Abstract
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The 27-gene IO score is associated with molecular features and response to immune checkpoint inhibitors (ICIs) in patients with gastric cancer

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Introduction

- Gastric cancer (GC) is the 3rd leading cause of cancer-related death worldwide.
- A variety of biomarkers have been used to identify patients most likely to benefit from ICI therapies such as high PD-L1 expression, MSI-high, Epstein Barr virus (EBV) positive, or TMB high.
- ICIs + chemo have recently been approved as a first line treatment in advanced GC for patients with CPS> 5%.
- Among GCs, there is extensive molecular heterogeneity, tumor immune microenvironments (TIME), and patient factors that may be associated with outcomes
- Despite these potential biomarkers, most patients with advanced GC do not respond to ICI treatment.

The 27-Gene IO Assay

- The CLIA-validated 27-gene immuno-oncology (IO) assay was developed to assess both inflammatory effector cell and surrounding cancer associated fibroblasts phenotypes in the TIME **(Figure 1).**

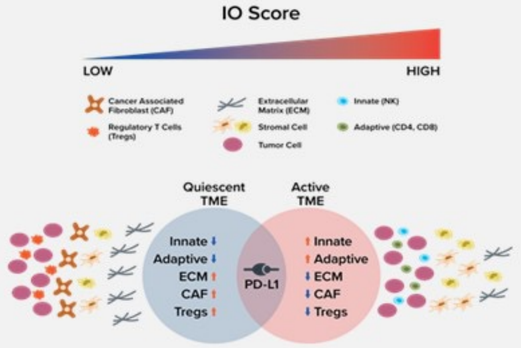

Figure 1: The IO score is a measure of both the quiescent and active states of the TIME

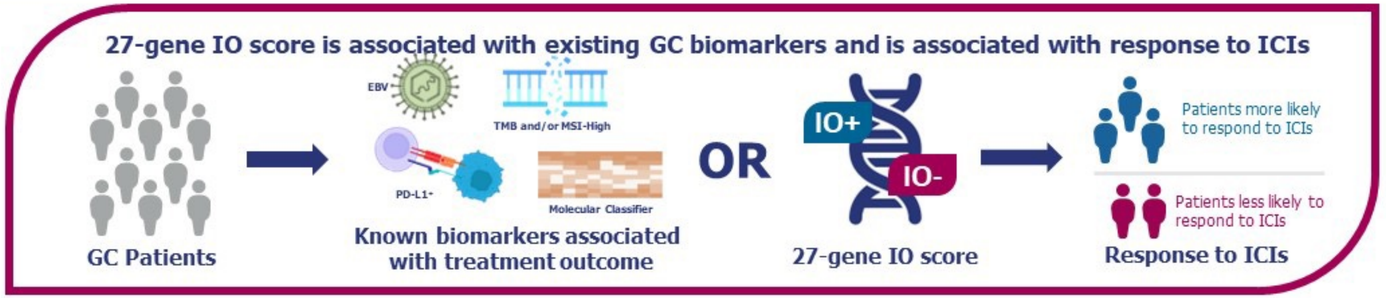

Table 1: Cohort Characteristics of TCGA and ACRG

Feature	TCGA (STAD) N	TCGA (STAD) IO+ (n,%)	TCGA (STAD) IO− (n,%)	ACRG N	ACRG IO+ n (%)	ACRG IO− n (%)
All Patients	135	59 (44%)	76 (56%)	294	125 (43%)	169 (57%)
Age			0.49			0.56
<64 years	55	22 (40%)	33 (60%)	151	67 (44%)	84 (56%)
≥ 64 years	80	37 (46%)	43 (54%)	143	58 (41%)	85 (59%)
Sex			0.097			0.90
Female	47	25 (53%)	22 (47%)	99	43 (43%)	56 (57%)
Male	88	34 (39%)	54 (61%)	195	82 (42%)	113 (58%)
Stage			0.40			0.05
I	18	10 (56%)	8 (44%)	30	19 (63%)	11 (37%)
II	59	25 (42%)	34 (58%)	92	40 (43%)	52 (57%)
III	55	24 (44%)	31 (56%)	94	38 (40%)	56 (60%)
IV	3	0 (0%)	3 (100%)	76	28 (37%)	48 (63%)
Lauren Type			0.26			1
Intestinal	97	45 (46%)	52 (54%)	141	57 (40%)	84 (60%)
Diffuse	23	7 (30%)	16 (70%)	133	58 (44%)	75 (56%)
Mixed	11	3 (27%)	8 (73%)	17	7 (41%)	10 (59%)
Tumor Location			0.89			0.24
Antrum	52	21 (40%)	31 (60%)	152	69 (45%)	83 (55%)
Body	39	19 (49%)	20 (51%)	104	37 (36%)	67 (64%)
Cardia	25	11 (44%)	14 (56%)	32	17 (53%)	15 (47%)
Fundus	17	7 (41%)	10 (59%)	6	2 (33%)	4 (66%)

