Identification and characterization of immunogenic neoantigens in biliary cancer (BC) and pancreatic cancer (PC)

Francesca Battaglin[1], Andrew Elliott[2], Joanne Xiu[2], Sandra Algaze[1], Jingyuan Wang[1], Priya Jayachandran[1], Shivani Soni[1], Karam Ashouri[1], Alexandra Wong[1], Pooja Mittal[1], Jae Ho Lo[1], Lesly Torres-Gonzalez[1], Wu Zhang[1], Benjamin A. Weinberg[3], Sanjay Goel[4], Emil Lou[5], Anthony El-Khoueiry[1], Heinz-Josef Lenz[1]

1 Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA; 2 Caris Life Sciences, Phoenix, AZ; 3 Ruesch Center for The Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC; 4 Department of Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ ; 5 Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, MN

Abstract ID: 552
fbattagl@usc.edu

Introduction

- Recognition of tumor neoantigens by autologous T cells activates immune surveillance and has been reported to promote sensitivity to immune checkpoint inhibitors (ICI) in mismatch repair deficient (MMRd)/microsatellite instability high (MSI-H) tumors.
- Neoantigen-targeted reactivity has also been reported in microsatellite stable (MSS) tumors.
- Neoantigens are emerging targets for novel immunotherapy strategies, including tumor vaccines, in BC and PC.
- We aimed to comprehensively assess the spectrum of immunogenic neoantigens in BC and PC.

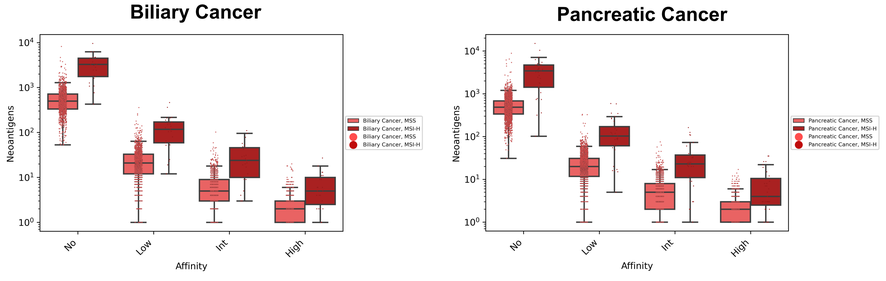
Methods

- A total of 3728 tumor specimens (1389 BC; 2339 PC) tested at Caris Life Sciences (Phoenix, AZ) with NextGen Sequencing on DNA (720-gene panel) and RNA (whole transcriptome) were analyzed.
- 9-mer peptides were generated from protein sequences surrounding detected mutations and downstream of frameshift mutations.
- Immune epitope prediction was performed on translated peptide sequences harboring detected mutations using the NetMHCpan v4.0 method in the Immune Epitope Database, with HLA genotyping performed using arcasHLA.
- Binding affinity for patient-specific HLA alleles was classified as: IC50 < 5000 nM: total binding peptides (IC50 < 50 nM: High affinity; 50 nM ≤ IC50 < 500 nM: Intermediate affinity; 500 nM ≤ IC50 < 5000: Low affinity); IC50 ≥ 5000 nM: no affinity.
- Immune/stromal cell abundance in the tumor microenvironment (TME) was estimated from RNA expression profiles using MCP-Counter.
- Gene expression profiles were analyzed for a transcriptional signature predictive of response to immunotherapy (T cell-inflamed signature, TIS).

Study Population	Biliary Cancer	Pancreatic Cancer
Sample size		
- Tumors, N samples	1389	2339
Age		
- Years, Median (range)	67 (19-90+)	67 (26-90+)
Gender		
- Male, N (%)	628 (45.2%)	1215 (51.9%)
- Female, N (%)	761 (54.8%)	1870 (48.1%)
TMB-High, N (%)	71 (5.1%)	50 (2.1%)
MSI-High, N (%)	25 (1.8%)	33 (1.4%)

Results

Figure 1. Neoantigen Load and Affinity Level in BC and PC.



117219 unique peptide:allele interactions with predicted binding-level affinity for patient-specific HLA alleles were identified (48781 in BC; 71182 in PC). MMRd/MSI-H tumors had higher neoantigen load at all affinity levels compared to MMRp/MSS.

Figure 2. Top 20 Recurrent Neoantigens in BC and PC.

