Consensus Gene Expression Analysis to Identify Key Hallmarks of Cancer in Malignant Melanoma

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Summary

Using clara^T we have identified novel molecular subtypes. Using the combined analyses in this retrospective study we demonstrate:

- Significant separation of patients outcome depending on their Immune status
- clara^T reveals significant interactions with other hallmarks
- Immune OFF: Proliferation and EMT ON had the worst outcomes of all
- Immune ON but specifically IFNg alone had better outcomes of all

Introduction

Traditionally gene expression signatures (GES) are used individually to classify patients into subgroups. Signatures targeting the same biology are often developed independently and may not classify identically. We developed the clara^T software tool that uses consensus between multiple published GES categorised by the Hallmarks of Cancer (Hanahan & Weinberg, 2011) to classify cancers. As metastatic melanoma represents poor prognostic disease (5-yr survival 15-20%), we applied clara^T to the TCGA melanoma dataset to identify targetable biologies, validated in a cohort of melanoma patients treated with Ipilimumab.

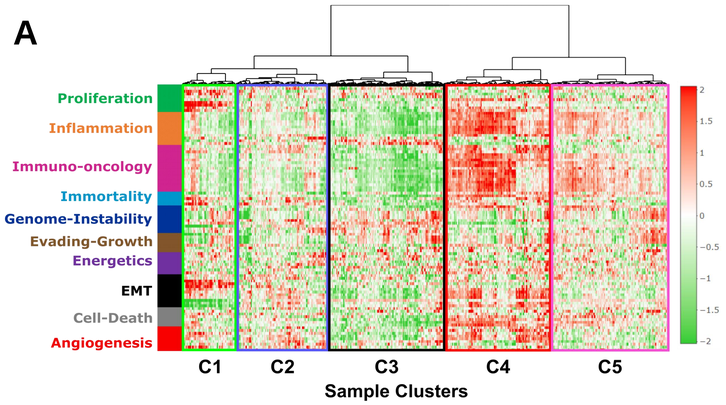
Methodology

TCGA RNA-seq data (n=472) was analysed using the clara^T platform including 92 GES across 10 Cancer Hallmarks. Samples were clustered for the combined and individual Hallmarks, using agglomerative hierarchical clustering.

Each sample group was given a putative label based on the pattern of GES scoring by hallmark. Kaplan Meier curves together with Cox proportional hazard regression analysis were used to assess differences in survival across the different sample groups.

Validation of the survival differences observed within the immune hallmark signatures was performed in the Van Allen Ipilimumab treated melanoma dataset (n=42) (Van Allen, 2015).

Immune-negative tumours have significantly worse outcomes in TCGA



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Sample Cluster	Observed hallmark pattern	HR	CI	P-value
C1	Proliferation, Immortality & EMT	-	-	-
C2	Weak Angio & Energetics	0.307	0.610-0.155	0.0007
C3	Inflammation, GI & Evading Growth	0.540	0.978-0.299	0.0419
C4	Inflammation, Immune, EMT, Cell Death & Angio	0.169	0.357-0.080	<0.0001
C5	Inflammation & Immune (IFNγ only)	0.175	0.368-0.083	<0.0001

A: clara^T gene expression signature heatmap, with signatures grouped by associated hallmark of cancer, which are colour coded on the y axis. The 5 identified sample clusters with similar biologies are highlighted.

B: Overall survival (OS) analysis for each of the clusters identified in figure A.

C: Table detailing the observed Hallmark pattern for each cluster, as well as the hazard ratio (HR) and confidence interval (CI) and p-value for the OS analysis of each cluster using cluster 1 as a reference.

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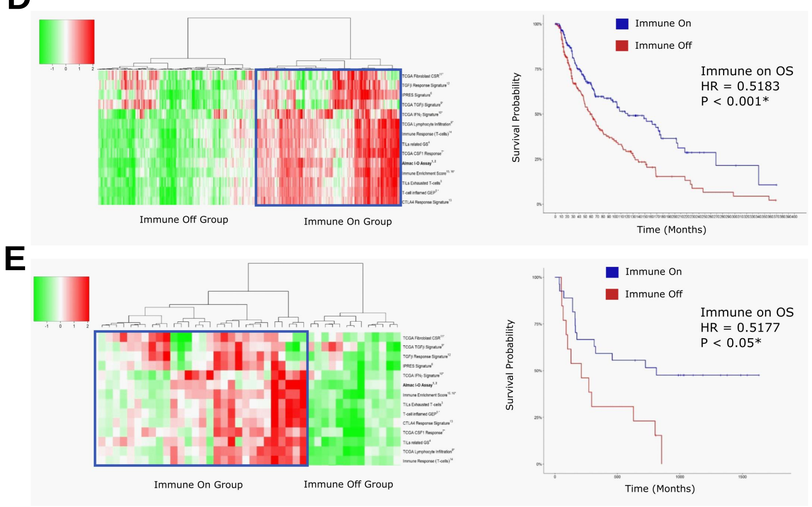

Immune signature analysis comparing TCGA melanoma (n=472) (fig. D) and an Ipilimumab treated melanoma dataset (n=42) (Van Allen, 2015) (Fig E).

The heatmaps show Clara^T immuno-oncology signature scores across samples, with immune-on and immune-off groups highlighted.

The survival plots to the right show OS analysis for the immune-on and immune of-groups. Hazard ratios (HR) and p-values are displayed on both plots.

Results

Clustering the combined Hallmarks identified 5 subgroups in the TCGA cohort summarised in the table below.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Proliferation	Active				
Inflammation			Active (macrophage)	Active (non-macrophage)	Active (non-macrophage)
Immune activation				Active	Active
Immortality	Active				
Genome instability			Active		
Energetics					
Evading growth			Active		
EMT	Active			Active	
Cell death				Active	
Angiogenesis				Active	

Groups 4&5 had significantly improved OS compared to Groups 1,2&3 (HR=0.50, p<0.0001), which were differentiated strongly by activation of immune and inflammation hallmark signalling. Clustering using single Hallmarks revealed that immune-positive tumours had significantly improved OS (HR=0.52, p<0.0001) compared to immune-negative tumours. When validated in the Ipilimumab treated dataset, patients classified as immune-positive had improved OS (HR= 0.52, p=0.05) when compared to immune-negative.

Conclusion

This study demonstrates how simultaneous analysis of multiple gene expression signatures can identify robust biologies through consensus expression. This platform may have value in the identification of reliable biomarkers for clinical trials and could inform how combination therapies targeting key biologies may be used in cancer treatment. **Contact: Jonathan.young@almacgroup.com**

References

The TCGA dataset referenced in this work is available at https://www.cbioportal.org/

The Van Allen dataset referenced in this work is available in dbGAP under accession code phs000452.v3.p1

Van Allen *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science (New York, N.Y.) vol. 350, 6257 (2015): 207-221. doi:10.1126/science.aad0095*