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Case series

Genomic profiling of endometrial cancer and relationship with volume of endometrial cancer disease spread

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ABSTRACT

Objectives: Lymph node (LN) metastasis and genomic profiles are important prognostic factors in endometrial cancer (EMCA). However, the prognostic significance of low volume metastasis found in sentinel lymph nodes (SLN) is unknown. We sought to determine if genomic mutations were associated with metastatic volume.
Methods: Surgically staged women with EC who were enrolled in both a SLN clinical trial and tumor sequencing protocol were eligible. Relevant targets were enriched by a custom designed Agilent SureSelect hybrid capture enrichment library using standard protocols. Three specific gene mutations were evaluated, *TP53, PTEN and PIK3CA* in the primary tumor of patients with LN negative, LN positive and ITC disease.
Results: 42 patients were eligible; of these, 7 (16.7%) had ITC only and 7 (16.7%) had micrometastatic or macrometastatic (LN positive) disease. No differences were seen in TP53, *PIK3CA or PTEN* between groups. All ITC patients with *TP53* mutations were of non-endometrioid histology (2/7). Deeper myometrial invasion and lymph vascular space invasion were more likely to occur in the LN positive group ($p < 0.01$ for both). No patients with ITC had a recurrence in a median 67.7 months of follow-up since surgery.
Conclusions: This pilot investigation did not identify differences between frequency of *PIK3CA, PTEN or TP53* mutations in tumors and volume of LN metastasis. Low number of ITC limited the ability to detect genomic differences, however mutations appeared to align with expected histology. More work is needed to define the relationship between genomic mutations, histology, ITC, and prognosis.

1. Introduction

Endometrial cancer (EMCA) is the most common gynecologic malignancy in the US and lymph node (LN) status is the most important prognostic factor in determining adjuvant treatment. The sentinel lymph node (SLN) technique, adapted from other malignancies, has been applied to EMCA and is now commonly used among providers (Rossi et al., 2017). LN metastasis are classified by size as either macrometastases (>2mm), micrometastases (MM) (0.2–2 mm), or isolated tumor cells (ITC) (<0.2 mm). Ultrastaging, which involves serial sectioning of the LN in conjunction with cytokeratin IHC staining, is only done for SLNs allowing detection of low volume disease. ITCs are overlooked by routine pathologic evaluation, therefore only macrometastasis, and on occasion MM can be detected in comprehensive lymphadenectomy. In EMCA, there is evidence that as many as half of all LN metastasis are detected through ultrastaging (Rossi et al., 2017; Kim et al., 2013). There are observational data suggesting that patients with ITC in historically low risk EMCA have an excellent prognosis (Plante et al., 2017); however there are no prospective data to guide adjuvant therapy and no standard treatment approach for ITCs.

The Cancer Genome Atlas (TCGA) identified distinct genomic subgroups with prognostic implications (Cancer Genome Atlas Research, 2013). Further investigations have validated the importance of molecular characteristics in prognosis of endometrial cancer and indeed these characteristics are being utilized in ongoing and future clinical studies to stratify patients in treatment groups (Raffone et al., 2019). There are no

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large investigations regarding molecular characteristics of EMCA tumors and association with size of LN metastasis.

The Sensitivity of Sentinel Lymph Node Identification with Robotic Fluorescence Imaging for Detecting Metastatic Endometrial and Cervical Cancer (FIRES) is a prospective, multi-institution trial investigating the use of SLN technique (Rossi et al., 2017). This trial collected tumors from comprehensively staged women with EMCA at our institution which can be utilized to evaluate the genomic profiles of EMCA that may shed light on the biologic importance of small volume disease.

Using specimens from the FIRES clinical trial, we sought to characterize the primary endometrial tumors using next generation sequencing. Our primary objective was to describe the prevalence of three primary gene mutations common to EMCA, *TP53, PTEN, PIK3CA*, in tumors of patients with LN negative (LN−) disease, ITC, and LN positive (LN+) disease. Both MM and macrometastases were considered LN+ in this investigation as both can be detected on routine sectioning. Our secondary objective was to identify any associations between these mutations and clinicopathologic factors in patients within the three groups previously identified. We hypothesize that the genomic profiles of the endometrial tumors in patients with ITC will more closely mirror profiles of tumors with similar histology rather than tumors with macrometastatic disease, further supporting that the presence of ITC should not independently direct adjuvant therapeutic decisions.

