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**AWARD NUMBER:** W81XWH-16-1-0271

**TITLE:** Somatic Mosaicism for Cancer Predisposition Genes and Pancreatic Cancer

**PRINCIPAL INVESTIGATOR:** Christine A. Iacobuzio-Donahue

**RECIPIENT:** Sloan Kettering **Institute for Cancer Research**
**New York NY 10065**

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**TYPE OF REPORT:** Final

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

# REPORT DOCUMENTATION PAGE

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| October 2019 | Final Progress | 15 Jun 2016-14 Jun 2019 |

**4. TITLE AND SUBTITLE**

Somatic Mosaicism for Cancer Predisposition Genes and Pancreatic Cancer

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**5b. GRANT NUMBER**
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**6. AUTHOR(S)**
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**Sloan Kettering Institute for Cancer Research**

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U.S. Army Medical Research and Materiel Command
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**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Somatic mosaicism refers to the occurrence of two genetically distinct populations of cells within an individual, derived from a postzygotic mutation. Unlike inherited mutations in which the variant allele is present in all cells in the body, somatic mosaic mutations may affect only a subset of cells and may not be passed on to their progeny. The extent to which somatic mosaicism for PDA cancer predisposition genes accounts for PDA incidence is unknown. We performed targeted sequencing using the MSK-IMPACT platform containing 468 cancer genes to sequence 256 normal tissues from 35 patients, including their matched tumor tissues. We note that somatic mosaicism is indeed present in our patient cohort as evidenced by mutations private to a single normal tissue. These variants are in known driver genes and some are predicted to have deleterious consequences. We have also found recurrent somatic alterations in a subset in genes, most often in the *Androgen Receptor*. The significance of this findings is unknown but quite provocative, as they are confined to solid tissues only and not the blood in the patients they are found in. The most common variant identified affects G473 as an inframe deletion of various sizes (i.e. AR p.G473del, or AR p. G465_G473del). Skin and lung contains on average double the number of mutations than other normal tissues, consistent with UV and environmental damage over the lifetime of the patients.

**15. SUBJECT TERMS**
pancreatic cancer, mutation, genetics, screening, somatic mosaicism, hereditary cancer, clonal hematopoesis

| 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 | | 19b. TELEPHONE NUMBER *(include area code)* |

## TABLE OF CONTENTS

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

   Somatic mosaicism refers to the occurrence of two genetically distinct populations of cells within an individual, derived from a postzygotic mutation. Unlike inherited mutations in which the variant allele is present in all cells in the body, somatic mosaic mutations may affect only a subset of cells and may not be passed on to their progeny. Somatic mosaicism is a clinically relevant phenomenon for a variety of human diseases, including rare and common cancers. Our work being performed under this award aims to derive a better understanding of the prevalence and influence of somatic mosaicism in PDA incidence and recurrence. Should somatic mosaicism account for a subset of PDA it could dramatically change screening approaches, patient management and genetic counseling.

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

   pancreatic cancer, mutation, genetics, screening, somatic mosaicism, hereditary cancer, clonal hematopoesis

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

   **What were the major goals of the project?**

   As outlined in our Statement of Work, Major Task 1 was to Determine the Complete List of Variants in Normal Tissues in our Patient Cohort (100% complete), Major Task 2 was for Data Interpretation of this complex dataset (80% complete) and Major Task 3 was Manuscript Preparation (10% complete)

   **What was accomplished under these goals?**

   We performed targeted sequencing using the MSK-IMPACT platform containing 468 cancer genes to sequence 256 normal tissues from 35 patients, including their matched tumor tissues. Analysis is complete and reveals ~600 unique variants per patient. As expected, the majority of variants identified were in non-coding regions or silent. Moreover, for each patient the majority of variants were present in all tissues at allele frequencies of 25% or greater consistent with germline status. **Figure 1a** (next page) illustrates the histograms of mutant allele frequency for all high quality variants identified per sample.

