Comprehensive profiling of *MDM2* amplified gastrointestinal (GI) cancers

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Introduction

- The *MDM2* proto-oncogene encodes a nuclear localized E3 ubiquitin ligase with the core function of inhibiting the tumor suppressor p53. *MDM2* amplification has been reported in multiple tumor types and is a hallmark of tumorigenesis [1].
- In certain tumor types, such as glioblastoma and well-differentiated liposarcoma, *MDM2* amplification and *TP53* alterations are mutually exclusive, however, in other tumors (i.e. osteosarcoma, esophageal cancer), *MDM2* amplification and *TP53* alterations co-occur.
- Notably, preclinical studies have suggested several noncanonical p53-independent roles for *MDM2*, including a functional angiogenesis effect [2], disruption of the G1/S checkpoint [3], promotion of genomic instability [4], and negative regulation of T cell activation through ubiquitin-dependent degradation of NFATc2 [5].
- While MDM2 inhibitors are currently in early-phase clinical development, recently *MDM2* amplification also has been implicated as a potential marker for accelerated tumor growth after checkpoint inhibitors (ICIs) treatment, a phenomenon known as hyperprogression, affecting approximately 9% of patients who receive PD-1/PD-L1 inhibitors [6, 7].
- Here, we aimed to characterize the molecular and gene expression profile of *MDM2* amplified (a-*MDM2*) GI cancers.

Methods

- 23632 samples: 11692 colorectal, 3830 gastric/esophageal, 3960 pancreatic, 1860 biliary cancers, 2330 other GI, collected between August of 2015 to December of 2019 were included in the analysis.
- Samples were analyzed using NextGen DNA seq (Illumina NextSeq, 592 gene panel) for gene mutations and amplification (copy number > 6) and immunohistochemistry. The Illumina NovaSeq 6500 was used to sequence the whole transcriptome from patients to an average of 30M paired end reads. NGS RNA sequencing captures 22192 exonic regions. For transcription counting, transcripts per million molecules (TPM) was used (Caris Life Sciences , Phoenix, AZ).
- Molecular alterations were compared using Chi-square or Fisher Exact tests and a *P*-value of < 0.05 was considered a trending difference. Due to the large sample size of this study, *P*-values were further corrected for multiple comparison using Benjamini-Hochberg method and an adjusted *P*-value (i.e., *Q*-value) of < 0.05 was considered a significant difference. Continuous variables were compared using Oneway Anova.
- EBseq was used to identify differentially expressed genes based on *MDM2* expression levels (above vs below median) with control for false discovery rate (FDR, *Q* < 0.2).
- Pathway and functional enrichment analysis was performed using Reactome.

References

1. Wade et al. Nat Rev Cancer 2013; 2. Zhou et al. Mol Cell Biol 2011; 3. Tang et al. Clin Cancer Res 2012; 4. Eischen. J Mol Cell Biol 2017; 5. Zou et al. Nat Immunol 2014; 6. Champiat et al. Clin Cancer Res 2017; 7. Kato et al. Clin Cancer Res 2017.

Results

Figure 1. *MDM2* Amplification in GI Cancers.

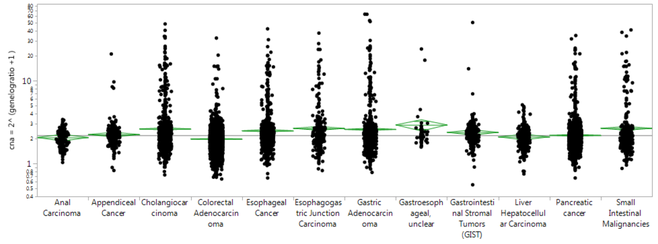


Table 1. *MDM2* Amplification Frequency According to Cancer Type.

