

2023 Newcastle University Vacation Research Scholarship,
George Brown Endowment, George Henderson Endowment and
Florence Kirkby Endowment.

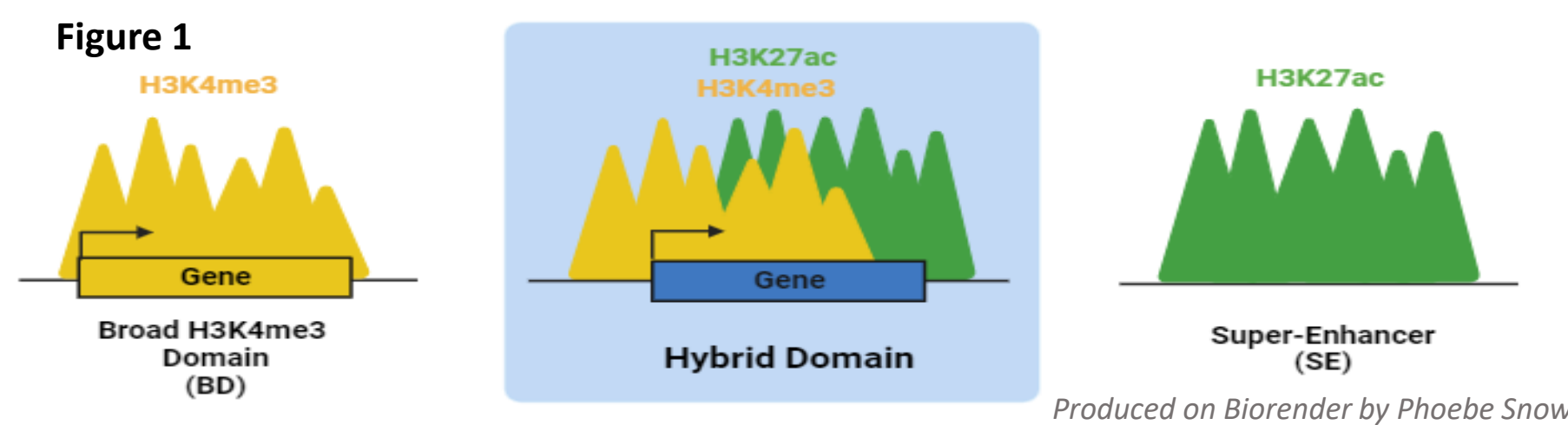
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Supervised by Dr Lisa Russell, with thanks to Dr Letizia Marchetti and Phoebe Snow

Background

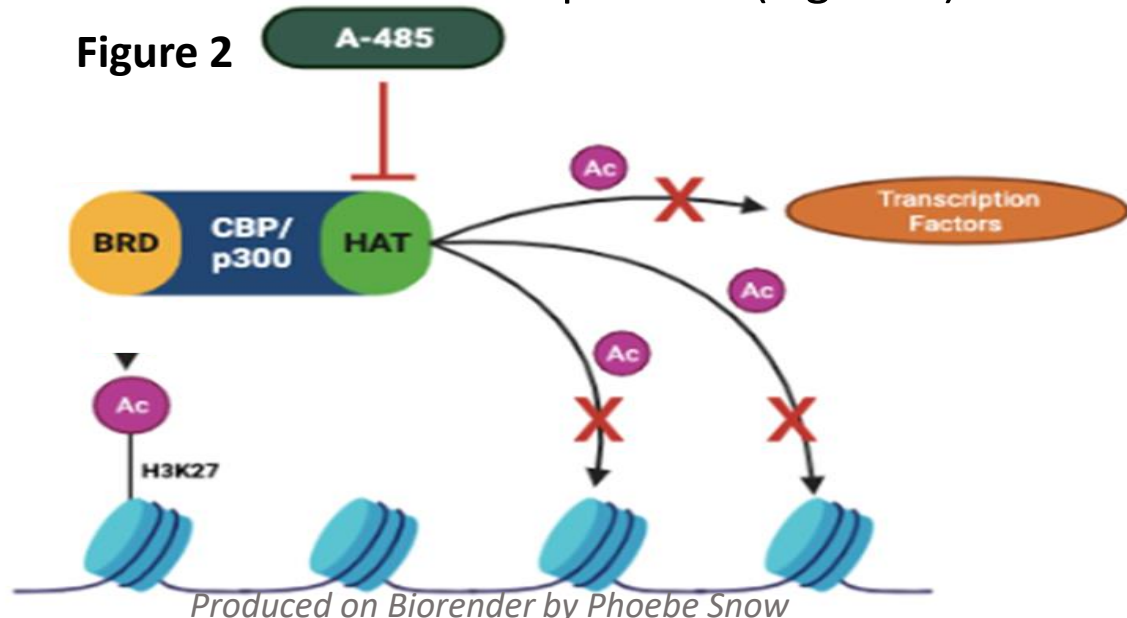
- Acute lymphoblastic leukaemia (ALL) is a cancer of lymphoid cells in the blood and bone marrow.
- ALL is treated with chemotherapy and efforts are being made to increase our understanding of the genetics fuelling the disease.
- The epigenetic landscape forms a layer (marks) over the genetic code, altering the level of activity of certain genes.
- 'Marks' on this landscape, are linked to the activity of a gene – H3K4me3 denotes promoter activity. When these marks are stretched, they form a 'broad domain', H3K27ac denotes a 'super enhancer'.
- These two marks can overlap – to form a hybrid domain (Figure 1).

