Final report form
Cancer Council ACT Research Grant 2022

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Report due date	15-02-2023	
Project Lay Title	Cellular stress as a cause of cancer	
Grant Amount	$64.985,00	
Chief Investigator	Dr. Anne Steins	
Project dates	Start: 01-06-2021	End: 01-06-2022

Project description Please explain the purpose of your research (including background and rationale). Please use language that the general public will understand. Word limit is approximately 250 words.	The formation of cancers is driven by a delicate sequence of events where failure to undergo effective cell death is a common denominator. A key player in cell death is the cellular stress response. The FUS and DDIT3 genes play an important role in the cellular stress response. Remarkably, genome rearrangements can occur that form the single fusion gene FUS-DDIT3 by joining parts of both genes together, which by itself is capable of inducing myxoid liposarcoma (MLS), a rare cancer type that arises in the body fat. This provides a unique model for how cancer forms as a result of stress to the cell. In this proposal, we aim to elucidate how the FUS-DDIT3 fusion gene in MLS cells allows evasion of cell death through hijacking of the production of ribosomes, which are small molecular machines that make new proteins. This will be investigated in MLS cells, following the activation of the nucleolar stress response by therapeutic stressors. We will perform comprehensive analysis of how the FUS-DDIT3 fusion gene impacts the production of RNA molecules and their translation into proteins that contribute to apoptosis evasion. The findings of this study may lead to the first-time identification and targeting of the master regulator of apoptosis evasion in tumour cells under stress. As a large number of solid tumours are exposed to and driven by cellular stress, the findings of this proposal could have an impact in many other cancer types.

Major results of this research project As this project is now complete, please explain the major results of your research, and what it means for advancing cancer control. Please use language that the general public will understand. Word limit is approximately 500 words.	In this project we have studied the behaviour of the FUS-DDIT3 fusion gene in MLS and how it can result in cancer formation under the influence of cellular stress. To this end, we used cell lines derived from patients, as well as stem cells that were genetically modified to carry the fusion gene. Intriguingly, when we activate cellular stress in these cell models, using a broad panel of therapies, the **FUS-DDIT3 fusion gene rapidly accumulates and forms droplets around/on ribosomal DNA**. We think it might interfere here with ribosome production, repair of DNA breaks and/or cell death signals. These FUS-DDIT3 droplets create an environment that acts as a sponge by attracting other specific proteins. Other studies suggest that these droplets promote cancer cell survival. We are currently investigating whether this is the case in our cellular models. We prevent droplet formation by making genetic alterations to the 'droplet forming part' of the protein and will use these models to answer this question. We also found that incorporation of the fusion gene in normal stem cells results in a substantial increased production of specific proteins that usually instruct cell death. In our cells it seems these proteins are now being used to *enhance* cell growth and survival, the so-called gain-of-function (GOF) activity. We have performed RNA sequencing to elucidate how cell death is bypassed, and how these signalling pathways can eventually support survival. Interestingly, when we inhibit the formation of FUS-DDIT3 droplets we see that these specific cell death proteins rapidly decrease and cells die. This suggests that; **1. the FUS-DDIT3 droplet formation is required for the GOF activity of these proteins, and 2. without these GOF signals MLS cells rapidly die.** We are, to our knowledge, the first to show this and now focus on further elucidating the mechanisms at play. To identify new therapies in MLS we have performed a high-throughput drug screen and found **a novel drug combination that has substantial synergy at very low doses**. These two compounds directly target ribosome biogenesis, FUS-DDIT3 droplet formation and DNA repair and coincide with all our previous findings which is very exciting. We are now establishing mouse models of MLS to validate our most potent drug combinations identified in the drug screen. In conclusion, our findings open up new avenues for effectively treating MLS with reduced therapy-induced toxicity. More importantly, we propose that this treatment strategy might also be beneficial in other sarcomas (e.g. bone and soft tissue) that arise from fusion genes and have a high incidence.

Moderating Issues Please describe any challenges that you faced and/or that have impacted upon intended activity, progress and outcomes. Please explain your strategies for any aspects of the project that are incomplete. (Limit 300 words)	Covid-19 lockdowns greatly impacted the work output during the majority of 2021 making it challenging to achieve our goals in the previously set-up time frame. Fortunately, our hypotheses were mostly correct and we were able to achieve most of our planned work during 2022. We foresee that in the coming 6-12 months the project will be complemented with *in vivo* validation and thorough analysis of sequencing datasets. An additional postdoctoral researcher has been hired to quickly advance these experiments.

Publications and presentations Please list any publications and/or abstracts produced as a result of the project. Include manuscripts in preparation or in submission/under review.	A first manuscript is in preparation regarding a specific subset of compounds that induce FUS-DDIT3 droplets in MLS. We expect the main paper to be drafted by the end of this year when the *in vivo* data is finished.

Further studies and/or funding Please outline any further studies or funding which have arisen as a result of the project.	Our identified compounds will be taken to clinical trials in collaboration with The Royal Marsden's Sarcoma Unit (UK). Moreover, we are testing all other commonly prevalent fusion genes in sarcoma for similar droplet forming effects and efficacy of compounds.

Other Comments Please outline any other items of general interest which have arisen as a result of the project.	This project also shows how anti-cancerous pathways/proteins can become pro-cancerous by the presence or absence of membraneless organelles within the cell.

Signed Chief Investigator	Anne Steins
Date	13-2-2023