Biliary Cancer

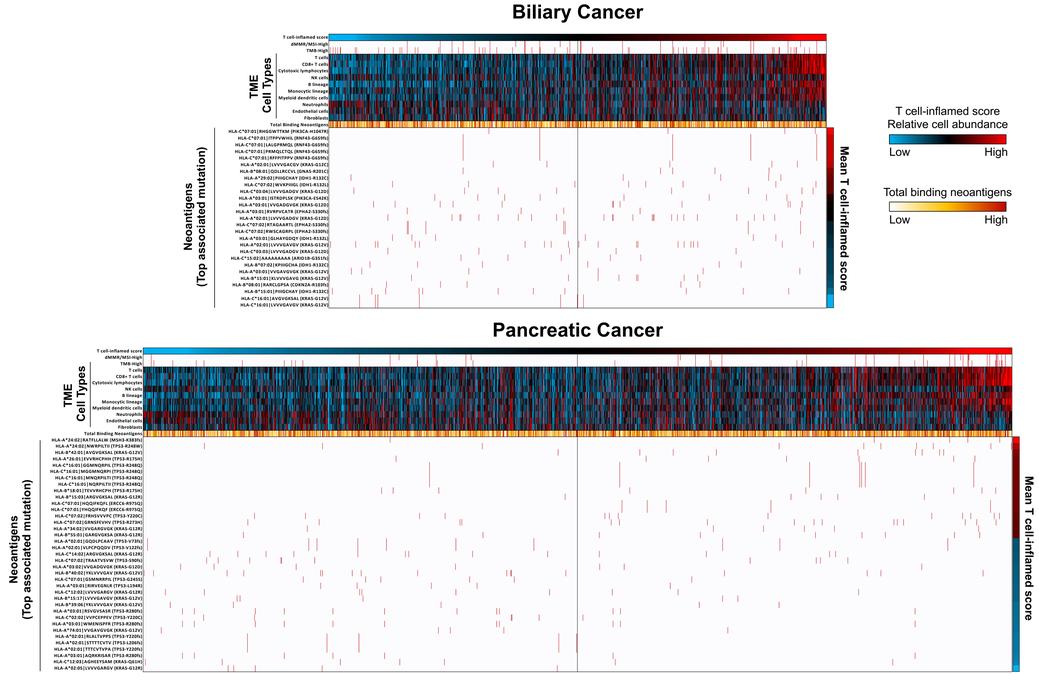
Neoantigen	N Samples with Neoantigen by Affinity Level: Binding	High	Int	Low	No	Total	Binding Prevalence	Top associated mutation	
HLA-A*02:01|LVVVGADGV	24	0	0	24	0	24	1.73%	KRAS	G12D
HLA-A*02:01|LVVVGAVGV	21	0	0	21	0	21	1.51%	KRAS	G12V
HLA-A*03:01|VVGADGVGK	12	0	0	12	0	12	0.86%	KRAS	G12D
HLA-B*15:01|PIIIGCHAY	11	0	0	11	0	11	0.79%	IDH1	R132C
HLA-B*15:01|KLVVVGAVG	9	0	0	9	0	9	0.65%	KRAS	G12V
HLA-C*07:02|WVKPIIIGL	8	0	0	8	0	8	0.58%	IDH1	R132L
HLA-A*03:01|ISTRDPLSK	8	0	0	8	0	8	0.58%	PIK3CA	E542K
HLA-C*03:04|LVVVGADGV	8	0	0	8	0	8	0.58%	KRAS	G12D
HLA-C*07:02|RTAGAAARTL	7	0	0	7	0	7	0.50%	EPHA2	S330fs
HLA-C*07:02|RWSCAGRPL	7	0	0	7	0	7	0.50%	EPHA2	S330fs
HLA-C*16:01|LVVVGAVGV	7	0	0	7	0	7	0.50%	KRAS	G12V
HLA-C*16:01|AVGVGKSAL	7	0	0	7	0	7	0.50%	KRAS	G12V
HLA-A*03:01|GLHAYGDQY	6	0	0	6	0	6	0.43%	IDH1	R132L
HLA-A*03:01|VVGAVGVGK	6	0	6	0	0	6	0.43%	KRAS	G12V
HLA-B*07:02|KPIIIGCHA	6	0	0	6	0	6	0.43%	IDH1	R132C
HLA-B*08:01|QDLLRCCVL	5	0	0	5	0	5	0.36%	GNAS	R201C
HLA-C*07:01|PRMQLCTQL	5	0	0	5	0	5	0.36%	RNF43	G659fs
HLA-C*07:01|RHGGWTTKM	5	0	0	5	0	5	0.36%	PIK3CA	H1047R
HLA-A*03:01|RVRPVCATR	5	0	0	5	0	5	0.36%	EPHA2	S330fs
HLA-C*03:03|LVVVGADGV	5	0	0	5	0	5	0.36%	KRAS	G12D

