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White Paper

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

ALMAC

Partnering to Advance Human Health

Introduction

Traditionally, gene expression signatures (GES) are used individually to classify patients into molecular subgroups. Signatures targeting the same biology are often developed independently and therefore may not classify identically. We therefore developed the clara^{T} software solution that uses consensus between multiple published and proprietary GES categorised by the Hallmarks of Cancer (Hanahan & Weinberg. 2011) to classify cancers in a more robust manner.

clara^{T*} is a pan-cancer solution, based on a powerful proprietary bioinformatics pipeline, used to automatically generate interactive cohort and sample reports that provide simplified visualisation of key discriminating biologies within both the study cohort and individual tumour samples.

Within this article we provide evidence of the ability of clara^{T} to robustly identify the biologies underlying several Hallmarks of Cancer. This is shown by a retrospective case study in malignant melanoma which represents poor prognostic disease (5-yr survival 15-20%). We applied clara^{T} to the TCGA melanoma dataset to identify targetable biologies and validated these in a cohort of malignant melanoma patients treated with Ipilimumab.

Methods

RNA-Sequencing data from the TCGA malignant melanoma dataset (*n*=472) were processed using clara^{T} software. A total of 62 gene expression signatures are represented within the clara^{T} V2.0.0 release classified as immuno-oncology (IO), epithelial to mesenchymal transition (EMT), angiogenesis, genome instability, proliferation and cell death hallmarks. For each gene expression signature, continuous scores and associated percentile ranks were calculated using the clara^{T} V2.0.0 analysis pipeline. Hierarchical clustering using these normalised percentile ranked scores was then performed on the sample data with the number of sample clusters guided by the gap statistic. Samples were grouped based upon clustering of the Hallmarks combined and each Hallmark individually. Hallmark activation labels were assigned to sample clusters based on the mean score of the Hallmark signatures in each cluster compared to that across the cohort. Cox proportional hazards regression for overall (OS) and progression free survival (PFS) was analysed across the identified subgroups and within Hallmark labels. This analysis was repeated in anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) targeted agent, Ipilimumab treated malignant melanoma samples (*n*=42) (Van Allen et al. 2015).

Results

Clustering of the TCGA Malignant Melanoma Dataset simultaneously using Hallmark Biologies

The clara^{T} V2.0.0 analysis software tool was applied to the TCGA malignant melanoma cohort of 472 patients (Figure 1A).

A

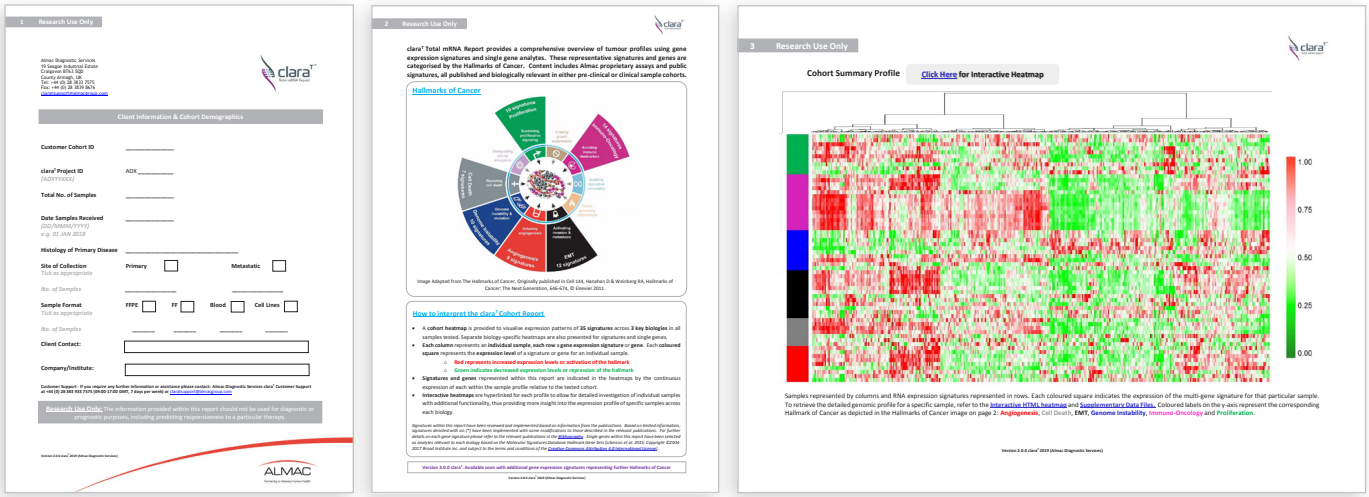

Figure 1: A) clara^{T} Cohort Report for TCGA Melanoma.

