Mr Simon Hughes
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Penguins Against Cancer
Farrs
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22 November 2019

Dear Mr Hughes

Project report: Novel therapeutic combination strategies for patients with Ewing sarcoma (The Fergus Scholefield Cancer Research Fund)

We were honoured to receive the award of £5,000 from Penguins Against Cancer this year in support of our sarcoma research. I have pleasure in enclosing a progress report, prepared by Antonio Romo-Morales, PhD student at ICR's Sarcoma Molecular Pathology Team, which is led by Professor Janet Shipley.

With the support from Penguins Against Cancer, Antonio has been able to identify two successful drug combinations that have a greater effect inhibiting Ewing sarcoma cell growth than either drug alone. The most promising combination has been investigated further to gain better insights into its efficacy and use that knowledge when planning future clinical trials with patients.

We are very grateful for the tireless work you are carrying out to continue Fergus' legacy and on behalf of the Sarcoma Molecular Pathology Team, I would like to thank you once again for making such a valuable contribution towards our work into the greater understanding of Ewing sarcoma, here at the ICR.

I hope you will be pleased with the report, but if you need any further information, please do contact me. I will be very happy to help.

As always, please do send our best wishes to all the Penguin flock!

Yours sincerely

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Novel therapeutic combination strategies for patients with Ewing sarcoma

Report for: Penguins Against Cancer - Fergus Scholefield Cancer Research Fund

Prepared by Antonio Romo-Morales, PhD student, Sarcoma Molecular Pathology Team at the Institute of Cancer Research, London

November 2019

Project summary

Sarcomas grow in connective tissues – cells that connect or support other kind of tissue in the body. These tumours are most common in the bones, muscles, tendons, cartilage, nerves, fat, and blood vessels of arms and legs, but they can also happen in other areas of the body.

Ewing Sarcoma (ES) is a rare, highly aggressive sarcoma affecting children and young adults. The current five-year survival rate for patients with a localised tumour is approximately 70%. However this number drops dramatically to 20% in patients where ES cells have spread to other parts of the body (1-2). Current treatments available are not specific and have long-term toxicities that can have a detrimental impact on patients' quality of life.

For these reasons, the purpose of this project was to identify more effective and kinder targeted therapies for ES, which could be more readily translated into the clinic.

ES cells are characterised by a translocation - a type of chromosomal rearrangement in their DNA, resulting in the fusion of the *EWSR1* gene to an ETS gene family member. In 85% of cases, this occurs between genes *EWSR1* and *FLI1* (3). Recently, the EWS-FLI1 fusion gene product in Ewing sarcomas has been linked to problems in the process of replicating DNA, crucial step in cell division, which causes damage to the genetic material in ES cells. In turn, this creates a dependency on the mechanisms allowing DNA repair to occur (4-5).

We hypothesise that inhibitors of these molecular checkpoints and DNA repair proteins can exploit the dependency of ES cells on these mechanisms as a therapeutic strategy. Based on this, inhibitors of DNA repair proteins and checkpoint inhibitors are expected to enhance the effectiveness of chemotherapy (given it normally exerts its effects by causing damage to the DNA), potentially enabling a decrease in the dose intensity of standard treatments, thus minimising short and long-term toxicities.

Project objectives

- To identify clinically relevant drugs that enhance response to current standard chemotherapy in *in vitro* 3D models (e.g. cellular models or cells in the lab) of Ewing sarcoma tumors.
- To assess the clinical potential of this combination by directly measuring the concentration of the drugs in our *in vitro* models and comparing this with data of concentrations achievable in patients.

To ensure that novel treatments can be repurposed for Ewing sarcoma patients in the clinic, the inhibitors of DNA repair proteins tested were limited to drugs already undergoing clinical trials for other cancer types.

Scientific progress

Clinical drug candidates inhibiting DNA repair proteins were selected and tested as single-agents in 3D models of ES. To verify that the drug was active in our 3D models we measured biomarkers (e.g. molecules that can be measured to give information about a process happening in the cell) specific to each clinical drug candidate.

The concentrations of the agents used in our experiments were determined based on published clinical data on what could be an achievable dose in patients. Five clinical drug candidates were then tested alone and in combination with chemotherapy in 3D models. The chemotherapeutic agent chosen is part of the standard of care administered to ES patients that relapse. It is at this stage where new treatments are initially tested in clinical trials, therefore exploring the combined effect with novel clinical drug candidates was our priority in this project.

We have identified two successful combination strategies that are able to inhibit growth of 3D models of ES (Fig. 1). For the purposes of this project, we focused on one clinical drug candidate that had the greatest effect. We have carried out validation of this successful combination in five different 3D models of ES, which exhibited different degrees of response to the combination treatment (Fig. 2). Whilst all of them respond to treatment, we are particularly interested in exploring what makes their responses different. Identifying the characteristics of models determining sensitivity can provide insight into the profile of ES cells, and to an extent of patients, that could respond better to such treatment.

