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TITLE: *Targeting Satellite Repeat RNAs in High-Grade Serous Ovarian Cancer*

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Targeting Satellite Repeat RNAs in High-Grade Serous Ovarian Cancer

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6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Massachusetts General Hospital
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A. Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: There is a critical need for novel therapeutic strategies in HGSOC, specifically for platinum-resistant disease. Recent work from our lab and others has discovered aberrant expression of repeat non-coding RNAs across many cancers, and these repeat RNAs have active reverse transcription (RT) and expansion in cancer genomes, are recognized by pathogen recognition receptors and can trigger cancer cell death and alterations in the immune microenvironment. Thus, the goals of this project to 1) define the spectrum of repeat RNAs expressed across epithelial ovarian cancers and link these to immune characteristics of the tumor and response to therapies and 2) target repeat RNA reverse transcription as a potential therapeutic strategy in HGSOC. To date, Total RNASeq was performed on 32 patient-derived ovarian cancer cell lines, 11 HGSOC PDX, 11 additional ovarian cancer cell lines, revealing abundant repeat RNA expression from all three major subclasses. Comparison of repeatome data from HGSOC models with previously generated RNA-seq data from colorectal cancer and pancreatic ductal adenocarcinoma models shows that repeat RNA profiles are unique across epithelial cancers. In HGSOC models, we find SAT and HERV repeats display the most variable expression. In particular, HSATII, a cancer specific satellite, is strongly expressed in HGSOC and displays highly variable expression across different models. Preliminary data targeting HSATII RNA levels shows cytotoxicity in some HGSOC cell lines, and combinatorial therapies are currently being investigated in vitro and in vivo using xenograft models.

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**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	20	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Although the majority of women with HGSOC will enter remission following cytoreductive surgery and platinum-based chemotherapy, most experience disease relapse and ultimately die from increasingly platinum- and treatment-resistant disease. More recently, poly ADP ribose polymerase inhibitors (PARPi) and immune-based therapies have been and are continuing to be explored as additional therapies in HGSOC. However, PARPi are less effective in platinum-resistant disease, and the immunosuppressive tumor microenvironment (TME) has limited the activity of immunotherapies in HGSOC to date. Thus, there is a critical need for novel therapeutic strategies in HGSOC, specifically for platinum-resistant disease. Recent work from our lab and others has discovered aberrant expression of repeat non-coding RNAs across many cancers, and these repeat RNAs are now known to behave like viruses with active reverse transcription (RT) and expansion in cancer genomes, and they are recognized by pathogen recognition receptors, triggering cancer cell death and alterations in the immune tumor microenvironment. Given this, the overall goals of this project were to 1) define the spectrum of repeat non-coding RNAs expressed across epithelial ovarian cancers and to link these to immune characteristics of the tumor and response to therapies and 2) target repeat RNA reverse transcription as a potential therapeutic strategy in high grade serous ovarian cancer.

2. KEYWORDS:

high grade serous ovarian cancer, repeat non-coding RNAs, satellite repeats, reverse transcription, tumor immune microenvironment, locked nucleic acids

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Quantify repeat RNAs in HGSOC cell lines and PDX models – months 1-6
- 100% completed

Major Task 2: Perform RNA-ISH/IHC on Epithelial Ovarian Cancer (EOC) TMAs – months 0-12
- 100% completed

Major Task 3: Exosome isolation and RNA-seq of exosomes – months 9-12
- 75% completed

Major Task 4: Inhibit repeat RNA RT with NRTIs in HGSOC cell lines and PDX models – months 12-15
- 100% completed

Major Task 5: Evaluate response to NRTI therapy in xenograft tumors – months 15-24
- 75% completed

What was accomplished under these goals?

Specific Aim 1: Define the expression of non-coding repeat RNAs in HGSOC cell lines and human tumors using total RNA-sequencing

Major Task 1: Quantify repeat RNAs in HGSOC cell lines and PDX models

Major Activities: We have utilized our Total RNASeq platform and novel computational pipelines to quantify the repeatome in HGSOC model systems. To date, Total RNASeq has been performed on 32 patient-derived ovarian cancer cell lines, 11 HGSOC PDX, 11 additional ovarian cancer cell lines and several fallopian tube epithelial cell lines as normal controls.

Key outcomes: Overall, we detect abundant repeat RNA expression from all three major subclasses, endogenous retroviruses (HERV), and satellites (**Fig. 1A; see appendix**). Comparison of repeatome data from EOC models with previously generated RNA-seq data from colorectal cancer (CRC) and pancreatic ductal adenocarcinoma (PDAC) preclinical models reveals that repeat RNA profiles are unique across epithelial cancers and repeat RNA profiles can be used to cluster these models by cancer type, identifying discrete clusters of models based on repeat RNA expression (**Fig. 1B**). Across HGSOC models, we find variable expression of each subclass of repeats, with SAT and HERV displaying the most variable expression (**Fig. 1C, D**). In particular, *Human satellite II* (HSATII), a cancer specific satellite, is strongly expressed in HGSOC and displays highly variable expression across different models (**Fig. 1A**).

Major Task 2: Perform RNA-ISH/IHC on Epithelial Ovarian Cancer (EOC) TMAs

Major Activities: We selected repeat RNAs from three distinct subsets that demonstrated high and/or variable expression in our preclinical models for validation in human tumors. Therefore, we obtained RNA-ISH probes complimentary to HSATII (SAT), HERV-H (ERV) and LINE-1 (retrotransposon) sequences and applied them to a tissue microarray (TMA) of >150 cases of human epithelial ovarian cancers using RNA-in situ hybridization (RNA-ISH). In addition, the TMA was stained with antibodies to CD8 and CD163 to quantify cytotoxic T cells and tumor-associated macrophages, respectively. RNA and protein signal was quantified using digital image analysis on the Halo software (Indica Labs).

Key outcomes: These experiments confirmed that repeat RNAs are indeed expressed in human EOC and their expression levels vary from patient to patient (**Fig 2A**). Combined RNA-ISH and immunohistochemistry for cytotoxic T cells and tumor-associated macrophages were also analyzed to identify any correlations between repeat RNA expression levels with immune cell infiltrates in the tumor. Thus far, we find no correlation between CD163+ macrophages or CD8+ cytotoxic T cells with HSATII, HERV-H and LINE-1 (**Fig 2B).** We are also actively investigating the relationship between repeat RNA expression and clinical outcomes using available clinical data from tumors included in the TMA.

