Society of Medical Education & Research

2024, Volume 4, Issue 1, Page No: 67-80

ISSN: 3108-4834

Archive of International Journal of Cancer and Allied Science

Genomic Landscape and Clinical Implications of MTAP Loss in Advanced Gastrointestinal Malignancies

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Abstract

Deletion of the 9p21 chromosomal region is among the most frequently observed homozygous genomic events in human cancers. This locus contains the methylthioadenosine phosphorylase (MTAP) gene along with CDKN2A and CDKN2B, and its loss has been associated with inferior clinical outcomes and diminished benefit from immune checkpoint blockade. Recent therapeutic strategies have highlighted MTAP deficiency as a targetable vulnerability through synthetic lethal interactions involving MAT2A and PRMT5 inhibition. The present study was designed to determine the incidence of MTAP loss across advanced gastrointestinal (GI) malignancies, characterize its accompanying genomic features, and evaluate its relevance as a prognostic biomarker. Comprehensive next-generation sequencing was conducted on a large dataset comprising 64,860 tumor specimens derived from five distinct GI cancer types. Integrated genomic and clinical analyses were performed, and patient outcomes were retrospectively compared between tumors harboring MTAP loss and those retaining MTAP. Across all GI cancers examined, MTAP loss was detected in 8.30% of cases. The alteration was most frequently observed in pancreatic ductal adenocarcinoma (PDAC), affecting 21.7% of tumors, while colorectal carcinoma (CRC) exhibited the lowest frequency at 1.1%. A higher proportion of MTAP-deficient tumors was identified among East Asian patients with PDAC (4.4% vs 3.2%, P = .005) and intrahepatic cholangiocarcinoma (IHCC; 6.4% vs 4.3%, P = .036). The presence of MTAP loss was associated with tumor type–specific differences in the distribution of potentially actionable genomic alterations, including ATM, BRAF, BRCA2, ERBB2, IDH1, PIK3CA, and PTEN. In PDAC, IHCC, and CRC, MTAP-deficient tumors demonstrated lower rates of microsatellite instability and reduced tumor mutational burden. Furthermore, tumor cell PD-L1 expression was less commonly observed in MTAP-loss IHCC compared with MTAP-intact counterparts (23.2% vs 31.2%, P = .017).MTAP deficiency in gastrointestinal cancers predominantly arises in conjunction with deletion of the 9p21 locus and is present in approximately 8% of cases overall. The frequency of MTAP loss varies considerably by tumor subtype, occurring in 22% of PDAC, 15% of IHCC, 8.7% of gastroesophageal adenocarcinoma, 2.4% of hepatocellular carcinoma, and 1.1% of CRC. Importantly, MTAP loss does not preclude the coexistence of other clinically relevant, targetable genomic alterations.

Keywords: MTAP deficiency, 9p21 deletion, Gastrointestinal malignancies, Genomic profiling, Predictive biomarkers, Cholangiocarcinoma

Introduction

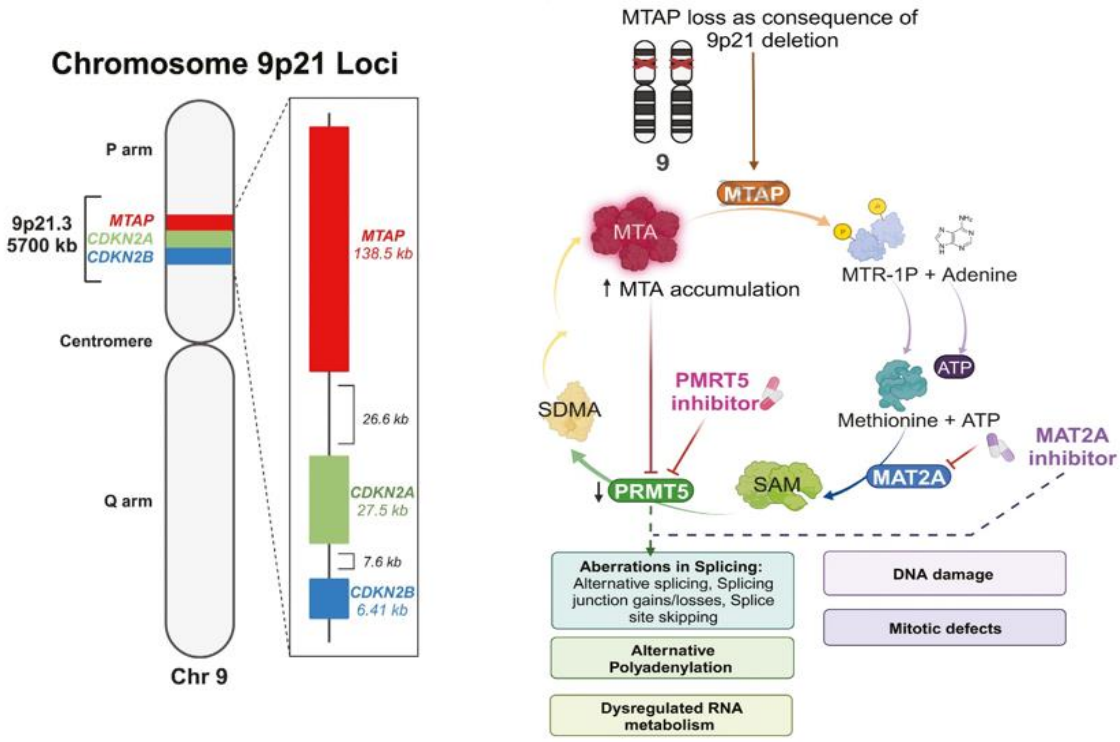
The detection of recurrent somatic homozygous deletions within cancer genomes has long been employed as an effective approach for discovering tumor suppressor genes [1–3]. Cytogenetic analyses conducted during the 1990s identified the p21 region of chromosome 9 as a hotspot for homozygous deletions across multiple tumor types, ultimately leading to the identification of the CDKN2A tumor suppressor gene in 1994 [4–6]. Homozygous loss of tumor suppressor loci is a critical driver of malignant transformation, and deletion of the 9p21 region (hereafter referred to as 9p21 loss) has been shown to represent an early event in cancer evolution [7, 8].

