The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Functional characterisation of DNA damage response deficiency in cancer**

SUPERVISORY TEAM	
Primary Supervisor(s):	Anderson T Wang
Backup Supervisor:	Olivia W Rossanese
Lead contact person for the project:	Anderson T Wang

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics Unit
Primary Team:	Target biology and genomic instability

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

DNA damage response (DDR) is a cellular stress response to DNA damage and encompasses a multitude of DNA repair pathways and cell cycle checkpoints necessary to maintain genomic stability. A hallmark of cancer is genomic instability, which can arise as a consequence of mutations in the components of the DDR (Negrini et al., 2010). Recent analysis of TCGA PanCanAtlas has revealed that approximately one third of cancer types showed significant enrichment of somatic mutations in DDR genes (Knijnenburg et al., 2018). Genomic instability is therefore suggested to be a common vulnerability of cancer cells that can lead to dependency of remaining functional DDR pathways for survival (O'Connor, 2015). The synthetic lethality concept, whereby simultaneous abrogation of 2 redundant pathways conferring lethality, offers a therapeutic approach for targeting these cancer cells with DDR gene mutations. The recent approval of PARP inhibitors (PARPi) for the treatment of ovarian and breast cancer patients with mutation in *BRCA* genes demonstrates that the concept of synthetic lethality can be successfully exploited.

Although synthetic lethality offers a strategy for selective killing of tumour cells with genetic aberrations in DDR genes, the therapeutic efficacy will be dictated by the penetrance of the synthetic lethality (Ryan et al., 2018). Whether the DDR gene mutations identified in cancer cells have functional impact on the pathway, which will affect the synthetic lethal penetrance, remains largely uncharacterised. In addition, it is likely that during tumourigenesis and upon chemotherapeutic agent treatment, cancer cells have undergone adaptation by 'rewiring' DDR pathways, which can result in suppression of the reported synthetic lethal interaction (Ryan et al., 2018). A successful exploitation of synthetic lethality for targeting DDR pathways in cancer cells showing DDR deficiency requires a full understanding of the functional impact of the mutations and the potential adaptive mechanisms.

PROJECT AIMS

1. To characterise ATM functionality in cell line models with ATM mutations.
2. To complement ATM functionality in ATM loss of function (LoF) cell lines and examine synthetic lethality with inhibitors of DNA damage response.
3. Proteomics profiling of ATM LoF cell lines to identify novel biomarkers for ATM deficiency.
4. *To perform functional genomic and chemical screens to identify suppressors and novel partners of synthetic lethal interaction. (stretch goal)*

RESEARCH PROPOSAL

ATM is one of the three PI3K-related protein kinases that play an integral role in co-ordinating the DNA damage response (Blackford and Jackson, 2017). ATM functions as a master regulator of double strand break repair (Blackford and Jackson, 2017). *ATM* mutation is found to be prevalent in multiple cancer types and is associated with poor prognosis (Choi et al., 2016). Therefore, it represents a significant patient population of high unmet need. Synthetic lethal interactions have been reported for ATM deficiency whereby siRNA-mediated depletion or deletion of ATM sensitises cells to inhibitors against PARP, ATR and DNA-PK (Min et al., 2017, Balmus et al., 2019, Riabinska et al., 2013). ATM deficiency is currently being explored as a patient selection biomarker for these DDR inhibitors under clinical testing. Although there is high prevalence of ATM in multiple cancer types, whether the ATM pathway is functionally lost in these cancer cells remains to be determined. A full understanding of the functional impact of the mutations in ATM will be critical to define clinical actionable ATM deficiency in cancer.

The objective of the proposed research is to establish a platform for functional profiling of ATM mutations in cancer cells in order to identify clinically actionable synthetic lethal interactions and novel targets to suppress the adaptive rescue mechanisms.

Aim 1: To characterise ATM functionality in cell line models with ATM mutations.

To address the question of whether *ATM* mutations in cancer result in highly penetrant synthetic lethality that can be therapeutically targeted, we will first attempt to characterise whether these mutations have functional consequences on ATM pathway activation. Panels of cell lines carrying *ATM* mutation from specific cancer indications with high prevalence of *ATM* mutations, such as non-small cell lung, stomach and prostate cancers, will be created by surveying the COSMIC and CCLE database. We will next characterise the ATM pathway functionality in these cell lines on two levels.

