Genomic landscape of metastatic breast cancer (MBC) patients with methylthioadenosine phosphorylase (MTAP) loss

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ABSTRACT

Introduction: Homozygous deletion of MTAP upregulates de novo synthesis of purine (DNSP) and increases the proliferation of neoplastic cells. This increases the sensitivity of breast cancer cells to DNSP inhibitors such as methotrexate, L-alanosine and pemetrexed.

Materials and Methods: 7,301 cases of MBC underwent hybrid-capture based comprehensive genomic profiling (CGP). Tumor mutational burden (TMB) was determined on up to 1.1 Mb of sequenced DNA and microsatellite instability (MSI) was determined on 114 loci. Tumor cell PD-L1 expression was determined by IHC (Dako 22C3).

Results: 208 (2.84%) of MBC featured MTAP loss. MTAP loss patients were younger (p = 0.002) and were more frequently ER− (30% vs. 50%; p < 0.0001), triple negative (TNBC) (47% vs. 27%; p < 0.0001) and less frequently HER2+ (2% vs. 8%; p = 0.0001) than MTAP intact MBC. Lobular histology and CDH1 mutations were more frequent in MTAP intact (14%) than MTAP loss MBC (p < 0.0001). CDKN2A (100%) and CDKN2B (97%) loss (9p21 co-deletion) were significantly associated with MTAP loss (p < 0.0001). Likely associated with the increased TNBC cases, BRCA1 mutation was also more frequent in MTAP loss MBC (10% vs. 4%; p < 0.0001). As for immune checkpoint inhibitors biomarkers, higher TMB >20 mut/Mb levels in the MTAP intact MBC (p < 0.0001) and higher PD-L1 low expression (1–49% TPS) in the MTAP loss MTAP (p = 0.002) were observed.

Conclusions: MTAP loss in MBC has distinct clinical features with genomic alterations (GA) affecting both targeted and immunotherapies. Further efforts are necessary to identify alternative means of targeting PRMT5 and MTA2 in MTAP-ve cancers to benefit from the high-MTA environment of MTAP-deficient cancers.

INTRODUCTION

Breast cancer is the most diagnosed malignancy worldwide and a leading cause of cancer-related death in women [1]. Recent advancements in diagnostic and therapeutic modalities have led to improved survival and prognosis. Tumor metastasis is one of the driving factors for treatment failure and mortality from cancer with underlying molecular mechanism still poorly understood [2, 3]. One of such alterations includes loss of tumor suppressor gene [4]. Treatment modalities aimed at halting or possibly reversing the molecular pathway
leading to metastasis hold promise for effectively treating cancers.

Breast cancers can be broadly divided as per their hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status into HR positive, HER2 positive and triple negative breast cancer (TNBC) [5]. In addition to surgery, radiation therapy, endocrine therapy and hormone therapy, tumor-tailored treatment can be provided with therapies targeting HER2, PIK3CA, TRK, CDK4/6, BRCA1/2, and VEGF and PDL1 receptors. Research is underway for multiple other promising therapies [6, 7].

5′Methylthioadenosine phosphorylase (MTAP) is a key enzyme in the polyamine pathway and aids in catabolism of 5′Deoxy-5′-Methythioadenosine (MTA) leading to formation of methionine and adenine. MTAP gene is located at 9P21 surrounded by miR-31 and CDK2NA and has been reported to serve as a tumor suppressor gene [8–10]. MTAP deletion leads to low levels of adenine leading to cellular dependence on de novo purine synthesis and accumulation of MTA which in turn inhibits PRMT5 [11]. Most tumor cells have MTAP, P16 and other tumor suppressor genes located on 9P21 such as CDKN2A and CDKN2B making it a poor target for therapeutic regimens [11]. MTA accumulation in MTAP deleted cells creates a hypomorphic PRMT5 state that is sensitized towards further PRMT5 inhibition making PRMT5 inhibitors a potential therapy for MTAP deleted cancers [8]. PRMT5 inhibition leads to reduced histone methylation of which eventually leads to decrease FOXP1 expression (Figure 1). This not only creates sensitivity to PRMT5 targeting, but also leads to cell apoptosis and decreased metastasis [12, 13]. MTAP downregulation also promotes tumor metastasis by activating the GSK3B/slug/E-cadherin axis in esophageal squamous cell carcinoma [14]. In breast cancer, MTAP downregulation activates ornithine decarboxylase (ODC) which in turn leads to formation of putrescine which promotes tumor migration, invasion and angiogenesis [15]. Cytotoxicity assays with inhibitors of de novo adenine synthesis, 5-fluorouracil (5-FU), methotrexate (MTX) and 5′aza-deoxycytidine (AZA) after MTAP gene knockdown in breast cancer cell lines have shown an increased sensitivity to 5-FU [4].