Access this article online https://smerpub.com/

Received: 03 November 2023; **Accepted:** 26 January 2024

How to cite this article: Lee MH, Choi JW. Genomic Landscape and Clinical Implications of MTAP Loss in Advanced Gastrointestinal Malignancies. Arch Int J Cancer Allied Sci. 2024;4(1):67-80. https://doi.org/10.51847/1wgE2vWBFG

The methylthioadenosine phosphorylase (MTAP) gene is located immediately adjacent to CDKN2A within the 9p21 locus and is frequently eliminated through the same interstitial chromosomal deletion that removes both CDKN2A and CDKN2B, a genomic alteration referred to in this study as MTAP loss [9] **(Figure 1a).** Pan-cancer analyses using The Cancer Genome Atlas dataset have reported homozygous MTAP deletion in approximately 9.3% of tumors, with an additional 27.8% exhibiting loss of heterozygosity at 9p21 due to hemizygous MTAP deletion [10]. Despite the relatively common occurrence of 9p21 and MTAP loss across malignancies, progress in this area has been hindered by the inherent difficulty of therapeutically targeting loss-of-function genetic alterations.



a) b)

Figure 1. Genomic localization of 9p21 and biological consequences of MTAP loss.

Scientific interest in the biology of MTAP deficiency has re-emerged following seminal studies published in 2016 demonstrating that MTAP loss creates a synthetic lethal vulnerability to inhibition of methionine adenosyltransferase 2A (MAT2A) and protein arginine methyltransferase 5 (PRMT5) [11–13]. MTAP functions as a rate-limiting enzyme in the terminal step of the methionine salvage pathway, facilitating the regeneration of intracellular adenine and methionine pools and supporting the metabolic demands of rapidly proliferating, metabolically stressed tumor cells [14] **(Figure 1b).** Loss of MTAP results in intracellular accumulation of methylthioadenosine (MTA), a metabolite that has been linked to more aggressive tumor behavior [15, 16]. Although the precise molecular basis underlying the synthetic lethal interaction between MTAP deficiency and MAT2A or PRMT5 inhibition has not been fully elucidated, emerging data suggest that dysregulation of alternative splicing and polyadenylation may contribute to this vulnerability [17, 18]. Notably, early-phase clinical trials have provided proof-of-concept evidence that MTAP-deficient tumors can be therapeutically targeted using PRMT5 inhibitors, with encouraging signals of clinical activity [19]

Despite these advances, the relevance of MTAP loss as a therapeutic target in advanced gastrointestinal (GI) malignancies has not been systematically evaluated. Existing studies describing the genomic features and clinical significance of MTAP loss have largely been conducted in pan-cancer cohorts, with relatively small

representations of GI tumors. In these broader analyses, MTAP loss has been associated with inferior overall survival (OS) [10]. In addition, deletion of the 9p21 locus encompassing MTAP has emerged as a predictive marker of an immunologically "cold" tumor microenvironment, characterized by altered immune cell infiltration, decreased T-cell receptor diversity, reduced PD-L1 expression, and shifts in immunoregulatory gene expression profiles [10, 20]. Across cohorts treated with anti–PD-(L)1 therapies—primarily consisting of patients with melanoma, lung, and urothelial cancers—tumors harboring 9p21 loss demonstrated significantly shorter progression-free survival (PFS) and disease-specific survival compared with 9p21-intact tumors [10].

In contrast, the frequency, associated genomic alterations, immune features, and clinical implications of MTAP loss in advanced GI cancers have not been previously defined. The present study therefore aimed to determine the prevalence of MTAP loss across major GI malignancies, characterize differences in genomic coalterations between MTAP-deficient and MTAP-intact tumors, and explore associations with immune-related biomarkers and clinical outcomes in common GI cancer subtypes.

Materials and Methods

Study populations and clinical data

Genomic Profiling Cohort (N = 64,860)

This investigation was performed following approval by the Western Institutional Review Board (Protocol No. 20152817). A large cross-sectional dataset was assembled consisting of patients diagnosed between January 1, 2018 and July 15, 2022 with one of five gastrointestinal (GI) malignancies: pancreatic ductal adenocarcinoma (PDAC), intrahepatic cholangiocarcinoma (IHCC), hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), or gastroesophageal adenocarcinoma (GEAC). All included patients had previously received comprehensive genomic testing as part of routine clinical management at a centralized molecular diagnostics facility accredited by the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP) (Foundation Medicine, Inc.).

Tumor classification was assigned based on the histopathologic diagnosis provided by the submitting clinician and subsequently confirmed through centralized pathology review. Available clinicopathologic variables, including age at testing, sex, histologic subtype, immunohistochemical findings, and diagnostic verification, were abstracted from pathology documentation and corresponding medical records.

Clinical Outcomes Cohort (N = 102)

To assess the prognostic relevance of homozygous MTAP deletion in advanced GI malignancies, a separate retrospective cohort was identified at The University of Texas MD Anderson Cancer Center (MDACC). This cohort included 102 patients with advanced-stage PDAC or IHCC diagnosed between January 11, 2018 and August 2, 2022 whose tumors had clearly defined MTAP copy number status, categorized as either homozygous loss or intact based on next-generation sequencing (NGS) results.

Patients with MTAP-intact tumors were matched to those with MTAP loss according to age, sex, and ethnic background. Clinical variables, including baseline demographics, treatment exposure, and therapeutic outcomes, were obtained through systematic review of electronic health records. Progression-free survival (PFS) was calculated from the start date of any systemic treatment line to documented clinical or radiographic progression or discontinuation of therapy for that treatment line, as determined by the treating oncologist. Overall survival (OS) was defined as the interval from initial cancer diagnosis to death from any cause.