   Tumor specific mutations include *KRAS, TP53, CDKN2A* and *SMAD4* as the most frequent driver events consistent with the known genetics of pancreatic cancer (**Figure 1b, next page**). The need for inclusion of the tumor tissues was only realized after the project commenced which resulting in a delay of this work. However, this step has greatly clarified those variants

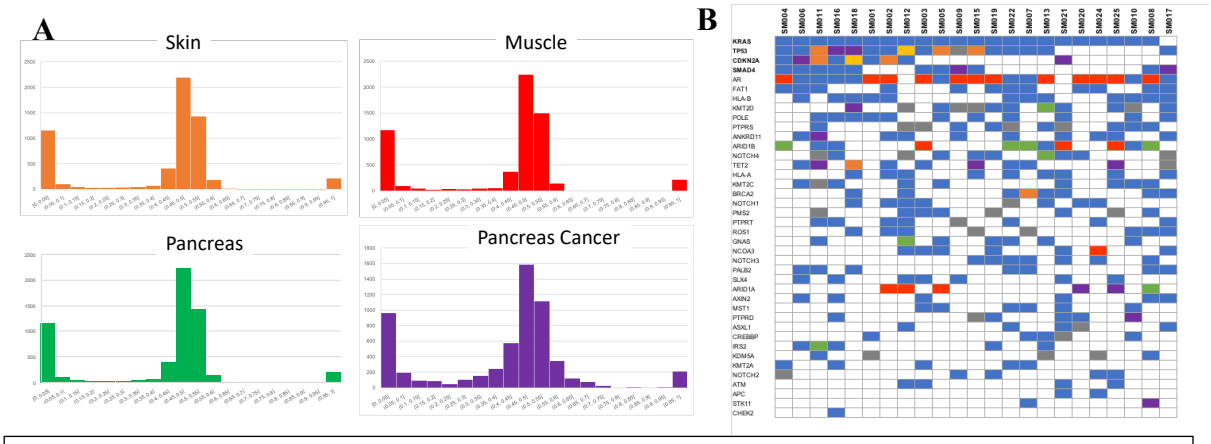


**Figure 1. Summary of High Quality Variants in Representative Normal Tissues and Pancreatic Cancer.** A. Each histogram represents a summary of all variants across all patients. Allele frequencies (Y axis) were binned into 0.05% increments with 0% on the left and 100% at the right (X axis). B. Oncoprint of the tumor-specific variants for the first 23 patients analyzed.

indicating contamination of the normal tissues by circulating tumor cells and improved the quality of the dataset.

Once accounting for both germline and tumor-specific variants and excluding them from further study, we analyzed the variants in normal tissues specifically. These variants fell into two major categories that we are referring to as somatic mosaic pattern 1 and 2 (SM1 and SM2). SM1 variants are those present in one or more normal tissues but not all for the patient (candidate somatic mosaics), whereas SM2 variants were present in all normal tissues but at allele frequencies <25%. We hypothesized that SM2 variants are enriched for hematopoietic specific mutations because blood cells would be present within vascular spaces of the other normal tissues examined of that patient. We therefore evaluated the blood samples available for these patients, resulting in another delay while these samples were analyzed and included in the dataset. However, this was again worth pursuing as it indeed revealed mutations in blood that were "contaminating" the other tissues. We are confident in this interpretation as a subset of patients were found to have clonal hematopoiesis associated mutations in genes such as *TET2, ARID1A*, and *DNMT3A*; these mutations had the highest allele frequencies in blood compared to the other normal tissues or tumor for that patient**. Table 1** illustrates these mutations found.

**Table 1: Representative Blood-Specific Mutations.**

| Gene | Variant Class | Consequence | silent_non_s | HGVSc | HGVSp | HGVSp_Shor |
|---|---|---|---|---|---|---|
| ARID1A | In_Frame_D | inframe_del | non-silent | c.261_278de | p.Ala88_Gly | A88_G93del |
| BCL2 | Missense_M | missense_va | non-silent | c.163C>A | p.His55Asn | H55N |
| ARID1A | In_Frame_D | inframe_del | non-silent | c.261_278de | p.Ala88_Gly | A88_G93del |
| PGR | Missense_M | missense_va | non-silent | c.2362C>T | p.Arg788Trp | R788W |
| MST1R | Missense_M | missense_va | non-silent | c.1706C>T | p.Pro569Leu | P569L |
| MPL | Missense_M | missense_va | non-silent | c.1544G>T | p.Trp515Leu | W515L |
| TET2 | Missense_M | missense_va | non-silent | c.5704T>C | p.Tyr1902His | Y1902H |
| BCOR | Splice_Regic | splice_regior | non-silent | c.86+8C>T | | |
| TOP1 | Splice_Regic | splice_regior | non-silent | c.1708-7C>T | | |
| DNMT3A | Missense_M | missense_va | non-silent | c.2645G>A | p.Arg882His | R882H |
| SDHA | Missense_M | missense_va | non-silent | c.17G>A | p.Gly6Asp | G6D |
| TSHR | Missense_M | missense_va | non-silent | c.1601G>T | p.Arg534Leu | R534L |
| MSH2 | Missense_M | missense_va | non-silent | c.2039G>A | p.Arg680Gln | R680Q |
| RAD54L | Missense_M | missense_va | non-silent | c.317G>A | p.Arg106His | R106H |
| ALK | Missense_M | missense_va | non-silent | c.563G>A | p.Arg188His | R188H |

