AWARD NUMBER: **W81XWH-18-1-0343**

TITLE: **The MexTAg Collaborative Cross: Understanding Genetic Modifiers in Mesothelioma**

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REPORT DATE: **August 2021**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2021	Annual report	15Jul2020-14Jul2021

4. TITLE AND SUBTITLE

The MexTAg Collaborative Cross: Understanding Genetic Modifiers in Mesothelioma

5a. CONTRACT NUMBER
W81XWH-18-1-0343

5b. GRANT NUMBER
CA170299

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

3

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This is the third progress report for award CA170299. In the last 12 months we have overcome a major setback associated with breeding the last 20 CC-MexTAg groups and have successfully bred and asbestos exposed all 70 groups CC-MexTAg groups onto the asbestos exposure study. Moreover, we now have complete data from 60 of 70 groups and are able to perform interim analysis. The final 10 CCMT groups remain on study and we will have complete data by November 2021. Interim analysis demonstrates significant variation in disease phenotype, with a 3-fold change in median survival and disease latency between groups. We have identified a variety of qualitative trait loci (QTL) for each of 5 traits (phenotypes) being assessed and are currently investigating genes and regulatory elements associated with each QTL. These data will be used to interrogate human mesothelioma datasets once complete CCMT data has been obtained. To date, we have completed or will soon complete Aims 1 and 2. Aim 3 is currently underway as we have now received US DoD human ethics approval. We are on track to achieve all stated aims by the end of December 2021. A no-cost extension has been approved through to January 31st 2022.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	24	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Mesothelioma is an incurable cancer caused by asbestos exposure. However, why some people develop disease, while others do not, despite similar exposures remains unknown. There is strong evidence that a person's genes can affect their chance of contracting mesothelioma, but the power of conventional human genetic studies are hindered by small sample sizes and various environmental and lifestyle factors associated with this rare cancer, meaning how a person's genetic makeup affects disease development remains unknown. In this project we have combined two powerful mouse models to discover genes (and their associated biological pathways) that prevent or delay mesothelioma developed after asbestos exposure. To confirm the importance of candidate modifier genes identified in our study we will compare our results against large human mesothelioma genetic data sets.

2. KEYWORDS:

Mesothelioma, asbestos, Collaborative Cross, MexTAg, CC MexTAg, host genetics, genetic predisposition, asbestos related disease, disease susceptibility, disease resistance.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Generate CC-MexTAg mice, expose them to asbestos and assess mesothelioma latency, disease progression and survival in CC-MexTAg mice*.*

Subtask 1: MexTAg mice crossed with CC lines (months 3-12)

Subtask 2: Expose MexTAg controls and CCMT progeny to asbestos (months 3-23)

Subtask 3: Local IRB/IACUC Approval (month 3)

Aim 2: Identify candidate modifier genes associated with these traits.

Subtask 1: Genotype and haplotype analysis using a combination of collaborative cross-specific bioinformatics programs commonly referred to as the GeneMiner platform (months 18-36). Subtask 2: Gene mapping performed by using phenotype traits such as ARD overall survival as a quantitative trait using our GeneMiner pipeline (months 3-36).

Aim 3: Identify human orthologs and interrogate human mesothelioma datasets.

Subtask 1: Identify human orthologues using BLAST of DNA sequences encompassing the peak SNPs and/or best candidate causal SNPs identified in Aim 2 (months 36-41).

Subtask 2: Human orthologues will be interrogated against publicly available mesothelioma data sets (TCGA) and additional human mesothelioma datasets available via CI Bueno (months 36-41).

Publication and presentation of data:
Major publications months 36-41 (pub 1 in review month 34). Pubs 2 & 3 in preparation (months 36-41)
Local, national, and international data (conference) presentations: months 1-41.

What was accomplished under these goals?

Summary of previous progress reports. In the stage 1 report (15th July 2018-14th July 2019) we reported successful completion or initiation of all Aim 1 subtasks; Local IACUC approval was obtained (subtask 3), 50 of 70 CC-MexTAg groups had been successfully bred (subtask 1) and progeny mice exposed to asbestos (subtask 2). At the time of submission limited data was available as the majority of CC-MexTAg groups remained on study with only a few CC-MexTAg mice having reached an experimental endpoint, thus Aims 2 and 3 remained to be implemented. We also acknowledged that we had experienced a significant, unforeseen delay in setting up breeding the remaining 20 CC-MexTAg groups, but had implemented a strategy to overcome this delay, which was progressing as planned. We also noted that consistent with the original timeline, time and effort are heavily weighted toward Aim 1 of this study, *viz*., generation and asbestos exposure of CC-MexTAg groups and subsequent collection of phenotypic data and biological samples.

During the stage 2 reporting period (15th July 2019-14th July 2020), we reported successful completion of Aim 1 subtasks 1, 2, and 3 (generation and asbestos exposure of all proposed 70 CC-MexTAg groups). At the time of submission 55 of 70 asbestos exposed CC-MexTAg groups (including a MexTAg control group on the parental B6 background) had completed the study, for which we have complete survival and phenotypic data, with 16 of the last 20 groups remaining on study. Data from the 55 completed groups were used in preliminary analyses outlined in Aim 2, subtasks 1 and 2 above and were described in detail in the 2nd progress report (Figure 4, Table 1.2). At submission of the 2nd progress report we expect complete survival and phenotypic data for all 70 CC-MexTAg groups to be available by end of May 2021.

In addition to completion of Aims 1 and 2, we had also initiated work on Aims 3 and 4. We have received local Human Ethics approval (University of Western Australia) and were awaiting on the US DoD Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval (initial application submitted 19th March 2020), prior to commencing interrogation of human mesothelioma datasets to analyse the relevance of human orthologs of candidate modifier genes identified in data generated from Aim 2. We presumed the delay in response was related to COVID-19, and we finally received HROP human ethics approval in May 2021.