Figure 1



- Super enhancers are known to be hijacked in cancer cells and can fuel the progression of disease.
- The drug A-485 acts to inhibit the enzyme EP300, which has a region that deposits H3K27ac onto histone proteins (Figure 2).

Figure 2



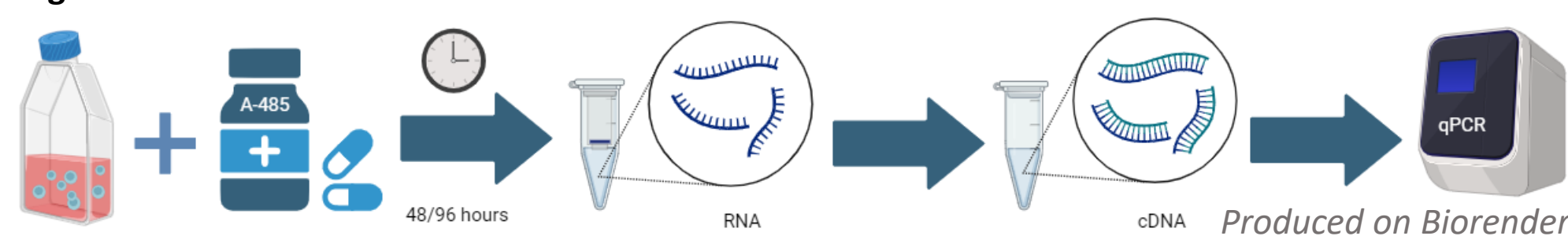
Aims

Assess and characterise the effect A-485 has on genes depending on their epigenetic landscape

Methods

- 4 flasks of GM12878 cells (healthy lymphoblasts) were set up in sterile conditions – a negative control and increasing concentrations of A-485. these flasks were left for 48/96 hours.
- Cell viability was measured, and RNA extracted.
- RNA was converted to cDNA, and gene quantities analysed by qPCR.

Figure 3



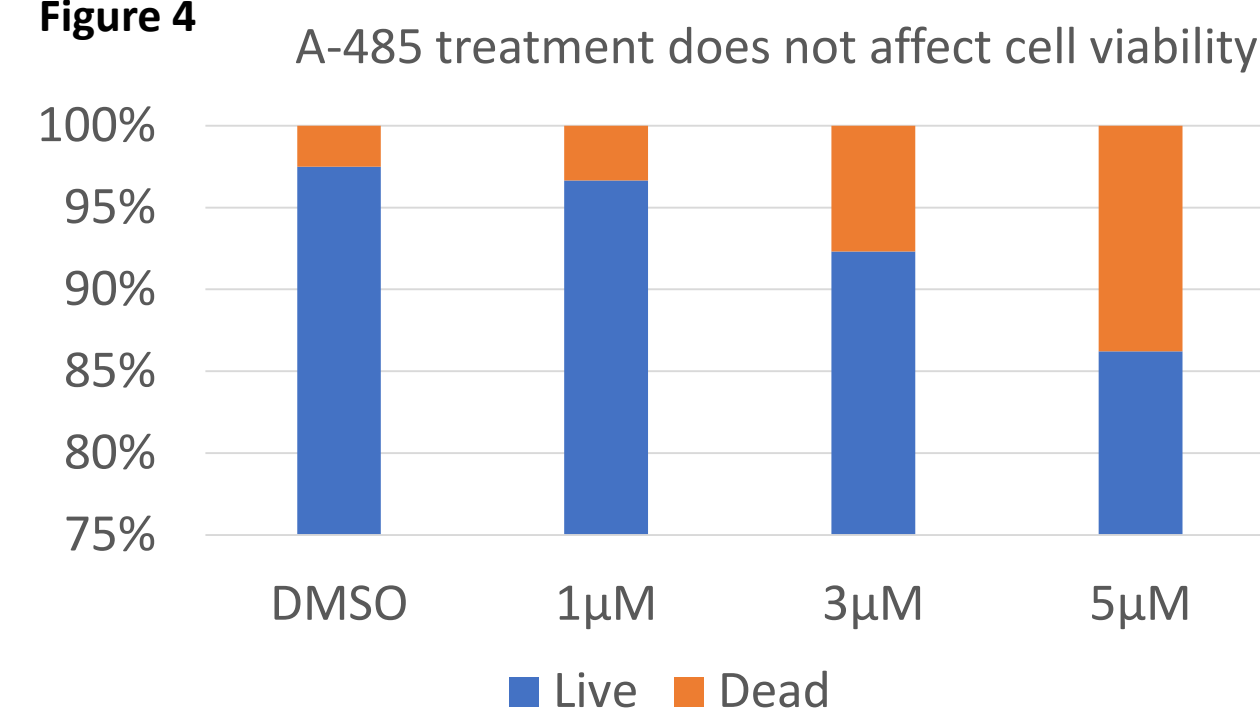
- The entire experiment was performed three times – to produce a set of biological triplicates and analysed by the 2-way-Anova test.