We further refined our dataset by removing all blood-specific mutations in the SM1 category so as to identify somatic mosaics in other normal tissues. Our rationale is that we will enrich for true somatic mosaic mutations in the non-hematopoietic tissues from the dataset. While our analysis is ongoing we can already make some conclusions related to our goals of this work. First, somatic mosaicism is present in our patient cohort

as evidenced by mutations private to a single normal tissue. The mean allele frequency of these variants is ~3% across all normal tissues suggesting the vast majority occurred much later than the gestational period of the individual. Determinations of the number of clones per individual is ongoing. Second, these variants are in known driver genes and some are predicted to have deleterious consequences (i.e. nonsense mutations, frameshift mutations). While our targeted assay was biased towards cancer genes, we nonetheless can conclude that functionally deleterious somatic mutations in cancer genes occur. Third, we have found recurrent somatic alterations in a subset in genes, most often in the *Androgen Receptor*. The significance of this findings is unknown but quite provocative, as they are confined to solid tissues only and not the blood in the patients they are found in. The most common variant identified affects G473 as an inframe deletion of various sizes (i.e. AR p.G473del, or AR p. G465_G473del). Fourth, Skin and lung contains on average double the number of mutations than other normal tissues, consistent with UV and environmental damage over the lifetime of the patients.

In summary, we are pleased to report that despite two technical setbacks that delayed our progress, we have ultimately achieved our primary goal of creating a dataset to explore the extent that somatic mosaicism is associated with pancreatic cancer. The analysis is complex and challenging but not insurmountable and we anticipate manuscript submission in six-8 months to a high impact journal.

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

In the course of this work a high school student worked with the postdoctoral fellow in charge of this project and learned how to extract and quantify genomic DNA from tissues.

**How were the results disseminated to communities of interest?**

Nothing to report.

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

Nothing to report.

4. **IMPACT:** Describe 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?**

Nothing to report.

**What was the impact on other disciplines?**

Nothing to report.

**What was the impact on technology transfer?**

Nothing to report.

**What was the impact on society beyond science and technology?**

Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

   Nothing to repor

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

   Nothing to report.

   **Changes that had a significant impact on expenditures.**

   Nothing to report.

   **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**

   Nothing to report.

   **Significant changes in use or care of vertebrate animals.**

   Not applicable.

   **Significant changes in use of biohazards and/or select agents**

   Nothing 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**

Nothing to report.

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

Nothing to report.

**Other publications, conference papers, and presentations**.

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

Nothing to report.

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

Nothing to report.

- **Other Products**

Nothing to report.

## 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."*

| | |
|---|---|
| Name: | Christine A. Iacobuzio-Donahue |
| Project Role: | Principal Investigator |
| Researcher Identifier: | orcid.org/0000-0002-4672-302 |
| Nearest Person Month Worked: | 1 |
| Contribution to Project: | Dr. Iacobuzio has overseen all sample processing, sample submissions and analyses of the data. |

| | |
|---|---|
| Name: | Peter Allen |
| Project Role: | Co-Investigator |
| Researcher Identifier: | not available |
| Nearest Person Month Worked: | 1 |
| Contribution to Project: | Dr. Allen has worked with Dr. Iacobuzio to select samples for initial submission to the Genomics Core. |

| | |
|---|---|
| Name: | Hitomi Sakamoto |
| Project Role: | Postdoctoral Fellow |
| Researcher Identifier: | not available |
| Nearest Person Month Worked: | 6 |
| Contribution to Project: | Dr. Sakamoto has performed all genomic DNA extractions of all tissues, managed all sample submissions to the Genomics Core and analyzed all data under Dr. Iacobuzio's supervision. |

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

Nothing to report.

**What other organizations were involved as partners?**

Nothing to report.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** For collaborative awards, independent reports are required from BOTH the Initiating 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.

## 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.

None.