Impact of COVID-19 pandemic.
The COVID-19 pandemic has had a significant global impact, with many countries implementing strict restrictions that have impacted scientific research. Fortunately, to date, Australia, and Western Australia in particular, have not been affected to the same degree as the United States or many other European countries, although we did experience an extended period of lock down between March and June 2020 and again in February-April 2021, that slowed our research output. However, our CC-MexTAg work remained relatively unaffected; daily welfare monitoring and experimental procedures continued as normal, with variation to staff rostering to avoid being in the same place and the same time the only significant change during this time. Delays to some administrative/reporting procedures (i.e. HRPO approval) were experienced, and our ability to report findings at local, national and international meetings was significantly affected. However, while COVID-19 has delayed many aspects or our work, it will not prevent us from completing this study. We will continue to manage the situation and adapt our research as required, should the situation in Western Australia change and will inform the US DoD if any significant delays to the remaining aims are experienced.

Progress July 15th 2020-July 14th 2021
Significant outcomes:
Aim 1: Generate CC-MexTAg mice, expose them to asbestos and assess mesothelioma latency, disease progression and survival in CC-MexTAg mice.
Subtask 1: MexTAg mice crossed with CC lines (months 3-12)
Subtask 2: Expose MexTAg controls and CCMT progeny to asbestos (months 3-23)
Subtask 3: Local IRB/IACUC Approval (month 3)

To date we have successfully generated and asbestos exposed 70 different CC-MexTAg groups. Furthermore, we have complete data on 61 groups; 60 CC-MexTAg groups and a MexTAg control group, while the other 11 CC-MexTAg groups remain on study. Along with overall survival, we have complete data on four additional phenotypic traits: disease latency (time from asbestos exposure to first signs of disease), disease progression (time from first signs of disease to cull), ascites volume and the total number of mice with tumours for each group. We have also collected tissue samples (ascites, spleen, kidney, liver, diaphragm) and tumour if present from each animal when they reached an experimental endpoint; either disease development (usually ascites related abdominal distention, or disease related loss of condition), or when animals survived to 18 months from first asbestos exposure. There are currently 210 mice left on study, with the last group scheduled to reach the maximum experimental end point on November 26th 2021. However, mice may succumb to disease at any time prior to this time point. In addition to this report, we will be applying for a no-cost extension to ensure that data from the final 210 mice can be collected and Aim 1 of this study can be completed entirely.

Each mouse was given two intraperitoneal injections of 3 mg asbestos suspended in 0.5 ml PBS at weeks 0 and 4 and survival calculated from the day of injection. We monitored asbestos related disease (ARD) using three metrics: disease latency (time from first injection to first signs of disease); time to progression (time from first signs of disease to cull); and overall survival (time from first asbestos exposure to cull), which is the sum of the first two measurements (Figure 1A). To date, we have observed considerable variation (three-fold range) in median overall survival between different asbestos exposed CC-MexTAg groups (Figure 1B, C).

