BRITISH LUNG FOUNDATION

Final Report

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

1. Grant-holder details

Grant Holder(s) and positions held	Dr Edward Hollox, University Reader.	Grant no	MESOUK17-8
Research worker(s)	n/a		
Place at which research is carried out	University of Leicester		

2. Grant details

Title of Research	MEDUSA – <u>M</u>esothelioma <u>E</u>volution: Deciphering dr<u>U</u>gable <u>S</u>omatic <u>A</u>lterations as potential targets for synthetic lethal therapy

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
MEDUSA - Finding the Achilles' heel(s) of mesothelioma by interrogating genomic evolution

3b. Lung diseases relevant to the research

Malignant pleural mesothelioma

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

First generation DNA sequencing projects, although revealing the population level extent of mutations in mesothelioma, cannot decipher the intra-tumour spatial and temporal heterogeneity of these mutations. Intra tumour heterogeneity (ITH) underpins poor survival and drug resistance in cancer, by fuelling subclonal expansion under selection pressure exerted by drug treatments. Understanding the nature of ITH mesothelioma, will allow define a new hierarchy of genetic vulnerabilities, to underpin the discovery of new treatments most likely to target the whole cancer. In addition, this will allow the inference of mechanisms that underpin the formation, and natural history of mesothelioma. In order to advance treatment discovery, MEDUSA will therefore employ a second generation sequencing methodology (m-WES) with the goal of uncovering new, relevant drug targets to support synthetic lethal drug discovery, and accelerate development of new medicines to effectively treat mesothelioma.

We sampled four regions of the mesothelioma from 40 patients, together with peripheral whole blood. Following DNA extraction, the exomes of each sample were sequenced, and tumour cell fraction, ploidy and copy number changes calculated for each sample, using the matching exome from whole blood as a non-tumour control. These data were then used to construct a phylogenetic tree of each tumour from the pattern of shared copy number breakpoints between different regions of the tumour.

This allowed us to identify key truncal driver copy number losses that occurred early in tumour formation. These affected known driver genes such as NF2 and BAP1 important in tumour formation, and were sometimes double hit, with one copy of the gene being lost by gene deletion, the other being inactivated by nucleotide mutation. Across the different patients, each clearly showed a different mutational profile, but with losses of certain genes shared across a minority of tumours. This highlights the intertumour heterogeneity of mesothelioma. Nevertheless,

60% of tumours had a truncal copy loss of a known driver gene early in evolution, emphasising the importance of gene loss in addition to nucleotide mutation in mesothelioma.

The identification of early driver gene losses in certain tumours shows that, for a subset of patients, drugs targeting the vulnerabilities generated by those gene losses are potential therapies for the cancer. Importantly, these changes occurred early and are clonal, occurring throughout the tumour, and therefore generate a vulnerability for the whole tumour. Also importantly, not all tumours have the same vulnerabilities, so treatments must be targeted to particular tumours based ont their genomic profile and evolutionary history. This work will lead to personalised approaches to treating mesothelioma in patients.

3d. Background / reasons for the research

There are currently limited treatment options for mesothelioma. Analysis of the genomes of mesothelioma, and other cancers, show that it the genomes show significant amounts of heterogeneity between patients (inter-tumour heterogeneity) and within patients (intra-tumour heterogeneity). This has two consequences. Firstly, *inter*tumour heterogeneity suggests that, because certain genomic mutations will render certain tumours susceptible to particular drugs, one drug may not be effective against all tumours, and therefore a personalised medicine approach, i.e. tailoring a drug to the genomic profile of a tumour, is needed. Secondly, *intra*tumour heterogeneity suggests that a particular mutation may not occur throughout the tumour, having arisen later in tumour evolution. To target a drug to a particular patient, not only must the genomic mutation that confers vulnerability to that drug be in the patients tumour, but it must be throughout the tumour, rather than just part of it, to ensure effective treatment.

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

First generation DNA sequencing projects, although revealing the population level extent of mutations in mesothelioma and inter-tumour heterogeneity, cannot decipher the intra-tumour spatial and temporal heterogeneity of these mutations. Intra tumour heterogeneity (ITH) underpins poor survival and drug resistance in cancer, by fuelling subclonal expansion under the selection pressure exerted by drug treatments. Understanding the nature of ITH mesothelioma allows us to define a new hierarchy of genetic vulnerabilities and to underpin the discovery of new treatments most likely to target the whole cancer. In addition, this will allow the inference of mechanisms that underpin the formation, and natural history of mesothelioma. In order to advance treatment discovery, MEDUSA will therefore employ a second generation sequencing methodology (multiregional whole exome sequencing) with the goal of uncovering new, relevant drug targets to support synthetic lethal drug discovery, and accelerate development of new medicines to effectively treat mesothelioma.

