# Identifying New Biomarkers and Targeted Molecules for Immunotherapy Using Targeted RNA Next Generation Sequencing

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# Background

Predictive biomarkers for selecting patients who may benefit or experience serious adverse effects from immune checkpoint blockade therapy (imRx) are urgently needed. These biomarkers may vary from one type of tumor to next. Developing a broad approach for the discovery of new tissue-specific predictive biomarkers may also help guide combination therapy. We used targeted RNA sequencing for the discovery of biomarkers that are co-regulated with PD-L1.

## Methods

We used RNA sequencing of 1385 genes to profile tissues from solid tumors and lymphomas, and correlated RNA levels with PD-L1 expression as detected by IHC in tumor and inflammatory cells.

## **Key Points**

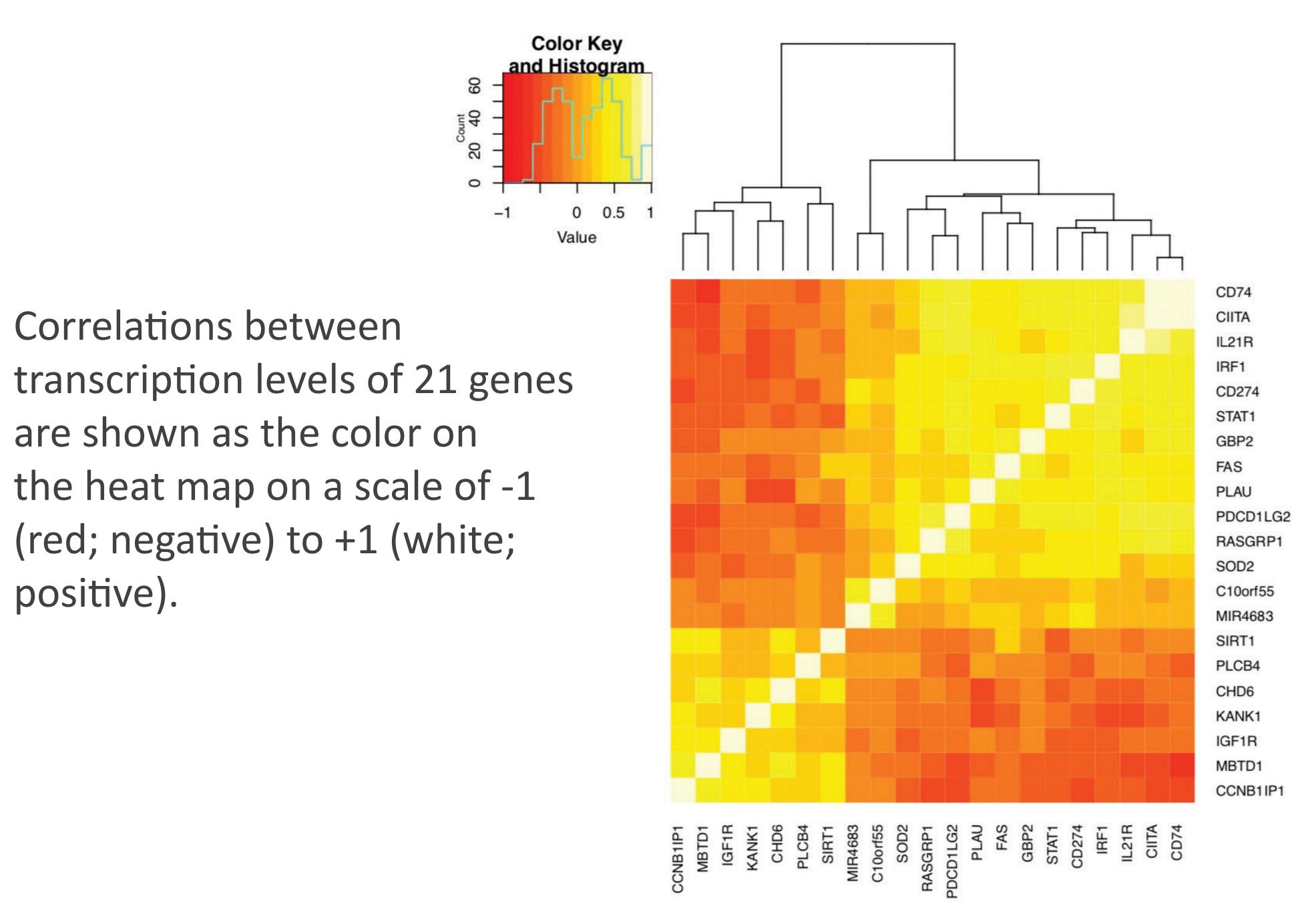
- Targeted RNA NGS can reliably be used in predicting PD-L1 IHC expression.
- 20 genes in addition to CD274 (PD-L1) showed high correlation with PD-L1 protein expression and potentially can be used in predicting PD-L1 expression.
- CD274, PLAU, and RAC1 are independent predictors of PD-L1 protein expression.
- A model incorporating CD274, RAC1, PLAU, and tumor type can significantly improve prediction of PD-L1 expression.
- Targeting PLAU and RAC1 in combination with PD-L1 inhibitors may potentially augment the therapeutic effects of anti-PD-L1.

