BRITISH LUNG FOUNDATION

Final Report

Please return to Ian.Jarrold@blf.org.uk

1. Grant-holder details

Grant Holder(s) and positions held	Professor Dean A Fennell Chair and Consultant in Thoracic Medical Oncology	**Grant no**	MESOUK15-11
Research worker(s)	Dr Sara Busacca PhD		
Place at which research is carried out	Leicester cancer Research Centre, University of Leicester		

Contact details of the Host Institution's press or publicity officer			
Name			
Address	Enkalon House, Regent Road, LE1 7DD, Leicester		
Tel. no.	**+44 (0) 116 252 5761**	**E-mail**	**pressoffice@le.ac.uk**

2. Grant details

Title of Research	Synthetic lethal targeting of the CDK4/6 pathway in CDKN2A mutated mesothelioma: A proof of concept Study to support a phase IIA clinical trial

3. Report in lay language

This is particularly important as portions will be used to inform members of the public and our supporters about the research we fund and its achievements. It will also be used by lay members of the British Lung Foundation

Research Committee and Board of Trustees to help assess the outcomes of the grant. Please answer the following questions in language and style that will be understandable to people who have a limited understanding of science. Considerable attention should be given to this section. It is recommended that you show your report to non-scientists, local carers and/or people with lung disease for their comments. The British Lung Foundation reserves the right to request a redraft of this section if it is not deemed acceptable.

3a. Lay title
A new approach for personalising therapy for mesothelioma patients using a targeted, drug-based strategy

3b. Lung diseases relevant to the research
Malignant pleural mesothelioma (cancer)

3c. Summary – please write a 'lay abstract' of your work in language that a non-scientist can understand.

Focus on:

- **Questions being addressed by the work and why the work was needed**
- **Describe how the work was carried out**
- **Describe the results found**
- **Describe how the results might help people with lung disease in the short/longer term**

This section may be used as the basis of communications to the general public and MUST be understandable to a non-scientist. Please minimise the use of technical terms and explain those used.

500 words maximum

This section is very important for our funders! Please give details in lay language

BACKGROUND

Malignant pleural mesothelioma is a lethal cancer of the lining tissue that covers the lungs (pleura), and it is caused by exposure to asbestos. The UK has the highest rate of mesothelioma in the world and the number of cases increases each year, here in the UK and worldwide. Some patients may respond to initial chemotherapy, but then the cancer will grow back. At present, there is no approved therapy to offer to these patients. There is clearly a need for new, effective treatments in order to improve survival outcomes. CDKN2A/MTAP is commonly co-deleted in mesothelioma. Approaches to target this subtype include inhibition of i. CDK4/6 (which was accelerated and is currently being explored in a BLF funded phase II trial, MiST arm 2), and ii. PRMT5, which has been shown to be a dependency in MTAP negative mesothelioma.

AIM

The goal of this project is to explore whether a common subtype of mesothelioma, that harbours a specific mutation in the gene known as CDKN2A/MTAP, will respond to a new type of therapy – blocking another protein called PRMT5

HYPOTHESIS

It is predicted that loss of CDKN2A/MTAP will be associated with response inhibition of PRMT5

SPECIFIC OBJECTIVES OF THE RESEARCH

The research objectives of this project are

1) To determine the effect of MTAP expression on survival
2) To determine the role of MTAP in regulating response to PRMT5 inhibition
3) To identify new strategies to target PRMT5

RESULTS

Genetic analysis showed that CDKN2A/MTAP deletion is associated with shorter survival and more aggressive mesothelioma compared to MTAP positive patients

Using the connectivity map, we identified that an old (off patent) drug known as Quinacrine, is a novel inhibitor of PRMT5, capable of transcriptionally suppressing PRMT5 in common with siRNA.

Quinacrine phenocopies siRNA silencing of PRMT5 leading to an arrest of growth in MTAP negative mesothelioma cells but not in MTAP positive cells, suggesting a targeted effect.

IMPLICATIONS
We have identified quinacrine as a novel agents with potential to target PRMT5 expression in MTAP negative mesothelioma. This suggests a repurposing therapeutic potential either alone or in combination with CDK4/6 or small molecule PRMT5 inhibitors. Quinacrine is a well tolerated antimicrobial that may have a role as a novel anti-cancer agent in a specific somatic mutational context.