1.NHS Acute ;lymphoblastic leukaemia. <https://www.nhs.uk/conditions/acute-lymphoblastic-leukaemia/>

2.. Transcriptional regulation by Fos and Jun in vitro: interaction among multiple activator and regulatory domains. Mol Cell Biol. 1991 Jul; 11(7): 3624–3632

Results

Figure 4



Comparison of the percentage of live cells and the percentage of dead cells after drug treatment.

Figure 5

Gene without hybrids, broad domains or enhancers. Significant results are indicated by bars with stars. Significance was calculated using the 2-way-Anova test.

'Normal' genes show no change or increase in response to A-485

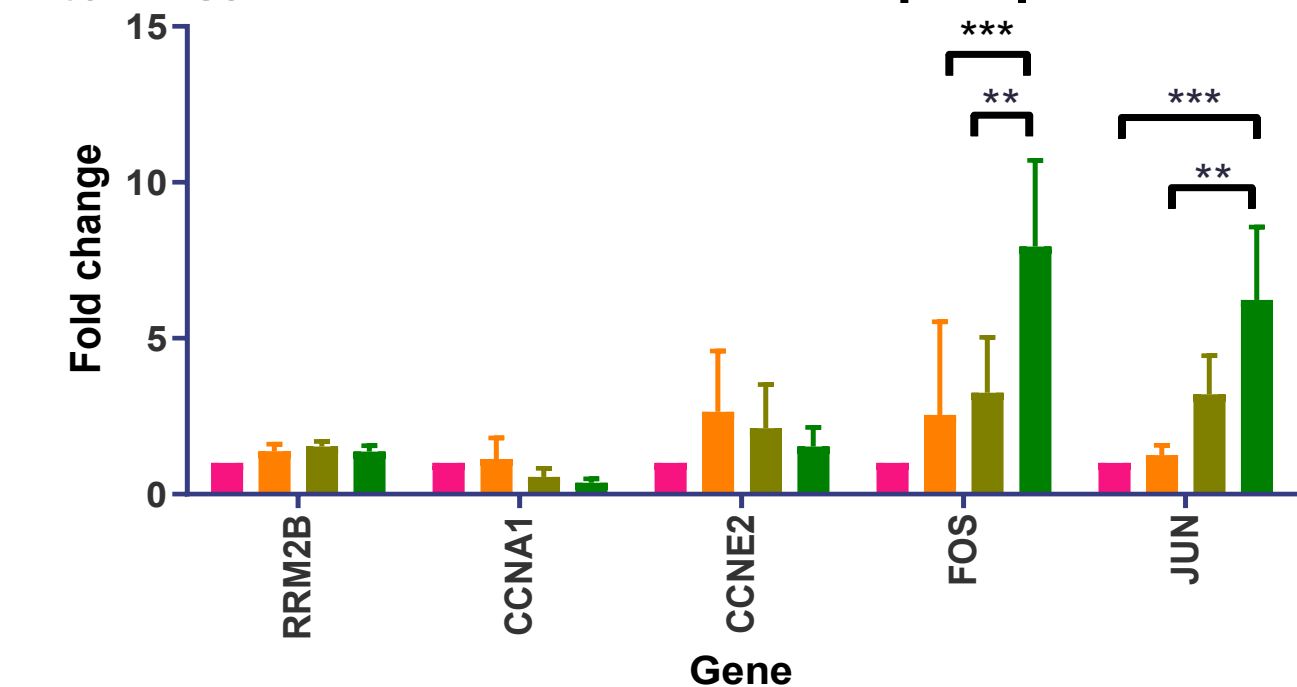
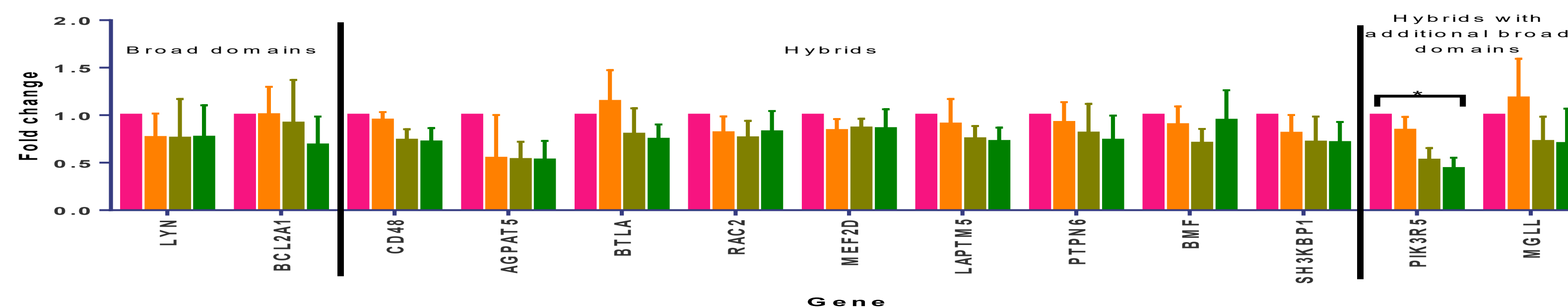