Major Task 3: Exosome isolation and RNA-seq of exosomes

Major Activities: We have isolated extracellular vesicles (EVs) from 4 HGSOC cell lines by collecting supernatant from 3D cultures and performing standard filtering and ultracentrifugation procedures. We have also developed a column-based purification method of isolating EVs from EOC cell line 3D culture supernatant and confirmed the yield based on protein expression of published EV surface markers (**Fig 3A**). Total RNA was purified from the collected EVs and analyzed for repeat RNA expression. As is parental cells, a diverse array of repeat RNA species are detected in the EV RNA (**Fig 3B)**. In fact, EOC EVs were found to be enriched for repeat RNAs compared to their parental cell counterpart (**Fig 3C**). Based on this work, we are currently testing the transcriptional and functional effects of exposing human monocytes to EOC cell line derived EVs.

Specific Aim 2: Test the effect of repeat RNA RT inhibition as a therapeutic strategy in HGSOC
Major Task 4: Inhibit repeat RNA RT with NRTIs in HGSOC cell lines and PDX
Major Task 5: Evaluate response to NRTI therapy in xenograft tumors
Major Activities and Key Outcomes: In Aim 2, we hypothesized that targeting repeat RNA reverse transcription using NRTIs may be an effective therapeutic strategy in EOC. Thus, we treated a panel of EOC cell lines with a selection of NRTIs *in vitro* and tested cytotoxicity and effect on repeat RNA expression. None of the NRTIs that were tested have demonstrated cytotoxicity as a single agent in EOC cell lines. Next, we tested NRTIs combined with epigenetic therapies (azacytidine and HDAC inhibitor) and cytotoxic chemotherapy (carboplatin and paclitaxel). While chemotherapy agents and HDAC inhibitors demonstrate single-agent cytotoxicity in the majority of EOC cell lines, no additive or synergistic activity was observed when combined with NRTIs, suggesting that NRTIs are likely not effective in EOC models, at least as single agents or in the combinations we have tested. Given this, we are now attempting to target satellite repeat RNA reverse transcription using locked nucleic acids (LNAs). We chose to focus first on HSATII, given its known immunomodulatory properties and its variable expression across different EOC models, providing a system in which to study cell lines with high and low HSATII expression. Significant cytotoxicity was observed in several cell lines transfected with LNAs designed specifically to target *HSATII* RNA (**Fig. 4A**). RNA-Seq results show that LNA increases HSATII RNA levels early after transfection (**Fig. 4B**), suggesting that increased SAT RNA may be toxic to EOC cells. In addition, cell lines treated with HSATII LNA demonstrate upregulation of innate immune response genes and activation of the interferon pathway (**Fig. 4C**), which may play a role in the cytotoxicity observed with this therapy. Interestingly, HSATII LNA also increases expression of MHC Class I genes and PDL1 on tumor cells (**Fig. 4D)**, and this was confirmed at the protein level by flow cytometry (**Fig 4E**). We therefore hypothesize that increasing SAT repeat RNAs in tumor cells may be a method to sensitize tumor cells to immunotherapies, which to date have shown only minimal activity in ovarian cancer. Currently, we are working to further investigate these findings in human tumors by correlating HSATII RNA expression in tumor cells with immune cellular infiltrates in tumor, as well as PD-L1 expression on tumor and immune cells. In parallel, we are testing the effect of HSATII LNA *in vivo*, utilizing murine xenograft models to determine the cell autonomous effects of HSATII LNA. In the future, syngeneic immunocompetent models will be necessary to test the *in vivo* effects of HSATII RNA modulation.

A manuscript containing these results is currently nearly fully drafted and ready for submission in the next 2-3 weeks.

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

Training: during the 24 months of this award, I have mentored three different technicians on this project. For each, this has included one-on-one training in areas of cell culture, RNA and DNA isolation, total RNA-sequencing preparation and data analysis, immunohistochemistry, RNA in situ hybridization, digital image analysis, and xenograft generation. One has gone on to a PhD program in the U.K., one is now a first year MSTP student at Harvard Medical School and one is just beginning her first year in medical school at Cornell University. In addition, I have worked individually with clinical fellows, residents and physician assistances on the clinical service, teaching about gynecologic cancers, specifically delivering lectures focused on “Genetics/genomics of ovarian cancer”, “Areas of clinical need in ovarian cancer” and “Immunotherapy in ovarian cancer”.

Professional Development: I have attended two conferences during this report period at which I presented our preliminary findings in poster format. These were the AACR Advances in Ovarian Cancer Research Biannual Meeting in Atlanta, GA in Sept 2019 and the Stand Up to Cancer Scientific Summit in Santa Monica, CA in Jan 2020. A third abstract was also chosen for an oral presentation at a local

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:

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

The potential impact of a more comprehensive understanding of the "repeatome" in ovarian cancer is twofold. First, characterization of the repeatome would allow for the development of more precise molecular subtyping and/or specific cancer biomarkers, given that some repeat RNAs are specific for cancer cells. Second, the fact that cancer cells reverse transcribe repeat RNAs similar to retroviruses may represent a unique therapeutic opportunity to exploit. Indeed, the data we have generated in the first 12 months of this project confirm abundant and variable expression of repeat RNAs in ovarian cancer and some evidence of cytotoxicity upon modulation of HSATII levels in the cancer cells. Importantly, since work by others in different cancers has demonstrated that repression of repeat RNAs appears to be important in surviving death via cytotoxic agents (Guler et al.*, Cancer Cell*, 2017), resistant cells may be particularly vulnerable to this strategy of increasing repeat RNA stress, specifically addressing the critical need for therapies in treatment-resistant disease. To this end, we are observing cytotoxicity from acutely increasing HSATII RNA in EOC cell lines, which may translate to a novel therapeutic strategy to target chemotherapy- and immunotherapy-resistant cells. Altogether, this work may not only allow for more precise subtyping and/or identification of novel biomarkers for HGSOC, but may also unveil a novel therapeutic strategy to specifically target cancer cells, particularly in the setting of treatment-resistant disease.