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

The heterogeneity of mesothelioma tumours is not well-understood, yet is important for individualised selection of drugs for treatment, so we are using multiregional sampling and DNA sequencing to understand the heterogeneity of tumours, their evolution, and key early mutations in cancer genes that can be targeted for treatments.

3g. How was the research carried out?

We have exome-sequenced multiple regions of malignant pleural mesothelioma and matching whole blood-derived DNA from 30 patients. A further 10 will be sequenced when samples become available (sample extraction from tissue is delayed due to COVID-19-related reassignment of duties). We have analysed 25 so far, and the results presented relate to these 25. We have used called copy number changes based on sequencing read-depth and allelic ratio, then constructed a phylogeny of the cancer for each of the twenty patients. This has allowed us to identify clonal, early homozygous and heterozygous changes in these patients.

We have presented part of these data as preliminary data at the American Society of Cancer Research Conference in Atlanta in April 2019 (Prof Fennell was an invited speaker), at the American Society of Human Genetics meeting in October 2019 (funded by University of Leicester College of Life Sciences), and at the World Conference on Lung Cancer in September 2019 (Dr Hollox was an invited speaker).

3h. Please summarise the findings of your research project

Our initial analysis has identified extensive intra-tumour and inter-tumour copy number heterogeneity in mesothelioma genomes. In 60% of the 25 patients analysed so far, we have identified clonal, early, copy number losses in known tumour-suppressor genes that drive the formation of mesothelioma. In two, we have identified a complete gene loss known to confer sensitivity to drugs acting against the PRMT5 methylation pathway. In other patients we have identified gene inactivation caused by two hits - a gene loss and a somatic DNA nucleotide mutation, affecting genes including NF2 and BAP1. Inactivation of these genes are known to confer susceptibility to drugs that target the Hippo signalling pathway in cells, and recombination processes in cells, respectively.

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?

The results from this project do not have immediate impact for patients, but they do have the real potential to change patients' lives in the future. The Mesothelioma Research Programme at Leicester is using cell lines derived from patients with known clonal mutations in NF2 and BAP1 to examine the efficacy of drugs targeting those particular vulnerabilities – defects in homologous repair and the Hippo signalling pathway, in particular. Therefore there is an avenue from the fundamental discovery research of this project through translational aspects to improved treatments and disease management for patients.

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

Yes, it has deepened our understanding of malignant pleural mesothelioma. In particular, we now have an understanding of the extent and intratumour heterogeneity of copy number alterations of the tumour genome across patients. We also know that some key driver gene deletions occur early in tumour formation, and that in some patients heterozygous loss of a driver gene is complemented by a nucleotide mutation in the other copy of that gene, resulting in two "hits" that removes that gene from the genome, and therefore its protein product from the tomour cell. This can render the tumour cell vulnerable to targeting the biological pathways damaged by that loss of protein.

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.

- Malignant pleural mesothelioma shows extensive heterogeneity in the genomes of the tumour cells within a tumour.

- Key genes that control the development of the tumour are lost early in mesothelioma evolution, but different patients can differ in the key genes that are lost early.

- Identification of the genes lost early in mesothelioma evolution can potentially inform us which drugs will be most effective against that particular tumour, therefore developing a personalised medicine approach for malignant pleural mesothelioma.

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

This funding provided a case for further support from the University of Leicester, in the form of a PhD studentship (started October 2018). This PhD student is focusing on the clonal deletions identified as part of the MEDUSA project by carrying out targeted resequencing of common clonal deletions to finely map deletion breakpoints and investigate potential mechanisms of early breakpoint generation in mesothelioma.

We have also generated preliminary data showing many potential gene fusion events in mesothelioma, and we believe this aspect has great potential to understanding the molecular development of mesothelioma and the potential to provide biomarkers for early development. This is the basis for a further grant application to BLF to be submitted in October 2020 (delayed from April 2020 due to COVID19).

3m. Glossary of terms used

Clonal – occurring in all parts of the tumour.

Truncal – occurring early in the evolution of the tumour (in the trunk of the phylogenetic tree).

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 given me the opportunity to be selected as a faculty invited speaker at the World Conference of Lung Cancers in Barcelona in September, presenting my work to a new, broad, audience of clinicians and scientists.