3d. Background / reasons for the research

Malignant Pleural Mesothelioma (MPM) is an incurable cancer of the lining tissue that covers the lungs (pleura). Treatment options are limited and currently there is only one therapy that has been proven to give a survival benefit, platinum/pemetrexed chemotherapy. There is no standard care in patients with mesothelioma, particularly in the relapse setting. Personalised therapy is lacking but has been cited by the James Lind alliance as a top research priority.

Deletion of chromosome 9p21.2 involving the genes CDKN2A and methylthioadenosine phosphorylase (MTAP) occurs frequently in mesothelioma (> 50%). Strategies to target this subset of mesotheliomas could have a significant impact on the population.

MTAP deletion leads to a build up of the metabolite methylthioadenosine, which binds to and inhibits protein arginine methyltransferase 5 (PRMT5). This reduces the functional pool of this epigenetic regulator, leading to a reduction in histone H4 arginine demethylation, and altered gene expression. PRMT5 is therefore a vulnerability/molecular target in MTAP negative mesothelioma, and further inhibition cannot be tolerated as PRMT5 is an essential protein. We therefore proposed that PRMT5 would be a target for suppressing mesotheliomas that harbour MTAP and sought to identify novel, translationally relevant approaches to target this protein.

3e. What problem/need/question does this research address?

Personalised therapy for mesothelioma is lacking and there is no licenced treatment in the relapsed setting.

This work addresses the development of personalized therapy for the common CDKN2A/MTAP deleted subset of mesotheliomas. The goal of this research is to establish proof of concept data to support translational development of CDKN2A/MTAP inhibition.

3f. Can you summarise the need for your research in one sentence?

Defining a new therapeutic strategy for treating CDKN2A/MTAP negative mesothelioma, in the relapsed setting

3g. How was the research carried out?

Please provide details of methodology in lay language

I. To determine how MTAP expression impacts clinical outcome we have carried out genetic analysis on DNA from 79 patients with malignant pleural mesothelioma who had complete macroscopic surgical resection. We have then divided patients into two groups: “MTAP positive” and “MTAP negative” and compared their time-to progression and overall survival.

II. We then silenced PRMT5 from the cells using RNA interference to test if loss of PRMT5 would lead to an arrest of growth of mesothelioma cells.

III. By using a computational approach we have identified an old drug that can decrease the amount of PRMT5 that is transcribed (a so-called perturbagen), and tested to see if this phenocopied PRMT5 siRNA.

IV. To understand the transcriptional silencing mechanism of the perturbagen we analysed its effect on different “transcription factors” which are proteins that regulate the production of other proteins.

3h. Please summarise the findings of your research project

This section is very important for our funders! Please give comprehensive details in lay language.

- MTAP confers worse outcomes for patients with mesothelioma. Genetic analysis showed that MTAP negative patients had a shorter survival compared to MTAP positive patients (336 vs 430 days respectively). There was also a significantly shorter time to disease progression following surgical resection, suggesting that CDKN2A/MTAP may be a useful biomarker for identifying patients unlikely to benefit from surgery.

- Silencing of PRMT5 was associated with growth arrest in MTAP negative mesothelioma cells. This effect was selective, and not associated with programmed cell death. We identified quinacrine as an off-patent antimicrobial drug with PRMT5 perturbagen activity. Quinacine suppressed expression of PRMT5 at low micromolar concentration, leading to loss of protein expression. The effect was due to suppression of mRNA in common with siRNA.

- Using a panel of siRNAs to target predicted PRMT5 transcription factors, we identified c-jun as the critical transcriptional regulator, implicating Quinacrine an indirect inhibitor of c-jun. Our results suggested that a c-jun inhibitor drug might also copy the effects of quinacrine, as an indirect PRMT5 inhibitor. Quinacrine induced MTAP specific growth arrest. This was prevented by introducing a normal PRMT5 gene back into the mesothelioma cell, that could not be suppressed by the quinacrine. However, a mutant PRMT5 lacking activity was incapable of rescuing cells in the presence of quinacrine.
-

3i. How do your research results contribute to or change the diagnosis, prevention, treatment and/or outcome of lung disease? Are there any impacts on quality of life? If there is no immediate impact for patients, do they have the potential for change in the future?