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:

Changes in approach and reasons for change

As above, in Aim 2 we hypothesized that targeting repeat RNA reverse transcription using NRTIs may be an effective therapeutic strategy in EOC. This was based on preliminary data generated in colorectal and pancreatic cancer models. Thus, we treated a panel of EOC cell lines with a selection of NRTIs *in vitro* and tested cytotoxicity and effect on repeat RNA expression. None of the NRTIs tested demonstrated cytotoxicity as a single agent in EOC cell lines. Next, we tested NRTIs combined with epigenetic therapies (azacytidine and HDAC inhibitor) and cytotoxic chemotherapy (carboplatin and paclitaxel). While chemotherapy agents and HDAC inhibitors demonstrate single-agent cytotoxicity in the majority of EOC cell lines, no additive or synergistic activity was observed when combined with NRTIs, suggesting that NRTIs are likely not effective in EOC models, at least as single agents or in the combinations we have tested. Given this, we shifted our focus for Aim 2 to targeting satellite repeat RNA reverse transcription using locked nucleic acids (LNAs) as described under Accomplishments.

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

The COVID-19 pandemic resulted in full shut down of laboratory operations for several weeks of this funding period, which has delayed scientific progress significantly. Data analysis and computational work has continued, but laboratory-based work was halted for a period of time and led to delays in experiments, especially animal based experiments. Given this, Major task 5 activities utilizing xenograft models are still ongoing and not completed.

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

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Porter RL and Matulonis UA, **Checkpoint blockade: Not yet NINJA status in Ovarian Cancer.** *Journal of Clinical Oncology* (2021)*, accepted, awaiting publication.*

Porter RL, Sun S, Flores MN, Berzolla E, Magnus NKC, Desai N, Tai E, Szabolcs A, Lang E, Pankaj A, You E, Thapar V, Kolin D, Pepin D, Deshpande V, Solovyov A, Greenbaum B, Ting DT. **Satellite repeat RNA expression in epithelial ovarian cancer is linked to a tumor immunosuppressive phenotype.** (2022) *Under revisions at Journal of Clinical Investigation.*

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

Porter RL and Matulonis UA, **Ovarian Cancer Therapy,** Cold Spring Harbor Perspectives (2021), *under review.*

Other publications, conference papers and presentations.

1. **Porter RL,** Szabolcs A, Desai N, Thapar V, Pepin D, Solovyov A, Greenbaum B, Ting DT. Repeatome profiling in high grade serous ovarian cancer reveals abundant repeat non-coding RNA. AACR Advances in Ovarian Cancer Research Biannual Meeting, September 13-16, 2019, Atlanta, GA

2. **Porter RL,** Thapar V, Pepin D, Solovyov A, Szabolcs A, Flores M, Desai N, D, , Greenbaum B, Ting DT. Repeatome profiling in high grade serous ovarian cancer reveals abundant repeat non-coding RNA expression. Stand Up to Cancer Scientific Summit, Santa Monica, CA, Jan 26-28, 2020

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

Nothing to report

- **Technologies or techniques**

Nothing to report

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

An invention disclosure has been submitted for HSATII LNA technology.

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: **Micayla Flores** - no change
Name: **Neelima KC Magnus** - no change
Name: **Rebecca Porter, MD, PhD** – no change

Name: **Emily Berzolla**
Project Role: Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Ms. Berzolla performed work in the areas of cell culture, RNA isolation, preparation for RNA-Seq, in vitro cytotoxicity assays and in vivo LNA therapy in murine models.
Funding Support:

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?

Organization Name: Memorial Sloan Kettering Cancer Center
Location of Organization: (if foreign location list country) New York, NY
Partner's contribution to the project (identify one or more)
Collaboration: Drs. Benjamin Greenbaum and Alexander Solovyov are long-time collaborators working on RNA-seq projects in PDAC and CRC (Solovyov A et al, Cell Reports, 2018), and have assisted with development of programs to map repetitive elements in the genome.

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.*

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.*

Appendix 1: Figures 1-4
Appendix 2: Quad Chart
Appendix 3: Award Expiration Transition Plan