2. Methods

2.1. Data sources and patient population

Patients with EMCA who enrolled in the FIRES trial at our institution as well as our institutional genomic sequencing protocol were eligible for inclusion (Rossi et al., 2017), see Fig. 1. Patients underwent hysterectomy, SLN biopsy, and full lymphadenectomy as part of the FIRES trial. Whole exome sequencing of their uterine tumor was performed. *TP53, PTEN* and *PIK3CA* were chosen to be described based on reliability and impact on prognosis (Cancer Genome Atlas Research, 2013). Clinicopathology data from patients was gathered from their electronic medical record and verified by two independent investigators. This study was approved by our IRB, #14-2098 and informed consent obtained.

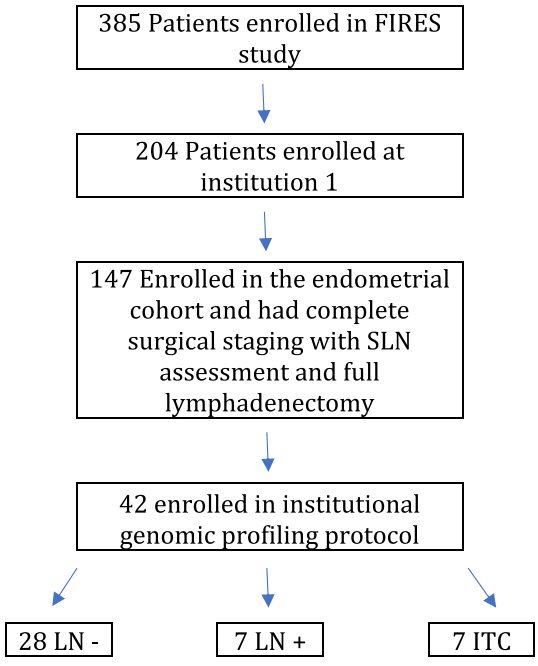

Fig. 1. Patient population who enrolled in both FIRES trial and Institutional genomic profiling protocol. Enrollment diagram of patients who enrolled in both the FIRES trial and our institutional genomic profiling protocol.

Extracted DNA from archival formalin-fixed, paraffin-embedded, or frozen endometrial tumor was performed using the Qiagen DNEasy column. Tumor DNA sequencing was performed using a hybrid capture approach and the Agilent custom SureSelect protocol in conjunction with next generation sequencing using the Illumina HiSeq 2000 platform (Zhao et al., 2015). The analysis was done in collaboration with the UNC-CH High Throughput Sequencing Facility, which is also utilized for the UNCseq clinical study.

2.2. Statistical analysis

Exploratory analysis was performed of all unique mutations found after sequencing. We defined the collection of mutations for a particular sample as that sample's "genomic profile". The prevalence of *TP53, PIK3CA* and *PTEN* mutations was described for each tumor in the following groups, LN−, ITC, and LN+.

Descriptive statistics were used to describe continuous data and discrete data using Fisher's exact test for categorial variables and Wilcoxon rank-sum test for continuous variables. Patient characteristics such as age, histology, grade was further assessed for their association with volume of disease in the LN using regression methods (linear, logistic and cox proportional hazards).

Progression free survival (PFS) was calculated from date of surgery to date of progression, date of death, or date of last follow-up. Overall survival (OS) was calculated from date of surgery to date of death from any cause. Patients known to be alive without recurrent disease or lost to follow up at analysis were censored at the time of their last follow up.

3. Results

A total of 42 patients with EMCA completed surgical staging with SLN biopsy as part of the FIRES trial in addition to the institutional genomic sequencing protocol, see Fig. 1. The median age at diagnosis was 61 yrs and median BMI was 32 kg/m^2. Most patients, 34/42 (81.0%) had endometrioid histology, and 14/42 (33.3%) had lymph vascular space invasion (LVSI). Only 8/42 patients had a grade 1 tumor, while 18/42 had grade 2 and 16/42 had grade 3 tumors.