This section is very important for our funders! Please give details in lay language

The results from this work could lead to the development of new personalised treatment of malignant mesothelioma

3j. Has your work increased the understanding of lung disease? If so, how?

This section is very important for our funders! Please give details in lay language

This work has allowed the identification of a new strategy to effectively targeting MTAP negative mesotheliomas in the relapse setting based on identification of the CDKN2A/MTAP subtype as being vulnerable to inhibition of PRMT5.

3k. Can you summarise the outcomes of your work in 3 bullet point 'take home' messages for our supporters? This should focus on why your work has been beneficial to people with lung disease.

This section is very important for our funders! Please give details in lay language

- CDKN2A/MTAP loss is associated with worse clinical outcome
- PRMT5 is required for viability of CDKN2A/MTAP mesothelioma
- PRMT5 can be inhibited by Quinacrine, drug originally developed as an antimicrobial. Quinacrine can be "repurposed" to inhibit PRMT5 selectively in MTAP negative mesotheliomas

3l. Developments/ new directions for research - what do you want to do next? Has this research resulted in further research funding?

This section is very important for our funders! Please give details in lay language

Quinacrine is an off-patent drug that is well tolerated. Our work suggests that quinacrine could potentially be used to treat CDKN2A/MTAP negative mesothelioma. The next stage of this work is to explore whether or not quinacrine can synergise with other S-methyladenosine (SAM) competitive PRMT5 inhibitors which are not MTAP selective, in order to optimize personalised therapy. The data generated by this project provides proof of concept data to support further clinical development in a molecularly stratified treatment setting (eg. MiST trial).

3m. Glossary of terms used

- CDKN2A: cyclin-dependent kinase Inhibitor 2A, gene on Chromosome 9, encodes for tumor suppressor proteins p16 and p14
- MTAP: Methylthioadenosine Phosphorylase, gene on Chromosome 9, encodes for a protein essential for metabolism in cells
- PRMT5: Protein arginine N-methyltransferase 5, gene on chromosome 14, encodes PRMT5 protein essential for transcription

3n. How has this research grant helped you? (e.g. influence on career progression, providing data to help you access further funding etc.) (in lay language)

This grant has been of significant benefit and enabled discovery and exploration of a novel strategy for treatment of malignant mesothelioma. This work has led to a potentially new, translationally relevant finding that could lead to new way of treating MTAP negative mesothelioma.

Are you happy for portions of this lay content to be used publically the BLF immediately?
If not, please provide a date when we can do so

YES

DATE: 25/11/19

4. Scientific outcomes of your research Please summarise the research findings from your project on not more than one side of A4 paper (single spaced, two sides if double spaced) (font size no less than 10).

- MTAP deletion is associated with poor prognosis

To determine the prognostic impact of MTAP deletion, array based CNV analysis was conducted in a total of 79 MPM samples. MTAP homozygous deletion frequency was 41.77 % and co-deletion of MTAP and CDKN2A was observed in 100% of the MTAP negative patients. Homozygous MTAP deletion was associated with lower overall survival (OS) from time of surgery compared to wild type (median OS= 336 vs 430 days respectively, p=0.024, HR=1.753)

- PRMT5 silencing mediates growth arrest in MTAP negative mesotheliomas

To assess whether MTAP deletion creates a dependency on PRMT5 inhibition, we silenced PRMT5 in MTAP positive and MTAP negative mesothelioma cell lines, as well as in MTAP CRiSPR HAP1 isogenic models. PRMT5 siRNA selectively targeted MTAP negative cell lines, reducing clonogenic growth with reduction in symmetrical di-methylation of the PRMT5 substrate Histone H4 arginine 3 (H4R3me2S). To investigate the kinetics of growth arrest we conducted real time analysis in cells following PRMT5 silencing. Longitudinal analysis of response to PRMT5 revealed significant growth arrest after 120 hours. Neither apoptosis nor cell cycle perturbation were observed in these cells. We then hypothesized that reduction in global histone methylation (H4R3me2S) might lead to upregulation of genes associated with disruption of cell cycling. To test this hypothesis we examined transcriptionally induced genes following PRMT5 downregulation (at 120 hours), in MTAP negative cell lines. PRMT5 silencing was phenocopied by silencing of PRMT5 interactor WDR77 but not consistently across all cell lines by inhibition of the atypical kinase Rio1.