This research grant has funded the generation of multiregionally sampled exome sequences. These have already led to insights beyond the analysis of copy number alterations that was the remit of this grant. We have used the data to compare different SCNA detection algorithms beyond Sequenza (ABSOLUTE, ASCAT, GISTIC), analyse single nucleotide changes using Mutect2 and VarScan2. The data have also been used to analyse evolution using an alternative approach implemented by the program PhyloWGS. We have also generated preliminary data showing many potential gene fusion events in mesothelioma, and we believe this aspect has great potential to understanding the molecular development of mesothelioma and the potential to provide biomarkers for early development. This is the basis for a further grant application to BLF to be submitted in October 2020 (delayed from April 2020 due to COVID19).

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:

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).

The Mesothelioma evolution: Drugging somatic alterations (MEDUSA) project aims to investigate the genomic evolution and heterogeneity of malignant pleural mesothelioma and identify genomic changes early in mesothelioma evolution that can be targeted by drugs. For 25 malignant pleural mesothelioma patients, we have analysed the exomes of at least four regions of the tumour and paired whole blood.

Using paired tumour-normal analysis with the software Sequenza, we have called somatic copy number alterations (SCNAs) specific to the tumour, and used the software Tumult to reconstruct a phylogeny of the tumour for each of the 25 patients. By using Sequenza, we found that SCNA was an important feature of the tumour genome. The proportion of the genome affected by SCNA varied from tumour to tumour, with a range of 7% to 94%, and a mean of 32%. Following reconstruction of the SCNA phylogeny using Tumult, we found that in general more SCNA occurred subclonally (more recently in tumour evolution, on the branches of the tree) rather than clonally (at the start of tumour evolution, on the trunk of the tree) – with the proportion of the genome affected being on average 10% and 22% respectively.

We identified particular tumour-suppressor "driver" genes that are repeatedly lost across patients early in mesothelioma evolution. These are shown in table 1. Furthermore, using the exome sequences we could identify tumour-suppressor genes that had been completely inactivated on both chromosomes either by a homozygous deletion or a compound heterozygote of a deletion of one allele and a point mutation.

Tumour suppressor gene	Number of patients with heterozygous truncal loss (total 25)	Number of patients with homozygous truncal loss (total 25)	Number of patients with truncal compound heterozygous loss (total 25)
MTAP/CDKN2A	4	2	2 (*CDKN2A*)
BAP1	9	0	1
NF2	9	0	4
LATS1	5	0	0
LATS2	5	0	0
SETD2	9	0	1
FBXW7	1	0	1
MTOR	3	0	0

Table 1 : Numbers of tumours with truncal losses in the MEDUSA cohort

It is clear that there is not a truncal loss that is present in all tumours. Individually, a particular loss at one of these genes will be present at a maximum of 36% of tumours truncally, however 60% of tumours have a truncal loss in at least one of the genes in table 1. We are currently identifying truncal nucleotide mutations in these genes to see if single nucleotide mutations account for the remaining 40% of tumours. Mesothelioma tumours are clearly

heterogeneous between patients, but some commonalities across a proportion of tumours in terms of gene loss are beginning to emerge.

Gene loss, either heterozygous reducing expression or homozygous abolishing expression, can be an Achilles' heel for the tumour, rendering it susceptible to particular drug treatments. This work has confirmed that personalisation of this approach is critical – a drug targeting vulnerabilities exposed by *BAP1* loss, for example, will be effective in some but not all patients. The Mesothelioma Research Group in Leicester is currently pursuing drug treatment options that target *BAP1* loss and *NF2* loss using cell models.

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

1. The DNA extraction and exome sequencing was delayed due to a key member of staff supporting (but not directly funded by) the project taking parental leave. However the delay was mitigated during the second half of the grant.

2. Originally, our multiregional sampling of mesotheliomas included sampling from the apex region. However, in many of the mesotheliomas undergoing surgery this region is not tumour. We have therefore decided to sample from four locations consistently across all patients, rather than five. This still gives us a good representation of the truncal mutations in the tumour, and the money saved in exome sequencing a fifth region will allow our patient cohort size to increase to more than 30 patients.

3. Due to cost savings in exome sequencing, we were able to complete the exome sequencing under budget. We requested, and were granted, a no-cost extension to sequence another 10 patients. We have managed to secure this, but sampling is delayed due to key personnel being reassigned to clinical duties during the COVID-19 outbreak.

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

Original papers and publications

A paper describing the analysis described here, together with further analyses on the exome data, is in preparation.

Abstracts presented at meetings

Hollox et al. MEDUSA: Phylogenetic analysis of mesothelioma tumours by multiregional sampling, whole exome sequencing, and copy number analysis. American Society of Human Genetics Meeting, October 2019, Houston, Texas

Hollox et al. MEDUSA: Phylogenetic analysis of mesothelioma tumours by multiregional sampling, whole exome sequencing, and copy number analysis. IASLC World Congress on Lung Cancer, September 2019, Barcelona, Spain

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.

Not applicable