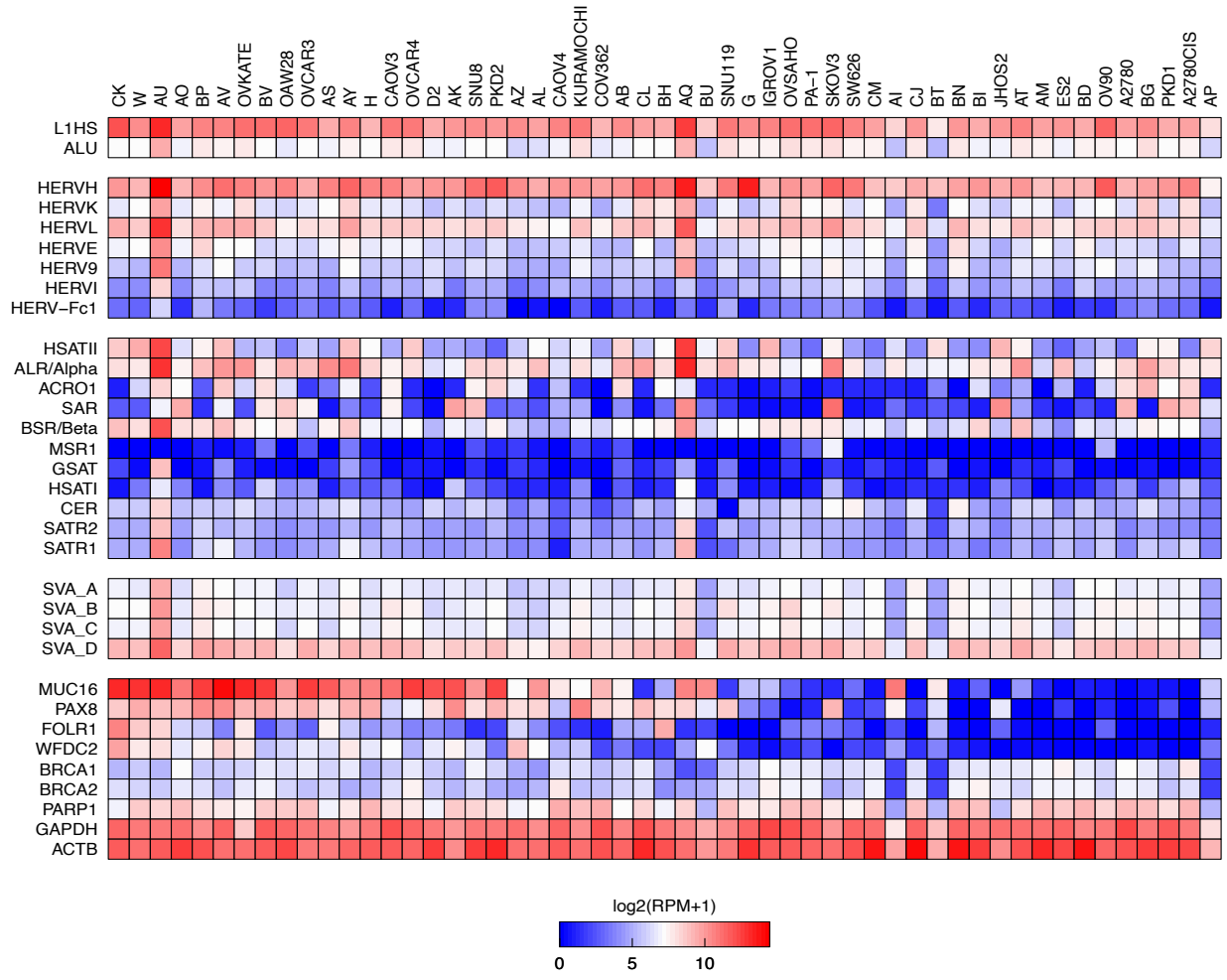


Figure 1A. Expression heat map showing log10(RPM) in each cell line/patient sample for a selection of repeat RNAs (including retrotransposons, ERVs and satellites), as well as EOC-associated coding genes and housekeeping genes.

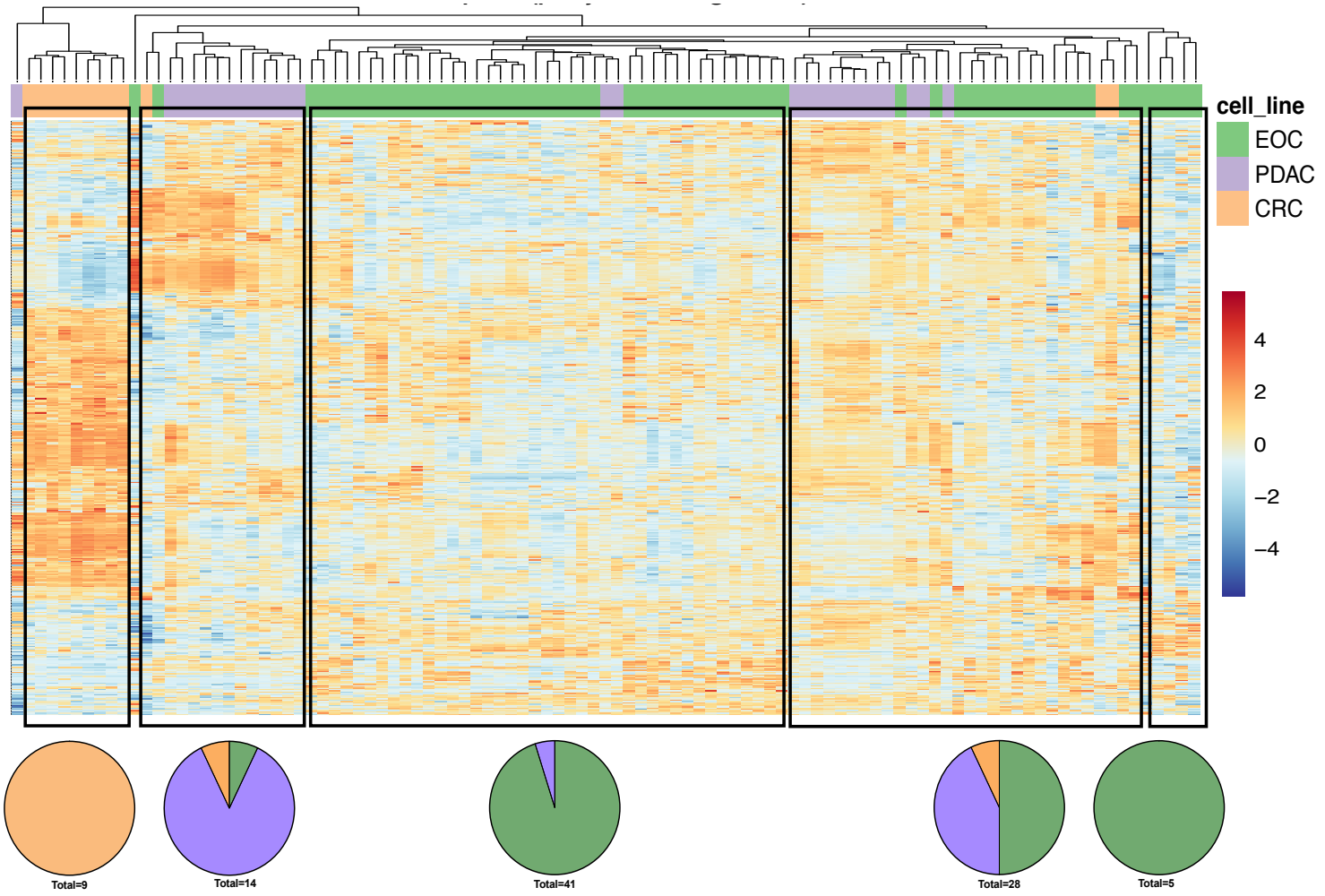
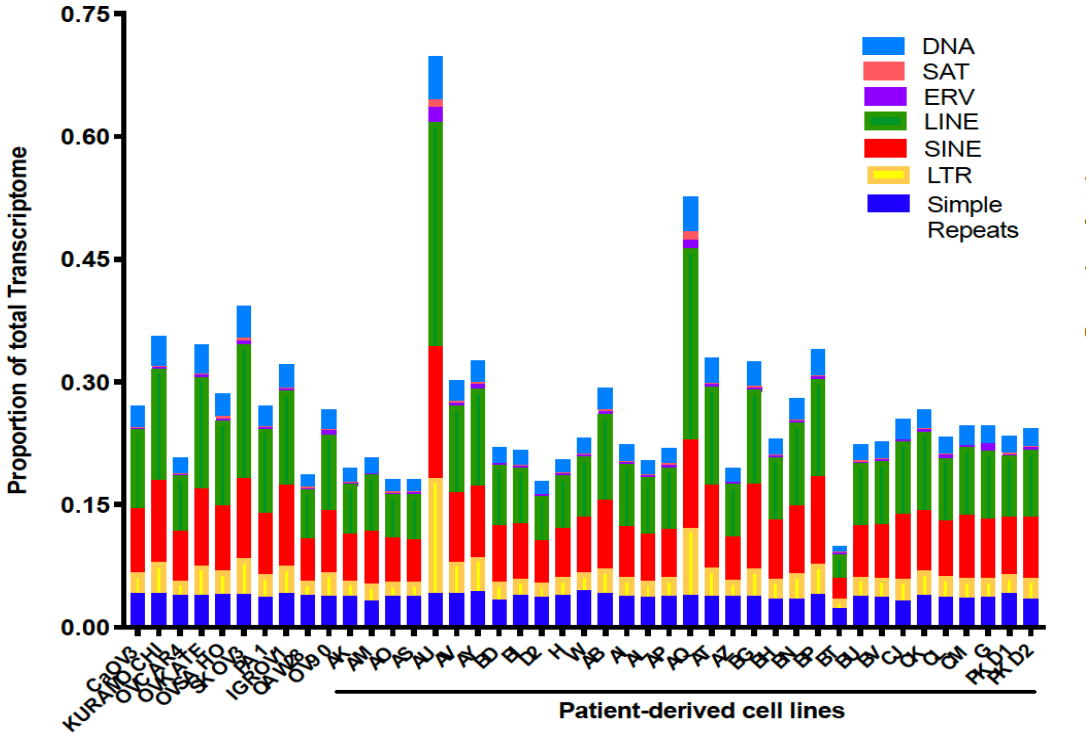