- Identification of Quinacrine Hydrochloride (QH) as a PRMT5 perturbagen

Enzymatic inhibition of PRMT5 by elevated methylthioadenosine (MTA) is thought to be limited through negative feedback by s-adenosylmethionine on PRMT5.
Accordingly, the small molecule PRMT5 inhibitor EPZ01566 showed a significantly lower potency (IC_{50} > 5μM), compared to the effect of siRNA. Consequently novel methods of inhibiting PRMT5 are required. We have conducted connectivity map analysis to identify novel transcriptional suppressors of PRMT5. Among the top 5 predicted molecules Quinacrine Hydrochloride (QH) led to significant suppression of PRMT5 mRNA levels and induced growth arrest (1μM) in MTAP negative cells only as confirmed by clonogenic assay, essentially phenocopying PRMT5 siRNA. QH also led to PRMT5 protein downregulation and consequent reduction of H4R3me2S, without any effect on PRMT5 enzymatic activity. QH inhibited PRMT5 promoter activity as confirmed by reporter assay.
Neither apoptosis nor cell cycle perturbation were observed after treatment with QH.
To confirm that QH mediated a PRMT5-dependent growth arrest we performed rescue studies While transfection of $PRMT5^{(WT)}$ led to rescue after treatment with QH, this was not observed with the methyltransferase dead mutant $PRMT5^{(E444Q)}$, compared to cells transfected with EV $^{(GFP)}$ control.
This finding is consistent with QH's modulation of endogenous PRMT5 transcription as essential mechanism underpinning its effect in MTAP negative context.

- PRMT5 is transcriptionally regulated by c-JUN

We utilized PROMO to map PRMT5 putative transcription factors. CEBP1, c-JUN and NF-YA were predicted to bind to the PRMT5 promoter. C-JUN silencing in MTAP negative cells, caused downregulation of both PRMT5 mRNA and protein, loss of H4R3me2S and clonogenicity but not in MTAP positive cells.
QH suppressed c-JUN mRNA suggesting that it targets PRMT5 transcription indirectly via this transcription factor.

5. Problems encountered during the research. If the start date of your work was delayed, please clarify reasons.

This section is very important for our funders! Please give details in lay language

This project was originally focused on targeting CDK4/6 as a strategy for targeting CDKN2A negative mesothelioma. However as this concept evolved rapidly, it became possible during the lifetime of the project to develop a phase II trial, which became arm 2 of the MiST trial. During the lifetime of this project, it emerged that PRMT5 was a critical vulnerability in CDKN2A/MTAP negative cancer, and therefore strategies to target PRMT5 became the primary focus.

6. Publications arising or planned from this grant (please enclose a copy of each) - please acknowledge the BLF in all publications that arise from our funding and forward copies of future publications

Original papers and publications

Manuscript in preparation

Abstracts presented at meetings

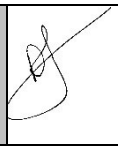
IMIG, 2018, Ottawa, Canada:
Small Molecule Transcriptional Suppression of PRMT5 induces synthetic lethality in MTAP Negative Mesothelioma
Sara Busacca, Qi Zhang, Alan G. Dawson, Annabel Sharkey, David A. Waller, Apostolos Nakas, Shu-Dong Zhang, Dean A. Fennell

7. If the research results have commercial potential please provide details below including the name and address of the appropriate authority within the Host Institution with whom the Foundation should liaise.

This section is very important for our funders! Please give details in lay language

N/A

Please sign to confirm that, as stated in the BLF Grant Regulations and Conditions document, you are willing to assist the BLF in any press/PR activities relating to your grant. This may include participating in interviews, providing patient case studies and anecdotes, helping to construct press releases etc. Electronic signatures are acceptable.

Signed		**Date**	25/11/2019

I certify that to the best of my knowledge all the information given in this report is correct and I will continue to acknowledge the British Lung Foundation and keep them informed about future publications and developments arising from this work. Electronic signatures are acceptable.

Signed		**Date**	25/11/2019