In terms of characterisation of the effects of this treatment, we have observed an increase in cell death in the combination treatment (Fig. 3). The donation from the Fergus Scholefield Cancer Research Fund Grant has enabled us to extensively assess the DNA damage response using several markers following treatment in order to shed light into the molecular mechanism pushing ES cells towards cell death (Fig. 4). These molecular markers are also biomarkers of successful treatment. The support from Penguins Against Cancer has also enabled us to extend these successful findings into assessing the effects of different scheduling of the drug inhibiting the DNA damage response with the standard of care drug. This became an important priority as the top hit drug has effects on the cell cycle which has implications on drug scheduling for translation into the clinical setting. This additional work has taken priority over the original proposal to assess drug concentrations in cells, which will now be assessed in the final phase of the PhD studies next year using the most effective scheduling identified.

Impact

In this project, we have established an *in vitro* system with a clinical focus to assess combination therapies for ES models. With the support from Penguins Against Cancer, this has led to identifying two successful drug combinations that have a greater effect inhibiting ES cell growth than either drug alone. The most promising combination has been investigated further to derive molecular mechanistic insights, biomarkers of efficacy and optimised scheduling. This novel translational research is expected to lead into benefitting future ES patients.

Figures

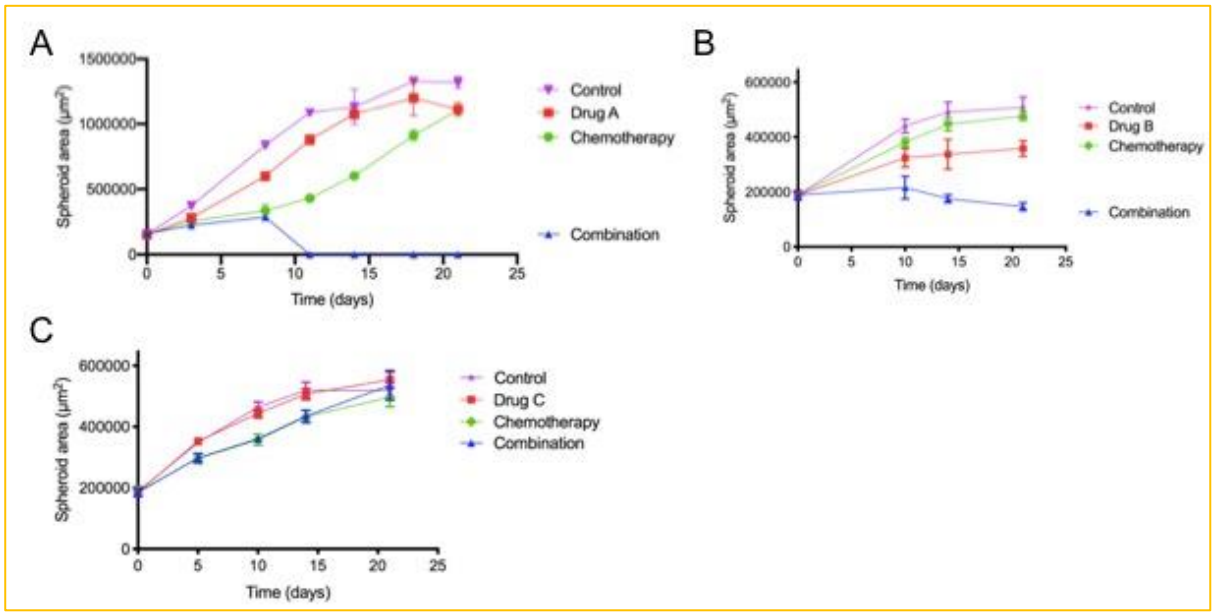

Figure 1 Combination treatments in Ewing sarcoma 3D models. (A-C) Two successful drug combinations between chemotherapeutic drug (green) and different clinical drug candidates A and B. Combination between drug C and chemotherapy did not reduce ES cells growth.

