The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	The Institute of Cancer Research (ICR)
PROJECT DETAILS	
Studentship start date:	Ideally March 2022 – if the successful candidate is able. If not, October 2022
Project Title:	**Understanding myeloma cell – microenvironment interactions and their role in immunomodulatory drug resistance**
SUPERVISORY TEAM	
Primary Supervisor:	Dr Charlotte Pawlyn
Secondary Supervisor:	Dr Olivia Rossanese
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Myeloma Biology and Therapeutics

SHORT ABSTRACT

The bone marrow cancer multiple myeloma remains incurable despite advances in therapy. Immunomodulatory drugs (IMiDs) are the current backbone of standard and experimental combination therapies at all stages of disease. Resistance to IMiDs is likely to be multifactorial with both myeloma cell intrinsic and extrinsic factors playing a role. This project will examine the impact of the extrinsic stromal cell compartment of the bone marrow microenvironment on resistance generation.

BACKGROUND TO THE PROJECT

The bone marrow cancer multiple myeloma remains incurable despite advances in therapy. Immunomodulatory drugs (IMiDs) are the current backbone of standard and experimental combination therapies at all stages of disease. Understanding IMiD resistant and refractory states is therefore imperative to help us improve patient outcomes.

The mechanism of action of the IMiDs in myeloma has been partly elucidated(Ito et al., 2010, Lopez-Girona et al., 2012, Kronke et al., 2014). On IMiD binding, C2H2 domain containing zinc finger transcription factors including Ikaros (IKZF1) and Aiolos (IKZF3), become neosubstrates of cereblon (CRBN) and are degraded. Ikaros and Aiolos degradation results in the subsequent downregulation of target genes, including interferon regulatory factor 4 (IRF4) and c-Myc which are transcription factors regulating stages of B cell development.

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Resistance to IMiDs is likely to be multifactorial with both myeloma cell intrinsic and extrinsic factors playing a role. Myeloma cell intrinsic mechanisms such as mutations and deletion of CRBN have been described and other factors such as epigenetic modifications are thought to play a role (Gooding et al., 2021, Jones et al., 2021, Gandhi et al., 2014). We have generated *in vitro* cell line tools that mimic both CRBN mutated and unmutated resistant states. In patients, however, myeloma cells exist in a complex ecosystem in the myeloma bone marrow microenvironment. Previous studies suggest that the interaction with the bone marrow stroma/fibroblasts may also contribute to therapy resistance by direct and indirect communication leading to re-wiring of signalling pathways, but the protein level changes that are responsible for this are not well understood. Whether resistance results from soluble factor transmission, exchange or direct cell-cell contact is also unclear.

This project aims to study these myeloma cell - stroma interactions and how they contribute to IMiD resistance.

PROJECT AIMS

1. **Analyse proteomic, transcriptomic and epigenetic changes induced in myeloma cells by direct and indirect co-culture with bone marrow stroma.**
2. **Assess the impact of co-culture on IMiD induced neosubstrate degradation and myeloma cell viability responses to IMiDs.**
3. **Explore findings from Aims 1 and 2 in patient derived stroma and myeloma cell models.**

RESEARCH PROPOSAL

Depending on the interests and qualifications of the candidate as well as progress within the group prior to the start date, the scope and aims of the PhD will be tailored accordingly.

Aim 1:

To understand the changes induced by interaction between myeloma cells and bone marrow stroma, myeloma cell lines will be cultured in direct and indirect co-culture with the bone marrow stromal cell line HS5. These are techniques that have already been established in the lab. The stromal cell line HS5 has stable GFP expression to enable flow sorting of the direct co-culture. Indirect co-culture will be achieved using trans-well inserts in a plate format. Different cell lines from the various molecular subgroups of myeloma will be used so the changes with co-culture can be compared between subgroups. Proteomic, transcriptomic and epigenetic techniques will be used to identify changes induced in the co-culture systems. These will then be validated using a wider panel of cell lines and differences between subgroups examined.

Aim 2:

To examine the effect of co-culture on IMiD induced neo-substrate degradation and viability response, IMiD sensitive and resistant cell lines will be co-cultured (direct and indirect) with bone marrow stroma as before but now with vehicle control (DMSO) or a range of IMiDs including lenalidomide, pomalidomide and iberdomide. Differences in neo-substrate degradation will be examined using Western blotting and downstream effects using qRT-PCR.

Aim 3:

Pathways or individual genes/proteins that have been prioritised after identification in Aim 1 and 2 will be validated using cell line models and then explored in patient derived stroma and myeloma cell models. Isolation of CD138 positive myeloma cells from patient bone marrow aspirates and the culture of adherent patient stroma enables the

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study of both compartments of the myeloma bone marrow microenvironment to achieve this. Potential targets for combinatorial therapeutic strategies will be explored with available inhibitors or other approaches.

The project will be conducted in a lab led by a physician-scientist exposing the candidate to translational cancer research from the bedside problem of IMiD drug resistance to the laboratory to interrogate mechanisms which can then be translated back into the clinic. The project will also include multidisciplinary working with other teams specialising in proteomics, bioinformatics and drug discovery.

LITERATURE REFERENCES

GANDHI, A. K., MENDY, D., WALDMAN, M., CHEN, G., RYCHAK, E., MILLER, K., GAIDAROVA, S., REN, Y., WANG, M., BREIDER, M., CARMEL, G., MAHMOUDI, A., JACKSON, P., ABBASIAN, M., CATHERS, B. E., SCHAFER, P. H., DANIEL, T. O., LOPEZ-GIRONA, A., THAKURTA, A. & CHOPRA, R. 2014. Measuring cereblon as a biomarker of response or resistance to lenalidomide and pomalidomide requires use of standardized reagents and understanding of gene complexity. *Br J Haematol,* 164**,** 233-244.

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ITO, T., ANDO, H., SUZUKI, T., OGURA, T., HOTTA, K., IMAMURA, Y., YAMAGUCHI, Y. & HANDA, H. 2010. Identification of a primary target of thalidomide teratogenicity. *Science,* 327**,** 1345-50.

JONES, J. R., BARBER, A., LE BIHAN, Y. V., WEINHOLD, N., ASHBY, C., WALKER, B. A., WARDELL, C. P., WANG, H., KAISER, M. F., JACKSON, G. H., DAVIES, F. E., CHOPRA, R., MORGAN, G. J. & PAWLYN, C. 2021. Mutations in CRBN and other cereblon pathway genes are infrequently associated with acquired resistance to immunomodulatory drugs. *Leukemia*.

KRONKE, J., HURST, S. N. & EBERT, B. L. 2014. Lenalidomide induces degradation of IKZF1 and IKZF3. *Oncoimmunology,* 3**,** e941742.

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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:	Candidates must have a first class or upper second class honours BSc Honours/MSc in Biological Sciences.
Intended learning outcomes:	• Training in translational research in cancer medicine including discovering and validating markers of drug resistance • Experience and knowledge specific to myeloma / blood cancer research • Training in laboratory techniques, including cell culture, cell viability assays, antibody-based protein detection techniques, mass spectrometry and flow cytometry • Broad training in basic bioinformatic approaches

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. Mechanisms of drug resistance**
	2. Microenvironment interactions
	3. Proteomics
	4. Blood cancer