Genomic analysis and sequencing methodology

Comprehensive genomic characterization of tumors in the genomics-only cohort was conducted using U.S. Food and Drug Administration–approved hybrid capture–based NGS assays. DNA was extracted from formalin-fixed, paraffin-embedded tumor specimens, each containing a minimum tumor cellularity of 20%. Library construction involved adaptor ligation followed by hybridization-based enrichment targeting the complete coding sequences of up to 324 cancer-associated genes, in addition to selected intronic regions from up to 31 genes commonly involved in oncogenic rearrangements.

Sequencing output was interrogated for a broad range of genomic alterations, including single nucleotide substitutions, short insertions and deletions, copy number changes (both amplifications and homozygous deletions), and select gene rearrangements or fusions, using previously validated methodologies [21]. Variant detection employed Bayesian statistical modeling for base substitutions, localized de novo assembly for short

insertions and deletions, comparative analysis against process-matched normal controls for copy number assessment, and evaluation of chimeric sequencing reads to identify rearrangement events [22].

For downstream analyses, short variants were included only if annotated as "Known Pathogenic" or "Likely Pathogenic," unless otherwise specified. MTAP status was assigned based on copy number estimates generated by the NGS pipeline; tumors designated as MTAP loss uniformly demonstrated a copy number of zero, consistent with homozygous deletion of the MTAP locus.

PD-L1 immunohistochemical analysis

Evaluation of programmed death-ligand 1 (PD-L1) expression was conducted in selected tumor samples from the Foundation Medicine cohort using the Dako 22C3 PharmDx assay. All staining procedures were performed in a CLIA- and CAP-certified reference laboratory in strict accordance with the manufacturer's protocol. PD-L1 expression was assessed by a board-certified pathologist and quantified using the tumor proportion score (TPS). TPS was calculated as the percentage of viable tumor cells exhibiting any definitive partial or complete linear membranous staining, excluding purely cytoplasmic staining, relative to the total number of viable tumor cells, multiplied by 100. Tumors were categorized as PD-L1 "low positive" when TPS ranged from 1% to 49%, and as PD-L1 "high positive" when TPS exceeded 50%.

Tumor mutational burden and microsatellite instability

Tumor mutational burden (TMB) for cases in the genomics-only cohort was calculated using sequencing data spanning approximately 0.83–1.14 megabases (Mb) of interrogated genomic regions. A validated computational algorithm was applied to estimate mutation burden by extrapolating detected genomic alterations to the exome or whole-genome level, as previously described [23]. Microsatellite instability (MSI) status was determined through analysis of sequencing data from 114 microsatellite loci, each consisting of repeat lengths between 7 and 39 base pairs [24]. An NGS-derived MSI score was generated and subsequently classified as MSI-high, MSI-intermediate, or microsatellite stable using unsupervised clustering based on samples with MSI status previously defined by gold-standard testing methods.

Inference of genomic ancestry

Because self-reported or physician-documented race information was unavailable in the genomics-only cohort, patient ancestry was inferred computationally using ancestry-informative genetic markers. Genomic ancestry assignment was performed with a random forest classification model based on single nucleotide polymorphism (SNP) variation, categorizing samples into one of six ancestral groups: East Asian, European, South Asian, African, Admixed American, or South Asian [25].

COSMIC trinucleotide mutational signature analysis

Mutational signature profiling was conducted in accordance with previously established methodologies [26]. The overall distribution of mutational burden was first evaluated to determine an appropriate cutoff for reliable signature detection [27]. Analyses focused on six major COSMIC trinucleotide mutational signatures: mismatch repair deficiency (signatures 6, 15, 20, and 26), APOBEC-mediated mutagenesis (signatures 2 and 13), ultraviolet light exposure (signature 7), polymerase epsilon deficiency (signature 10), tobacco-associated mutagenesis (signature 4), and alkylating agent exposure (signature 11).

Statistical methods

All statistical analyses were performed using SPSS version 28.1.1 (IBM Corp., Armonk, NY, USA). Differences between categorical variables were evaluated using Fisher's exact test. Adjustment for multiple comparisons was performed using Bonferroni correction to control the false discovery rate. All statistical tests were two-sided, and a P-value of less than .05 was considered statistically significant. Graphical data representation was generated using R version 4.3.1. Survival outcomes, including overall survival (OS) and progression-free survival (PFS), were analyzed using the Kaplan–Meier method, with survival curves generated in Prism version 10. Group comparisons were performed using the log-rank test. Multivariable survival analyses were conducted using Cox proportional hazards regression models to estimate hazard ratios (HRs), 95% confidence intervals (CIs), and corresponding P-values.

Results and Discussion

Genomics-only cohort

Analysis of the genomics-only cohort revealed that MTAP loss was observed in 21.6% of pancreatic ductal

adenocarcinoma (PDAC) cases (3401/12,319 tumors), 15.3% of intrahepatic cholangiocarcinoma (IHCC) cases (785/4352 tumors), 8.7% of gastroesophageal adenocarcinoma (GEAC) cases (589/6143 tumors), 2.4% of hepatocellular carcinoma (HCC) cases (32/1306 tumors), and 1.1% of colorectal carcinoma (CRC) cases (396/35,537 tumors) (**Figures 2a and 1**). Across the five gastrointestinal tumor types profiled, MTAP loss was present in 8.7% of tumors overall (5203/59,657). The distribution of MTAP-loss cases was similar with respect to patient age and sex **(Table 1).**

Genetic ancestry analysis indicated that the majority of tumors across all types were of European descent (PDAC: 78.2%; IHCC: 73.7%; GEAC: 90.7%; HCC: 65.3%; CRC: 72.4%). Notably, MTAP-loss tumors were enriched among patients of East Asian (EAS) genomic ancestry in PDAC (4.4% versus 3.2%, P = .005) and IHCC (6.4% versus 4.3%, P = .036) when compared with tumors retaining MTAP.