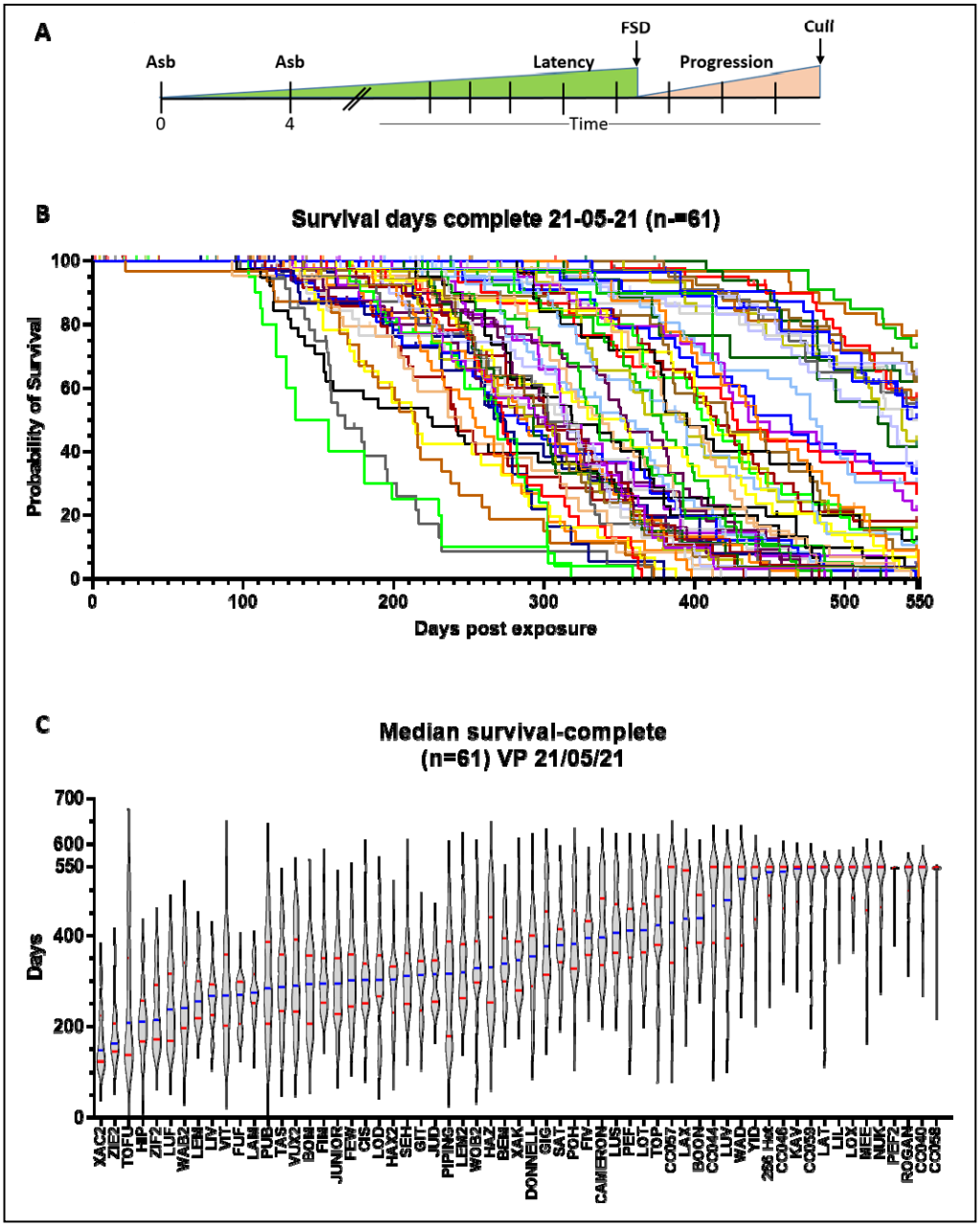

Figure 1: Variation in median survival in asbestos exposed CC-MexTAg mice. Groups of CC-MexTAg mice (n=55, median 36 mice/group) were exposed to a total of 6 mg of asbestos via two consecutive intraperitoneal injections, 4 weeks apart and assessed for disease development over time. (A) Experimental schematic. (B) Kaplan Myer survival plot censored for asbestos related disease (ARD). Each line represents a unique asbestos exposed CC-MexTAg group (n=61). (C) Violin plot showing median overall ARD survival (ranked) for CC-MexTAg groups. Red bars = median, blue bars = quartiles.

Further analysis indicated that the observed variation in overall survival was driven by disease latency, but not disease progression (Figure 2A, B). This is supported by the strong correlation between overall survival and latency ($r = 0.9985$, $p < 0.001$), but not overall survival and disease progression ($r = -0.05112$, $p =$ ns; Figure 2).