## Results

correlated with PD-L1 expression; 14 genes correlated positively and 7 correlated negatively. Using the first principle component, we demonstrated that these 21 genes are highly

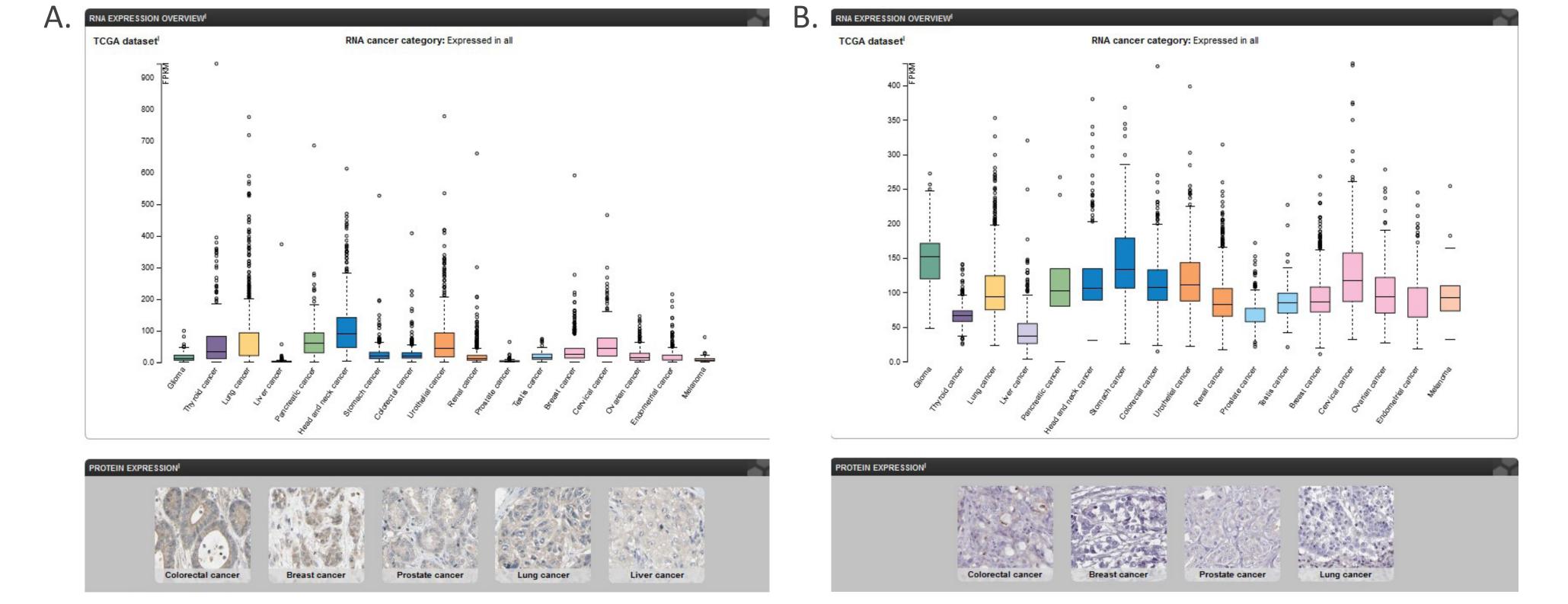
After normalization, adjusting for group effect and multiple hypothesis testing, 21 genes redundant in predicting levels of IHC PD-L1 expression and practically any one can be used as a biomarker. Using LASSO to develop a multivariate model, we demonstrated that RNA levels of CD274, PLAU (uPA), and RAC1 are independent biomarkers predictive of IHC PD-L1 expression.

