectal data in the second panel).

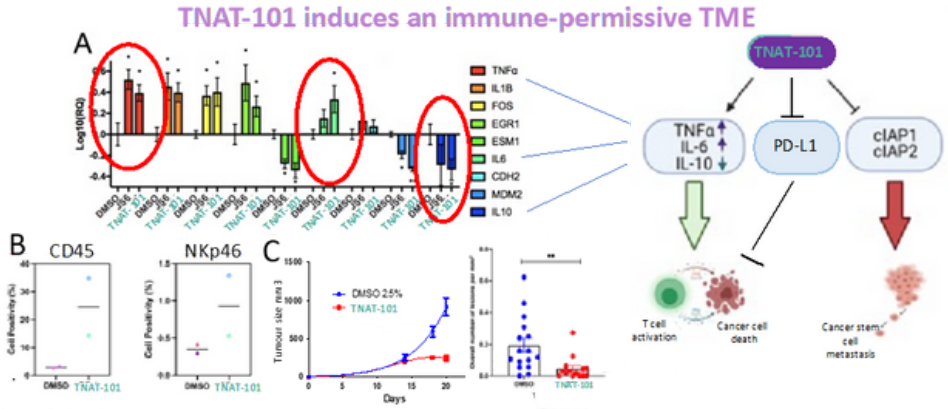

Figure 7. ***TNAT-101 enhances the immune tumour micro-environment and potently suppresses immune competent tumours.*** (A) Three of the TNAT-101 target gene responses identified in Figure 5 correlate with the activation of effector T cell mediated killing of tumour cells. (B) Preliminary analysis of the proportion of CD45+ve immune cells and Natural Killer cells in TNAT-101 treated tumours by IHC supports this hypothesis as it demonstrates early evidence of an increase in immune surveillance following treatment. (C) This correlated with a profound tumour efficacy of TNAT-101 (10mg/Kg) in an immune competent allograft model of aggressive metastatic breast cancer (4T1.2). Tumour stasis was observed at 10mg/Kg along with a significant reduction in spontaneous metastatic burden

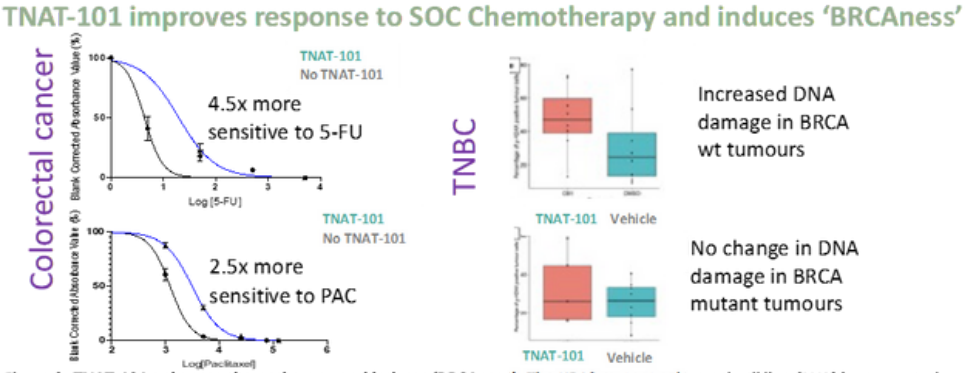

Figure 8. ***TNAT-101 enhances chemotherapy and induces 'BRCAness'.*** The KRAS mutant colorectal cell line SW480 was treated with increasing doses of 5-fluorouracil or paclitaxel in the presence or absence of TNAT-101. ***TNAT-101 enhanced the chemo-cytotoxicity 4.5-fold and 2.5-fold respectively.*** Analysis of PDX tumours treated with oral daily dosing of TNAT-101 (10mg/Kg) demonstrated a non-significant trend for an increase in gamma-H2AX positive tumour cells – denoting increased DNA damage – in BRCA wild-type, but not in BRCA mutant tumours. This is consistent with the recent published report that Bcl3 promotes DNA damage repair through homologous recombination (Parker, 2021). Thus TNAT-101 induces a BRCAness phenotype in cells, that promotes DNA damage, leading to loss of viability.