RESULTS

Overall, 7301 cases of metastatic breast cancer underwent hybrid capture based comprehensive genomic profiling (CGP). 208 patients out of 7301 (2.84%) were noted to have MTAP loss (Table 1). The median age of patients with MTAP loss was 54.5 years compared to 57.8 years in MTAP intact (p = 0.002). Tumors with MTAP loss were noted to have lesser ER expression (50%) compared to MTAP intact (70%) (p < 0.001). Similarly, HER2 expression was less frequently noted in MTAP loss tumors as well (1.92% vs 7.8% in MTAP intact, p < 0.001). Triple negative status was noted more frequently noted in MTAP loss (47.28%) than MTAP intact (27%) (p < 0.05).

Among currently non-targetable mutations, MTAP loss tumors had higher frequency of TP53 (61.30 % vs.
Table 1: Targetable and non-targetable GA along with number of cases in our cohort and their characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cases with MTAP Intact</th>
<th>Cases with MTAP Loss</th>
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<tbody>
<tr>
<td>Number of Cases</td>
<td>7093</td>
<td>208</td>
</tr>
<tr>
<td>Mean Age*</td>
<td>57.8</td>
<td>54.5</td>
</tr>
<tr>
<td>ER+/PR+ Status by IHC**</td>
<td>70.0%/49.0%</td>
<td>50.00%/29.90%</td>
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<tr>
<td>HER2+ Amplification by CGP</td>
<td>7.80%</td>
<td>1.92%</td>
</tr>
<tr>
<td>TNBC Status*</td>
<td>27.00%</td>
<td>47.28%</td>
</tr>
<tr>
<td>Driver Alterations/sample**</td>
<td>5.7</td>
<td>8.81</td>
</tr>
<tr>
<td>Non-targetable GA (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53*</td>
<td>51.70</td>
<td>61.30</td>
</tr>
<tr>
<td>CDKN2A**</td>
<td>3.10</td>
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<tr>
<td>CDKN2B**</td>
<td>1.30</td>
<td>96.70</td>
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<tr>
<td>RB1*</td>
<td>7.20</td>
<td>1.40</td>
</tr>
<tr>
<td>CDH1*</td>
<td>14.30</td>
<td>0.90</td>
</tr>
<tr>
<td>Targetable GA (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN*</td>
<td>13.10</td>
<td>21.70</td>
</tr>
<tr>
<td>PIK3CA**</td>
<td>36.8</td>
<td>23.60</td>
</tr>
<tr>
<td>NF1</td>
<td>6.40</td>
<td>9.90</td>
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<tr>
<td>BRCA1**</td>
<td>3.70</td>
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<td>ERBB2 amplification*</td>
<td>7.80</td>
<td>1.92</td>
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<td>ERBB2 sequence mutation*</td>
<td>11.20</td>
<td>6.60</td>
</tr>
<tr>
<td>EGFR*</td>
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<td>5.20</td>
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<tr>
<td>Immuno-Oncology Drug Biomarkers</td>
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<tr>
<td>MSI High</td>
<td>Frequency</td>
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</tr>
<tr>
<td></td>
<td>Cases Tested</td>
<td></td>
</tr>
<tr>
<td>CD274 (PD-L1) Amp</td>
<td>1.10%</td>
<td>2.80%</td>
</tr>
<tr>
<td>STK11 Inactivating GA</td>
<td>1.50%</td>
<td>4.20%</td>
</tr>
<tr>
<td>Median TMB</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TMB &gt;10%/&gt;20%</td>
<td>7.84%/7.40%</td>
<td>5.32%/0.96%</td>
</tr>
<tr>
<td>PD-L1 Positive IC Expression (Dako 22C3)</td>
<td>Low (1–49%)*</td>
<td>11.45%</td>
</tr>
<tr>
<td></td>
<td>High (&gt; 50%)</td>
<td>2.86%</td>
</tr>
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</table>