Figure 1B. Hierarchical clustering of EOC (green), PDAC (purple) and CRC (gold) cell lines by repeat RNA expression based on the 500 most variant repeats. Expression is plotted as median-polished log2(TTM repeats +1). Major clusters are outlined in black boxes. Pie graphs below depict the cancer-type composition of each cluster.

C)



D)

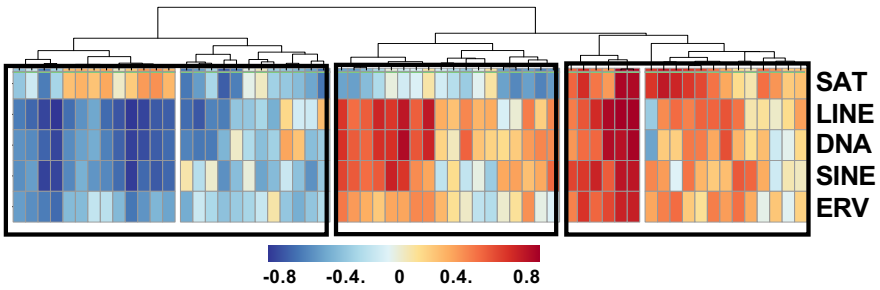


Figure 1C-D. C) Quantification of individual classes of repeat RNAs across EOC models using total RNA-Seq. Expressed as proportion of total transcription, including coding and non-coding reads in each cell line or patient-derived cells. D) Hierarchical clustering of EOC cell lines based on total repeat expression by subclass.