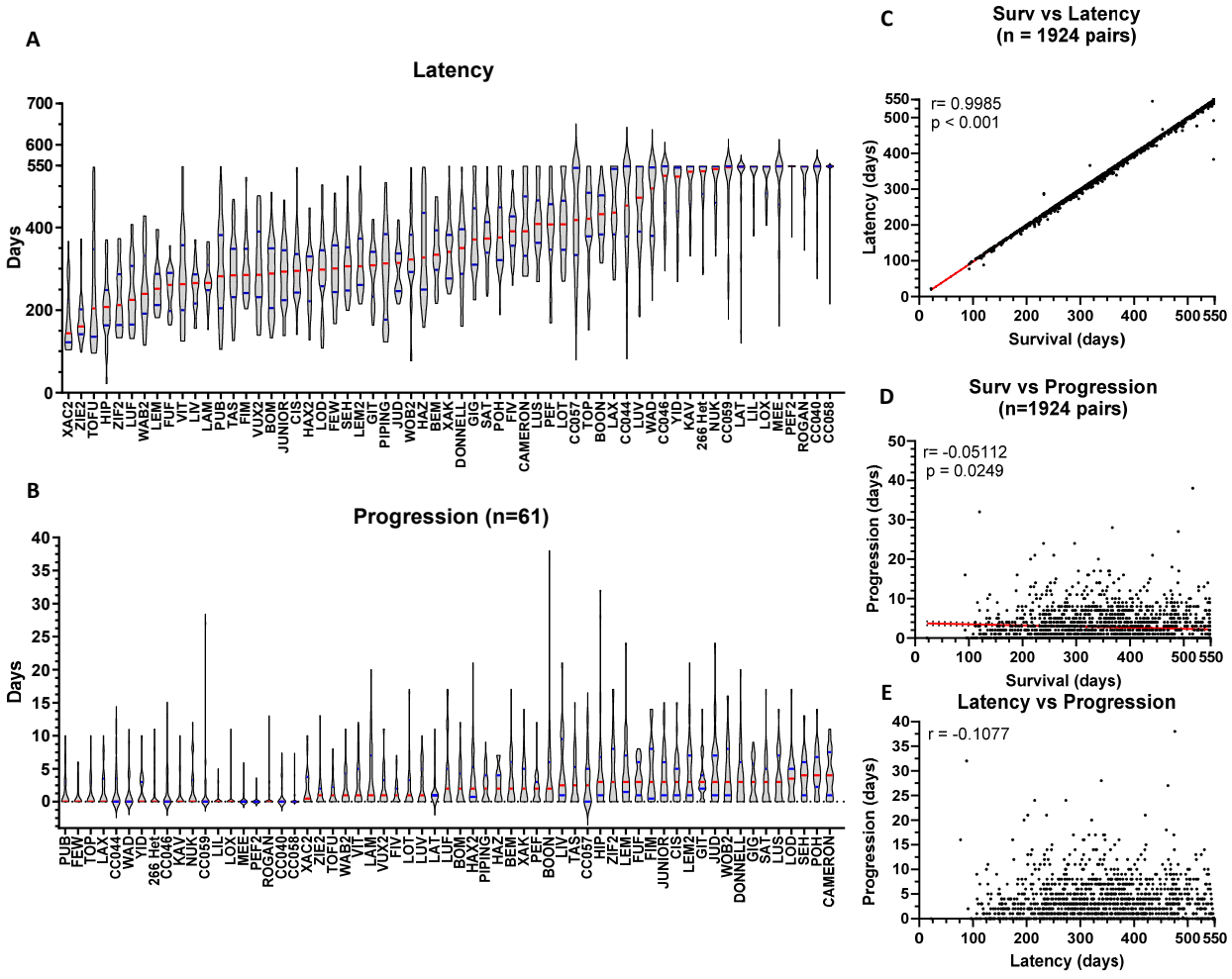

Figure 2: Asbestos related disease latency drives variation in overall survival in asbestos exposed CC-MexTAg mice. (A-B) Violin plots depicting variation in ARD latency and progression (ranked) respectively (median, red line and interquartile range, blue lines) for each of the 61 asbestos exposed CC-MexTAg groups. (C-E) Scatter plots depicting correlations (Pearson's) between individual phenotypes for individual mice.

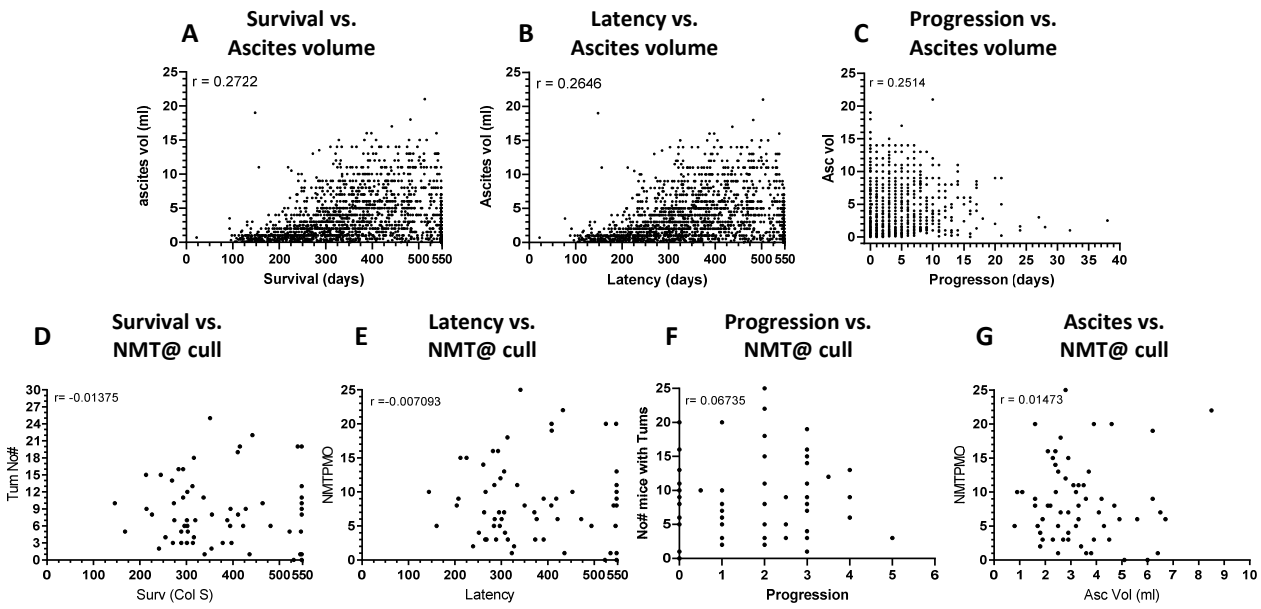

Figure 3: Asbestos related disease phenotype correlations. (A-C) Plots depicting correlations between survival, latency and progression relative to ascites volume for individual mice. (D-G) Correlations between the numbers of mice with tumour at cull (NMT@ cull) for each CC MexTAg group and respective ARD phenotypes. Red line = line of best fit. r = Pearson's correlation.

Taken together, overall survival and disease phenotype data suggest that in asbestos exposed CC-MexTAg mice, as with the human disease, ARD manifests over time with a strong, significant correlation between overall survival and both ascites volume and the number of mice with tumours. Furthermore, the discordance between disease progression and all other ARD phenotypes suggests that the influence of host genetics on ARD occurs during the latent period from asbestos exposure prior to disease manifestation, but has limited influence on survival once ARD is established.

Histological confirmation of disease and establishment of a of tissue sample biobank

Throughout Aim 1 we have collected tissue and tumour samples from each mouse as they complete the study. An up to date list of samples from the 61 different asbestos exposed groups are shown in Table 1. We have collected and prepared 2357 histological blocks each containing the kidney, spleen, liver and diaphragm from a single animal. The process of cutting and staining sections continues as outlined in the original grant application, with a focus on the CC-MexTAg groups located in the upper and lower 10% of survival. A full summary of all histological analyses will be included when the study is complete.

Table 1 also shows the number of samples collected from the first 61 asbestos exposed groups to complete the study, including the number of ascites derived cells lines made from each group and the total number of histology samples in each group. To date, we have generated a total of 1003 ascites derived cell lines, 60 tumour derived cell lines and 430 tumour samples for RNA sequencing and immunofluorescence / histological analyses. The wealth of data from this program is a significant resource that will undoubtedly prove valuable in future research into ARD. Indeed, the biobank of CC-MexTAg samples is currently being exploited as part of a separate Australian

Commonwealth National Health and Medical Research Council funded grant investigating the interplay between host genetics and gene expression profiles and immune cell infiltration of the tumour microenvironment of different CC-MexTAg groups.

Table 1: Samples generated from the CC MexTAg study. Data indicates the number of mice per group for each type of sample. PF = Pleural effusion. TC = Tissue culture. Sample collection ongoing for remaining 11 CC-MexTAg groups.