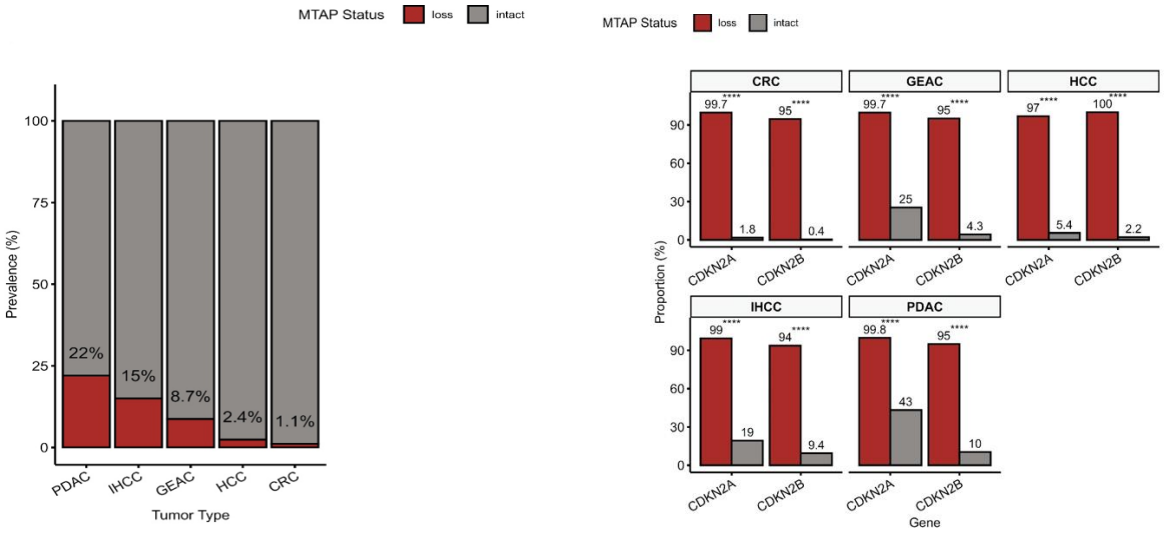


Figure 2. Frequency of MTAP loss and 9p21 alterations in gastrointestinal cancers.

(a) Distribution of MTAP loss across various GI tumor types as determined by immunohistochemistry (IHC).

(b) (b) Rates of co-alterations in 9p21-associated genes CDKN2A and CDKN2B, comparing MTAP-loss versus MTAP-intact tumors.

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; IHCC, intrahepatic cholangiocarcinoma; CRC, colorectal carcinoma; GEAC, gastroesophageal adenocarcinoma; HCC, hepatocellular carcinoma

Statistical significance: *P ≤ .05; **P ≤ .01; ***P ≤ .001; ****P ≤ .0001.

Table 1. Baseline demographic and clinical characteristics of patients in the genomics-only and clinical outcomes cohorts.

Cohort	PDAC: MTAP Intact	PDAC: MTAP Loss	IHCC: MTAP Intact	IHCC: MTAP Loss	HCC: MTAP Intact	HCC: MTAP Loss	CRC: MTAP Intact	CRC: MTAP Loss	GEAC: MTAP Intact	GEAC: MTAP Loss
Genomics-only cohort[a]										
Number of cases (% within tumor type)	12,319 (78.4)	3,401 (21.6)	4,352 (84.7)	785 (15.3)	1,306 (97.6)	32 (2.4)	35,537 (98.9)	396 (1.1)	6,143 (91.3)	589 (8.7)

Gender (% male)	52.9	52.4	48.9	51.1	75.3	59.4	55.6	58.3	86.4	83.7
Age, median, years (range)	67 (23-89)	67 (25-89)	66 (18-89)	66 (18-89+)	67 (5-89)	61.5 (34-83)	61 (10-89)	61.5 (21-89)	66 (22-89)	65 (21-89)
Genomic ancestry, %										
AFR	10.1	9.8	8.8	8.0	13.9	12.5	12.5	10.9	2.8	2.0
AMR	7.5	8.0	11.9	10.8	12.5	12.5	10.1	10.9	5.1	5.6
EAS	3.2	4.4	4.3	6.4	6.7	9.4	40.5	45.5	1.1	0.8
EUR	78.5	77.1	73.6	74.2	65.3	65.6	72.4	73.5	90.6	91.0
SAS	0.7	0.7	1.4	0.5	1.6	0	0.9	0.3	0.4	0.5
Clinical outcomes cohort										
Number of cases (% within tumor type)	11	21	49	21	—	—	—	—	—	—
Gender (% male)	72.7	33.3	50.0	33.3	—	—	—	—	—	—
Age, median, years (range)	62.7 (52.4-74.4)	60.8 (19.0-70.9)	57.3 (23.6-77.2)	54.0 (23.3-80.5)	—	—	—	—	—	—
Ethnicity										
Caucasian	9	14	40	17	—	—	—	—	—	—
African	1	5	1	1	—	—	—	—	—	—
Hispanic	1	2	3	1	—	—	—	—	—	—
Asian	0	0	5	2	—	—	—	—	—	—
NGS panel used, %										
FoundationOne CDx	5	17	43	21	—	—	—	—	—	—
Perthera	2	1	0	0	—	—	—	—	—	—
Tempus xT	3	3	6	0	—	—	—	—	—	—
MSK IMPACT	1	0	0	0	—	—	—	—	—	—

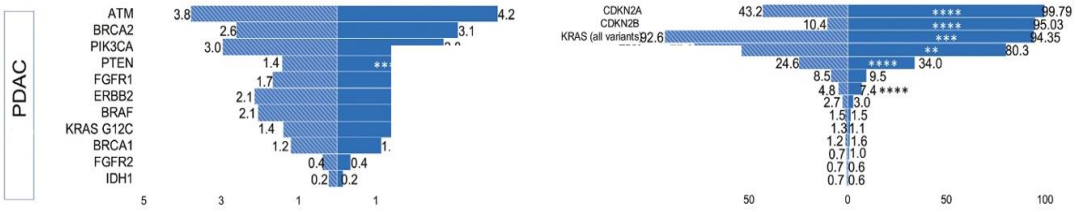
[a]The FoundationOne CDx NGS panel was used for the entire Genomics-only cohort: 64 860 (100%).