*Significant (P < 0.05), **(P < 0.0001).

51.70, p < 0.05), CDKN2A (100% vs. 3.10%, p < 0.001) and CDKN2B (96.70% vs. 1.30%, p < 0.001) MTAP intact tumors had higher frequency of RB1 (7.20% vs. 1.40%, p < 0.05) and CDH1 (14.30% vs. 0.90%, p < 0.05).

Among targetable mutations, MTAP loss tumors had higher frequency of PTEN (21.70% vs. 13.10%, p < 0.05), BRCA1 (9.90% vs. 3.70%, p < 0.001) and EGFR mutation (5.20% vs. 2.60%, p < 0.05). MTAP intact tumors had higher frequency of PIK3CA (36.8% vs. 23.60%, p < 0.001), ERBB2 amplification (7.80% vs. 1.92%, p < 0.05) and ERBB2 sequence mutation (11.20% vs. 6.60%, p < 0.05). The expression of NF1 was not statistically significant with 9.90% in MTAP loss vs. 6.40 in MTAP intact.

The tumors were also tested for checkpoint inhibitor biomarkers. The analysis included 205 out of 208 MTAP loss and 7077 out of 7093 MTAP intact tumors. The PDL-1 low status analyzed by Dako 22C3 was statistically higher in MTAP loss compared to MTAP intact tumors (42.90% vs. 11.45%, p < 0.05).
vs. 11.45%, \( p < 0.05 \)). PDL-1 high status (>50%) was not noted in the \textit{MTAP} loss tumors and 2.86% in \textit{MTAP} intact, however this finding was not statistically significant. These results can be seen in Figure 2.

\section*{DISCUSSION}

\textit{MTAP} is an important enzyme found in almost all tissues in the body. It is an important enzyme in the

\begin{figure}[h]
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\caption{(A) Long Tail Plot of Genomic Alterations in MTAP Intact clinically advanced breast cancer. (B) Long Tail Plot of Genomic Alterations in ER+/HER2− clinically advanced breast cancer with MTAP Loss. (C) Long Tail Plot of Genomic Alterations in ERBB2 Amplified (HER2+) clinically advanced breast cancer with MTAP Loss. (D) Long Tail Plot of Genomic Alterations in Triple Negative clinically advanced breast cancer with MTAP Loss.}
\end{figure}
methionine salvage pathway, responsible for regenerating methionine and adenine, which is in turn essential for the cell cycle [13]. Following the concepts of synthetic lethality, when MTAP is lost in a tumor cell, MTA will build up inside the cell leading to more suppression of PRMT5, thereby increasing their vulnerability to inhibition [16, 17]. PRMT5 inhibitor molecules like GSK3326595, PRT81114 and JNJ-6461917815 are currently under investigation in advanced solid malignancies including breast cancer. Safety data on GSK3326595, were recently reported by the phase 1 METEOR-1 trial (NCT02783300) which included breast cancer patients. Phase 2 portion of the study is currently underway [18]. PRMT5 is an inhibitor of tumor suppressor genes and thus enables the unchecked proliferation of cancer cells. PRMT5 induces methylation of p53 and disrupts its ability to cause death of malignant cells. It also promotes cyclin kinase-dependent neoplastic growth. The clinical paradigm of MTAP deficient cells, by building up MTA, which is a potent inhibitor of PRMT5, was studied as early as 1981 [18]. Another evolving target of interest are the methionine adenosyltransferases MAT1a and MAT2a. They are important cofactors in the polyamine biosynthesis cycle and play an essential role in the growth and survival of cells. In vivo models have shown that MAT2a knockdown reduced the growth and development of MTAP deficient tumor cells [19]. IDE397, a small molecule inhibitor of MAT2A, is under investigation as a part of a Phase 1 trial for advanced solid tumors with MTAP deletion [20].