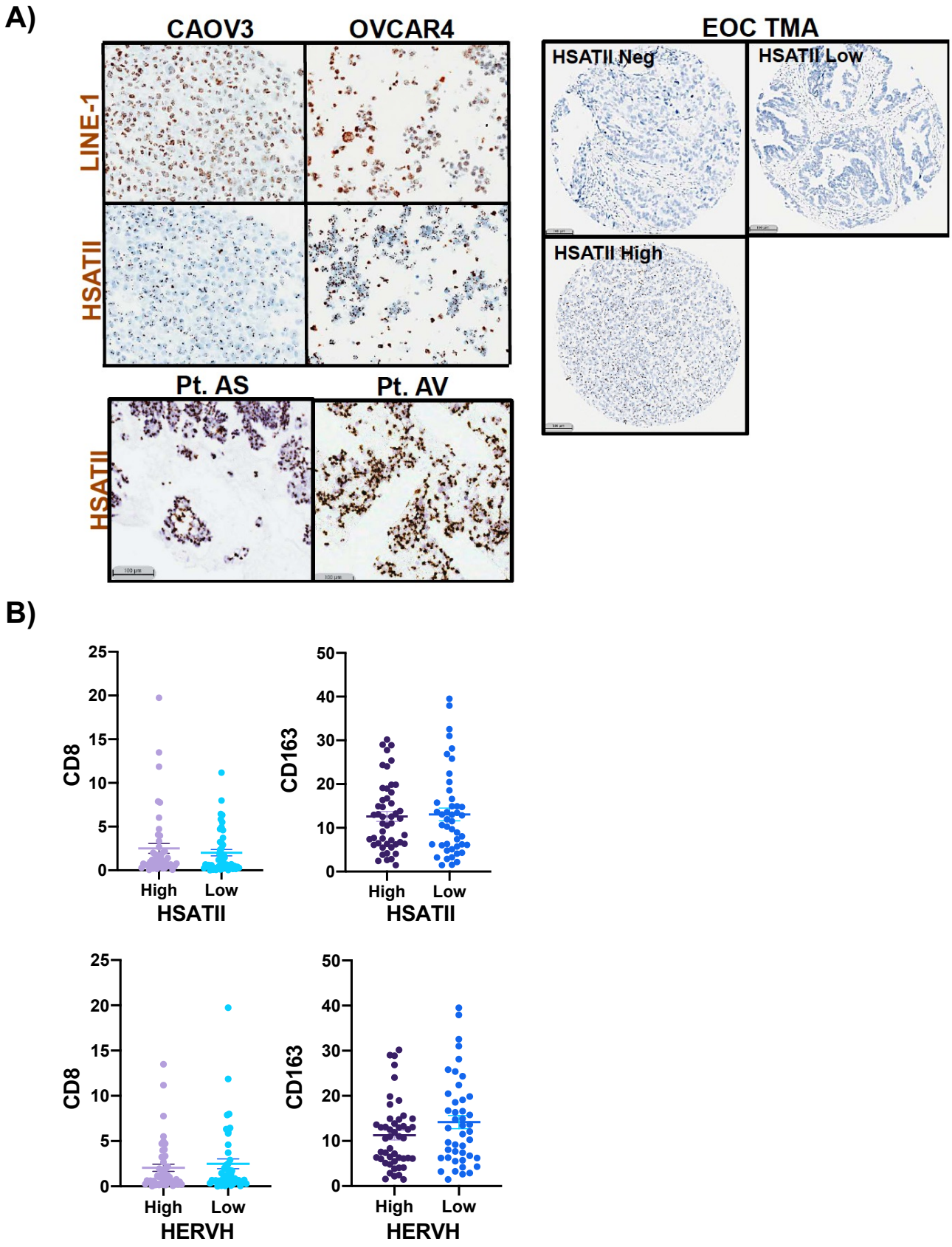
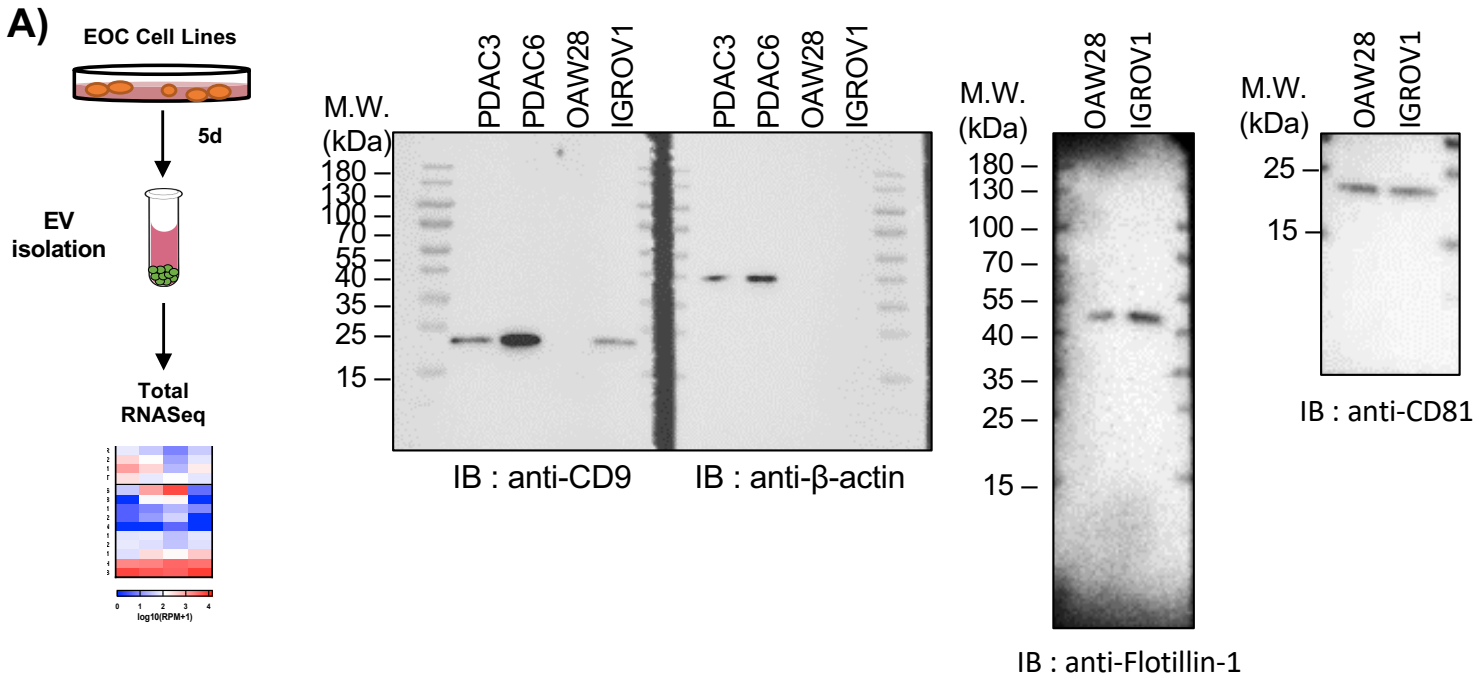


Figure 2. A) RNA-ISH with LINE1- and HSATII-specific probes applied to FFPE pellets from cell lines or patient-derived cell lines (left) or EOC TMA cores (right). B Quantification of CD8 (left panels) and CD163 (right panels) positive cells by IHC in HSATII (top) and HERVH (bottom) high or low tumor cores.



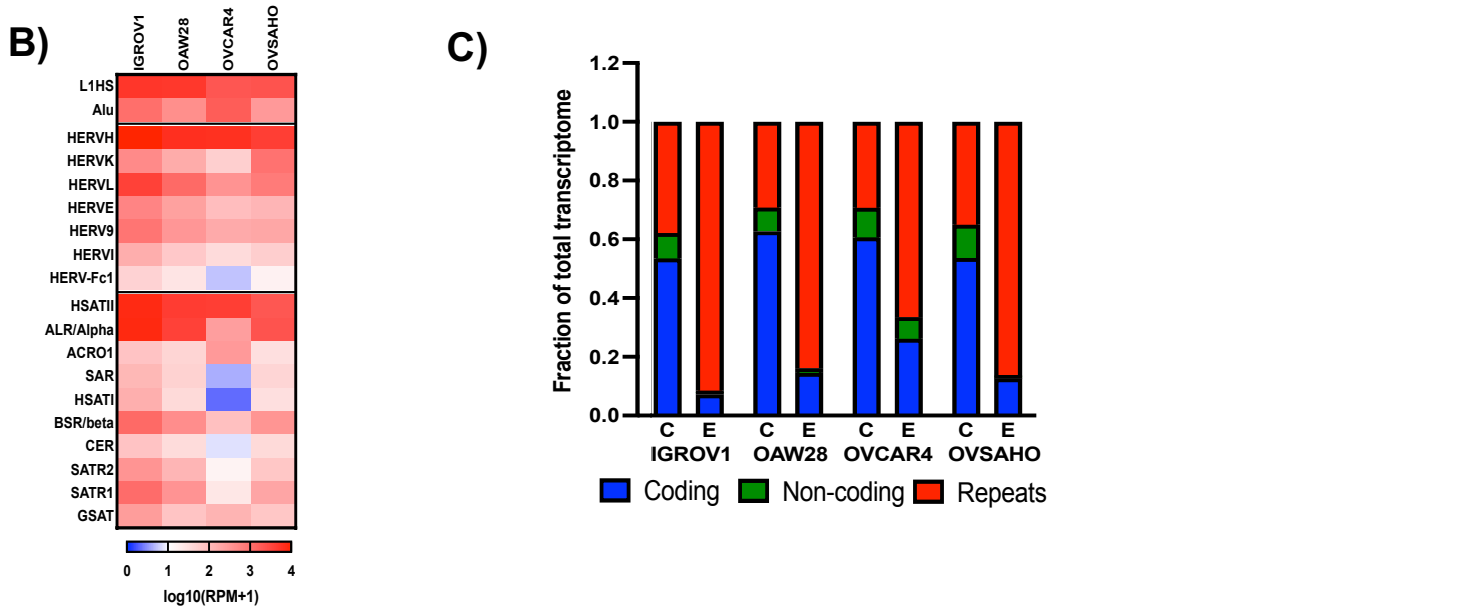


Figure 3. A. Schema of experimental design and western blots confirming EV yield based on expression of EV surface markers CD9, CD81 and flotillin. B. Expression heatmap of representative repetitive elements in EVs released by EOC cell lines. C. RNA content of tumor cells (C) and tumor cell-derived EVs (E) in EOC cell lines as determined by total RNASeq and plotted as fraction of total transcriptome.