1. Protein expression: we will use different antibodies to identify whether full-length or any potential truncated hypomorphic isoforms are being expressed.
2. ATM pathway activation: ATM pathway biomarkers (ATM pS1981, CHK2 pT68, KAP1 pS821, RAD50 pS635) in response to ionising radiation will be tested.

Results from these experiments will allow classification of ATM mutations as ATM proficient (in cell lines showing intact ATM protein expression and pathway activation) or as ATM pathway inactivated. This profiling will also contribute to the understanding of whether there is cell context dependency of specific *ATM* mutations manifesting in deleterious impact on pathway activation.

Aim 2: To complement ATM functionality in ATM loss of function (LoF) cell lines and examine synthetic lethality with inhibitors of DNA damage response.

For cell lines that show impaired ATM expression and/or pathway activation, we will complement ATM activity by either overexpressing wildtype ATM protein by lentiviral transduction or editing the ATM mutation back to wildtype sequence. Mutations in cell lines where ATM protein expression and pathway deficiency can be rescued by wildtype ATM protein expression will be classified as *ATM* loss-of-function (ATM LoF) mutations. We will then

examine the relative sensitivity of the isogenic cell lines with or without complemented ATM function to ionising radiation, DNA damaging chemotherapeutic agents and inhibitors of PARP, ATR and DNA-PK. The sensitivity profiling will identify the best treatment options for tumours with specific ATM mutations. In addition, the *ATM* LoF mutations that confer cellular sensitivity to DDR inhibitors will represent a more robust set of patient selection biomarkers for the clinical development of these agents.

Aim 3: Proteomics profiling of ATM LoF cell lines to identify novel biomarkers for ATM deficiency.

As described in Aim 1, characterisation of ATM functionality relies on induction of the pathway with exogenous DNA damaging agents. Significant practical challenges are associated with applying this approach to assess ATM functionality in fixed clinical samples. This highlights the need of an alternative approach to characterise ATM functionality without exogenous stimulation. Using cell lines characterised to be ATM LoF and the complemented counterpart generated from the above aims, we will perform reversed phase protein arrays profiling on these cell lines to uncover common biomarkers preferentially enriched with ATM LoF at baseline samples (Spurrier et al., 2008).

Aim 4 (stretch goal): To perform functional genomic and chemical screens to identify suppressors and novel partners of synthetic lethal interaction

We hypothesise that cell lines with ATM LoF mutations but no sensitivity to DDRi treatment have likely undergone adaptation. Using the isogenic cell line pairs, we will perform functional genomic and/or chemical screens in the presence of DDR inhibitors to identify suppressors of synthetic lethality. Hits identified from the screen will be functionally validated by genetic loss-of-function approaches, such as RNAi or CRISPR, and further mechanistic profiling will be conducted to understand the adaptation mechanisms through which these factors suppress synthetic lethality. These suppressors, once functionally validated, will be potential therapeutic targets that can lead to more effective combination therapies in cancers with ATM mutations.

Expected outcome

The results from the proposed research will provide molecular insight into the functional consequences of ATM gene mutations in cancer. Upon successful completion of the proposed research, it is anticipated that we will uncover the following:

1. Clinically actionable ATM mutations associated with pathway functionality defect in cancer.
2. ATM mutations that can be targeted with specific DDRi through synthetic lethality.
3. Novel protein biomarkers for identifying tumours with ATM deficiency.
4. Potential therapeutic targets that can lead to more effective therapies against tumours with ATM deficiency.