Pancreatic Cancer

Neoantigen	N Samples with Neoantigen by Affinity Level: Binding	High	Int	Low	No	Total	Binding Prevalence	Top associated mutation	
HLA-A*02:01|LVVVGADGV	267	0	0	267	0	267	11.42%	KRAS	G12D
HLA-A*03:01|VVGADGVGK	178	0	0	178	0	178	7.61%	KRAS	G12D
HLA-A*02:01|LVVVGAVGV	169	0	0	169	0	169	7.23%	KRAS	G12V
HLA-A*03:01|VVGAVGVGK	140	0	140	0	0	140	5.99%	KRAS	G12V
HLA-A*03:01|VVGARGVGK	65	0	65	0	0	65	2.78%	KRAS	G12R
HLA-C*12:03|LVVVGADGV	64	0	0	64	0	64	2.74%	KRAS	G12D
HLA-C*07:02|ARGVGKSAL	63	0	0	63	0	63	2.69%	KRAS	G12R
HLA-C*16:01|AVGVGKSAL	59	0	0	59	0	59	2.52%	KRAS	G12V
HLA-C*16:01|LVVVGAVGV	59	0	0	59	0	59	2.52%	KRAS	G12V
HLA-B*15:01|KLVVVGAVG	55	0	0	55	0	55	2.35%	KRAS	G12V
HLA-B*07:02|AVGVGKSAL	51	0	0	51	0	51	2.18%	KRAS	G12V
HLA-C*03:04|LVVVGADGV	50	0	0	50	0	50	2.14%	KRAS	G12D
HLA-C*12:03|GAVGVGKSA	46	0	0	46	0	46	1.97%	KRAS	G12V
HLA-C*12:03|AVGVGKSAL	46	0	0	46	0	46	1.97%	KRAS	G12V
HLA-C*03:03|LVVVGADGV	46	0	0	46	0	46	1.97%	KRAS	G12D
HLA-C*12:03|YKLVVVGAV	46	0	0	46	0	46	1.97%	KRAS	G12V
HLA-C*12:03|LVVVGAVGV	46	0	0	46	0	46	1.97%	KRAS	G12V
HLA-A*30:01|VVGAVGVGK	41	0	0	41	0	41	1.75%	KRAS	G12V
HLA-C*03:04|LVVVGAVGV	39	0	0	39	0	39	1.67%	KRAS	G12V
HLA-C*03:04|AVGVGKSAL	39	0	0	39	0	39	1.67%	KRAS	G12V

Only 4 recurrent neoantigens with binding affinity (observed in >10 samples) were identified in BC and derived from mutations in *KRAS* (3/4) and *IDH1* (1/4), while 78 were found in PC, mostly associated with mutations in *KRAS* (53/78) and *TP53* (16/78). The frequency of individual neoantigens was particularly low in MMRp/MSS BC (< 2%).

Figure 3. Association with TME cell infiltration and T cell-inflamed signature in BC and PC.



Across both cancer types, TIS scores positively correlated with the abundance of immune cell populations in the TME, notably cytotoxic lymphocytes (r > 0.40). Recurrent neoantigens associated with highest average
TIS scores resulted from mutation of *KRAS* (G12D/V, 1.8%/1.5% of samples, respectively) in BC yet mean TIS scores were low (~60th percentile overall). Similarly, *TP53* (Y220C/R273C, 0.6%/0.7%, respectively) and *CDKN2A* (multiple variants, 0.5%) were associated with the highest yet relatively low mean TIS scores in MMRp/MSS PC (~73th percentile) compared to mean TIS scores associated with *MSH3* (K383fs, 12%) and *KRAS* (G12D, 12%) in MMRd/MSI-H PC (~90th percentile).

CONCLUSIONS

This is the largest study to investigate the landscape of immunogenic neoantigens in BC and PC.

The frequency of high-level binding affinity neoantigens was relatively low and associated with relatively lower TIS scores in MSS tumors, which may contribute to the immunogenic cold TME characterizing these tumor types.