In our cohort of MBC, the frequency of MTAP loss was 2.84% (208/7301). In the COSMIC gene database, MTAP copy number variation loss was reported in 2.55% (38/1492) breast cancer samples [21]. In the AACR GENIE portal, MTAP deletion was reported in 2.9% (138/15210) breast cancer specimens [4, 22]. These findings are consistent with our analysis. Although literature on MTAP loss in breast cancer is scarce, MTAP and CDKN2A loss co-occur with concordance in up to 90% of the cases, enabling indirect estimation of MTAP loss. A study evaluating this, estimated MTAP loss at around 16% (19/119) from frozen section specimens. Larger datasets as mentioned above revealed a lower number, suggesting that the small size could be the limitation of this study [4].

We provide one of the first large analyses of the spectrum of GA occurring in MTAP deleted MBC with the hope that this would enable identifying potential therapeutic agents in the future.

Even though breast cancer is becoming more common worldwide, its prognosis has improved thanks to advances in early detection and treatment. Currently, distant metastasis has the largest influence on a breast cancer patient’s prognosis. The five-year survival rate of breast cancer patients without metastasis is over 80% [23], but that of patients with metastasis is only around 25% [2, 3]. Yet, the molecular basis for the spread of breast cancer remains poorly understood.

In many human malignancies, such as leukemia [24], lymphoma [25], lung cancer [26], pancreatic cancer [27], and melanoma [28, 29], MTAP is frequently suppressed or absent, making it a potential target for cancer treatment. However, uncertainty still exists regarding MTAP’s clinical and biological impact on breast cancer metastasis.

Similarly, Zhang et al. revealed for the first time a positive correlation between reduced MTAP expression and tumor recurrence in breast cancer patients, indicating that MTAP may be crucial to the malignant development of breast cancer [15]. This study showed that, in an orthotopic breast cancer model using BT20 cells, MTAP downregulation could greatly accelerate both tumor development and metastasis. MTAP expression also differs by breast cancer type: studies have shown that TNBC cells express significantly less MTAP than the more differentiated group made up of Luminal-A breast tumors, this would open the door for novel therapeutical strategies for the treatment of TNBC where endocrine or targeted therapy are usually ineffective [30].

The results of cytotoxicity assays using inhibitors of de novo adenine synthesis (5-FU, AZA, and MTX) after MTAP gene knockdown showed an increased sensitivity, primarily to 5-FU [4]. Vieira de Oliveira also evaluated MTAP expression in two groups of breast cancer patient samples, including fresh tumors and paired normal breast tissue, as well as formalin-fixed paraffin embedded (FFPE) core breast cancer samples diagnosed as Luminal-A tumors and TNBC. Although the difference in MTAP expression between fresh tumors and normal tissue was not statistically significant, MTAP expression was significantly higher in Luminal-A breast tumors compared to TNBC. This suggests that a lack of MTAP expression is associated with more aggressive breast tumors and may support the development of new therapeutic approaches based on MTAP status in TNBC. In our study, BRCA1 mutation was more frequent in MTAP loss MBC (10% vs. 4%; p < 0.0001) which was likely associated with the increased TNBC cases.

There is growing evidence that MTAP can control tumor invasion and migration through many signaling mechanisms. In esophageal cancer, MTAP depletion can activate the GSK3/Slug/E-cadherin axis, promoting migration and invasion [14]. In colorectal cancer, downregulation of MTAP can also influence the epithelial-to-mesenchymal shift and stimulate tumor growth and metastasis [30]. When MTAP is downregulated in melanoma, 5′-methylthioadenosine (MTA) builds up and promotes tumor spread by preventing protein methylation and activating the extracellular signal-regulated kinase (ERK) signal [28]. Emerging studies show that MTAP overexpression dramatically changes the amounts of polyamine metabolites (particularly putrescine) in
MTAP in various malignancies

Apart from the previously mentioned malignancies such as melanoma, esophageal and colorectal carcinoma, MTAP has a role to play in different malignancies.