* **Note:** clara^{T} is for Research Use Only (RUO) and is not to be used for diagnostic or prognostic purposes, including predicting responsiveness to a particular therapy.

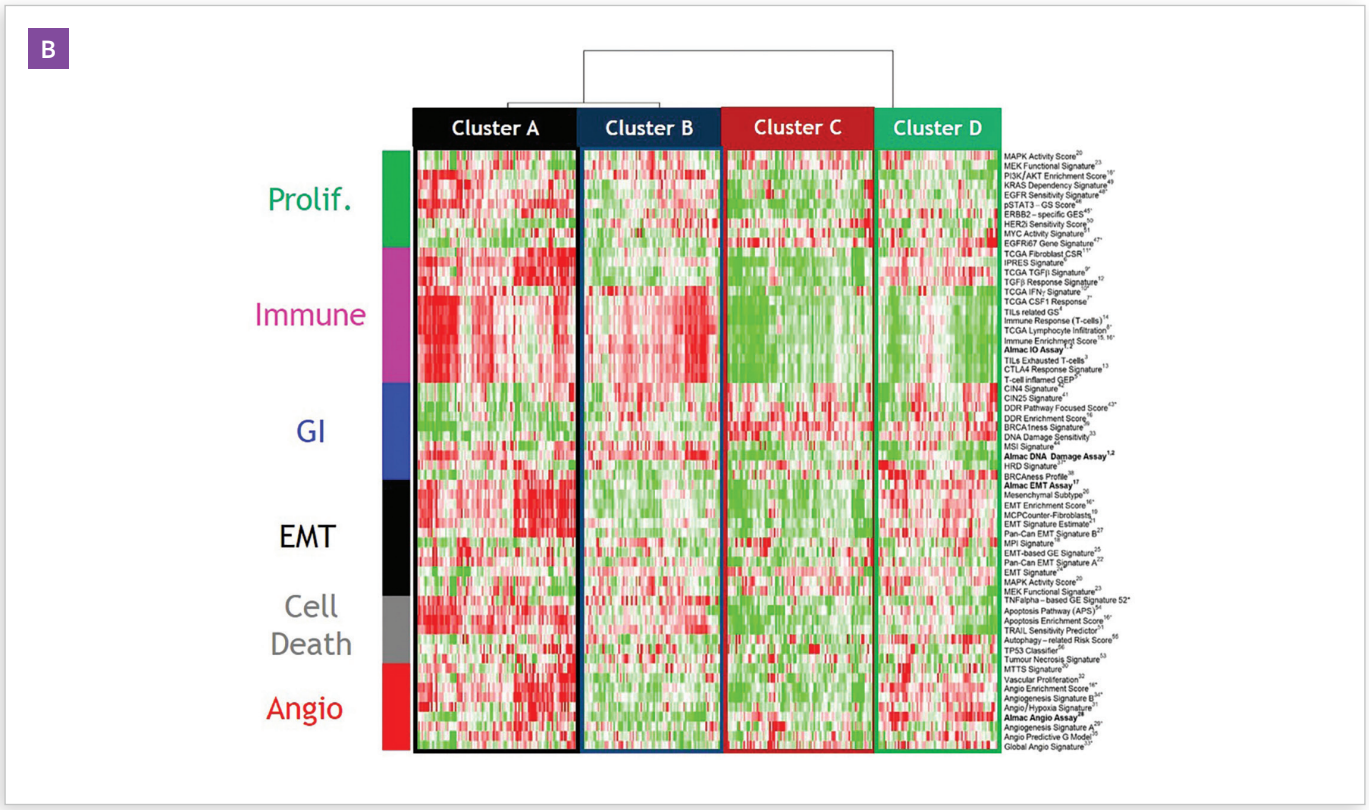

Figure 1: B) Combined clustering of TCGA melanoma identifies 4 molecular subgroups.