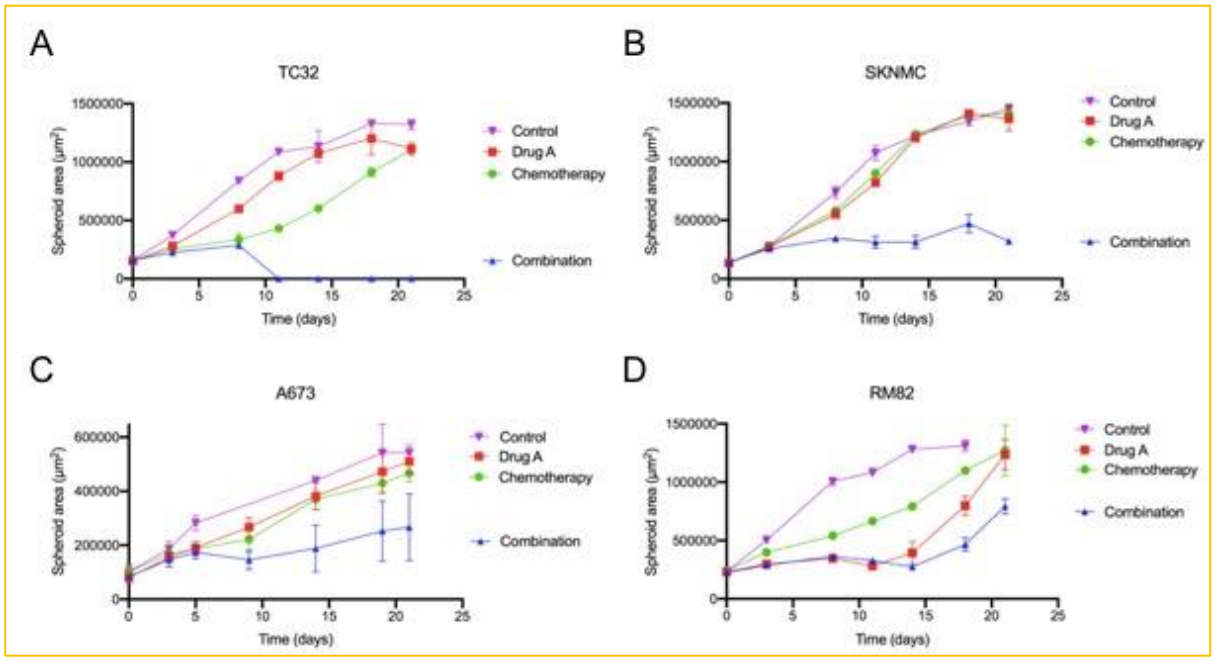

Figure 2 Validation of combination treatment between chemotherapy and Drug A in multiple Ewing sarcoma 3D models. (A-D) Different models of ES display varying degrees of response to the same doses of the drug combination.

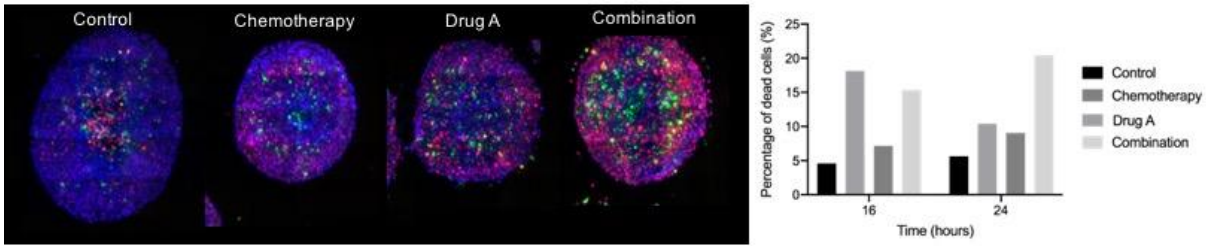

Figure 3 Assessment of cell death in Ewing sarcoma 3D models. Immunofluorescence analysis of apoptotic cell death markers indicating greater percentage of cell death in the combination treatment.

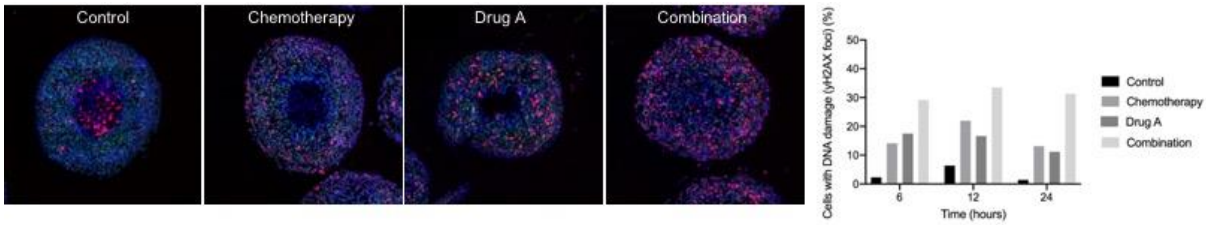

Figure 4 Assessment of DNA damage markers in Ewing sarcoma 3D models. Immunofluorescence analysis of two markers of DNA damage (red and green). Graph indicates percentages of cells positive for γH2AX in red, a marker of double strand breaks.

References:

1. Children with cancer. Incidence of Ewing sarcoma. 2018. https://www.childrenwithcancer.org.uk/childhood-cancer-info/cancertypes/ewing-sarcoma/
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5. Iniguez AB, Stolte B, Wang EJ, Conway AS, Alexe G, Dharia NV, et al. EWS/FLI Confers Tumor Cell Synthetic Lethality to CDK12 Inhibition in Ewing Sarcoma. Cancer Cell. 2018;33(2):202-16 e6.

About the ICR

The Institute of Cancer Research is one of the world's most influential cancer research institutes. We are passionate about our mission to make the discoveries that defeat cancer. We are a college of the University of London and charity. Our aim is to deliver excellent research for the benefit of patients, high quality training for the next generation of cancer researchers and clinicians, and a world-class environment for our science. In 2017, we were awarded a highly prestigious Queen's Anniversary Prize for Higher and Further Education, acknowledging our outstanding contribution to the discovery of new cancer drugs – including pioneering the transition from one-size-fits-all chemotherapy to targeted drug treatment.

For more information please contact:

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