Hellerband et al. [42] detected a decreased or even undetectable MTAP expression in three hepatocellular carcinoma lines and strong cytoplasmatic immunosignals were detectable in surrounding non-tumorous hepatocytes. These findings highlight that the downregulation or loss of MTAP expression in hepatocytes occurs during malignant transformation. Furthermore, Kirovski et al. [42] revealed that downregulation of MTAP in hepatocellular carcinoma increases MTA levels in hepatocellular carcinoma and can potentially be involved in HCC progression.

In osteosarcomas, Miyazaki et al. found that [43] MTAP deficiency was caused by MTAP gene deletion or promoter methylation in most MTAP-negative samples. In in vitro experiment, the MTAP-negative parental cell line was found to be more sensitive to inhibitors of de novo AMP synthesis, compared to the MTAP-positive transfectedoma. The authors suggested that the MTAP deficiency frequently observed in osteosarcoma can be targeted with inhibitors of de novo purine synthesis, as a potential chemotherapy strategy for MTAP-negative osteosarcoma patients [43].

Zimling, Jorgensen, and Santoni-Rugiu conducted a study where they analyzed MTAP reactivity in 99 cases of malignant pleural mesothelioma (MPM). They found that 65% of the tumors showed decreased MTAP reactivity. The authors suggested that this decrease in MTAP expression, along with other common markers, could be a valuable diagnostic tool for MPMs. Similarly, the reduced expression of MTAP in triple-negative breast cancer could serve as both a diagnostic and therapeutic marker. Low MTAP expression has been linked to a poor prognosis in glioblastoma [44], gastric cancer [45], and non-small cell lung cancer [46], according to earlier research.

Utilizing MTAP in treatment

Cytotoxic chemotherapy, radiotherapy, hormonal therapy, and immunotherapy have been shown to be effective in the treatment of breast cancer [47]. However, some breast cancer types, and especially TNBC, have no ongoing or maintenance treatment available. This might be due to the metabolic flexibility of cancer cells, which enables compensatory adaptations. It is believed that only a small number of tumor-specific metabolic vulnerabilities have been successfully targeted [48], and that many potential targeted therapies are under investigation, including therapies targeting MTAP deficiency [49].

The rationale behind MTAP targeted therapy is that adenine and methionine cannot be salvaged from endogenous MTA in MTAP-deficient cells. As a result, methionine deprivation and inhibitors of de novo purine synthesis are more toxic to MTAP-deficient cells than to MTAP-positive ones [50, 51]. The difficulty has been in developing a targeted therapy that takes advantage of MTAP deficiency and its resulting alterations in metabolism.

Different strategies based on MTAP status have been proposed that utilize inhibitors of de novo purine synthesis and the enzyme substrate MTA to specifically target and eliminate MTAP-negative cells [52–54].

According to several studies, MTAP-negative tumor cells are up to 20 times more susceptible to purine biosynthesis inhibitors such as MTX, 6-mercaptopurine, azaserine (a powerful inhibitor of the first step in purine biosynthesis), and L-alanosine, than MTAP-positive cells are [50, 55, 56]. The study by Hori et al., which transfected MTAP complementary DNA (cDNA) into a lung cancer cell line lacking MTAP, may have been the most convincing one demonstrating the link between MTAP deficiency and sensitivity to purine and methionine depletion. MTAP deficient cells proved to be more sensitive to purine synthesis inhibitors 5,10-dideazafolate, L-alanosine, and to methionine depletion. The MTAP-containing cell lines, but not the MTAP-deficient cell lines, were entirely rescued from these inhibitors and methionine restriction by adding MTA [56].