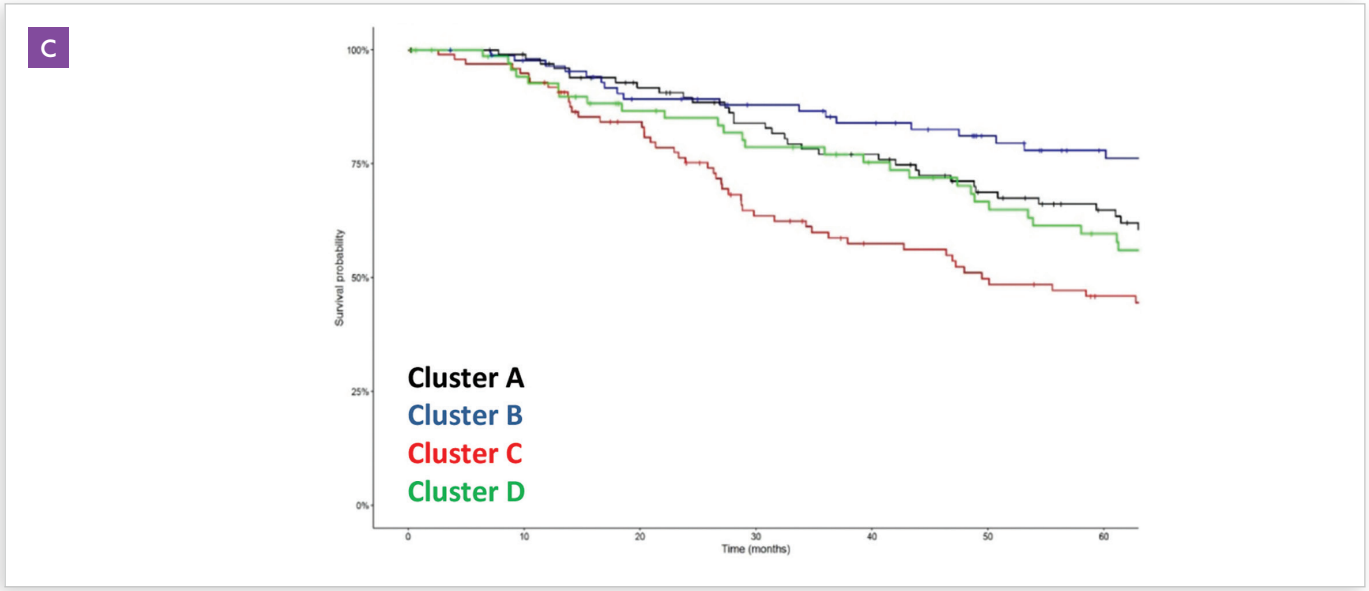

Figure 1: C) Improved overall survival in Cluster B (blue) compared and significantly decreased overall survival benefit in Cluster C (red) within TCGA melanoma patients.

Consensus clustering simultaneously using signatures representing the combined hallmarks identified 4 subgroups in the TCGA cohort: Cluster **A**) All biologies active except Genome Instability (**black**), Cluster **B**) Immune (IFNγ), Genome Instability and Cell Death biologies active (**blue**), Cluster **C**) Genome Instability, MAPK and Proliferation active (**red**) and Cluster **D**) TGFß, Genome Instability, EMT and Angiogenesis active (**green**) (Figure 1B). Cluster B (blue line) of the combined analysis had significantly improved OS (HR=0.38 [0.25-0.58]; $p<0.001$) compared to Cluster C (red line) that displayed the worst overall survival (Figure 1C). In addition, Clusters A&D (black and green lines) showed intermediate survival benefit compared to Cluster C (HR=0.51 [0.35-0.74]; $p<0.001$ and HR=0.72 [0.49-1.06]; $p=0.09$ respectively).

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Clustering of TCGA Malignant Melanoma individually using Hallmark Biologies

Next we clustered the TCGA malignant melanoma dataset using single Hallmarks individually to identify consensus between multiple gene expression signatures within a single biology (Figure 2A-F). A survival analysis revealed that immune-positive tumours (blue) had significantly improved OS (HR=0.53, $p<0.0001$) compared to immune-negative tumours (Figure 3A). Angiogenesis-negative (red) tumours displayed improved PFS (HR=0.73, $p=0.03$) and OS (HR=0.53, $p<0.0001$) compared to angiogenesis-positive tumours (Figure 3B). Finally cell death-active tumours also showed improved OS compared to cell death inactive tumours (HR=0.44 [0.31-0.63]; $p<0.0001$) (Figure 3C). Interestingly the other Hallmarks (EMT, Proliferation & Genome Instability) were not found to be individually prognostic.

Figure 2: Single biology clustering with **Angiogenesis (A), Cell Death (B), EMT (C), Genome Instability (D), Proliferation (E) and Immuno-oncology (F),** gene expression signatures separately in TCGA melanoma.