28 patients (66.7%) were LN−, while 7 (16.7%) were LN+, and 7 (16.7%) had ITC, see Fig. 1. Within the LN+ group, 1 tumor had MM, the other 6 had macrometastatic disease. There were no differences in age at diagnosis, BMI, race, grade, histology, or tumor size among the three groups ($p > 0.05$ for all). The LN+ group was more likely to have deeper myometrial invasion and LVSI than the LN− group ($p=<0.01$ for both). The LN+ group was more likely to have LVSI than the ITC group ($p = 0.02$) but there was no significant difference in myometrial invasion between these groups ($p > 0.05$). See Table 1 for clinicopathologic features of each cohort.

There were 25 tumors with *PIK3CA* mutations, 23 with *PTEN* and 10 with *TP53*. The LN+ group had the highest percent of *TP53* mutated tumors although this did not reach significance, see table 1. There were no statistically significant differences in mutation distribution among LN groups, see Table 1. Among the 10 tumors positive for *TP53,* 2 were grade 2 and 8 were grade 3; there were no grade 1 tumors positive for *TP53*. See Fig. 2 for details on copy number alterations by LN group and histology.

Regarding histology, 5/7 serous tumors were positive for *TP53,* 2 of which had ITC. One patient had carcinosarcoma, and this tumor did not have any of the three predefined mutations. Of the two serous tumors without a *TP53* mutation, both were LN−, neither had LVSI and neither recurred during this investigation with OS of 69.7 and 64.6 months.

Of the 14 patients with ITC or LN+, 13 received adjuvant chemotherapy (carboplatin and paclitaxel), and 6 of these patients also received external beam radiation therapy. Of the 7 that did not receive external beam, 2 received vaginal brachytherapy. There were 4

Table 1
Patient characteristics and mutation summary between lymph node groups.

	Lymph node negative (n = 28)	Lymph node positive (n = 7)	ITC (n = 7)	P value
Age (years)	59.9 ± 10.4	61.0 ± 7.7	64.3 ± 12.7	0.77
Body Mass Index (kg/m^2)	32.7 ± 7.9	32.0 ± 8.1	32.0 ± 5.0	0.89
Race				0.61
White	21 (75.0)	6 (85.7)	6 (85.7)	
Black	2 (7.1)	1 (14.3)	1 (14.3)	
Other or unknown	5 (17.9)	0 (0.0)	0 (0.0)	
Grade				0.58
Grade 1	6 (21.4)	1 (14.3)	1 (14.3)	
Grade 2	14 (50.0)	2 (28.6)	2 (28.6)	
Grade 3	8 (28.6)	4 (57.1)	4 (57.1)	
Histology				0.51
Endometrioid	24 (85.7)	5 (71.4)	5 (71.4)	
Serous	3 (10.7)	2 (28.5)	2 (28.5)	
Carcinosarcoma	1 (3.6)	0 (0.0)	0 (0.0)	
Tumor size (cm)	3.6 ± 2.8	5.1 ± 2.0	3.8 ± 1.6	0.15
Myometrial invasion (%)	15.0 ± 22.8	72.9 ± 27.8	48.3 ± 24.4	**<0.01**
LVSI	5 (17.9)	7 (100)	2 (28.6)	**<0.01**
PIK3CA	18 (64.3)	3 (42.9)	4 (57.1)	0.66
PTEN	16 (57.1)	3 (42.9)	4 (57.1)	0.90
TP53	5 (17.9)	3 (42.9)	2 (28.6)	0.42

Data presented as n (%) for categorical variables and mean (± SD) for continuous variables. Percentages may not sum to 100 due to rounding. Bolded text indicates statistical significance.

recurrences, 2 in the LN− group and 2 in the LN+ group. No patients with ITC had a recurrence or death. Within the LN− group, both patients originally had grade 1 endometrioid tumors and later died of non-EMCA related illnesses at 43 and 50 months after diagnosis. One patient had a *PTEN* mutation, the other had none of the three preidentified mutations. Both patients in the LN+ group that recurred had macrometastatic disease at diagnosis, serous histology, *TP53* mutations, and died due to disease. There were no significant difference in PFS or OS between groups, see Fig. 3.

