Abstract
ID: 439

Identification of Cancer Hallmarks Associated with Benefit in Advanced Gastroesophageal Adenocarcinoma Patients Treated with Checkpoint Blockade

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Background

- The benefit of checkpoint blockade in advanced gastric cancer is limited and biomarkers related to response are needed.
- Although the addition of andecaliximab to nivolumab (NCT02862535; ref) was not efficacious in advanced gastric cancer, biomarker analyses treating the entire population as a nivolumab monotherapy group can be informative in understanding underlying mechanisms of response to PD1i therapy.

Objective

- Identify subpopulations of patients with advanced gastroesophageal adenocarcinoma (GEA) treated with PD1i that experience a clinical benefit (clinical response or prolonged survival).

Methods

- Novel gene expression analysis software was used to identify Hallmarks of Cancer associated with clinical benefit following PD1i in >2nd line.
- RNA-sequencing data from baseline GEA patient diagnostic tumor samples (103 from NCT02862535; 5 from NCT02862535) were analyzed using the claraT platform (V2.0.0, Almac Diagnostic Services) using FPKM normalised RNA-sequencing data.
- 62 gene signatures were quantified with V2 claraT – representing 6 key Hallmarks of Cancer (Avoiding Immune Destruction, Activating Invasion and Metastases, Sustaining Proliferative Signaling, Inducing Angiogenesis, Resisting Cell Death and Genome Instability and Mutation).
- For each gene expression signature, continuous scores and associated percentile ranks were calculated using the claraT V2.0.0 analysis pipeline. Euclidean distance was calculated using the percentile ranked scores and hierarchical clustering using Ward's linkage criteria was performed on this matrix. Samples are clustered based upon signature outputs within individual Hallmarks and across all samples.
- HER2 status was identified from medical records.
- Clinical benefit (CB) was defined as tumor response (CR or PR) or overall survival (OS) > 1 year, Survival analyses were conducted using cox proportional hazards models

Results

- Gene expression signatures (GES) identified 5 molecular subgroups (C1-C5).
- The rate of clinical benefit in each molecular subtype are outlined in Table 1. (chi-square test p-values provided in Figure 1B)
- C3 and C4 had statistically significant improved OS compared to C2, (HR=0.45; p=0.02 and HR=0.42; p=0.02).
- Each of the clusters C4 and C3 had a greater proportions of HER2+ subjects relative to C2, with C3 reaching statistical significance (60% vs. 14%; p=0.012).
- Gene expression characterized by chromosomal instability (CIN) and homologous recombination repair deficiency (HRD) were associated with HER2(+) (wilcox p=<0.05).
- Patients selected by only using CIN & HRD had significant improvement in OS (HR=0.63; p=0.03).

Figure 1: A) Hierarchical clustering reveals 5 distinct patient subpopulations characterized by unique combinations of cancer hallmarks

A)

Results (cont'd)

Figure 1: B) Hierarchical clustering reveals 5 distinct patient subpopulations characterized by unique combinations of cancer hallmarks

B)

Cluster	Clinical Benefit (%)	Biology	p-value
C1	11.1	Potentially MSI-like	0.49
C2	0	Cold Tumours (All OFF)	-
C3	17.6	HR-deficient	0.16
C4	18.2	HR-deficient +IFNγ	0.12
C5	5.6	EMT/TGFb	1.00

- C1: group driven by IFNγ immune signalling and association with MSI signatures. Potentially an MSI group. Appear to have an intermediate response/outcome.
- C2: group with no active biologies including no immune signalling > cold tumours likely not to benefit for immune checkpoint inhibitors. The worst response group with poor overall survival.
- C3: group driven by Homologous Recombination DNA repair-deficient biology with no IFNγ immune signalling. First time across disease indications we have observed a benefit with no immune signalling > may suggest presence of neoantigens. Correlates with observed PD-L1 expression data from clinical trial It is subjective.
- C4: group driven by Homologous recombination-deficient biology plus IFNγ immune signalling. The best response group with improved overall survival.
- C5: group driven by TGFb, EMT and angiogenesis biologies. A poor response group