Group Number	Name	No# Tum in RNA later	No# Tum TC stocks	No# ascites TC stocks	No# histology samples
C1	266 Het (B6)	4	0	17	30
7	BEM	1	4	7	37
42	BOM	1	0	21	34
52	BOON	23	0	24	28
36	CAMERON	6	0	23	25
35	CIS	8	3	31	32
23	DONNELL	4	5	20	38
28	FEW	0	4	4	29
17	FIM	0	1	4	33
21	FIV	0	0 Tum +1PF	7	40
37	FUF	2	1	20	27
50	GIG	20	0	28	32
34	GIT	5	3	34	37
8	HAX2	0	0	16	34
25	HAZ	1	0	13	36
12	HIP	0	1	2	28
18	JUD	0	1	12	31
22	JUNIOR	0	0	4	33
53	KAV	14	0	8	27
14	LAM	0	0	15	31
59	LAT	13	0	15	30
47	LAX	16	0	23	33
20	LEM	0	0	13	34
11	LEM2	0	0	7	34
2	LIL	4	3	6	35
15	LIV	0	1	11	20
13	LOD	0	0	11	26
3	LOT	0	1	15	35
51	LOX	18	1	9	31

19	LUF	0	0	8	28
39	LUS	8	1	25	29
5	LUV	0	1	6	39
58	MEE	7	0	1	38
1	NUK	0	0	4	30
4	PEF	0	0	12	29
57	PEF2	9	0	0	30
26	PIPING	5	3	19	35
43	POH	8	0	26	28
49	PUB	2	0	27	41
48	ROGAN	15	0	18	36
24	SAT	3	3	20	39
10	SEH_	0	0	8	19
41	TAS	10	0	31	38
46	TOFU	2	0	15	34
9	TOP	0	0	13	33
33	VIT	2	1	21	27
31	VUX2	4	3	24	34
38	WAB2	3	0	23	30
55	WAD	2	0	0	17
32	WOB2	2	2	31	38
6	XAC2	0	0	4	20
16	XAK	0	4	14	40
45	YID	26	1	33	37
27	ZIE2	0	0	2	26
30	ZIF2	0	0	6	31
62	CC040	6	0	1	32
61	CC059	6	0	0	30
60	CC046	3	0	0	40
64	CC044/Unc	14	0	1	33
63	CC057/Unc	8	0	0	30
66	CC058/Unc	7	0	0	33

Aim 2: Identify candidate modifier genes associated with these traits
Subtask 1: Genotype and haplotype analysis using a combination of collaborative cross-specific bioinformatics programs commonly referred to as the GeneMiner platform (months 18-23).
Subtask 2: Gene mapping performed by using phenotype traits such as ARD overall survival as a quantitative trait using our GeneMiner pipeline (months 3-21).

Identification of candidate modifier genes associated with asbestos related disease development is achieved using the GeneMiner analysis platform developed by Professor Grant Morahan at The University of Western Australia. Upgrades to the GeneMiner platform to enable incorporation of the additional 20 CC strains obtained from UNC is scheduled to be completed mid-June 2021. This will allow full analysis of all 70 CC-MexTAg and the MexTAg (C57Bl/6) control groups once complete. Below are analyses of 55 completed CC-MexTAg groups, which should be considered preliminary, as at this stage there is still a high (<10%) false discovery rate (FDR). The FDR decreases significantly as data from more groups are added to the dataset, with little to no FDR once complete data from more than 60 groups are added. These data are subject to change as data from additional asbestos exposed CC-MexTAg groups are added the analysis. A comprehensive analysis will be performed once all 70 CC-MexTAg groups are completed.

Preliminary GeneMiner analysis indicates multiple 'suggestive' qualitative trait loci (QTL) observed for each ARD 'trait/phenotype' tested. The QTL are considered suggestive at this stage as a LOD score of > 7 is required for statistical significance. A representative analysis for the ARD Survival phenotype is shown in Figure 4, with complete data for all ARD phenotypes summarised in Table 2.