GI cancer type	MDM2 Amplified	Total N	Percent
Anal Carcinoma	0	311	0,0%
Appendiceal Cancer	4	558	0,7%
Cholangiocarcinoma	64	1860	3,4%
Colorectal Adenocarcinoma	15	11692	0,1%
Esophageal Cancer	48	1560	3,1%
Esophagogastric Junction Carcinoma	25	619	4,0%
Gastroesophageal, unclear	2	55	3,6%
Gastric Adenocarcinoma	50	1596	3,1%
Gastrointestinal Stromal Tumors (GIST)	3	398	0,8%
Liver Hepatocellular Carcinoma	0	436	0,0%
Pancreatic cancer	36	3920	0,9%
Small Intestinal Malignancies	28	627	4,5%

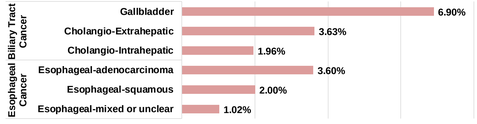
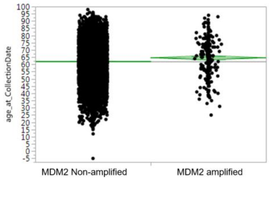


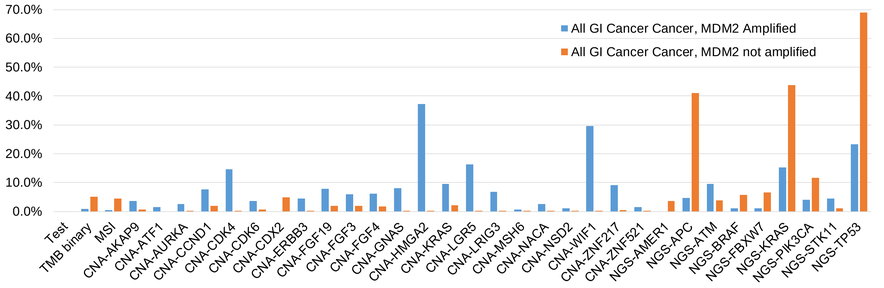
Table 2. Patient Demographics: association with gender and age.

Cancer type	Female %	Male %
Anal Carcinoma	0,0%	0,0%
Appendiceal Cancer	0,6%	0,8%
Cholangiocarcinoma	3,2%	3,8%
Colorectal Adenocarcinoma	0,2%	0,1%
Esophageal Cancer	3,1%	3,1%
Esophagogastric Junction Carcinoma	5,8%	3,6%
Gastric Adenocarcinoma	2,0%	3,9%
Gastroesophageal, unclear	0,0%	5,3%
Gastrointestinal Stromal Tumors (GIST)	0,0%	1,4%
Liver Hepatocellular Carcinoma	0,0%	0,0%
Pancreatic cancer	1,0%	0,8%
Small Intestinal Malignancies	2,7%	6,0%
All	**1,0%**	**1,3%**



- Male gender is more prevalent in *MDM2* amplified than non-amplified GI tumors (*P* = 0.0091).
- A slight increase of age is associated with MDM2 amplification (*P* = 0.0008).

Figure 2. Significant Molecular Differences between *MDM2* Amplified and Non-amplified GI Cancers.

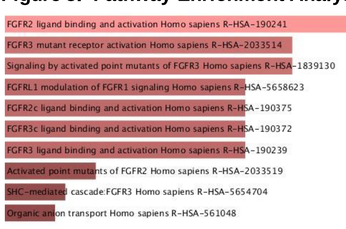


- Compared to *MDM2* not amplified (na), a-*MDM2* GI tumors showed lower mutation rates in ***TP53*** (23 vs 69%), ***KRAS*** (15 vs 44%), ***APC*** (5 vs 41%), and ***PIK3CA*** (4 vs 12%), whereas ***ATM*** mutations were higher (9.5 vs 4%) (*Q* < 0.001).
- Copy number alterations (CNA) were significantly higher in a-*MDM2* vs na, including ***CDK4***, ***ERBB3***, ***HMGA2***, ***LGR5***, ***NACA*** and ***WIF1*** (*Q* < 0.001).

Table 3. Summary of Main Molecular Differences According to Selected Tumor Types.