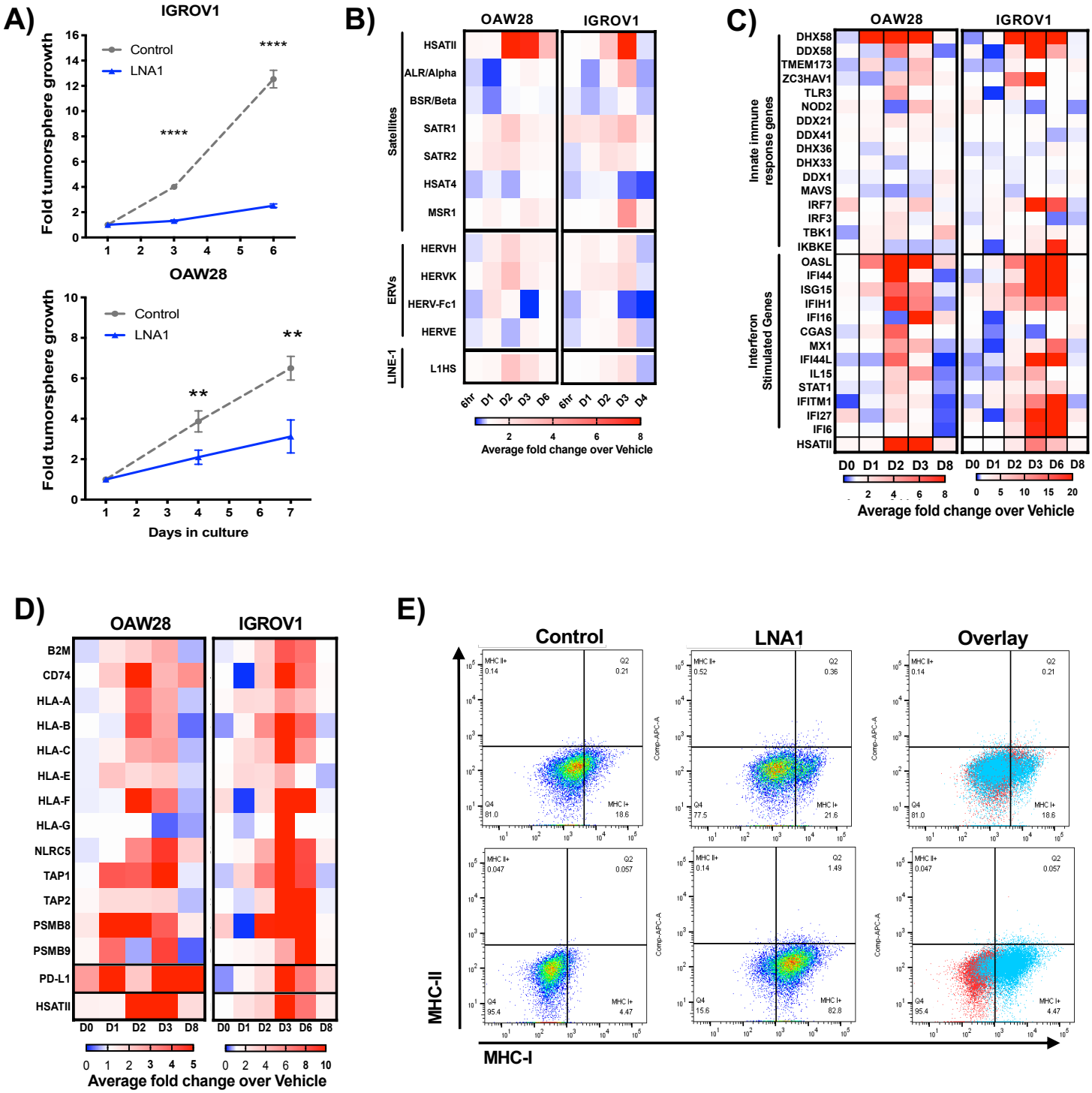


Figure 4. A. Effect of HSATII-specific LNAs on tumorsphere growth in EOC cell lines as determined by 3D CellTiterGlo viability assays. Plots represent 4 separate experiments for each cell line. B. Expression levels of HSATII and other repeats in EOC cell lines transfected with HSATII-specific LNA relative to scramble control over time. C. Expression heatmap depicting relative expression of innate immune response genes and interferon-stimulated genes (ISGs) in EOC cell lines transfected with HSATII-specific LNA (LNA1) relative to scramble control over time. D. Expression heatmap depicting relative expression of MHC-Class I genes and PD-L1 in EOC cell lines transfected with HSATII-specific LNA relative to scramble control over time E. Flow cytometric analysis of MHC-I and MHC-II cell surface protein expression on the cell surface of EOC cell lines transfected with HSATII-specific LNA.