LITERATURE REFERENCES

- Balmus, G., Pilger, D., Coates, J., Demir, M., Sczaniecka-Clift, M., Barros, A. C., Woods, M., Fu, B., Yang, F., Chen, E., Ostermaier, M., Stankovic, T., Ponstingl, H., Herzog, M., Yusa, K., Martinez, F. M., Durant, S. T., Galanty, Y., Beli, P., Adams, D. J., Bradley, A., Metzakopian, E., Forment, J. V. & Jackson, S. P. 2019. ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. *Nat Commun,* 10**,** 87.
- Blackford, A. N. & Jackson, S. P. 2017. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Mol Cell,* 66**,** 801-817.
- Choi, M., Kipps, T. & Kurzrock, R. 2016. ATM Mutations in Cancer: Therapeutic Implications. *Molecular Cancer Therapeutics,* 15**,** 1781.

- Knijnenburg, T. A., Wang, L., Zimmermann, M. T., Chambwe, N., Gao, G. F., Cherniack, A. D., Fan, H., Shen, H., Way, G. P., Greene, C. S., Liu, Y., Akbani, R., Feng, B., Donehower, L. A., Miller, C., Shen, Y., Karimi, M., Chen, H., Kim, P., Jia, P., Shinbrot, E., Zhang, S., Liu, J., Hu, H., Bailey, M. H., Yau, C., Wolf, D., Zhao, Z., Weinstein, J. N., Li, L., Ding, L., Mills, G. B., Laird, P. W., Wheeler, D. A., Shmulevich, I., Monnat, R. J., Jr., Xiao, Y. & Wang, C. 2018. Genomic and Molecular Landscape of DNA Damage Repair Deficiency across The Cancer Genome Atlas. *Cell Rep,* 23**,** 239-254.e6.
- Min, A., Im, S.-A., Jang, H., Kim, S., Lee, M., Kim, D. K., Yang, Y., Kim, H.-J., Lee, K.-H., Kim, J. W., Kim, T.-Y., Oh, D.-Y., Brown, J., Lau, A., O'connor, M. J. & Bang, Y.-J. 2017. AZD6738, A Novel Oral Inhibitor of ATR, Induces Synthetic Lethality with ATM Deficiency in Gastric Cancer Cells. *Molecular Cancer Therapeutics,* 16**,** 566-577.
- Negrini, S., Gorgoulis, V. G. & Halazonetis, T. D. 2010. Genomic instability — an evolving hallmark of cancer. *Nature Reviews Molecular Cell Biology,* 11**,** 220.
- O'connor, Mark J. 2015. Targeting the DNA Damage Response in Cancer. *Molecular Cell,* 60**,** 547-560.
- Riabinska, A., Daheim, M., Herter-Sprie, G. S., Winkler, J., Fritz, C., Hallek, M., Thomas, R. K., Kreuzer, K. A., Frenzel, L. P., Monfared, P., Martins-Boucas, J., Chen, S. & Reinhardt, H. C. 2013. Therapeutic targeting of a robust non-oncogene addiction to PRKDC in ATM-defective tumors. *Sci Transl Med,* 5**,** 189ra78.
- Ryan, C. J., Bajrami, I. & Lord, C. J. 2018. Synthetic Lethality and Cancer - Penetrance as the Major Barrier. *Trends Cancer,* 4**,** 671-683.
- Spurrier, B., Ramalingam, S. & Nishizuka, S. 2008. Reverse-phase protein lysate microarrays for cell signaling analysis. *Nat Protoc,* 3**,** 1796-808.

CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc or equivalent in specific subject area(s)
Intended learning outcomes:	• A wide range of technical skills in molecular and cell biology • In-depth knowledge in cancer biology and DNA damage response pathways • Experience in functional genomics. • Familiarity with the principles and practice of modern drug discovery in oncology. • Ability to formulate testable scientific hypotheses and devise experiments to test the hypotheses • Good scientific communication and presentation skills, including clear scientific writing skills.
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science

	☐ Other (provide details)
Keywords:	**1. Cancer biology and therapeutics**
	2. DNA damage response (DDR) inhibitors
	3. Genomic instability
	4. Synthetic lethality
	5. Cancer gene mutations
	6.
FUNDING (only complete this section if this project already has full/partial funding)	
☒ This project is fully funded	*Full-funding details (this must include stipend, fees and consumables) including the source of funding:* ICR.
☐ This project is partly funded	*Part-funding details* (Please set out how much you will provide, from what source, and how much is additionally required; detailing consumables and stipend payments):