#### Positive and Negative Correlations of 21 Genes



## TCGA Independent RNA Expression Predictive of PD-L1 Protein **Expression by Tumor Type**

A. PLAU vs tumor type; B. RAC vs tumor type



## **Transcripts Correlated with PD-L1 IHC Protein Expression**

Abbrev.	Gene Name	% PD-L1 in Tumor	
		p-value	q-value
C10orf55	Chromosome 10 Open Reading Frame 55	0.0001	0.0073
CD74	Major Histocompatibility Complex, Class II	0.0000	0.0019
CD274	PD-L1	0.0000	0.0000
CIITA	Class II MHC Transactivator	0.0000	0.0029
FAS	Fas Cell Surface Death Receptor	0.0000	0.0018
GBP2	Guanylate Binding Protein 2	0.0000	0.0018
IL21R	Interleukin 21 Receptor	0.0000	0.0018
IRF1	Interferon Regulatory Factor 1	0.0000	0.0013
MIR4683	MicroRNA 4683	0.0000	0.0014
PDCD1LG2	Programmed Cell Death 1 Ligand 2	0.0000	0.0001
PLAU	Plasminogen Activator, Urokinase	0.0000	0.0001
RASGRP1	RAS Guanyl Releasing Protein 1	0.0000	0.0020
SOD2	Superoxide Dismutase 2	0.0000	0.0014
STAT1	Signal Transducer and Activator of Transcription 1	0.0000	0.0007
CCNB1IP1	Cyclin B1 Interacting Protein 1	0.0000	0.0014
CHD6	Cadherin 6	0.0000	0.0020
IGF1R	Insulin Like Growth Factor 1 Receptor	0.0001	0.0079
KANK1	KN Motif and Ankyrin Repeat Domains 1	0.0000	0.0026
MBTD1	Mbt Domain Containing 1	0.0000	0.0030
PLCB4	Phospholipase C Beta 4	0.0001	0.0079
SIRT1	Sirtuin 1	0.0001	0.0079

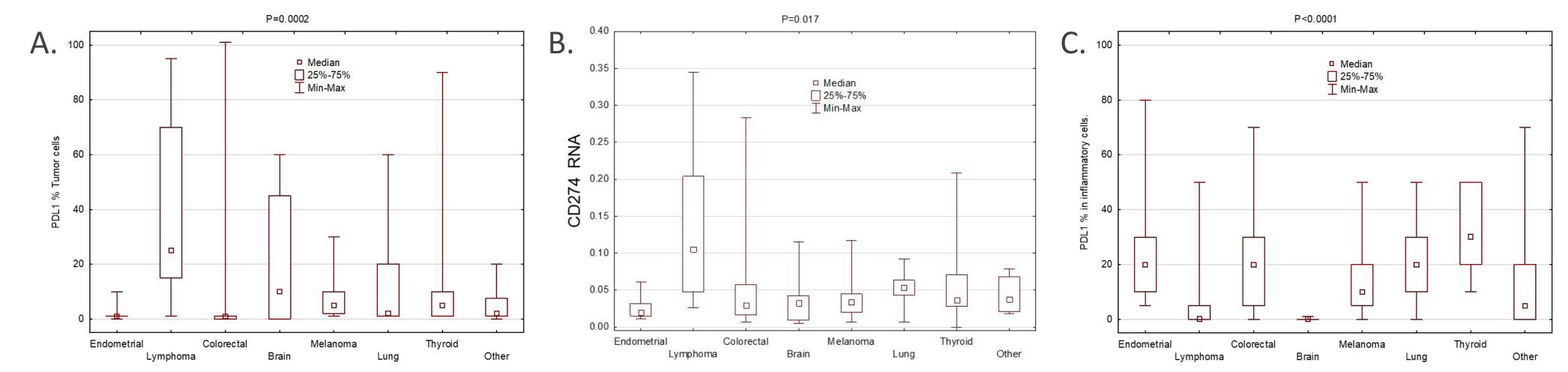
q-value cut-off of ≤0.01 after adjusting for group effect and multiple hypothesis testing

#### PD-L1 Expression as a Function of Tumor Type

A. Solid tumor type vs. % of PD-L1 protein in tumor samples;

B. Solid tumor type vs. CD274 (PD-L1) transcript in tumor samples; and

C. Solid tumor type vs. % of PD-L1 protein in immune cells.



#### LASSO Model Refinement – Predicting PD-L1 Expression

- A. Model 1 (CD274 alone). The predicted expression level is on the x-axis and the measured PD-L1 expression in tumor cells is on the y-axis;
- B. Model 2 (*CD274* + group);
- C. Model 3 (*CD274* + group + *CD274* + *PLAU* + *RAC1*); and
- D. Model 4 (*CD274* + group + *CD274* + *PLAU* + *RAC1* + 2 components).