Abbreviations: *MTAP*, methylthioadenosine phosphorylase; PDAC, pancreatic ductal adenocarcinoma; IHCC, intrahepatic cholangiocarcinoma; HCC, hepatocellular carcinoma; CRC, colorectal carcinoma; GEAC, gastroesophageal adenocarcinoma; AFR, African; AMR, admixed American; EAS, East Asian; EUR, European; SAS, South Asian.

Analysis of genomic profiles comparing MTAP-loss and MTAP-intact tumors across gastrointestinal cancers revealed that MTAP-deficient tumors almost always carried co-alterations in CDKN2A and CDKN2B (**Figure 2b**). Across all five tumor types, the prevalence of these co-alterations differed significantly depending on MTAP status. In colorectal carcinoma (CRC), only a small fraction of MTAP-intact tumors exhibited mutations in CDKN2A (1.8%) or CDKN2B (0.4%) among 35,537 cases. By contrast, nearly every CRC tumor with MTAP loss displayed concurrent CDKN2A (99.7%) and CDKN2B (95%) alterations, despite the low overall frequency of MTAP loss in CRC (1.1%). Similarly, in pancreatic ductal adenocarcinoma (PDAC), MTAP-intact tumors already demonstrated relatively high mutation rates in CDKN2A (43%) and CDKN2B (10%), whereas MTAP-loss PDAC tumors occurred almost exclusively as part of a 9p21 deletion, with 99.8% and 95% harboring co-alterations in CDKN2A and CDKN2B, respectively.

When examining mutations with potential clinical actionability, significant differences emerged between

MTAP-loss and MTAP-intact tumors **(Figures 3 and 2).** In CRC, ERBB2 alterations were enriched in MTAP-deficient tumors (8.8% vs 5.1%, P = .008), while PIK3CA mutations were less frequent (13.4% vs 19.0%, P = .015). In gastroesophageal adenocarcinoma (GEAC), ATM mutations were more common in MTAP-loss cases (6.1% vs 3.6%, P = .038). No significant disparities were observed in hepatocellular carcinoma (HCC). For intrahepatic cholangiocarcinoma (IHCC), MTAP-loss tumors had higher frequencies of BRCA2 (3.4% vs 2.0%, P = .043) and BRAF (9.2% vs 4.7%, P < .0001) mutations, while IDH1 mutations were less frequent (6.9% vs 15%, P < .0001) compared with MTAP-intact tumors. FGFR2 mutations in IHCC did not differ significantly by MTAP status (12.7% vs 11.6%, P = .594). In PDAC, PTEN mutations were slightly more prevalent in MTAP-loss tumors (2.4% vs 1.4%, P = .001).



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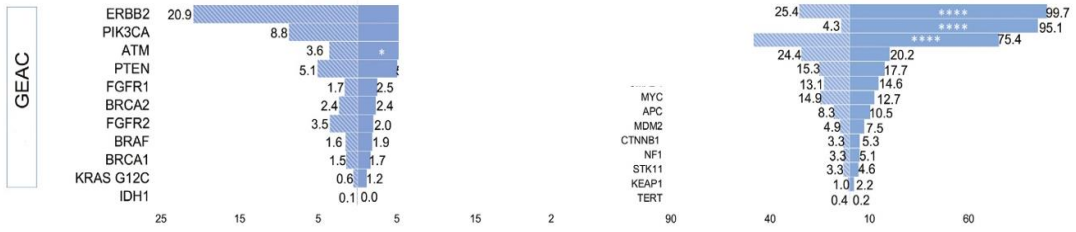

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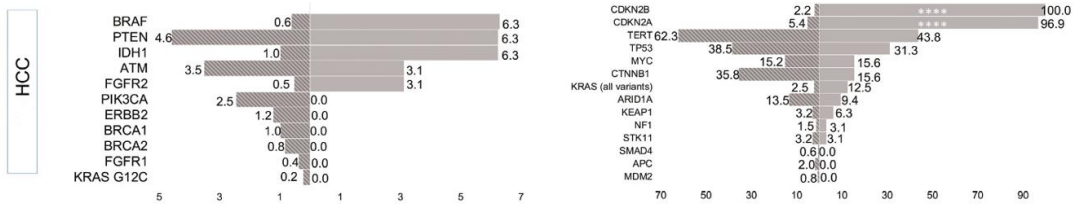


e)

Figure 3. Genomic alteration patterns in MTAP-loss versus MTAP-intact tumors in the genomics-only cohort. Panels display the prevalence of co-occurring mutations classified as either potentially targetable or untargetable across five GI cancer types: (a) pancreatic ductal adenocarcinoma (PDAC), (b) intrahepatic cholangiocarcinoma (IHCC), (c) colorectal carcinoma (CRC), (d) gastroesophageal adenocarcinoma (GEAC), and (e) hepatocellular carcinoma (HCC). In the graphs, solid bars represent MTAP-loss tumors, and striped bars indicate MTAP-intact tumors. Values shown correspond to the percentage of tumors with each alteration. Statistical significance is indicated as follows: *P ≤ .05; **P ≤ .01; ***P ≤ .001; ****P ≤ .0001.

The frequency of alterations in genes considered "undruggable" differed depending on tumor type and MTAP status (**Figure 3).** In CRC, MTAP-loss tumors had fewer APC mutations (48% vs 79%, P < .0001) and CTNNB1 mutations (1.5% vs 4.3%, P = .014) than MTAP-intact tumors, but showed higher rates of STK11 (3.3% vs 0.7%, P < .0001) and KEAP1 (1.3% vs 0.4%, P = .067) alterations. GEAC tumors with MTAP loss exhibited a lower proportion of TP53 mutations (75% vs 87%, P < .0001).