Other strategies to take advantage of MTAP-deficiency are also under investigation: MTA and adenine analogs such as 2,6-diaminopurine, 6-methylpurine, 2-fluoroadenine, 6-thioguanine (6-TG), and 5-FU that must undergo phosphoribosylation to transform into its toxic nucleosides are being studied to treat MTAP-deficient malignancies.
Future direction

When MTA is supplied to healthy host cells, MTAP produces a significant amount of adenine. After that, adenine successfully competes with these co-administered drugs for phosphoribosylation by 5-phosphoribosyl-1-pyrophosphate (PRPP). For the drug to have harmful activity, it must be transformed to its toxic nucleotide. However, tumor cells lacking MTAP are unable to convert MTA into adenine. Because of this, PRPP levels are sufficient, and the co-administered drug can easily be transformed to its harmful nucleoside [57]. The significant difference in MTAP activity between tumor and host cells ensures a high level of treatment selectivity, making it a promising therapy for MTAP deficient malignancies in general, and MTAP deficient breast cancer. MTAP loss is associated with ER-, HER2- and TNBC status, features a distinctive GL with potential to impact both targeted and immunotherapies and enables emerging clinical trials testing MTA2 and PRMT5 inhibitors for patients with clinically advanced breast cancer.

MATERIALS AND METHODS

The central laboratory (Foundation Medicine, Cambridge, MA, USA) used for comprehensive genomic profiling (CGP) is Clinical Laboratory Improvement Amendments (CLIA)-certified and accredited by the College of American Pathologists. Approval for this study, including a waiver of informed consent, was obtained from the Western Institutional Review Board (Protocol No. 20152817). A minimum of 50 ng of DNA was extracted from 7,301 cases of clinically advanced ductal and lobular breast cancers. Samples used for sequencing featured a minimum of 20% tumor nuclei. After DNA extraction and DNA library preparation, adaptor-ligation based hybrid capture was performed for all coding exons from 324 cancer-related genes plus select introns from 28 genes frequently rearranged in cancer. The Illumina HiSeq instrument was used for DNA sequencing to a mean exon coverage depth of ≥550X [58, 59]. Tumor mutational burden (TMB) was determined using 0.9 to 1.1 Mb of sequenced DNA [60]. Microsatellite instability (MSI) status was determined on 95 loci [61]. Given that no normal DNA sample was included from each patient, a computational approach was utilized to distinguish somatic vs. germline origin of genomic alterations [62]. PD-L1 expression was determined by immunohistochemistry using 5-micron tissue sections. Following the CDx assay guidelines a tumor proportion score (TPS) was determined for each sample stained with the DAKO 22C2 CDx assay. TPS = (positive tumor cells/total tumor cell) × 100. TPS of 0% was defined as negative, low-level staining defined as 1–49% TPS, and high-level staining defined as ≥50% TPS.

Differences in sample medians were assessed using the unpaired Mann–Whitney–Wilcoxon test. Differences among categorical variables were assessed using chi square test with Yates correction. Statistical tests were 2-sided and used a significance threshold of \( p < 0.05 \). Reported \( p \) values were not adjusted for multiple testing.

Abbreviations

5-FU: 5-fluourouracil; 6-TG: 6-thioguanine; AZA: 5’aza-deoxycytidine; cDNA: Complementary DNA; HR: hormone receptor; HER2: human epidermal growth factor receptor 2; ERK: Extracellular Signal-Regulated Kinase; MMP2: Matrix Metalloproteinase-2; MTA: 5’-Methylthioadenosine; MTAP: Methylthioadenosine Phosphorylase; MTR-1-P: Methylthioribose-1-Phosphate; MTX: Methotrexate; ODC: Ornithine decarboxylase; PRPP: 5-phosphoribosyl-1-pyrophosphate; TNBC: Triple-Negative Breast Cancer.

Author contributions

MBZ, JR, and AS contributed to the study concept and design. JR contributed to the acquisition of data. JR and PAK contributed to the analysis and interpretation of data. MBZ, NS, EH, and AS drafted the manuscript. All authors read, revised, and approved the final manuscript.

CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

Ethical statement and consent

Approval for this study, including a waiver of informed consent, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

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