#343: Angiogenic and T-effector subgroups identified by gene expression profiling (GEP) and propensity for PBRM1 and BAP1 alterations in clear cell renal cell carcinoma (ccRCC)

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Background:

Predictive biomarkers for optimal treatment selection in RCC are lacking. Gene expression data from IMmotion151 and Javelin Renal 101 clinical trials generated anti-angiogenic and immune signatures that warrant further validation. We aimed to describe the genomic and gene expression profiles in a multi-institutional database of patients with ccRCC, and its association with other biomarkers of interest.

Methods:

- Whole transcriptome sequencing was performed for ccRCC patient samples submitted to a commercial CLIA-certified laboratory (Caris Life Sciences, Phoenix, AZ) from February 2019 to September 2020.
- Tumor GEP and hierarchical clustering based on the validated 66-gene signature (D'Costa et al, 2020) were used to identify patient subgroups.
- Samples from both primary tumors and metastatic sites were included.

Results:

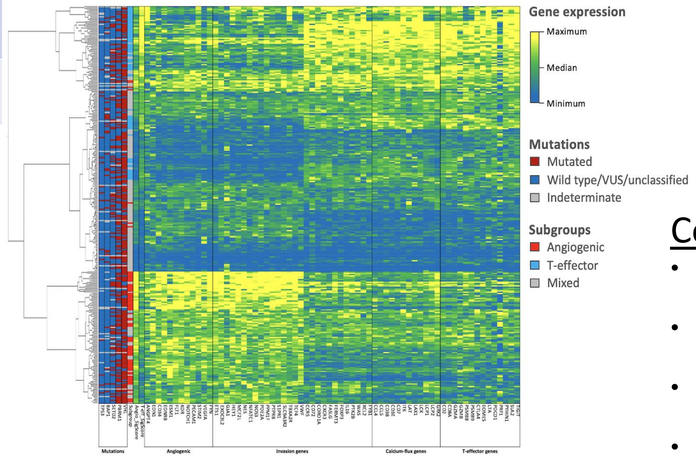
- A total of 316 patients with ccRCC, median age 62 (range 32-90), 71.8% male, were included. Tissue samples were obtained from primary tumor (46.5%), lung (12.3%), bone (9.5%), liver (4.7%) and other metastatic sites (27%).

Characteristic	All cases	'Angiogenic' subgroup	'Mixed' subgroup	'T-effector' subgroup	P-value (Test) 'Angio' vs 'T-eff'
Total, N cases (% of total)	316 (100%)	76 (24.1%)	162 (51.3%)	78 (24.7%)	--------
Median Age, years (SD) - Age Range, years	62 (10.6) 32-90	63 (10.3) 34-90	62 (10.5) 32-86	60 (10.7) 38-83	**0.0350** (Mann-Whitney U)
Female/Male, N cases - (% Female/% Male)	89/227 (28.2%/71.8 %)	31/45 (40.8%/59.2 %)	45/117 (27.8%/72.2 %)	13/65 (16.7%/83.3 %)	**0.0009** (Chi-square)
Metastatic/Primary, N cases - (% Metastatic/% Primary)	170/146 (53.8%/46.2 %)	41/35 (53.9%/46.1 %)	88/74 (54.3%/45.7 %)	41/37 (52.6%/47.4 %)	0.8634 (Chi-square)

Table 1 – Baseline patient and tumor characteristics.

- Gene expression analysis identified angiogenic (24.1%), mixed (51.3%) and T-effector (24.7%) subgroups (Figure 1)
- Angiogenic subgroup tumors compared to those with T-effector subgroup tumors were more likely to be older (63 versus 60 years, p=0.035) and female (40.8% versus 16.7%, p=0.0009) (Table 1).
- PBRM1 mutations were more common in the angiogenic subgroup (62.0% vs 37.5%, p=0.0034) while BAP1 mutations were more common in the T-effector subgroup (18.6% versus 3.0%, p= 0.0035) (Figure 1)

Figure 1 – Hierarchical clustering of gene expression signature



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Results:

- Markers associated with immune checkpoint inhibition such as PD-L1 (p=0.0021 [exploratory]), TMB (not significant), and dMMR/MSI-H status (not significant) were more frequent in the T-effector subgroup (Figure 2).
- Pancreatic/small bowel (Gastrointestinal; GI) metastases were more frequently 'angiogenic' compared to primary tumors (75% versus 23.8%, p=0.0103 [exploratory]) (Figure 3).