In IHCC, MTAP-loss tumors had slightly reduced TP53 (31% vs 35%, P < .05) and TERT (5% vs 8.1%) mutation frequencies, whereas KRAS (23% vs 19%, P < .05), SMAD4 (14% vs 6.6%, P < .0001), and KEAP1 (2.4% vs 1.1%, P < .05) mutations were more frequent. PDAC tumors with MTAP loss had higher rates of KRAS (94.4% vs 92.6%, P = .001), TP53 (80.3% vs 77.8%, P = .005), and MYC (7.4% vs 4.8%, P < .0001) mutations compared with MTAP-intact PDAC. SMAD4 alterations were consistently more common in MTAP-loss tumors across PDAC (34.1% vs 24.6%, P < .0001), IHCC (13.6% vs 6.6%, P < .0001), and CRC (24.2% vs 16.9%, P = .001).

Regarding immunotherapy biomarkers, differences by MTAP status were evident in CRC, IHCC, and PDAC. Microsatellite instability–high (MSI-H) was less common in MTAP-loss tumors in CRC (0.5% vs 5.7%, P < .0001), IHCC (0.4% vs 2.1%, P = .001), and PDAC (0.1% vs 0.6%, P = .0008; **Figure 4a**). PD-L1 immunohistochemistry data were available for a subset of tumors: 34% of PDAC, 17.5% of IHCC, 25% of HCC, 18.2% of CRC, and 12.3% of GEAC, with differences in expression observed depending on tumor type **(Figure 4b; Table 1).**

Tumor mutational burden (TMB) was significantly reduced in MTAP-loss tumors in CRC (4.6 vs 7.3 Mut/Mb, P < .0001) and IHCC (2.5 vs 3.0 Mut/Mb, P = .0015; **Figure 4c**). Additional analyses using TMB thresholds confirmed statistically meaningful differences in CRC, IHCC, and PDAC **(Figure 4d).**

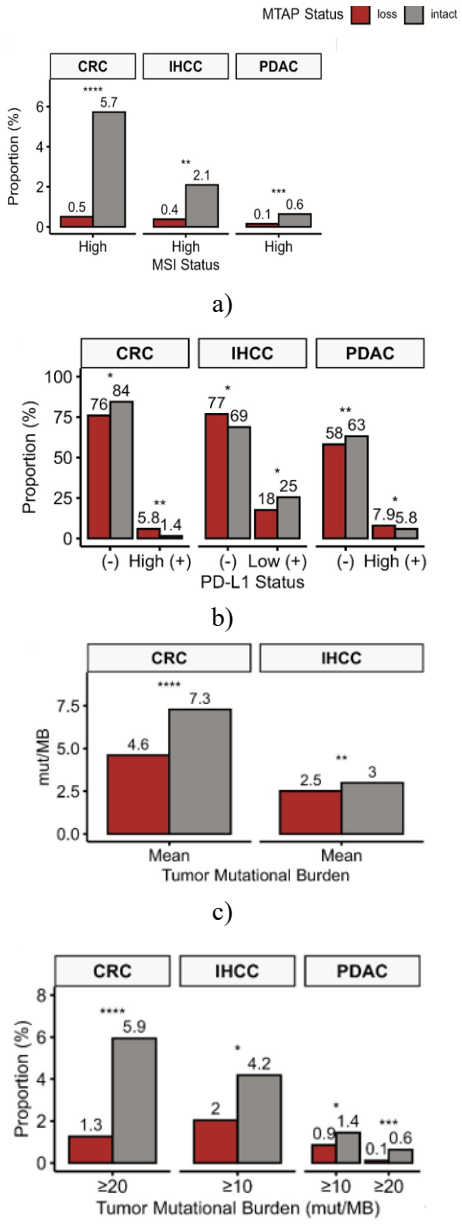


Figure 4. Immune biomarker comparisons between MTAP-loss and MTAP-intact tumors. Only statistically significant results are displayed for colorectal carcinoma (CRC), intrahepatic

cholangiocarcinoma (IHCC), and pancreatic ductal adenocarcinoma (PDAC). Panel A illustrates microsatellite instability (MSI) status, panel B shows PD-L1 expression, panel C reports mean tumor mutational burden (TMB), and panel D depicts TMB using standard clinical cutoffs. Symbols (-) and (+) represent negative and positive results, respectively. Statistical significance is indicated as follows: *P ≤ .05; **P ≤ .01; ***P ≤ .001; ****P ≤ .0001.

Among 5,234 tumors from the Genomics-only cohort, trinucleotide mutational signatures were evaluated using COSMIC-based analysis **(Table 2).** In CRC, the APOBEC-related mutational signature was more frequently detected in MTAP-loss tumors than in MTAP-intact cases (7.3% vs 1.3%, P = .054). A similar enrichment was observed in PDAC (16.7% vs 5.6%, P = .044). Additionally, UV-associated mutational patterns were more common in MTAP-loss CRC compared with MTAP-intact tumors (4.9% vs 0.2%, P = .015).

Table 2 summarizes key statistically significant genomic co-alterations and immunotherapy biomarkers identified in MTAP-loss GI tumors. This table highlights differences across tumor types, offering a concise overview of alterations that may have therapeutic or prognostic relevance.

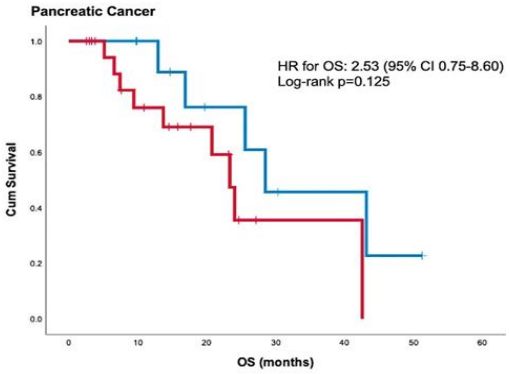
Table 2. Key co-alterations and immunotherapy markers in gastrointestinal tumors harboring MTAP loss.