Table 2: Molecular Features of TCGA and ACRG Cohorts

Feature	TCGA (STAD) N	TCGA (STAD) IO+ n (%)	TCGA (STAD) IO− n (%)	ACRG N	ACRG IO+ n (%)	ACRG IO− n (%)
EBV			p<0.001			p<0.001
EBV+	14	14 (100%)	0 (0%)	18	18 (100%)	0 (0%)
	121	45 (37%)	76 (63%)	251	94 (37%)	157 (63%)
MSI			p=0.02			p=0.016
MSI High	82	29 (35%)	53 (64%)	111	58 (53%)	53 (48%)
MSS	53	30 (57%)	23 (43%)	183	69 (38%)	114 (62%)
TMB			p<0.001			
High (≥10mut/MB)	36	26 (72%)	10 (28%)	Data Not Available in ACRG		
Low	99	33 (33%)	66 (67%)			
PDL1 mRNA Expression			p<0.001			p<0.001
High (≥75th quartile)	34	28 (82%)	6 (18%)	74	61 (82%)	13 (18%)
Low	101	31 (31%)	70 (69%)	74	3 (4%)	71 (96%)

Table 3: Molecular features associated with IO score in a clinical cohort

Feature	N	IO+ n (%)	IO− n (%)
All Patients	59	25 (42%)	34 (58%)
EBV			p=0.15
Positive	5	4 (80%)	1 (20%)
Negative	40	15 (37%)	2 (63%)
Missing	14	6 (43%)	8 (57%)
MSI			p=0.79
MSS	39	16 (41%)	23 (59%)
MSI-High	20	9 (45%)	11 (55%)
PDL-1 (CPS%)			p=0.02
Low (<1%)	20	3 (15%)	17 (85%)
High	32	16 (50%)	16 (50%)
Missing	7	6 (85%)	1 (15%)
TCGA Classifier			p=0.04
EBV	4	4 (100%)	0 (0%)
GS	20	8 (40%)	12 (60%)
CIN	16	4 (25%)	12 (75%)
MSI-H	5	3 (60%)	2 (40%)
Missing	14	6 (42%)	8 (57%)
Best Response			p=0.04
CR	4	3 (75%)	1 (25%)
PR	15	10 (67%)	5 (33%)
SD	21	7 (33%)	14 (67%)
PD	19	5 (26%)	14 (74%)

Table 4: Response status by molecular feature

Feature	Responder CR/PR	Non-Responder PD/SD
All Patients	19 (32%)	40 (68%)
MSI type		
MSI	21 (60%)	8 (40%)
MSS	7 (18%)	32 (82%)
IO Score		
Positive	13 (52%)	12 (48%)
Negative	6 (18%)	28 (82%)
EBV		
Positive	5 (100%)	0 (0%)
Negative	7 (18%)	33 (83%)
Missing	7 (50%)	7 (50%)
PD-L1 1%		
≥ 1%	16 (50%)	16 (50%)
< 1%	3 (11%)	24 (89%)
TCGA Classifier		
EBV	4 (100%)	0 (0%)
GS	2 (10%)	18 (90%)
CIN	1 (6%)	15 (94%)
MSI-H	5 (100%)	0 (0%)
Missing	7 (50%)	7 (50%)

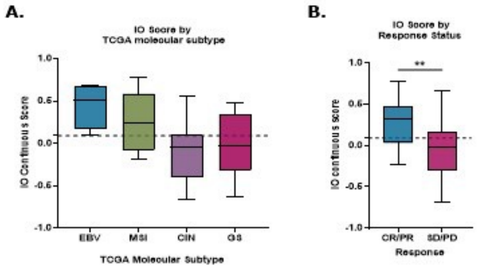

Figure 2:
A) IO continuous score plotted by TCGA classifier status

B) IO continuous score by objective response. Dotted line at 0.09, the binary threshold for IO positivity.

Aim

To demonstrate that the 27 gene IO score, a TIME classifier, is associated with the existing molecular markers of gastric cancer and with objective response to ICI therapy in a clinical cohort.

Methods

- RNA-seq expression data was obtained from 3 independent cohorts including TCGA (STAD), ACRG (GSE84437, GSE84426), and a clinical cohort with ICI response data (PRJEB25780, PRJEB40416)
- The 27 gene IO algorithm was applied to all available patient data to derive IO scores
- Fisher's exact test was used to examine the associations between IO score and clinical features and molecular subtypes.

Results

- In TCGA, IO score was associated with the molecular features EBV, MSI, TMB, and PD-L1
- In ACRG, the IO score was significantly associated with EBV, MSI, and PD-L1
- IO positive found 36 of 37 EBV+ patients across all cohorts
- In the clinical cohort of 59 patients, IO score was associated with ICI response ($p<0.05$)
- Association between IO score and ICI response OR= 5.3 (95% CI: 1.3 to 23.92, p=0.01).

Conclusions

Further studies are warranted to demonstrate that the 27-gene IO score may be a more comprehensive biomarker for assessing the TIME and provide complementary data to tumor-specific biomarkers for gastric cancers.