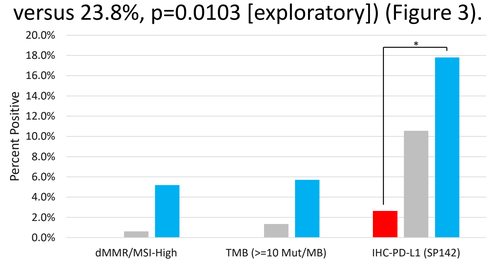

Figure 2 – Predictive biomarkers of Immunotherapy response.

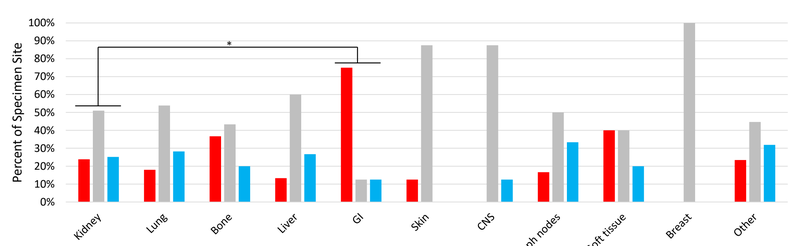

Figure 3 – Distribution of subtypes among specimen sites. *p-value (exploratory) < 0.05.

Figure 4A

Cell population/ Biomarker	Median TPM		P-value	Q-value
	Angio	T-effector		
T cells	3.72997	9.53754	4.4E-16	1.42E-15
CD8 T cells	1.98038	9.22734	0	4E-17
Cytotoxic lymphocytes	2.469843	7.186016	1.1E-15	2.96E-15
NK cells	1.110249	1.538543	0.0150	0.0200
B lineage	70.24514	276.1273	1.06E-07	2.12E-07
Monocytic lineage	29.47178	32.58231	0.4654	0.4964
Myeloid dendritic cells	2.375438	2.770685	0.0304	0.0348
Neutrophils	14.66841	11.21658	0.0015	0.0024
Endothelial cells	30.5171	12.11695	0	4E-17
Fibroblasts	183.6484	102.7199	0.01758	0.02164
PDCD1	0.437509	2.89322	0	4E-17
CD274	5.909935	7.940865	0.0025	0.0036
PDCD1LG2	1.644335	3.091815	5.26E-05	9.35E-05
CTLA4	0.885429	3.82667	4.5E-13	1.04E-12
HAVCR2	42.21655	44.1012	0.7975	0.7975
LAG3	0.989648	4.28201	0	4E-17

Figure 4B

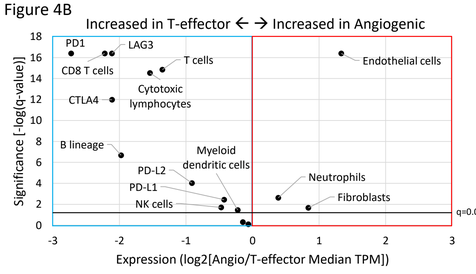

Figure 4 – Analysis of ccRCC tumor microenvironment by Microenvironment Cell Population (MCP)-counter. Immune cell population (e.g. T cells, cytotoxic lymphocytes) abundance and immune checkpoint genes (e.g. *PDCD1*, *CD274*, *CTLA4*, *LAG3*) were coordinately increased in the T-effector subgroup, while stromal cell population (endothelial cells, fibroblasts) abundance was increased in the Angiogenic subgroup.

Conclusions:

- Our hierarchical clustering results based on the 66-gene expression signature were concordant with results from prior studies;
- Angiogenic tumors were more likely to be found in patients who were older or female, and were more likely to harbor gastro-intestinal metastases, stromal cell population and PBRM1 mutations;
- BAP1 mutations and Immunotherapy markers such as TMB and dMMR/MSI-H (not significant), PD-L1 and Immune cell population were more frequent in the "T-effector" signature;
- These findings have potential predictive value and require further validation in prospective clinical trials