Figure 2: Subjects with HR deficiency (C3) and HR deficiency + higher IFNγ (C4) associated with a significantly improved OS relative to (C2)

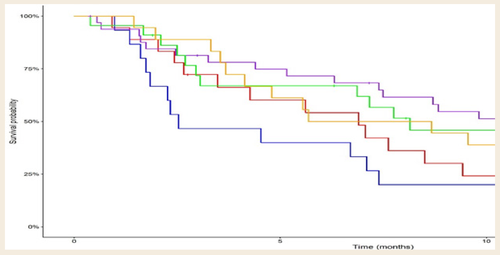


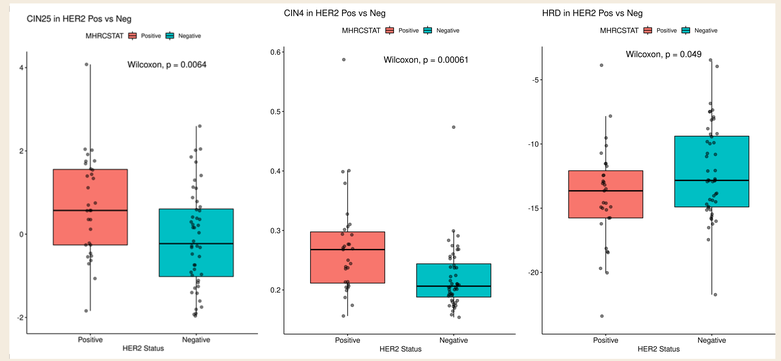
Table 1. Summary of hierarchical clustering

Molecular subgroup	C1	C2	C3	C4	C5
n	18	16	34	22	18
Rate of clinical benefit	11.1%	0%	17.6%	18.2%	5.6%
Associated GES	IFN/innate immune signaling, mismatch repair deficiency, epithelial-mesenchymal transition	None	CIN, HRD, HER2/EGFR/MEK	CIN, HRD, HER2/EGFR/MEK, IFN/innate immune signaling	epithelial-mesenchymal transition, TGFβ activation

- C2 (No biologies identified): worst response group, poor OS
- C3 (HR-deficient): good response and significantly improved OS (HR=0.45; p=0.02)
- C4 (HR-deficient + IFNγ): best response group, improved OS (HR=0.42; p=0.02)

Cluster ID	HR	P-value	Lower CI	Upper CI
C1	0.76	0.457	0.37	1.56
C2	-	-	-	-
C3	0.45	0.020	0.23	0.88
C4	0.42	0.020	0.20	0.87
C5	0.55	0.110	0.27	1.15

Figure 4: HER2 status significantly associated with genome instability signatures (CIN25 [1], CIN4 [2]) and HRD pathway activity [3]



- *For CIN signatures, higher score indicate greater chromosomal instability, while for the HRD signature, low scores indicate HR deficiency.

Table 2: Patient subpopulation experiencing greatest survival benefit (C3) included a significantly greater percentage of HER2 positive patients.

	HER2				
	-	+	N	+ freq	P-val
C1	8	5	13	0.38	0.321
C2	12	2	14	0.14	Ref
C3	12	18	30	0.60	0.012
C4	13	8	21	0.38	0.252
C5	15	2	17	0.12	1.00

- 13 subjects with unavailable HER2 status removed from this analysis

Conclusions

- Interferon-based GES did not predict benefit from immune checkpoint blockade. GES representing HRD and activation of HER2, EGFR and MAPK pathways (each enriched in CIN) were associated with improved survival upon checkpoint blockade in advanced GEA patients.

References & Acknowledgments

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4. Shah et al. ASCO GI 2019

- This study was funded by Gilead Sciences, Inc.