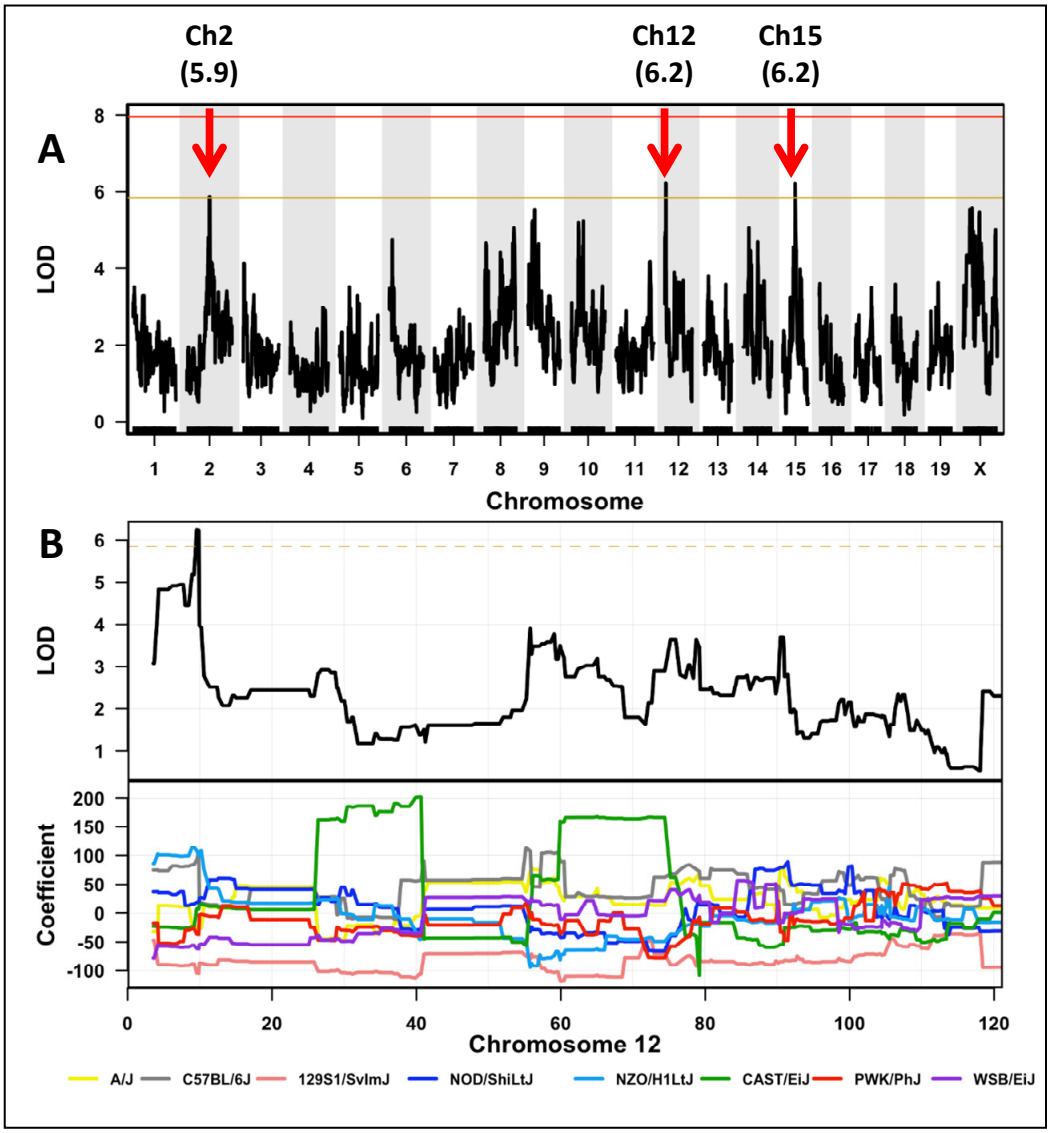

Figure 4. Qualitative trait loci associated with overall survival in asbestos exposed CC-MexTAg mice QTL analysis performed using the GeneMiner platform was used to analyse overall survival data from 55 completed CC-MexTAg groups. (A) Genome wide scan comparing overall survival between different CC-MexTAg groups. The X-axis shows chromosome position and the Y-axis shows the logarithm of odds values (LOD = -log10(P), where P values were derived from CC linkage haplotype data). The gold line indicates 'suggestive' threshold, red line indicates 'highly significant'. Suggestive QTLs identified on chromosomes 2, 12 and 15. (B) Founder coefficient plot. (Top) The -log10(P) values across chromosome 12. (Bottom) The plot of the calculated log odds ratio of eight founder alleles over chromosome 12, where the founders are color-coded.

The genome wide scan on data obtained from 55 completed CC-MexTAg groups indicated 3 suggestive QTL with LOD scores greater that 5.9 on chromosomes 2 (LOD 5.9), 12 (LOD 6.2) and 15 (LOD 6.2) associated with median survival as the phenotypic trait (Figure 4A). Closer analysis of each QTL was performed to identify exactly where each peak QTL was positioned along the respective chromosome. Focusing on Chromosome 12, our analysis indicates that the genes

associated with the peak QTL are located within a 300 kilo base region between nucleotide positions 9.5 mega base (Mb) and 9.8 Mb on chromosome 12 (Figure 4B top panel). The probability that gene variants (alleles) from any particular CC founder stain contributes to the peak QTL is represented as greater deviation in founder coefficient from 0 within the peak QTL region (figure 4B bottom panel). The data indicates that alleles from CC founder stains NZO (light blue line) and C57BL/6 (grey line) are positively associated with peak survival QTL (founder coefficient >. 100 on chromosome 12 at position 9.5-9.8 Mb), while the allele from CC founder stain 129S1 founder (pink line) are negatively associated with survival (founder coefficient <100).

Table 2: Quantity and location of ARD associated peak QTL

Phenotype/trait	Peak QTL location (chromosome) LOD >6
Survival	(2), 12, 15
Latency	(2), 12, 15
Progression	7, 14
Ascites volume	2, 4, 5*, 6, 8, 9*, 12, 16
No# mice/group with tumour at cull	10

Two statistically significant QTLs (*LOD >7) were identified on chromosomes 5 and 9 for ascites volume as a phenotypic trait. We are using the publicly available mouse genome informatics database (http://www.informatics.jax.org/) to identify both coding (known and predicted genes) and regulatory elements (regions that affect or regulate gene expression) associated with each peak QTL. These analyses will identify candidate modifier genes and regulatory elements associated with asbestos related disease development. These studies remain ongoing and a full report will be provided once data form all 70 CC-MexTAg lines have been analysed.

Aim 3: Identify human orthologs and interrogate human mesothelioma datasets.
Subtask 1: Identify human orthologues using BLAST of DNA sequences encompassing the peak SNPs and/or best candidate causal SNPs identified in Aim 2 (months 21-24).
Subtask 2: Human orthologues will be interrogated against publicly available mesothelioma data sets (TCGA) and additional human mesothelioma datasets available via CI Bueno (months 22-24).

Work on Aim 3 has been significantly delayed due to delays associated with obtaining US DoD Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval; initial application submitted 19th March 2020, final approval granted 19th May 2021. We are not sure why approval took so long. None-the-less, we are currently in the process of obtaining de-identified / anonymized human mesothelioma data and will be commencing interrogation of human mesothelioma datasets as soon as possible. We have confirmed with Prof Raphael Bueno that access to data derived from his mesothelioma tumour database is available. Similarly, we will seek access to publically available cancer databases such as The Cancer Genome Atlas Program (TCGA) supported by the National Cancer Institute (NCI, USA). We will interrogate these databases to identify and test the relevance of human orthologs (i.e. the human version of mouse genes) of any candidate genes or regulatory regions that we identify from our CC-MexTAg analyses in Aim 2. Data from these analyses will help identify the genes and their biological pathways associated with ARD, providing the necessary information for the rational design of new therapeutic modalities for the treatment of asbestos related diseases.

Publications & presentations
Throughout the duration of this research program we have had numerous opportunities to present our research data at local, national and international meetings. International conferences included;

2021: Invited speaker. Lung Club (Local), Perth, Western Australia.
2021: Invited speaker. 15th International Mesothelioma Interest Group (iMig). Brisbane. Australia.
2019: Invited speaker. Asbestos safety and eradication agency (ASEA) conference. Perth. Australia.
2019: Invited Speaker. Aust. & New Zealand Laboratory Animal Association. Perth, Australia.
2019: Invited speaker. UWA Animal Care Services Seminar series, Perth. WA.
2018: Invited speaker. 14th International Mesothelioma Interest Group (iMig). Ottawa. Canada.

With respect to publications; an invited article for a special edition of Frontiers in Oncology focusing on the immune-tumour microenvironment of thoracic cancers has been published (Behrouzfar K, Burton K, Mutsaers SE, Morahan G, Lake RA and Fisher SA (2021) How to Better Understand the Influence of Host Genetics on Developing an Effective Immune Response to Thoracic Cancers. Front. Oncol. 11:679609. doi: 10.3389/fonc.2021.679609). Additionally, a manuscript reporting on data form the first 30 CC-MexTAg group is currently in preparation (submit H2 2021) and progress on the full CC-MexTAg study is scheduled for H1 2022.

What opportunities for training and professional development has the project provided?

Training
This project has allowed the training of 2 research assistants (RAs) and a number of both Honours (a short 10 month post BSc research program) and PhD students with respect to *in vivo* (animal welfare monitoring / vivisection) and *in vitro* (cell culture, RNA sequencing and immunofluorescence) related work under the supervision of Dr Scott Fisher. We currently have 1 PhD student working on the NHMRC grant that utilised the CC-MexTAg biobank samples that were generated from this study. Additional grants utilizing the CCMT biobank samples are also in preparation.

Professional Development
All personnel associated with the daily running of this project (i.e. the students, RAs and Dr Fisher) have been afforded the opportunity for professional development primarily via presentation of work from this project at numerous local, national and international symposia and conferences (some listed above).

Dr Fisher has also undergone professional development with Prof Morahan's group with respect to the use and interpretation of the GeneMiner bioinformatics analysis platform.

How were the results disseminated to communities of interest?

Throughout the duration of this project and especially in the 12 months between July 2020-July 2021, we have had the opportunity to present updates on this project at both local (academic departments within the University of Western Australia and related Medical research institutions), and national meetings (Australian and New Zealand Laboratory Animal Association (ANZLAA), Sept. 2019 and the Asbestos Safety and Eradication Agency (ASEA), Nov 2019), and most recently at the 15th Meeting of the International Mesothelioma Interest Group (IMIG) in May 2021 (held virtually due to the initial conference scheduled in March 2020 being affected by COVID-19 restrictions). In addition to the above presentations, we are fortunate to be able to disseminate our work to a number of asbestos consumer/advocacy groups. These are often associated with the Asbestos Diseases Society of Australia (ADSA) and are mostly a non-scientific forum aimed at informing the general public and asbestos affected individuals and their families about the current progress of research related to mesothelioma and asbestos related diseases. This is usually via an annual event (Perth Mesothelioma symposium, held in October/ November each year), but may also include short invited talks at local ADSA meetings/branches on an ad hoc basis. We are also able to disseminate research updates to the asbestos consumer/ advocacy groups via our monthly National Centre for Asbestos Related Diseases (NCARD) newsletter, website www.NCARD.org.au and other social media platforms such as Twitter (@NCARD_research) and Facebook (NationalCentreforAsbestosRelatedDiseases).

What do you plan to do during the next reporting period to accomplish the goals?

We have recently applied for and receive a no cost extension from July 2021 to January 2022. During the final stages of this project (July 2021-January 2022) we will finalize the collection phenotypic data and tissue samples from the 8 remaining CC-MexTAg groups. Likewise, we will continue to process histological samples to confirm ARD diagnosis and determine histological subtype, while finalizing the collection, annotation and storage of all other samples, such as ascites and tumour derived cell lines and solid tumours for future study. The phenotypic data will be added to Aim 2 analyses as each group completes the study (maximum experimental endpoint is November 26th 2021). Additionally, we will continue to use the preliminary data derived from the 64 completed CC-MexTAg groups to identify candidate modifier genes and regulatory elements, which will be used to interrogate human mesothelioma datasets now that HRPO human ethics

approval has been granted. We will also submit the first CC-MexTAg paper highlighting the use of the CC-MexTAg model as a means to identify ARD associated genetic loci.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Identification of modifier genes affecting mesothelioma development provided by this study may have a significant impact in advancing our base knowledge of mesothelioma. Furthermore, the ongoing collection and storage of asbestos induced tumor samples from different CC-MexTAg groups with differential disease development (i.e. short vs. long overall survival, indolent vs. rapid tumour development, variable ascites development) provides a unique biological resource and dataset that will form the foundation for a long-term, asbestos induced mesothelioma research program. Indeed, the successful funding for this US Dept. Defense Ideas Award, we (CIs Lake, Morahan and Fisher) has already led to additional funding from the Australian National Health and Medical Research Council (NHMRC) for phase 2 of this study, in which we are undertaking comprehensive gene expression analysis and multiplex histological profiling on CC-MexTAg derived tumour samples. Collection of solid tumour samples from asbestos exposed mice with variable genetic backgrounds is an invaluable resource for a variety of future genetic based studies where we can assess the association between various genomic data (whole genome sequencing, somatic mutations, transcriptome, methylome etc.) with respective disease phenotypes (survival, latency, progression, ascites volume and presence of tumour.). Together, these data will provide the necessary information to better understand the biological pathways associated with mesothelioma susceptibility and progression; knowledge that is fundamental for the rational development of new diagnostic and therapeutic strategies for mesothelioma and which complements the molecular studies that are currently being pursued using clinically obtained human mesothelioma samples.

What was the impact on other disciplines?

This project brings together key disciplines including systems genetics and cancer biology to advance our understanding how an individual's genetic background affects the underlying biological processes associated with mesothelioma. To date we have established a unique animal model, in a 'first of kind' study for mesothelioma, to rapidly identify disease associated modifier genes; something that cannot be achieved using any other animal model. The unique approach of

this study and the data and resources it generates will enhance our knowledge in the fields of both mesothelioma and systems genetics as well as providing unique genetic and phenotypic information for publically available databases (i.e. mouse phenome database).

What was the impact on technology transfer?

Currently there is nothing to report. However, we will make all reasonable attempts to have the phenotypic data produced in this study entered into publically accessible data repositories such as the mouse phenome / mouse genome informatics databases when / where appropriate.

What was the impact on society beyond science and technology?

Nothing to report as yet. However, there is potential for identified modifier genes to be used in future screening of asbestos exposed individuals to assess the relative risk of developing mesothelima

5. CHANGES/PROBLEMS:

Apart from minor disruptions to our usual work environment due to COVID-19 related issues, there have been no significant delays that have impacted our ability to complete or perform this study as outlined in the original application and funding agreement. We have noted that many of the 20 additional CC-MexTAg groups have survived out to the maximum survival time, meaning that 11 groups remain on study as of May 31st 2021.

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

In the last 12 months we requested and received approval for a 'no cost extension' to carry over grant funding until May 31st 2021. The request was based on the delay to breeding the last 20 CC-MexTAg groups that we experience in early 2019 (during the previous reporting period).
The funding extension allows for payment of animal husbandry and agistment costs associated with completion of breeding and experimental work related to the last 20 CC-MexTAg groups.

As we still have 11 groups remaining on study, we will be requesting for an extension of the no-cost extension to December 31st 2021. This will allow 100% completion of the proposed 70 CC-MexTAg groups and will ensure the study remains adequately powered for the GeneMiner analyses We can assure that all animal work and analyses will be completed by Nov 26th 2021 at the latest and we will not be requesting any further extensions for this grant.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There have been change that have had a significant impact on expenditure, other than 'delays' associated with receiving invoices for animal related work (agistment, etc.). There is usually a 2-3 month delay in receiving invoices from UWA Animal Care Services although payment is promptly made when received.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

The only significant delay we experienced was related to obtaining HRPO approval for use of anonymized human mesothelioma patient datasets. Our initial application was submitted March 2020 and final approval was not granted until May 2021. We can only presume COVID-19 related issues in the US delayed to processing of our application.

Significant changes in use or care of vertebrate animals
In the 12 month reporting period for this progress report we have had to update the breeding and experimental animal ethics protocols related to the CC-MexTAg work. This was required as the local IACUC approval (UWA AEC protocols) had reached the 5 year expiry limit. The new CC-MexTAg breeding (RA/3/300/131) and experimental protocols (RA/3/100/1730) have received local IACUC and US DoD ACCURO approval.

Significant changes in use of biohazards and/or select agents

No changes to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

2021: Invited speaker. 15th International Mesothelioma Interest Group (iMig). Brisbane. Australia.

Journal publications. In the last 12 months we have published one review article and have one manuscript in preparation related to the US DoD funded CC-MexTAg project.

Peer Review

Behrouzfar K, Burton K, Mutsaers SE, Morahan G, Lake RA and Fisher SA (2021) How to Better *Understand the Influence of Host Genetics on Developing an Effective Immune Response to Thoracic Cancers*. Frontiers in Oncology. 11:679609.
doi: 10.3389/fonc.2021.679609

In preparation*:*

Asbestos related disease as a complex trait: host genetics affects disease onset after asbestos exposure. Scott A. Fisher, Kimberley Burton, Tracy Hoang, Pristina Goh, Sylvia Young, Anna K. Nowak, W. Joost Lesterhuis, Bruce W.S. Robinson, Grant Morahan, and Richard A. Lake.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Noting to report

- **Website(s) or other Internet site(s)**

  www.NCARD.org.au (Lab website, limited scientific data; URL links to published work).

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

Biobank of CC-MexTAg derived histological (tissue) and tumour samples, cell lines, RNA derived from CC-MexTAg tumours.

CC-MexTAg phenotype and GeneMiner analysis databases are generated as the study progresses.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Professor Richard Lake	No Change
Professor Grant Morahan	No Change
Dr Scott Fisher	No Change
Dr W. Joost Lesterhuis	No Change
Professor Anna Nowak	No Change
Professor Raphael Bueno	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No Change.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Not applicable. Nothing to report.

9. APPENDICES:

Nil