4. Discussion

This pilot investigation is the first to our knowledge to describe genomic mutation associations with size of lymphatic metastasis in EMCA. While we were unable to detect statistically significant differences in genomic mutations due to our small population size, we did appreciate that patients with LN+ disease have classic high-risk histologic features (more LVSI and deeper myometrial invasion) consistent with more aggressive disease (Baser et al., 2014). In patients with ITC, genomic mutations aligned closely with histology; both tumors with *TP53* mutations were serous histology, which is consistent with prior investigations (Raffone et al., 2019; Murali et al., 2019; Bell and Ellenson, 2019). We suspect that our inability to detect genomic differences between the LN groups is likely a result of small sample size and not a true lack of difference. Additionally, there were no PFS or OS differences between LN groups, however this small investigation was not powered for this outcome. Prior data sets mirror findings of ITC association with more favorable pathologic prognostic factors such as endometrioid histology and grade 1 disease while high grade tumors and serous tumors have higher rates of macrometastasis (Rossi et al., 2017; Kim et al., 2013; Plante et al., 2017).

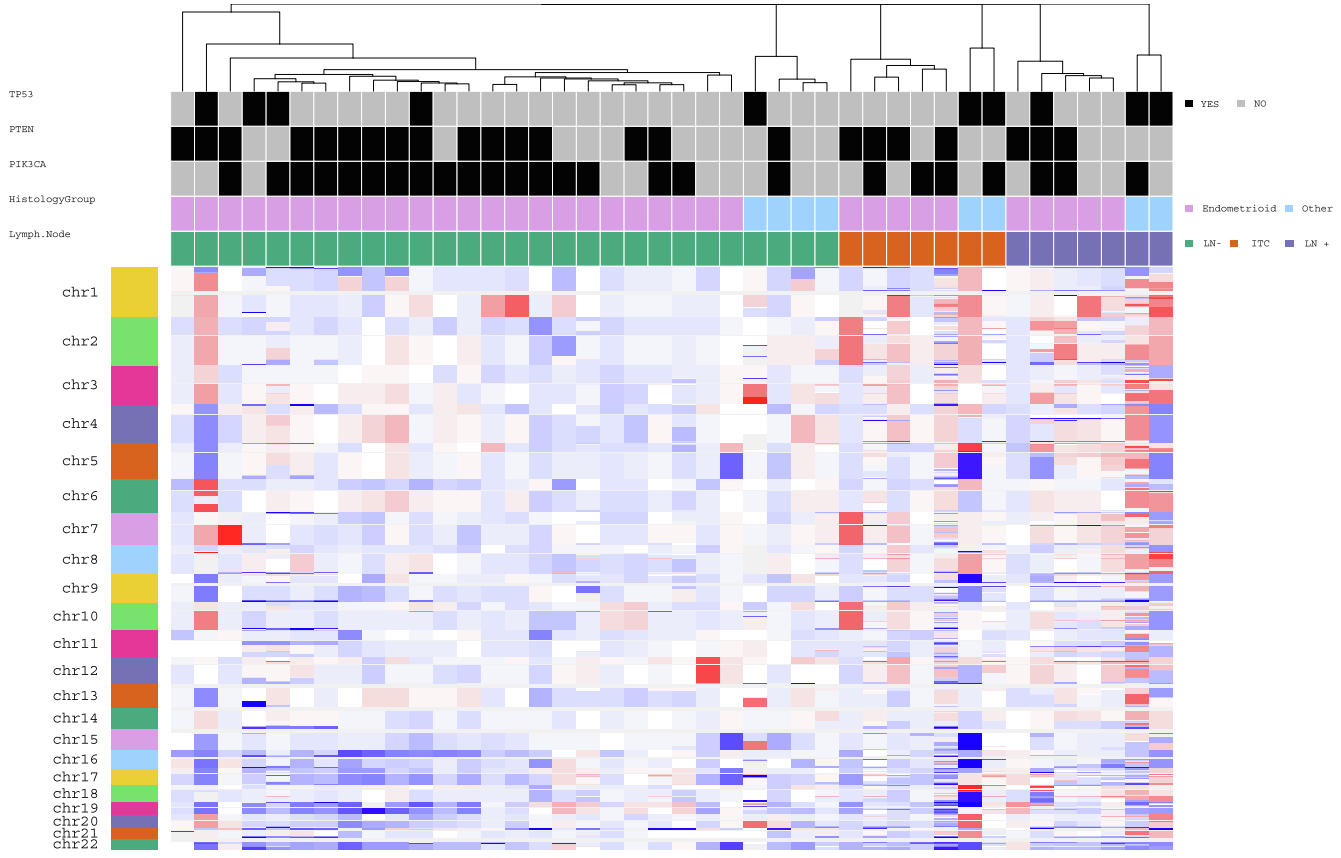

Fig. 2. Copy number alterations by lymph node group and histology. Heat map depicting copy number alterations of each patient. Annotation bar indicates patient group by lymph node status, histology and mutation.

