Structured Reports for the Jean Shanks/Pathological Society Grant Awards: Predoctoral Research Bursary, Clinical PhD Fellowship, Clinical Lecturer Support Grant.

Title: Therapeutic Targeting of Aneuploidy in Classical Hodgkin Lymphoma
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Background and aims:
A subset of patients with classical Hodgkin lymphoma (cHL) is characterised by aneuploidy associated with defects in the spindle assembly checkpoint (SAC). We have shown that SAC defects result from inactivating mutations or transcriptional downregulation mediated by the Epstein Barr virus. Transcriptional downregulation of SAC genes is also associated with shorter event-free survival and overall survival in cHL patients. SAC defects could lead to resistance to mitotic spindle poisons used to treat cHL patients rendering aneuploidy a novel therapeutic target.

Aims of the project: 1) Use our new in vitro model to identify novel therapeutic targets in aneuploid B cells, and: 2) Explore expression of these aneuploidy-associated targets in primary cHL

Results:
Establishing a model of aneuploidy in B-cells
B-cell maintenance and induction of aneuploidy
To establish a novel model of aneuploidy in B-cells, CD19+ B-cells were extracted from healthy donor peripheral blood using MACS Dynabeads. Extracted B-cells were maintained in culture with the IL4 cytokine and CD40L stimulation for seven days prior to the experiments. Following several days of stimulation with cytokines, the B-cells displayed blast-like morphology and formed large aggregates in suspension.

B-cell viability following MPSi/CENPEi
Following 7 days of maintenance with IL4 and CD40L, the B-cells were treated with MPS1 and CENPE inhibitors at time 0. Cells were harvested for viability assays at 24 and 72 hours. To assess the effects of MPS1/CENPE inhibition on cell viability, an Annexin/PI viability flow cytometry assay was performed. Annexin/PI staining at 24 and 72 hours showed an increasing proportion of cells transitioning from apoptosis to dead but the proportion of viable cells increased. There was no marked difference in cell viability between the MPS1/CENPE inhibitor treated and untreated suspensions.

Induction of aneuploidy
Metaphase spreads of untreated and treated suspensions were harvested at 24 and 72 hours incubation. One hundred metaphase spreads were counted for each suspension. No aneuploidy was seen in the untreated suspension. In contrast, induction of aneuploidy was seen in 2% of cells treated with MPS1 and CENPE inhibitors. Aneuploid cells showed duplications or near-duplications of the entire chromosome (~4n).

Single cell RNA sequencing
Single cell sequencing of aneuploid B-cells
Single cell sequencing was performed on MPS1/CENPE inhibitor treated suspensions of B-cells using the 10X platform (Figure-1). Aneuploid cells were inferred bioinformatically using our novel aneuploidy caller and existing published algorithms.

Comparison of genes upregulated in aneuploid B-cells and cHL
The gene expression profiles for aneuploid cells were compared to euploid cells from the treated and untreated suspensions. Based on gene ontology, genes involved with antigen presentation, interferon-γ signalling and protein stress were upregulated in aneuploidy cells (Figure-4). The genes differentially expressed in aneuploid cells were compared with a published dataset that compared HRS cells to CD30+ extrafollicular B-cells. Thirty seven genes upregulated in aneuploid B-cells induced by MPS1 and CENPE inhibitors accounted for 16.2% of the transcriptional profile of HRS cells.

Conclusions:
My findings demonstrate the utility of our novel aneuploidy model in B-cells and the feasibility of single cell RNA sequencing on these samples. The genes differentially expressed in aneuploidy...
cells accounted for a significant proportion of the transcriptional profile described in primary classical Hodgkin lymphoma. Key groups of upregulated genes in aneuploidy cells based on gene ontology included those associated with antigen presentation, interferon-γ signalling and proteotoxic stress. Interferon-γ signalling is known to play a key role in recruitment of inflammatory cells in cHL. These inflammatory cells play key role in the cHL infiltrate, ensuring the survival of the HRS cells, and paradoxically, aid in immune system evasion by expression via immune checkpoints. The increased expression of genes involved with proteotoxic stress is in keeping with that of the published literature on aneuploidy. Hyperploid cells with excess chromatin translate and excess of protein which can be toxic to the cells. This in turn induces a stress response. The increased expression of antigen presentation associated genes and proteotoxic genes, warrant the exploration of therapeutic strategies targeting these pathways. Two approaches can be envisioned. First, aneuploid cells that emerge following inactivation of the SAC can be recognised and eliminated by the immune system. Thus, hyperploid malignant cells over-express ligands for NKG2D and DNAM1 which stimulate NK cell cytotoxicity. Moreover, the bursary helped lay the groundwork for protein folding, activation and assembly. Aneuploid cells can also be recognised by adaptive immune cells. Thus, tumours generated from hyperploid cells are increased in mice depleted of CD4+ or CD8+ T lymphocytes. Hyperploid cancer cells also show increased levels of ER stress, resulting in over-expression of calreticulin, an ER chaperone required for rejection of hyperploid tumours by adaptive immunity. Second, aneuploid cells are characterised by energy and proteotoxic stress that increases their susceptibility to apoptosis. As a result aneuploid cells are more sensitive to specific small molecule compounds, such as AICAR, which allosterically activates AMP-activated protein kinase (AMPK) thereby mimicking energy stress, and 17-AAG, which inhibits Hsp90, a chaperone required for protein folding, activation and assembly. Such compounds could be employed to induce lethal levels of cell stress. Such approaches have been successful in colorectal cancer and lung cancer cell lines. Further in-vitro experiments are required to test these therapeutic approaches on cHL cell lines.

How Closely Have the Original Aims been Met:
The original aims have been met although comparison of aneuploidy gene expression profiles with primary cHL gene expression was done bioinformatically instead of experimentally.

Outputs (including meeting abstracts, oral presentations, original papers, review articles, further grants) from the study in which the Jean Shanks/Pathological Society has been acknowledged:
We are currently writing an original research article on the role of aneuploidy in classical Hodgkin lymphoma. The work funded by the Jean Shanks/Pathsoc bursary will form a significant component of this paper. Furthermore, the bursary helped lay the foundation for further funding applications, and I was successful in obtaining and MRC clinical research foundation.

References
Figure 1. Experimental workflow of a novel aneuploidy model. Aneuploidy is induced in CD19+ B-cells by MPS1 and CENPE inhibition. Following a period of incubation, ssRNAseq is performed on the Chromium 10X platform and aneuploidy is inferred from expression data.

Figure 2. Genes differentially expressed grouped by gene ontology in euploid vs aneuploidy cells at a single cell resolution.

Figure 3. Genes differentially expressed in aneuploidy vs euploid compared to genes differentially expressed in HRS vs CD30+ extra follicular B-cells show an overlap of 16.2%.