Spliceosome Mutations in Metastatic Breast Cancer: An Analysis of a De-centralized Clinical Trial and Large Clinical-Genomic Dataset

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Background

- Tumors that harbor spliceosome mutations have aberrant mRNA transcripts; when translated into proteins this can lead to diversified neoantigen landscapes potentially increasing susceptibility to immune checkpoint inhibitors
- Spliceosomes mutations such as SF3B1, U2AF1 and SRSF2 are rare in solid tumors
- Enrollment into clinical trials for rare mutations can be challenging; de-centralized clinical trials have the potential to reach patients who may not have access to clinical trials
- Limited clinical data exist that describe the molecular and genomic features of cancers harboring spliceosome mutations
- We present data from (1) a decentralized clinical trial (PRISMM) examining feasibility and describing outcomes in patients with metastatic solid tumors with spliceosome mutations, and (2) using a large clinical-genomic database (CARIS) we describe molecular features in patients with breast cancer harboring spliceosome mutations, as well as patient outcomes

Methods

Patient Response to Immunotherapy using Spliceosome Mutational Markers (PRISMM) study (NCT04447651)

- Patients were recruited via social media, informational oncology websites, collaborations with patient advocates, and commercial genomic partners and directed toward the PRISMM website where an online form was completed, and NGS reports were uploaded
- Eligible patients resided within mainland United States, had metastatic solid tumors, and harbored SF3B1, U2A1, or SRSF2 mutations. Once eligibility was confirmed they were consented remotely
- Consented patients had relevant medical information obtained and verified by local medical records, and their case was presented at the Johns Hopkins Molecular Tumor Board (MTB) for review, which rendered written recommendations provided to patients and local oncologists
- Patients and their oncologists would choose whether or not to proceed with MTB recommendations; patient and provider questionnaires were administered monthly for the first 6 months and then every 3 months thereafter
- A blood collection kit was sent to patients for research labs at baseline
- Primary endpoints for feasibility included enrolling 60 patients, conducting MTB reviews within 4 weeks of consent in at least 80% of patients, and achieving 80% patient response on at least 1 follow-up questionnaire

CARIS genomic dataset

- Patients with breast cancer and spliceosome mutations (SF3B1, U2AF1 and SRSF2) were identified via the CARIS database
- RNA sequencing data was used to identify immune cell populations (ie T cells, macrophages)
- NGS (DNA) was used to evaluate mutational profiles
- Immunohistochemistry was used to characterize ER/PR/HER2 and PDL1 status
- Outcome data available through the CARIS dataset was obtained
- Descriptive correlations were made between spliceosome mutations and molecular features and outcomes (Kaplan Meier Method)

PRISMM study

Table 1. Enrolled patient demographic data

Total Enrolled	N=6
Race/Ethnicity	
White (Non-Hispanic)	6/6 (100%)
Gender	
Female	4/6 (67%)
Male	2/6 (33%)
Age	
Median	69
Range	42-75
Location	
Northeast	3/6 (50%)
South	2/6 (33%)
Midwest	1/6 (17%)
Rurality	
Urban	5/6 (83%)
Rural	1/6 (17%)
Tumor Type	
Breast cancer	4/6 (67%)
Ampullary adenocarcinoma	1/6 (17%)
Prostate cancer	1/6 (17%)
Immunotherapy Initiation	
Yes	3/6 (50%)
No	3/6 (50%)

Figure 1. Patient enrollment process

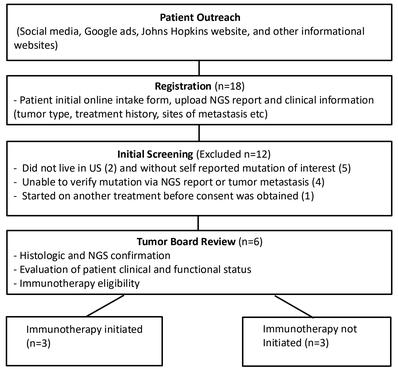


Table 2. (a) Feasibility and (b) efficacy outcomes

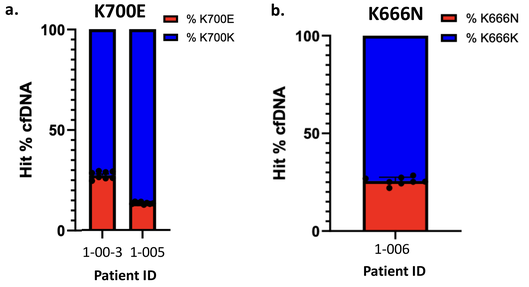
a. Feasibility data for the PRISMM trial. Six participants were eligible and were enrolled. Four participants underwent MTB review within one month of informed consent. All participants completed at least one follow up questionnaire.