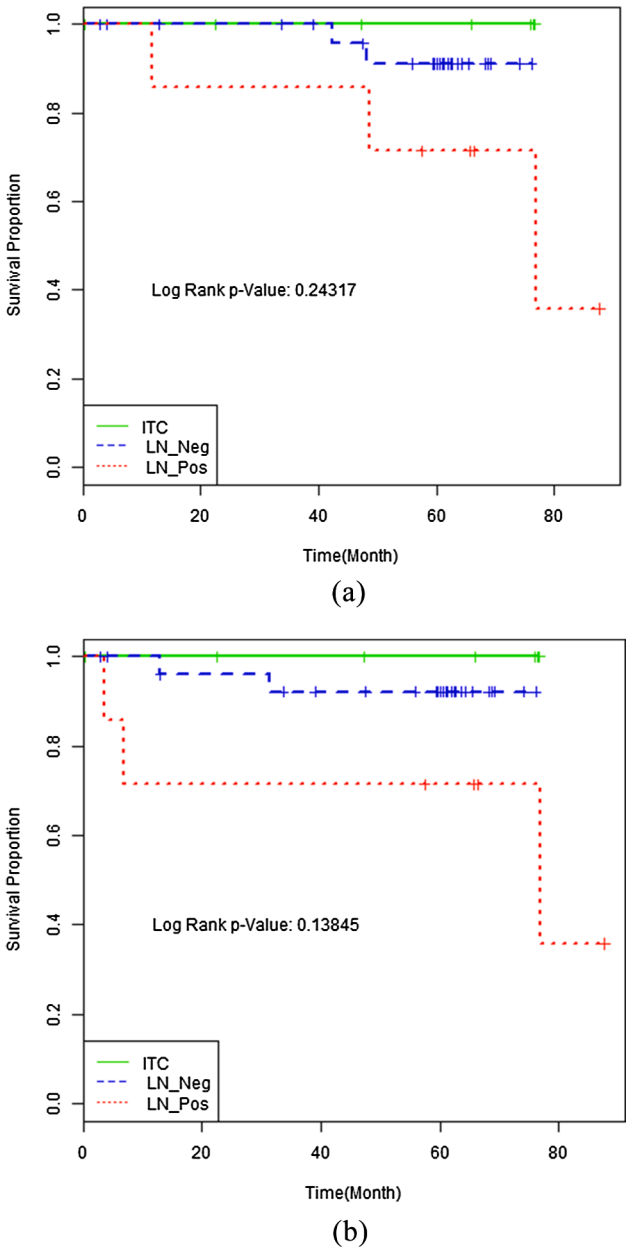

Fig. 3. Survival by lymph node group. Survival analysis performed between the three lymph node groups found no statistically significant difference in the distribution of overall survival (A) or progression free survival (B) between lymph node groups.