Figure 6

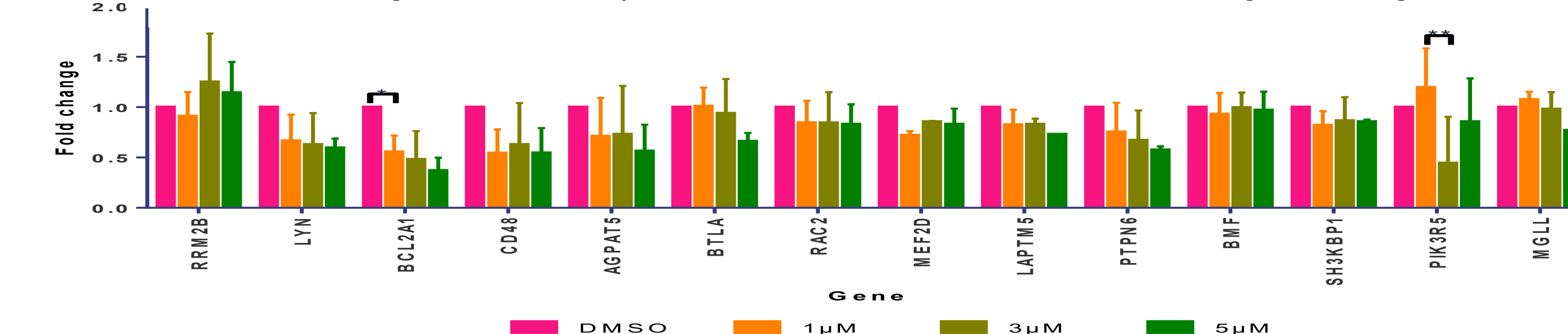
Some genes decrease in response to 48 hours of A-485 treatment. Others show not significant changes



Gene changes in response to 48 hours of A-485 treatment.

Figure 7

Some genes decrease in response to 96 hours of A-485 treatment. Others show not significant changes



Gene changes in response to 96 hours of A-485 treatment.

Discussion

- The results from qPCR analysis showed that some genes, such as PIK3R5 were significantly downregulated after 48/96 hours A-485 treatment, however there was no trend between the 'normal', broad domain only or hybrid groups. Neither was there significant changes in gene expression between 48 and 96 hours of drug treatment.
- The two genes that showed a significant increase in expression due to A-485 treatment are response genes that increase expression due to a range of cellular stimuli². The increase of these genes – JUN and FOS – show in this context, that there is indeed something occurring in the cells due to A-485 exposure.
- Promoter capture high seq data was used to profile the interactions each gene had with other areas of the genome. Preliminary data suggests that the genes that showed significant downregulation did not interaction with other broad domains and/or hybrid domains around the genome.
- Unfortunately, there was wide variation between the fold change of genes between the three biological replicate experiments, limiting the extent analysis can be performed.

Next Steps

- RNA from some of the samples have been sent off for RNA sequencing.
- Other members of the group will complete the analysis of A-485 treated Z-138 cells

Summary

Genes with different epigenetic profiles respond differently to EP300 inhibition. There is strong potential to develop this study further in the hope of improving treatment of ALL.