Targeting Satellite Repeat RNAs in High-Grade Serous Ovarian Cancer
Quad Chart
W81XWH1910058

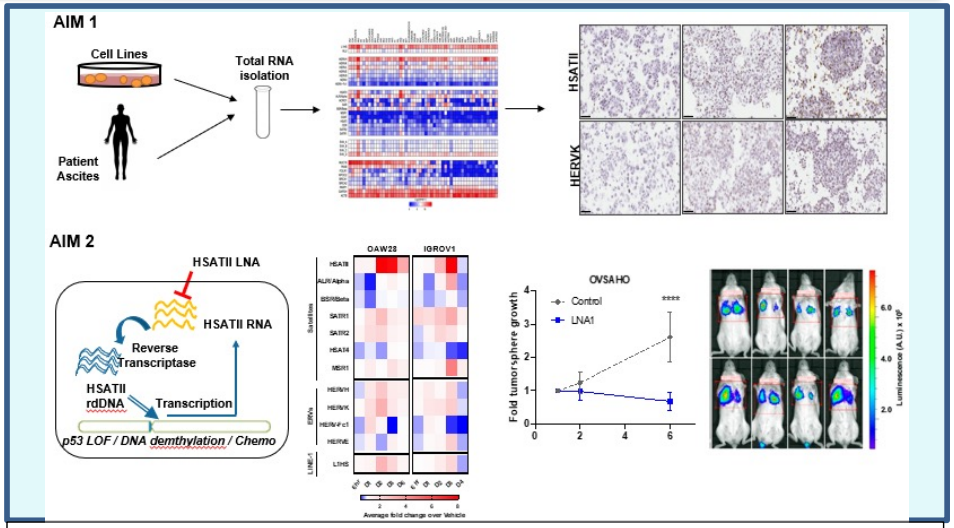
PI: Porter, Rebecca L **Org:** Massachusetts General Hospital **Award Amount:** $250,000

Study/Product Aim(s)

- **Aim 1:** Define the expression of non-coding repeat RNAs in HGSOC cell lines and human tumors using total RNA-sequencing
- **Aim 2:** Test the effect of repeat RNA RT inhibition as a therapeutic strategy in HGSOC

Approach

Aim 1: we will comprehensively define the "repeatome" in HGSOC by performing Total RNA-Seq on a panel of HGSOC cell lines and PDX models. Repeat RNA expression will be confirmed in human ovarian tissue microarrays (TMAs) by RNA-ISH. TMAs will be co-stained for relevant immune infiltrates to define the relationship between repeat RNAs and the immune TME.
Aim 2: we will target repeat RNAs by treating the HGSOC cell lines epigenetic agents, reverse transcriptase inhibitors and/or locked nucleic acids. We will perform cytotoxicity assays and Total RNA-Seq to determine changes in repeat RNAs and the effect on cell survival. We will then test active drugs/combinations in ovarian xenograft tumors from to determine in vivo efficacy.



Accomplishment: The results from this study have now been written into a manuscript that is currently under revisions at the *Journal of Clinical Investigation.*

Timeline and Cost

Activities	CY 19	20		
Quantify repeat RNAs in HGSOC cell lines and PDX models				
Perform RNA-ISH/IHC on Epithelial Ovarian Cancer (EOC) TMAs				
Exosome isolation and RNA-seq of exosomes				
Target repeat RNAs with NRTIs and LNAs in HGSOC models				
Evaluate response to NRTI therapy in xenograft tumors				

Updated: (3/2022)

Goals/Milestones

CY18 Goals: Repeat RNA Profiling in HGSOC Models & Human Tumors
☑ Comprehensive profiling of repeat RNAs in ovarian cancer models
☑ Validating repeat RNA expression in human tumors
CY18 Goals: Repeat RNA Profiling in HGSOC Exosomes
☑ Define repeat RNA content in HGSOC exosomes
CY19 Goals: Target Repeat RNAs Therapeutically
☑ Test effect of repeat RNA-modulating therapies in HGSOC cell lines
☑ Test effect of active drugs from cell line studies in xenograft murine models of ovarian cancer

Comments/Challenges/Issues/Concerns

- LNAs were the only active agents against repeat RNAs, so their development was continued and further investigation of reverse transcriptase inhibitors was aborted
- **Budget Expenditure to Date**

Projected Expenditure (non-personnel): $88,514
Actual Expenditure (non-personnel): $88,514

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?
Aberrant expression of viral-like repeat elements is a common feature in epithelial cancers, but the significant diversity of repeat species provides a distinct view of the cancer transcriptome. Repeatome profiling across ovarian, pancreatic, and colorectal cell lines identifies distinct clustering that is independent of tissue of origin that is seen with coding gene analysis. Deeper analysis of ovarian cancer cell lines demonstrated that HSATII satellite repeat expression was highly associated with epithelial mesenchymal transition (EMT) and anti-correlated with interferon (IFN) response genes indicative of a more aggressive phenotype. This relationship of HSATII with high EMT and low IFN response genes was also found in RNA-seq of primary ovarian cancers and associated with significantly shorter survival in a second independent cohort of ovarian cancer patients. Repeat RNAs were also found enriched in tumor derived extracellular vesicles that were capable of stimulating monocyte derived macrophages demonstrating a mechanism of altering the tumor microenvironment with these viral-like sequences. Targeting of HSATII with anti-sense locked nucleic acids (LNAs) stimulated IFN response and induced MHC I expression in ovarian cancer cells lines, highlighting a potential strategy of modulating the repeatome to re-establish anti-tumor cell immune surveillance.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.
Not at this time.

4. What will be **the next step(s)** for this project?
We have submitted this data for publication and the manuscript is under revisions at the Journal of Clinical Investigation. Following this, we will consider using this data to develop translational biomarker studies to incorporate into two different clinical trials utilizing immunotherapies in ovarian cancer.

5. How would you classify your **lead candidate product**? Knowledge Product
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Please choose, if applicable
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?
Ovarian cancer is the 5th leading cause of cancer deaths in women, with more than 230,000 women living with this disease in the US. While current standard of care therapies do induce remission in ~80% of women up front, nearly all of them eventually experience disease recurrence and will sucuumb to increasingly treatment-resistant disease. Although immunotherapies like immune checkpoint blockade are very successful in some solid tumors, these agents alone have produced very modest activity in ovarian cancer to date. Therefore, there is a huge unmet clinical need to improve how immune-based therapies may work for ovarian cancer patients. The work we performed there highlights the use of repeat non-coding RNAs as potential biomarkers and also therapeutic targets in ovarian cancer, and suggest that targeting these molecules may sensitize ovarian cancer tumors to immunotherapies.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

Our first next step will be in testing the performance of these repeat non-coding RNAs as predictive biomarkers for response to novel immunotherapy strategies in patients with ovarian cancer. To this end, we plan to incorporate sequencing of non-coding RNAs and quantification of repeats as correlative studies into our immunotherapy clinical trials in ovarian cancer.

In terms of therapeutic targets, next steps include the following:
1) Testing additional methods and strategies to target specific repeat RNA molecules in vitro and in murine models
2) Testing combinations of locked nucleic acids to specific repeats with epigenetic and/or immunotherapy agents in ovarian cancer models.

An invention disclosure form was filed for HSATII-targeting locked nucleic acids and is pending.