Somatic tumor testing has become a cornerstone in the care of EMCA and *TP53* mutation is recognized as a strong driver of biologic behavior (Raffone et al., 2019; Bosse et al., 2018). The four TCGA molecular subgroups, microsatellite unstable, POLE-mutant, copy number high (p53 mutant), and copy number low or some approximation of this grouping has been utilized to classify tumors in multiple investigations (Cancer Genome Atlas Research, 2013; Raffone et al., 2019; Talhouk et al., 2017). For example, integrating molecular classifications in early-stage EMCA from the PORTEC cohorts improved the risk assessment of these patients (Stelloo et al., 2016). Tumors from GOG 210 were evaluated for molecular subgroups with outcomes mirroring those from TCGA prognostic data, including worse outcomes among those with a *TP53* mutation (Cosgrove et al., 2018). We observed the lowest rate of *TP53* mutation in LN− group and highest in LN+. While frequency was not statistically different in our investigation, we expect this is a product of sample size. Our data mirror previously noted associations between serous tumors and *TP53* mutations (Raffone et al., 2019). Interestingly, our serous tumors that were not *TP53* mutated were also LN− suggesting that molecular subtyping may be a stronger driver for prognosis over histology alone. This finding is mirrored in PORTEC-3 where improved outcomes were noted in the p53 abnormal group who received chemotherapy in addition to radiation therapy, regardless of histology (León-Castillo et al., 2020). Future clinical trials are being designed with molecular considerations, and efforts are in place to better molecularly classify heterogeneous grade 3 endometrioid tumors (Bosse et al., 2018). Despite the development of molecular risk tools and integration into clinical trial design, none specifically address ITC, and management continues to be a clinical dilemma (Vermij et al., 2020). Of our serous tumors with *TP53* mutations, 2/5 had ITC. Given our small cohort and favorable outcome with ITC patients, further comparisons among ITC patients with and without TP53 are unable to made. This specific subgroup of patients is especially interesting, and we hope is a focus in future clinical trials.

One theory in EMCA recurrence is cell escape whereby small volume disease, such as ITC, are not recognized with traditional lymphadenectomy and increase risk of disease recurrence. The investigation by Plante et al and data presented by Backes et al find that classic high-risk features are the more important and reliable prognostic variables (Plante et al., 2017; Backes et al. 2017). Another investigation with 4/48 patients with ITC who sought to determine the incidence of low volume disease and association with relapse found no association between pathologic risk factors and size of lymphatic metastasis (Sawicki et al., 2015). These results, in conjunction to our data support that ITC may not be a good proxy for cell escape and predicting relapse.

While our primary objective, a descriptive outcome, was achievable, this pilot investigation is limited by its small sample size. Other limitations include liberal and non-standard adjuvant treatment, and our limited menu of high impact mutations. We hope to include data on mutations in the mismatch repair pathway and POLE exonuclease domain mutations in future investigations given prognostic importance however this data was not available for this unique cohort. Strengths include inclusion of high-risk histology; our cohort was enriched for high grade histologies with 1/3 of patients having a grade 3 tumors. All tumors were run through the same pipeline for consistency. This convenience sample was chosen for the unique data available; each patient included had both a SLN biopsy and completion lymphadenectomy eliminating patients being erroneously considered LN− or ITC only if simply one LN technique were employed. Prior investigations indicate high rates of positive non-SLNs when the SLNs have metastases (Rossi et al., 2017; Touhami et al., 2015). Given this association, it was critical to recognize patients with exclusively ITC-volume disease when defining molecular characteristics as these patients may be distinct from those with macrometastatic disease in the non-SLN. Our investigation is uniquely positioned to identify this group of patients.

This is the first paper to our knowledge that investigates the genomic profiles of tumors with exclusively ITC-volume metastases. This pilot investigation was feasible and adds to the collecting body of data emerging on genomic and molecular classification of EMCA while laying the foundation of genomic work to describe tumors with only ITCs. Genomic subgrouping of EMCA is a more precise method of classifying EMCA and future trials will undoubtedly utilize molecular profiles of tumors to guide therapy. Modern cohorts will benefit from multi-institutional collaboration given the rare nature of ITC and classification by TCGA subgroups to better expand our knowledge of how tumors with only ITC behave biologically, and most importantly whether EMCA patients with ITC should or should not be treated the same as those with macrometastases.

Presentations

Poster presentation at the American Society of Clinical Oncology

2018, Conquer Cancer Award Winner.

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CRediT authorship contribution statement

Stephanie A. Sullivan: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing - original draft, Writing - review & editing. **Gabriel Hawkins:** Data curation, Resources, Writing - review & editing. **Xiobai Zhao:** Data curation, Formal analysis, Software, Validation, Writing - review & editing. **Heejoon Jo:** Data curation, Resources, Writing - original draft, Writing - review & editing. **Neil Hayes:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Writing - review & editing. **Xiaoyan Deng:** Formal analysis, Methodology, Software, Writing - review & editing. **Dipankar Bandyopadhyay:** Formal analysis, Supervision, Methodology, Software, Writing - review & editing. **Victoria L. Bae-Jump:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing. **Emma C. Rossi:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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