	Biliary Cancer			Esophageal Cancer			Gastric Cancer			Small Bowel Cancer		
	a-MDM2%	na%	*Q*-value	a-MDM2%	na%	*Q*-value	a-MDM2%	na%	*Q*-value	a-MDM2%	na%	*Q*-value
NGS TP53	16	44	**0.002**	41	89	**< 0.0001**	9	57	**< 0.0001**	8	60	**< 0.0001**
CNA CDK4	19	0.2	**< 0.0001**	21	0.2	**< 0.0001**	6	0.1	**0.02**	11	0.3	**0.02**
CNA ERBB3	3	0	**0.05**	6	0.1	**0.01**	2	0	0.4	4	0.2	0.8
CNA GNAS	6	0.2	**0.005**	8	0.7	**0.03**	0	0.3	1	37	0.5	**< 0.0001**
CNA HMGA2	42	0.3	**< 0.0001**	35	0.5	**< 0.0001**	27	0.2	**< 0.0001**	52	0.3	**< 0.0001**
CNA LGR5	31	0.1	**0.01**	25	0.1	**< 0.0001**	6	0.1	**0.01**	18	0	**< 0.0001**
CNA NACA	5	0.1	**< 0.0001**	6	0	**0.002**	2	0	0.4	0	0.2	1
CNA WIF1	36	0.1	**< 0.0001**	27	0.4	**< 0.0001**	18	0.1	**< 0.0001**	38	0.2	**< 0.0001**

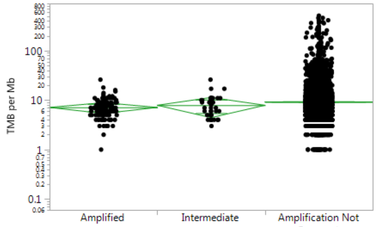
Figure 5. Pathway Enrichment Analysis based on *MDM2* Expression Levels.



Term	*P*-value	Adjusted *P*-value	Odds Ratio	Combined Score
FGFR2 ligand binding and activation	6.92E-06	0.01	8.9	105.9459641
FGFR3 ligand binding and activation	9.10E-05	0.02	9.8	91.17851172
FGFR3c ligand binding and activation	9.10E-05	0.02	9.8	91.17851172
FGFR2c ligand binding and activation	9.10E-05	0.02	9.8	91.17851172
Signaling by activated point mutants of FGFR3	5.79E-05	0.03	10.6	103.5822838
FGFRL1 modulation of FGFR1 signaling	9.10E-05	0.03	9.8	91.17851172
FGFR3 mutant receptor activation	5.79E-05	0.04	10.6	103.5822838

- A total of 785 genes were significantly differentially expressed based on *MDM2* levels, with an increase in FGF signaling related pathways in *MDM2* overexpressing tumors (*Q* < 0.05).

Figure 3. Tumor Mutational Burden According to *MDM2* Amplification in GI Cancers.

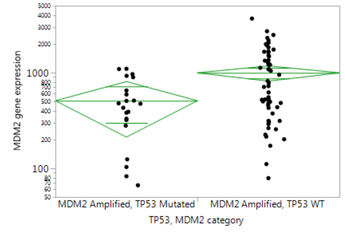


	Amplified	Intermediate	Amplification Not Detected
Mean:	7.13	7.88	9.20

- *MDM2* amplification had an inverse relationship with TMB (*P* = 0.01 for TMB as a continuous variable, *Q* = 0.04 for TMB-high* vs low) and MSI-H/dMMR (*Q* = 0.03).
- No association was found with PDL1 levels and CPS score [data not shown].

  * TMB-high > 17mut/Mb

Figure 4. *MDM2* Expression According to *TP53* Mutation in *MDM2* Amplified GI Cancers.



- Among *MDM2* amplified tumors, *TP53*-mutated had a lower copy number of *MDM2* compared to *TP53* wild type (11 copies vs 14, *P* < 0.05) and lower *MDM2* expression (*P* = 0.007).

Conclusions

- This is the most extensive profiling study to investigate *MDM2* amplified GI tumors.
- Our data show distinct molecular patterns of *MDM2* amplified GI cancers involving WNT pathway genes, upregulation of FGF signaling and inverse association with TMB and MSI which may explain the resistance mechanisms to ICIs.
- *TP53* mutations and *MDM2* amplification where not mutually exclusive in our cohort, however, lower *MDM2* expression was found in *TP53*-mutated tumors suggesting that TP53 mutational status may impact treatment with MDM2 inhibitors.