The Institute of Cancer Research
PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	The Institute of Cancer Research *(Professor Helin's lab start-up funding)*
PROJECT DETAILS	
Project title:	Generation of mouse models to identify and characterize novel therapeutic targets in chemo refractory AML disease
SUPERVISORY TEAM	
Primary Supervisor	Professor Kristian Helin
Associate Supervisor(s)	Dr. Robin Armstrong Dr. Rachael Natrajan
Secondary Supervisor	Professor Janet Shipley
DIVISIONAL AFFILIATION	
Primary Division	Cancer Biology
Primary Team	Epigenetics and Cancer
Site	Chelsea

BACKGROUND TO THE PROJECT

Acute Myeloid Leukaemia (AML) is the most frequent form of acute leukaemia in adults and has a 5-year survival rate ranging between 5 and 28,7% (DiNardo et al., 2016). AML is a disease of the hematopoietic stem and progenitor cells (HSPCs), which are the stem cells that give rise to other blood cells by their capacity to self-renew and differentiate (Dohner et al., 2015). In AML, mutations enhance self-renewal and promote a differentiation arrest, which leads to a myeloid lineage restriction and accumulation of immature blasts within the bone marrow and peripheral blood (Dohner et al., 2015, Khwaja et al., 2016, Yamashita et al., 2020).

Clinical outcomes in AML are associated with leukaemia-specific genetic features (Coombs et al., 2016, Morita et al., 2020). AML with inv(3)/q21q26.2)/t(3:3)/(q21q26.2) is a highly therapy refractory AML that is linked with an increased expression of the proto-oncogene EVI-1 (Gröschel et al., 2014). Whole exome sequencing efforts in inv(3)/t(3:3) AML patients have revealed several co-occurring mutations. The most frequent alterations are activating RAS mutations, of which NRAS is the most frequently mutated RAS gene, and -7/del(7q) (Gröschel et al., 2015). We hypothesize that in inv(3)/t(3:3) AML, EVI-1 over expression cooperates with activating RAS mutations and 7/del(7q) to drive pathogenesis of the disease. The aim of this project is to generate a mouse model that combines these alterations, in order to understand the mechanisms leading to AML and chemoresistance, and to identify potential new targets to treat chemo refractory AML.

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PROJECT AIMS

- Generation of a mouse model of chemo refractory AML subtype, inv(3)/t(3:3);
- Characterizing the mouse model;
- Elucidating the role of -7/del(7q) and EVI-1 overexpression in inv(3) AML pathogenesis; *and*
- Comparison of molecular targets between the inv(3)/t(3:3) and other CK-AML models

RESEARCH PROPOSAL

The first aim of the project will be the generation of the inv(3)/t(3:3) model. In mouse models of complex karyotype AML (CK-AML), -7/del(7q) can be mimicked by suppression of the tumour suppressor Mll3. Therefore, we hypothesize that 6/del(7q) in inv(3)/t(3:3) can be similarly mimicked (Chen et al., 2019). To establish the inv(3)/t(3:3) model, EVI-1 overexpression will be combined with an activating NRAS mutation and Mll3 suppression. To generate the model, c-KIT+ bone marrow derived cells from four cohorts of mice (WT, Mll3flox/+; Rosa26CreER/+, NRASLSL-G12D/+; Rosa26CreER/+ and Mll3flox/+; NRASLSL-G12D/+; Rosa26CreER/+) will be transduced with retrovirus expressing GFP tagged EVI-1.Transduced cells will then be FACS sorted and transplanted into lethally irradiated mice. To induce Mll3 disruption and NrasG12D activation, donor mice will be treated with tamoxifen to induce Cre-mediated recombination of the respective alleles. To determine whether the recipient mice develop leukaemia, complete blood counts, peripheral blood smears and survival analyses will be performed. Immunophenotyping on cells from the spleen, bone marrow, peripheral blood, thymus and lymph nodes of the generated transplants will be performed to elucidate the nature of the disease. We hypothesize that the disruption of Mll3 will alter the access to EVI-1 specific binding sites, as well as synergize with aberrant regulation of myeloid transcription factors that result from increased EVI-1 expression. ATAC-seq will be used to study the chromatin accessibility changes in HSPCs from pre-leukemic and leukemic stage mice from the transplants of the generated mice. To identify genes specifically regulated by EVI-1, CUT&RUN profiles (gene expression and DNA binding sites) will be generated. We expect that the inv(3)/t(3:3) will closely mimic the human disease, with features such as poor response to therapy and a gene expression signature consistent with atypical megakaryopoiesis.

The second aim of the project will be the identification of potential therapeutic targets for the treatment of refractory AML. Previously, a loss of function genetic screen was performed in our lab in an established chemo refractory CK-AML model (unpublished results). A similar screen will be performed in the inv(3)/t(3:3) model. For this ex vivo conditions for the maintenance of inv(3)/t(3:3) will be established and Cas9 expressing variants will be generated. The established cells will be transduced with the whole-genome gRNA library and a screen will be performed. Common and disease specific hits between both AML models will be tested for their therapeutic potential. By creating this new AML model, we expect to identify hits required for the maintenance of leukemic cells within the respective disease subtypes.

LITERATURE REFERENCES

Chen, T. J. K., Gilabert-Oriol, R., Bally, B. M., et al. Recent Treatment Advances and the Role of Nanotechnology, Combination Products, and Immunotherapy in Changing the Therapeutic Landscape of Acute Myeloid Leukemia. Pharm Res. 36 (9), 125 (2019)

Coombs, C. C., Tallman, S. M., Levine, L. R. Molecular therapy for acute myeloid leukaemia. Nat Rev Clin Oncol. 13 (5), 305 – 318 (2016).

DiNardo, D. C., Cortes, E. J. Mutations in AML: prognostic and therapeutic implications. Hematology Am Soc Hematol Educ Program 1, 348 – 355 (2016).

Döhner, H., Weisdorf, J. D., Bloomfield, D. C. Acute Myeloid Leukemia. N Eng J Med 373 (12), 1136 – 1152 (2015).

Gröschel, S., Sanders, A. M., Hoogenboezem, R., et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell 157 (2), 369 – 381 (2014).

Gröschel, S., Sanders, A. M., Hoogenboezem, R., et al. Mutational spectrum of myeloid malignancies with inv(3)/t(3:3) reveals a predominant involvement of RAS/RTK signaling pathways. Blood 125 (1), 133 – 139 (2015).

Khwaja, A., Bjorkholm, M., Gale, E. R., et al. Acute Myeloid Leukaemia. Nat Rev Dis Primers 2, 16011 (2016).

Morita, K., Wang, F., Jahn, K., et al., Clonal evolution of acute myeloid revealed by high-throughput single-cell genomics. Nat Commun. 11 (1), 5327 (2020).

Yamashita, M., Dellorusso, V. P., Olson, C. O., Passegué, E. Dysregulated haematopoietic stem cell behavior in myeloid leukaemogenesis. Nat Rev Cancer 20, 365 – 382 (2020).

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:	Master's degree in a relevant subject.
Intended learning outcomes:	• Generating and analysing data; • Experience in working with mice; • Gain presentation skills; *and* • Gain scientific writing skills.
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology