AWARD NUMBER: W81XWH-18-1-0343
CA170299

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

PRINCIPAL INVESTIGATOR: Richard Lake

CONTRACTING ORGANIZATION: The University of Western Australia.

REPORT DATE: August 2020

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel 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 2020	Annual	15 July 2019 – 14July 2020

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

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel 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 second 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 55 of 70 groups and are able to perform interim analysis. The additional 15 CCMT groups remain on study and we expect to have complete data by May 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 over the next 12 months. To date, we have completed or are about to complete Aims 1 and 2. Initiation of Aim 3 will occur in H2 2020, once US DoD human ethics approval has been received. We are on track to achieve all stated aims by the end of May 2021.

15. SUBJECT TERMS
CC-MexTAg, collaborative cross, MexTAg, mesothelioma, asbestos, genetic modifiers

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
U	U	U	UU	22	**19b. TELEPHONE NUMBER** *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   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 18-23).
   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).

   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).

   Publication and presentation of data:
   Major publications month 24. Local, national, and international data (conference) presentations: months 1-24.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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.

Summary of current progress report. We are pleased to report that during the stage 2 reporting period (15th July 2019-14th July 2020), we have successfully completed the generation and asbestos exposure of all proposed 70 CC-MexTAg groups and therefore have successfully completed Aim 1 subtasks 1, 2, and 3. While 16 of the last 20 groups remain on study, we are pleased to report that 55 of 70 CC-MexTAg groups, (including a MexTAg control group on the parental B6 background) have now completed the study, for which we have complete survival and phenotypic data. These data have been used in preliminary analyses outlined in Aim 2, subtasks 1 and 2 above and are described in detail below (Figure 4, Table 1.2). 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 have also initiated work on Aims 3 and 4. We have received local Human Ethics approval (University of Western Australia) and are currently waiting on the US DoD Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval (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 presume the delay in response is related to COVID-19, and are following up on the progress of our submission. With respect to Aim 4; we are preparing a manuscript reporting on data form the first 30 CC-MexTAg groups. This is planned for submission in October 2020. We had accepted an oral presentation of our work at the 15th Meeting of the International Mesothelioma Interest Group (IMIG) in March 2020. However, this was postponed for 12 months due to the COVID-19 pandemic. We still plan to present at IMIG in 2021, if the COVID-19 pandemic restrictions allow.

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 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, we do not envisage that COVID-19 will have a significant impact in our ability to complete this study. We will continue to manage the situation and adapt our research as required, should the situation in Western Australia change. We will inform the US DoD if any significant delays to the remaining aims are experienced.

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)

As mentioned above, we successfully generated and asbestos exposed 70 different CC-MexTAg groups. Furthermore, we have complete data on 55 groups; 54 CC-MexTAg groups and a MexTAg control group, while the other 16 CC-MexTAg groups remain on study. Along with overall survival, we collected 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 also collected tissue samples (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.

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 the different asbestos exposed groups (Figure 1B, C). 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 ($r^2 = 0.9973$, $p < 0.001$), but not overall survival and disease progression ($r^2 = 0.0083$, p = ns; Figure 2).

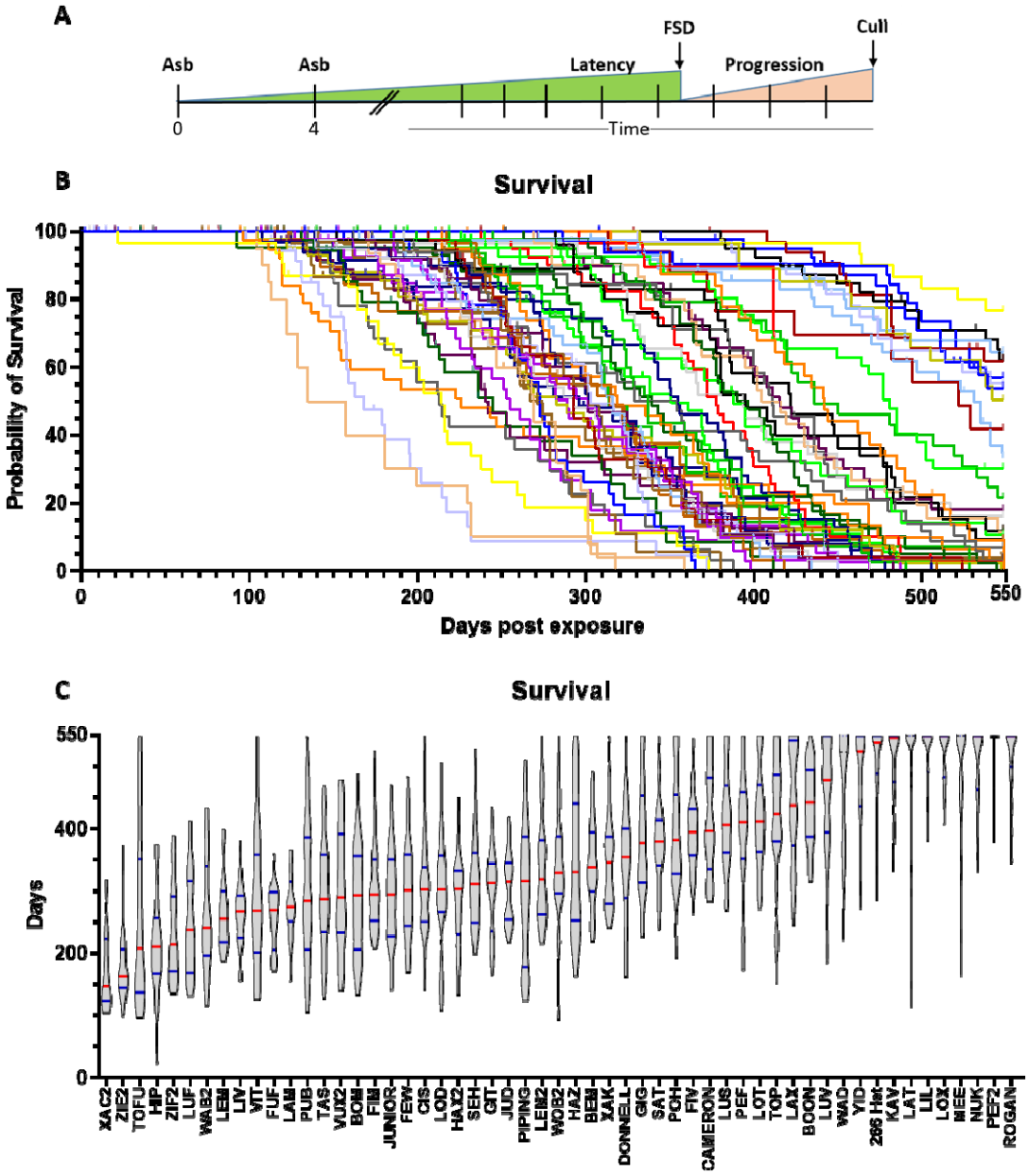

Figure 1: Variation in median survival in asbestos exposed CC-MexTAg mice. Groups of CC-MexTAg mice (n=55, median 36 mice/group) were exposed to a total of 6 mg of asbestos via two consecutive intraperitoneal injections, 4 weeks apart and assessed for disease development over time. (A) Experimental schematic.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

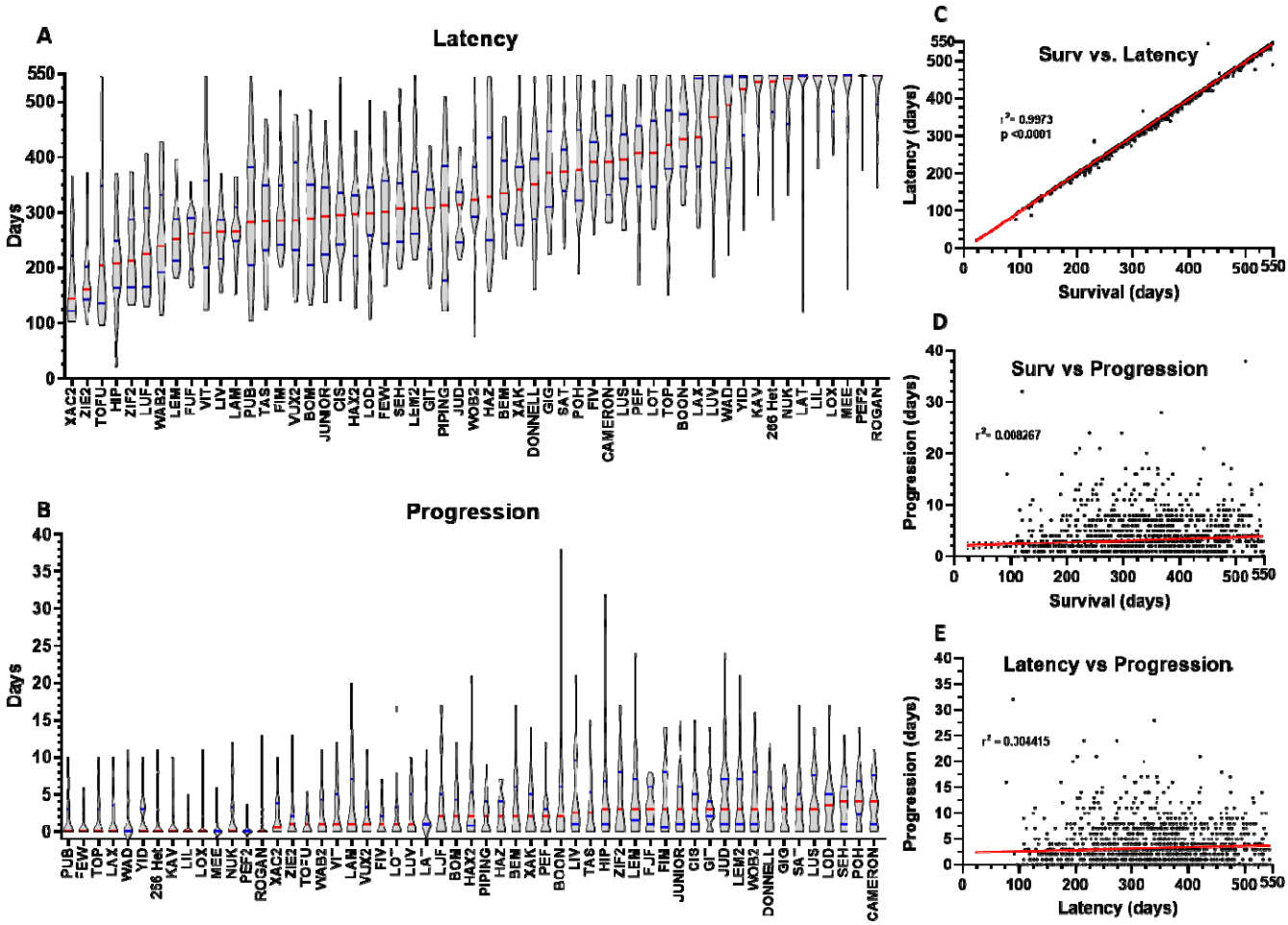

Figure 2: Asbestos related disease latency drives variation in overall survival in asbestos exposed CC-MexTAg mice. (A, B) Violin plots depicting variation in ARD latency and progression (ranked) respectively (median, red line and interquartile range, blue lines) for each of the 55 asbestos exposed CC-MexTAg groups. (C-E) Scatter plots depicting correlations (Pearson's) between individual phenotypes for individual mice.

Additional biological correlates related to ARD phenotype including mean ascites volume and the number of mice with tumours in each group also strongly correlate with survival and latency, but not progression (Figure 3).

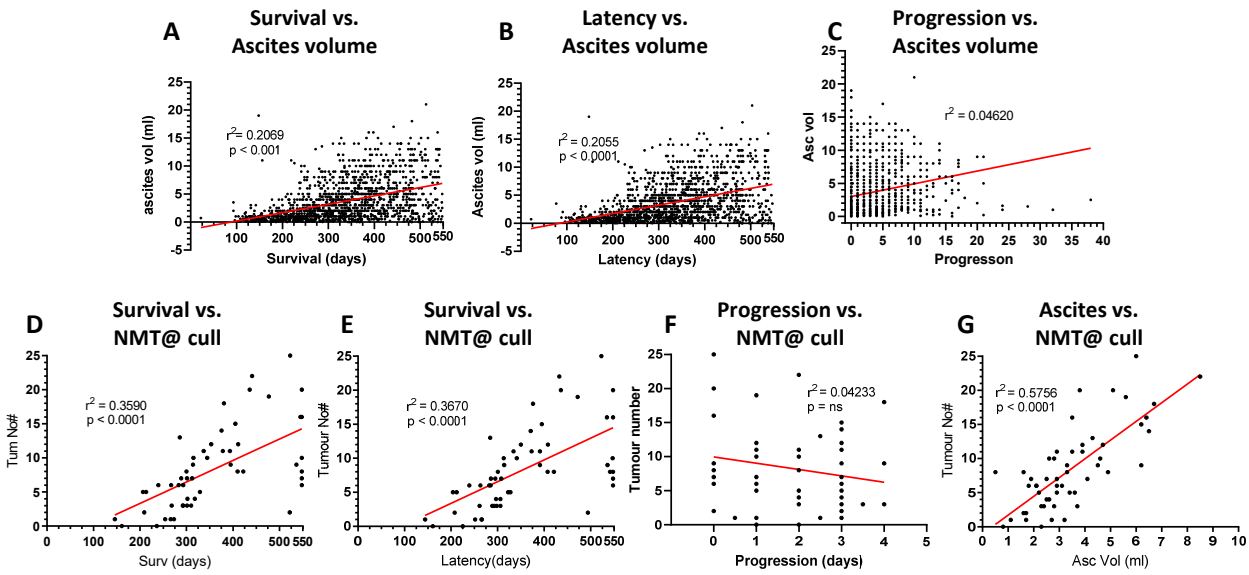

Figure 4.3: Asbestos related disease phenotype correlations (A-C) Plots depicting correlations between survival, latency and progression relative to ascites volume for individual mice. (D G) Correlations between the numbers of mice with tumour at cull (NMT@ cull) for each CC-MexTAg group and respective ARD phenotypes. Red line = line of best fit. r^2 = Pearson's correlation coefficient.

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 55 different asbestos exposed groups are shown in Table 1. We have collected and prepared 1704 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 55 CC-MexTAg groups to complete the study, including the number of ascites derived cells lines made from each group and the total number of histology samples (per animal) in each group. To date, we have generated a total of 803 ascites derived cell lines, 48 tumour derived cell lines and 249 tumour samples for RNA sequencing analysis. The wealth of data from this program is a significant resource that will undoubtedly prove valuable in future research into ARD.

Table 4.1: 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 15 CC MexTAg groups.

Group No#	Name	No# Tum in RNA later	No# Tum TC stocks	No# ascites TC stocks	No# histology samples
C1	**266 Het**	4	0	17	32
1	**NUK**	0	0	4	35
2	**LIL**	4	3	6	40
3	**LOT**	0	1	15	40
4	**PEF**	0	0	12	31
5	**LUV**	0	1	6	41
6	**XAC2(LID)**	0	0	4	25
7	**BEM**	1	4	7	38
8	**HAX2**	0	0	16	40
9	**TOP**	0	0	13	38
10	**SEH_**	0	0	8	38
11	**LEM2**	0	0	7	41
12	**HIP**	0	1	2	31
13	**LOD**	0	0	11	35
14	**LAM**	0	0	15	37
15	**LIV**	0	1	11	32
16	**XAK**	0	4	14	44
17	**FIM**	0	1	4	38
18	**JUD**	0	1	12	32
19	**LUF**	0	0	8	40
20	**LEM**	0	0	13	38
21	**FIV**	0	0 Tum +1PF	7	42
22	**JUNIOR**	0	0	4	39
23	**DONNELL**	4	5	20	39
24	**SAT**	3	3	20	40
25	**HAZ**	1	0	13	40
26	**PIPING**	5	3	19	40
27	**ZIE2**	0	0	2	39
28	**FEW**	0	4	4	37
30	**ZIF2**	0	0	6	33
31	**VUX2**	4	3	24	35
32	**WOB2**	2	2	31	43
33	**VIT**	2	1	21	30

34	**GIT**	5	3	34	40
35	**CIS**	8	3	31	35
36	**CAMERON**	6	0	23	25
37	**FUF**	2	1	20	31
38	**WAB2**	3	0	23	41
39	**LUS**	8	1	25	30
41	**TAS**	10	0	31	40
42	**BOM**	1	0	21	41
43	**POH**	8	0	26	30
45	**YID**	26	1	33	43
46	**TOFU**	2	0	15	39
47	**LAX**	16	0	23	40
48	**ROGAN**	15	0	18	44
49	**PUB**	2	0	27	43
50	**GIG**	20	0	28	43
51	**LOX**	18	1	7	34
52	**BOON**	23	0	24	35
53	KAV	14	0	5	28
55	**WAD**	2	0	11	18
57	**PEF2**	9	0	0	30
58	**MEE**	7	0	1	38
59	**LAT**	13	0	1	31

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. As only 55 CC-MexTAg groups are complete, the data below can only 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.

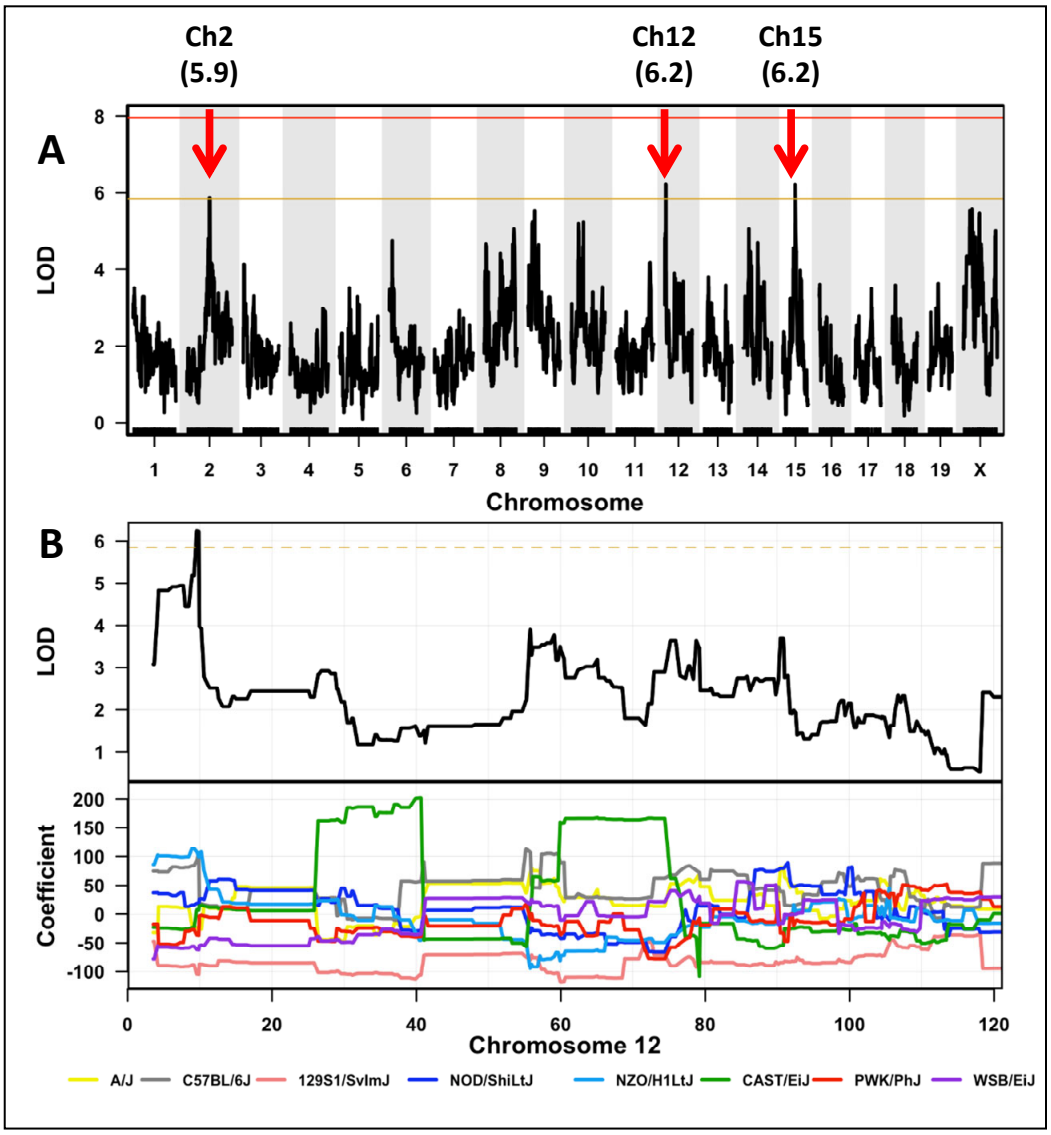

Figure 4. Qualitative trait loci associated with overall survival in asbestos exposed CC-MexTAg mice QTL analysis performed using the GeneMiner platform was used to analyse overall survival data from 55 completed CC-MexTAg groups. (A) Genome wide scan comparing overall survival between different CC-MexTAg 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 4.2: 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 is yet to begin as we are awaiting US DoD Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval (application submitted 19th March 2020), prior to commencing interrogation of human mesothelioma datasets. 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.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training
This project has allowed the training of 2 research assistants 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.

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.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

In the 12 months between July 2019-July 2020, we have had the opportunity to present updates on this project at both local (academic departments within the University of Western Australia), and national meetings (Australian and New Zealand Laboratory Animal Association (ANZLAA), Sept. 2019 and the Asbestos Safety and Eradication Agency (ASEA), Nov 2019). An additional presentation at the 15th Meeting of the International Mesothelioma Interest Group (IMIG) in March 2020 was postponed due to COVID-19 restrictions. In addition to the above presentations, we are also 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 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.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the final 12 months of this project we will continue with collecting phenotypic data and tissue samples from the 16 remaining CC-MexTAg groups. We will continue with processing of histological samples to confirm ARD diagnosis and determine histological subtype, while continuing to collect, annotate and store other samples, such as ascites derived tumour 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 May 2021). Additionally, we will continue to use the preliminary data derived from the 55 completed CC-MexTAg groups to identify candidate modifier genes and regulatory elements, which will be used to interrogate human mesothelioma datasets once HRPO approval is 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.

IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

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 data 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.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Apart from COVID-19 related issues mentioned above, 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.

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

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.

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.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes to report. Awaiting US DoD Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval (application submitted 19th March 2020),

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: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

2020*: Invited speaker. 15th International Mesothelioma Interest Group (iMig). Brisbane. Australia. (*Postponed until March 2021- COVID-19 permitting)
2019: Invited speaker. Asbestos safety and eradication agency (ASEA) conference. Perth. Australia.
2019: Invited Speaker. Aust. & New Zealand Laboratory Animal Association conference, Perth.
2019: Invited speaker. UWA Animal Care Services Seminar series, Perth. WA.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.
First CC-MexTAg Manuscript in preparation.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of*

publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*

- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

- 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?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No Change.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

No Change.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Not applicable. Nothing to report.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.