Tumor	Higher prevalence in MTAP-loss: Genomic alterations	Higher prevalence in MTAP-loss: Immunotherapy markers	Lower prevalence in MTAP-loss: Genomic alterations	Lower prevalence in MTAP-loss: Immunotherapy markers
CRC	CDKN2A, CDKN2B, ERBB2, KEAP1, SMAD4, STK11	PD-L1 high positive	APC, CTNNB1, PIK3CA	MSI-high, mean TMB, TMB ≥ 20 mut/Mb, PD-L1 negative
GEAC	ATM, CDKN2A, CDKN2B, KRAS (G12X)	—	TP53	—
HCC	CDKN2A, CDKN2B	—	—	—
IHCC	BRAF, BRCA2, CDKN2A, CDKN2B, KEAP1, KRAS (all variants), SMAD4	PD-L1 negative	IDH1, TERT, TP53	MSI-high, mean TMB, TMB ≥ 10 mut/Mb, PD-L1 low positive
PDAC	CDKN2A, CDKN2B, KRAS (all variants), MYC, PTEN, SMAD4, TP53	PD-L1 high positive	—	MSI-high, TMB ≥ 10 mut/Mb, TMB ≥ 20 mut/Mb, PD-L1 negative

Clinical outcomes cohort

At MD Anderson Cancer Center, 42 patients with advanced gastrointestinal tumors—equally split between pancreatic ductal adenocarcinoma (PDAC, n = 21) and intrahepatic cholangiocarcinoma (IHCC, n = 21)—were found to have homozygous MTAP loss. These cases were compared to a group of 60 patients with intact MTAP, matched for key demographic and clinical features **(Tables 1 and 3).** Patients with MTAP-deficient tumors showed a tendency toward shorter overall survival (OS) relative to MTAP-intact patients; however, this difference did not reach statistical significance **(Figure 5).**

When examining the impact of additional co-alterations on survival in IHCC, multivariate analysis highlighted several genes linked to poorer outcomes. Specifically, CDKN2A alterations were associated with a more than twofold increase in the hazard of death (HR 2.15; 95% CI 1.06–4.40; P = .035). Similarly, CCNE1 alterations conferred a markedly higher risk (HR 8.86; 95% CI 1.16–67,369; P = .035), and MYC alterations also correlated with reduced OS (HR 1.28; 95% CI 1.28–7.10; P = .012) **(Table 4).**



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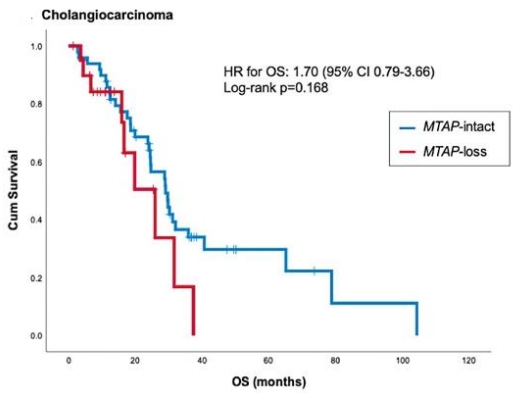

b)

Figure 5. Impact of MTAP status on overall survival in the Clinical Outcomes Cohort. (a) Kaplan-Meier survival analysis for patients with advanced pancreatic cancer. (b) Kaplan-Meier survival analysis for patients with advanced cholangiocarcinoma.

MTAP loss has recently emerged as a promising target for cancer therapy. In MTAP-deficient tumors, intracellular accumulation of methylthioadenosine (MTA) increases sensitivity to inhibition of PRMT5 and MAT2A, creating a synthetic lethal vulnerability [12, 28]. This relationship is being actively pursued in early-phase clinical trials for advanced solid tumors. For example, a Phase I study of MRTX1719, a PRMT5 inhibitor, has produced RECIST partial responses in patients with MTAP-loss mesothelioma, non-small cell lung cancer, melanoma, gallbladder adenocarcinoma, and malignant peripheral nerve sheath tumors [19]. The responses observed in gallbladder adenocarcinoma—a typically aggressive and therapy-resistant cancer—highlight the potential relevance of MTAP-targeted therapies in gastrointestinal (GI) malignancies.

Despite growing interest, data on MTAP loss in GI cancers remain limited. Fundamental epidemiologic information, such as prevalence and co-occurring genomic alterations, is largely unknown, yet is critical for guiding trial design. In this study, we analyzed over 64,000 tumor profiles from the five most common GI cancers to quantify MTAP-loss prevalence, explore genomic differences between MTAP-loss and MTAP-intact tumors, and evaluate overlap with established immunotherapy biomarkers. To our knowledge, this represents the largest dataset investigating MTAP status in GI tumors.

Our results show that MTAP loss is among the more common potentially targetable alterations in PDAC (22%), IHCC (15%), and gastroesophageal adenocarcinoma (GEAC, 8.7%) [29–31]. In contrast, MTAP loss is rare in hepatocellular carcinoma (HCC) and colorectal carcinoma (CRC), suggesting that dedicated clinical trials for these tumor types may have limited feasibility. Importantly, MTAP loss in GI cancers almost always occurs as part of 9p21 deletion, with concurrent CDKN2A and CDKN2B loss. Previous genomic studies and some commercial panels have not consistently tested for MTAP specifically, complicating retrospective outcome analyses [32, 33]. However, near-universal testing for CDKN2A and CDKN2B can serve as a surrogate for identifying MTAP loss or guide focused MTAP testing in select cases. Recognizing that MTAP loss effectively reflects 9p21 loss also facilitates interpretation of molecular and clinical studies on this chromosomal region [10, 20, 34]. In PDAC and GEAC, some MTAP-intact tumors still harbor CDKN2A and CDKN2B alterations, making direct confirmation of MTAP status particularly important in these cancers.

Demographic analysis revealed no significant differences in age or sex between patients with and without MTAP loss. Notably, tumors from individuals of East Asian genomic ancestry were more likely to exhibit MTAP loss, suggesting a potential genetic predisposition, reminiscent of patterns seen with EGFR mutations in East Asian lung cancer populations [35]. Further investigation is warranted, as existing studies report conflicting data regarding MTAP-loss prevalence in East Asian patients [36, 37].