	Target	Actual
Patient enrollment	60	6
MTB review within 4 weeks of consent	80%	4 (67%)
Patient response with at least 1 follow questionnaire	80%	100%
Baseline research blood collection	Exploratory	4 (67%)

b. Duration of treatment and overall survival of patients who underwent immunotherapy treatment. Median duration of treatment was 3 months (1-4 months) and median OS after start of immunotherapy was 4 months (1-32 months).

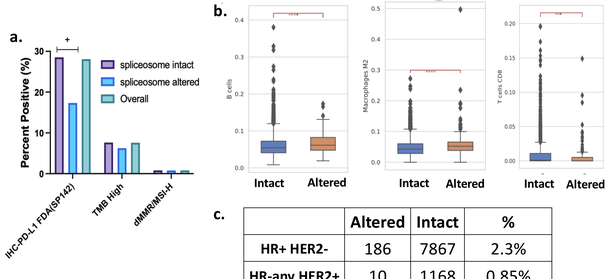
	Range (months)	Median (months)
Duration of Treatment	1 - 4	3
Overall Survival	1 - 32	4

Figure 2. Correlations between tissue-based NGS and baseline research blood ctDNA. (a) Two patients with SF3B1 K700E point mutation had variable allele frequency of 27.28% and 13.29%, respectively. (b) One patient with SF3B1 K666N mutation had variable allele frequency of 25.58%



CARIS analysis

Figure 3. Association of spliceosome mutations in breast cancer and (a) PDL1/TMB/MMR (b) immune cells by RNA seq, (c) subtype (d) genomic aberrations associated with markers of endocrine resistance



c.

	Altered	Intact	%
HR+ HER2-	186	7867	2.3%
HR-any HER2+	10	1168	0.85%
HR- HER2-	10	4429	0.23%

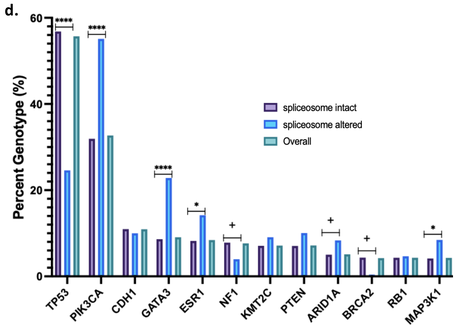
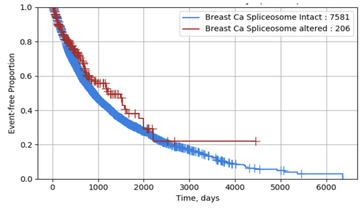


Figure 4. Overall survival in breast cancer in patients with spliceosome mutations. Breast cancer patients with mutated spliceosomes have improved OS (HR=1.251, 95% CI: 1.0-1.565, p <0.05).



Conclusions

- PRISMM trial did not meet its feasibility endpoints; however patient engagement was high and remote research blood collections were feasible
- No significant responses to immunotherapy in patients with spliceosome mutations in the PRISMM trial
- HR+/HER2- breast cancers had the highest frequency of spliceosome mutations
- Genomic aberrations in genes associated with endocrine resistance (ESR1, PIK3CA, MAP3K1, FGFR1) were observed in patients harboring spliceosome mutations
- Spliceosome mutations were associated with an immune-cold phenotype with higher M2 macrophages and CD8+ T-cell infiltration and lower expression PD-L1
- Breast cancers with spliceosome mutations demonstrated improved overall survival versus those that did not
- Future studies are need to understand the TME of tumors with spliceosome mutations in breast cancer and its potential associations with endocrine sensitivity/resistance