3048 Using Cell-Free RNA in Monitoring Immune System and the Demonstration of Significant Systemic Deficiency in Lymphoid and Myeloid Biomarkers in Patients with Cancer

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Background:

The immune system plays a major role in cancer initiation, control, progression and therapy. Significant work and efforts have been dedicated to understanding the tumor microenvironment (TME), but little work is focused on systemic immune response despite the fact that cancer is a systemic disease. Animal models and patients' actual data have shown that stem and mature hematopoietic cells are actively mobilized to contribute to the TME and this mobilization might represent the first response to oncogenic process. This includes monocytes, granulocytes, dendritic cells and lymphocytes. More importantly, the quantitative change in these immune cells is associated with significant changes in functional phenotype.

We have reported that cell-free RNA (cfRNA) can be evaluated reliably in peripheral blood of patients with cancer. The expression level of various genes can be quantified reliably using next generation sequencing of cfRNA. This includes RNA resulting from tumor cells as well as cells from the immune system that are responding to tumor. cfRNA may reflect the TME as well as the entire immune system including lymph node and bone marrow and has significant advantage over analyzing peripheral blood cells.

Methods:

cfRNA was extracted from plasma samples of 681 patients with various types of solid tumors, 113 patients with CHIP, and 34 normal individuals. cfRNA was sequenced via a hybrid capture based panel that includes approximately 16,000 genes. However, we focused in this study on only 54 genes reflecting immune cells including T-cells, B-cells, histiocytes, monocytes and myeloid cells. The RNA was quantified using TPM (transcript per million). In all samples, cfRNA is extracted from 3 ml of plasma. The 55 genes are :

CD13, CD57, CD14, CD19, CD1A, CD2, CD200, CD22, CD24, CD247, CD274, CD28, CD33, CD34, CD36, CD38, CD3D, CD3E, CD3G, CD4, CD40, CD40LG, CD44, CD47, CD5, CD52, CD58, CD59, CD68, CD7, CD70, CD74, CD79A, CD79B, CD81, CD8A, CD8B, CD9, DNTT(tdt), CD23, CD64, CD16, CD15, CD10, CD20, CD56, CD279, DCD1LG2 (PD-L2), CD133, CD45, CD138, BCMA, CD30, CD137.

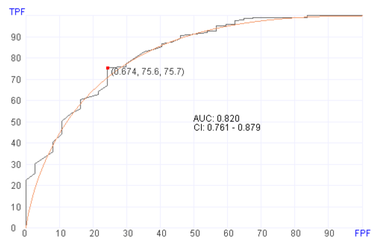
Results:

Immune Biomarker	Median in Solid Tumors (N=681)	Median in Normal (N=113)	P-Value (Cancer vs Normal)	Median in CHIP (N=34)	P-value (CHIP vs Normal)
CD19	27.049	48.426	<0.00001	55.476	0.4402
CD2	94.765	165.437	<0.00001	107.541	0.003
CD22	77.978	119.409	<0.00001	145.232	0.8291
CD247	29.987	75.927	<0.00001	50.939	0.0383
CD28	36.776	55.711	<0.00001	33.338	0.001
CD33	62.924	92.144	<0.00001	63.124	0.009
CD38	53.826	105.066	<0.00001	48.132	0.0006
CD3D	87.382	152.412	<0.00001	98.398	0.005
CD3E	95.437	177.635	<0.00001	109.246	0.004
CD3G	30.676	60.694	<0.00001	38.659	0.005
CD4	92.558	172.072	<0.00001	117.609	0.0689
CD40LG	174.412	207.249	<0.00001	199.271	0.5752
CD5	33.659	56.094	<0.00001	32.384	0.003
CD52	595.805	894.946	<0.00001	682.255	0.2359
CD7	70.059	149.399	<0.00001	75.833	0.0235
CD79A	122.357	256.126	<0.00001	231.027	0.7374
CD79B	57.788	111.471	<0.00001	96.392	0.3558
CD8A	25.736	50.863	<0.00001	25.866	0.008
CD8B	31.212	55.251	<0.00001	35.863	0.0196
CD9	2039.160	1133.485	<0.00001	1946.217	0.0008
FCER2(CD23)	35.736	52.847	<0.00001	42.436	0.3416
IL2RA(CD25, histiocytes)	7.563	9.145	<0.00001	3.894	0.0004
ITGAM(CD11B)	162.443	237.891	<0.00001	181.160	0.0305
ITGAX(CD11C)	229.972	384.458	<0.00001	273.285	0.0163
MS4A1(CD20)	81.823	171.780	<0.00001	185.288	0.9013
PTPRC(CD45)	688.220	1359.310	<0.00001	884.819	0.008

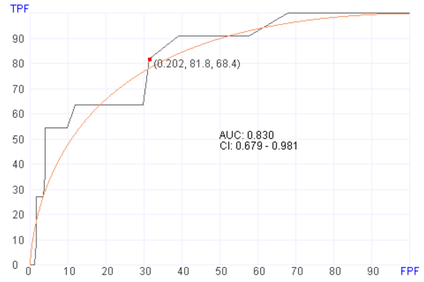
There was significant difference (P<0.0001) between normal individuals and patients with cancers in the levels of circulating biomarkers specific for immune cells. Surprisingly, expression of B-cell, T-cell, monocytic/histiocytic genes were significantly lower in patients with solid tumors when compared to normal individuals. This included CD19, CD20, CD2, CD22, CD3D, CD3E, CD3G, CD4, CD52, CD7, CD79A, CD79B, CD8A, CD8B,CD33, FCER2(CD23), IL2RA(CD25), ITGAM(CD11B), and ITGAX(CD11C) (see table).

Furthermore, using machine learning algorithm, we were able to predict the presence of absence of hematologic neoplasm or solid tumor using 2/3 of samples for training and 1/3 for testing.

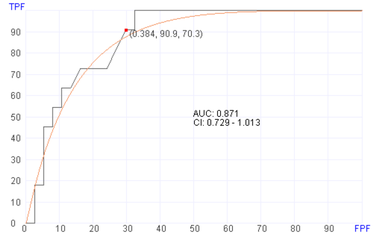
We predicted the presence of cancer vs no cancer (AUC =0.820) using 35 genes



We were able to distinguish between Cancer and CHIP with AUC =0.0830 using the same set of 35 genes



As for distinguishing between normal from CHIP, we were able to distinguish between the two groups with AUC of AUC =0.871.



Conclusions:

1) Limited number (55) of immune biomarkers detected in cfRNA provides information on the entire immune system and can be used to monitor patients and to predict the presence of cancer or CHIP.
2) Patients with cancer show significantly lower levels of myeloid and lymphoid elements in circulation as compared with normal control.

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