Our analysis indicates that MTAP-loss in gastrointestinal (GI) cancers does not occur exclusively from other targetable driver mutations. While current clinical trials for MTAP-loss focus on MAT2A or PRMT5 inhibitors as single agents, combination targeted approaches may ultimately offer superior efficacy [38]. The comprehensive mutational profiling provided in this study highlights the prevalence of co-occurring alterations that could potentially be exploited in combination therapy. For instance, in intrahepatic cholangiocarcinoma (IHCC), MTAP-loss tumors demonstrated a significantly higher frequency of BRCA2 and BRAF alterations, suggesting a potential biological interdependence that could be leveraged therapeutically. Prior research has linked breast cancer risk to defects in methionine metabolism and a methionine-dependence phenotype in BRCA1/2 mutation carriers [39]. These observations imply that a combination strategy involving poly(ADP)-ribose polymerase inhibitors and MAT2A or

PRMT5 inhibitors in MTAP-loss GI cancers warrants further preclinical investigation.

In pancreatic ductal adenocarcinoma (PDAC), PTEN mutations were enriched among MTAP-loss tumors. This finding is notable as several early-phase trials are evaluating PI3K/Akt/mTOR-targeted therapies, which could be considered for combinatorial regimens in this context [40]. Alternatively, a sequential treatment strategy may be appropriate for patients harboring both MTAP-loss and actionable genomic alterations, thereby broadening the therapeutic options. For example, approximately 36% of BRAF-mutated IHCC cases have been previously reported to co-occur with MTAP-loss, a trend recapitulated in our cohort [41]. These patients could potentially receive BRAF inhibitors, which have tumor-agnostic FDA approval, followed by PRMT5 or MAT2A inhibitors upon progression, or vice versa [42].

Previous studies have associated 9p21 or MTAP-loss with poor immunotherapy response, possibly due to mechanisms involving immune evasion through cell cycle regulation, metabolic pathways, and type I interferon responses [43]. In our Genomics-only cohort, MTAP-loss in CRC, IHCC, and PDAC correlated with a significantly lower likelihood of MSI-high status. PD-L1 expression was significantly reduced in MTAP-loss IHCC tumors, whereas it was unexpectedly elevated in MTAP-loss PDAC and CRC. Interestingly, MTAP-loss CRC also demonstrated increased alterations in STK11 and KEAP1, genes previously implicated in immunotherapy resistance in NSCLC and pan-cancer analyses [44, 45].

Mutational signature analysis provides insight into the processes shaping tumor genomes [27, 46]. In our cohort, MTAP-loss PDAC and CRC exhibited elevated APOBEC and UV-associated mutational signatures, respectively, suggesting potential differences in endogenous and exogenous mutational processes in MTAP-loss GI cancers that merit further study.

Regarding clinical outcomes, no robust survival data currently exist for MTAP-loss in GI cancers. In both advanced PDAC and IHCC, patients with MTAP-loss tumors showed shorter median overall survival (OS) compared to MTAP-intact cases, though these differences were not statistically significant. In IHCC, CDKN2A alterations, rather than MTAP-loss itself, were significantly associated with worse median OS in multivariate analysis. Prospective studies are needed to clarify the prognostic relevance of MTAP-loss in advanced PDAC and IHCC.

Limitations of this study include the relatively small sample size in the Clinical Outcomes Cohort (n = 102) and the fact that only approximately one-third of patients had PD-L1 immunohistochemistry and fewer than 10% had COSMIC trinucleotide mutational signature analysis. Additional limitations inherent to retrospective analyses include potential selection bias and variability in treatment, monitoring, and follow-up across the cohort. While NGS was used to reliably detect homozygous MTAP-loss, we did not assess alternative mechanisms of MTAP inactivation, such as epigenetic silencing via promoter methylation, which has been reported in melanoma and glioblastoma [47, 48].

Conclusion

MTAP-loss represents a promising therapeutic target, particularly in PDAC, IHCC, and gastroesophageal adenocarcinoma (GEAC), where it exhibits a relatively high prevalence and distinct genomic features. In gastrointestinal cancers, MTAP-loss almost always occurs as part of 9p21 deletion, and our comprehensive genomic and immunotherapy biomarker profiling provides a foundation for future translational and clinical research. The absence of mutual exclusivity with other actionable mutations, combined with the identification of co-occurring targetable alterations, highlights the potential for sequential or combination treatment strategies in patients with MTAP-loss GI tumors.

Acknowledgments: **Figure 1** was created with BioRender.com.

Conflict of Interest: Dean C. Pavlick, Vamsi Parimi, Richard S.P. Huang, Tyler Janovitz, Natalie Danziger, Mia A. Levy, and Jeffrey S. Ross are current or former employees of Foundation Medicine, a wholly owned subsidiary of Roche, with stock options. Shubham Pant reported consulting or advisory role with Zymeworks, Ipsen, Novartis, Janssen, AskGene Pharma, BPGBio, Jazz, AstraZeneca, Boehringer Ingelheim, USWorldmeds, Nihon Medi-Physics Co, Ltd, and Alligator Bioscience; and research funding (to institution) from Mirati Therapeutics, Lilly, Xencor, Novartis, Bristol-Myers Squibb, Astellas, Framewave, 4D Pharma, Boehringer Ingelheim, NGM Pharmaceuticals, Janssen, Arcus, Elicio, Biontech, Ipsen, Zymeworks, Pfizer, ImmunoMET, Immuneering, and Amal Therapeutics. Milind Javle reported advisory board

role or research funding from Abbvie, Array, Astellas, AstraZeneca, Bayer, Beigene, Biocartis, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Daiichi, GSK, Halozyme, Helsinn, Incyte, Ipsen, Janssen Research, Lilly, Merck Sharp & Dohme, EMD Serono, Novartis, Transthera, Meclun, Eli Lilly, Oncosil, QED, Taiho, Servier, and Agios. The other authors indicated no financial relationships.

Financial Support: None

Ethics Statement: None

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