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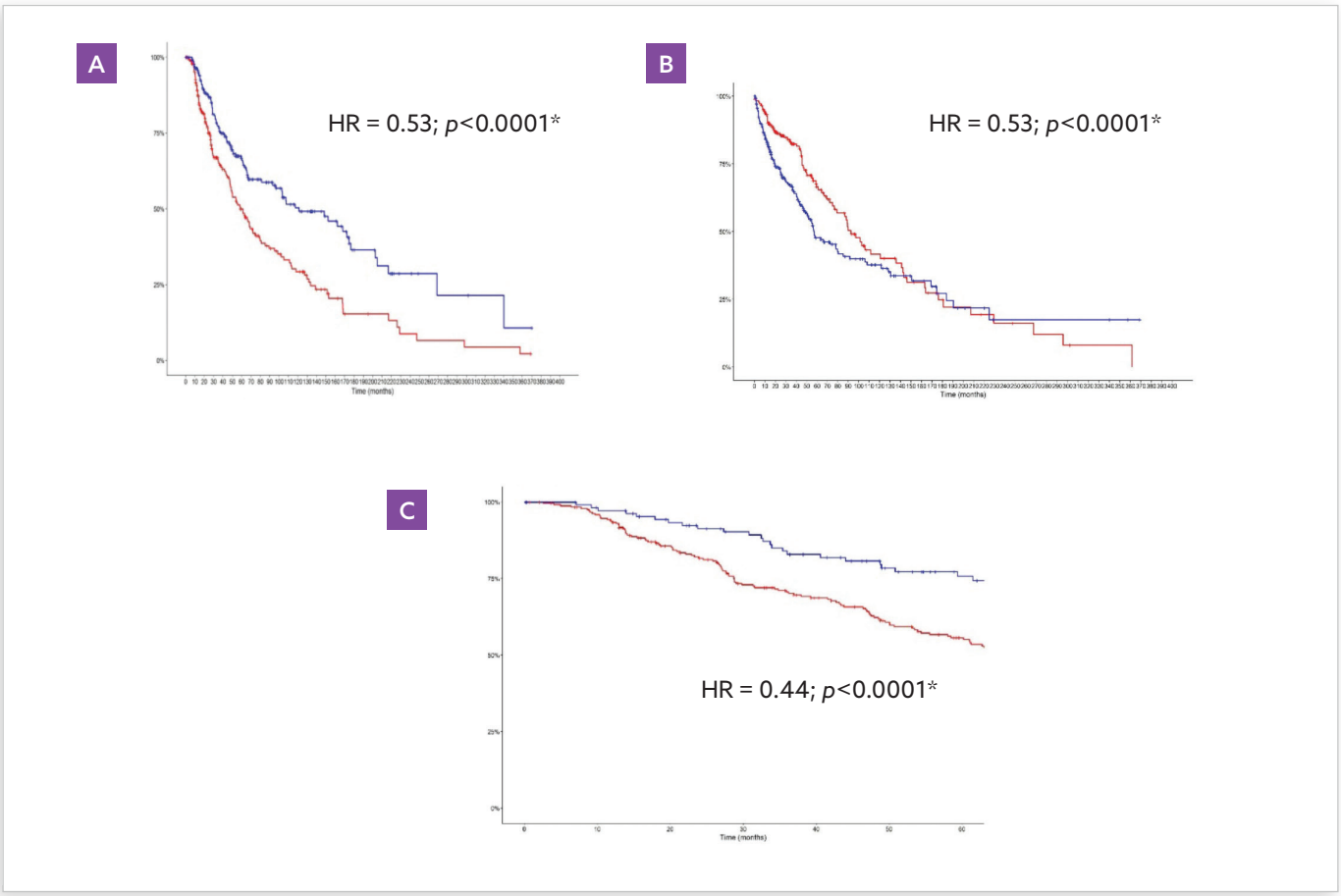

Figure 3: Single biology clustering with gene expression signatures shows immune-active tumours have improved OS **(A)**, angiogenesis-active tumours have poorer PFS **(B)** and cell death-active tumours have improved OS **(C)** in TCGA melanoma.

Validation of the Molecular Subgroups in an Ipilimumab Treated Dataset

Next we assessed the prognostic significance of the single hallmark molecular subgroups in a dataset of 42 patients treated with second-line Ipilimumab (Figure 4A-F). There was a consistent proportion of samples displaying consensus activation or repression of signatures within each hallmarks across both datasets. Only patients classified as immune-positive had improved OS (HR=0.357, p=0.010) when compared to immune-negative (Figure 5A). A trend towards improved survival was observed for angiogenesis (Figure 5B) but no effect was seen in the other Hallmarks.

Discussion

Multiple approaches can be used to generate GES which may differ in sample selection, technology and bioinformatics analysis pipelines; ultimately this can result in several different signatures being reported to detect similar biologies. Indeed we identified 14 signatures that were reported to detect an immune biology. We therefore developed a methodology to identify consensus calling between multiple signatures, the rationale being that these calls were likely to be more robust and reproducible with different approaches agreeing on the presence of a common Hallmark. We have further extended this approach by showing how these consensus Hallmarks could be analysed together to demonstrate the interaction of key biologies in large datasets. Importantly, these molecular subgroups were found to have clinical significance. We were able to demonstrate how immune, genome instability and EMT biologies interact in malignant melanoma. Moreover, we found that similar biological groups were reproduced in an independent clinical dataset, one of which also had prognostic significance following Ipilumumab treatment.

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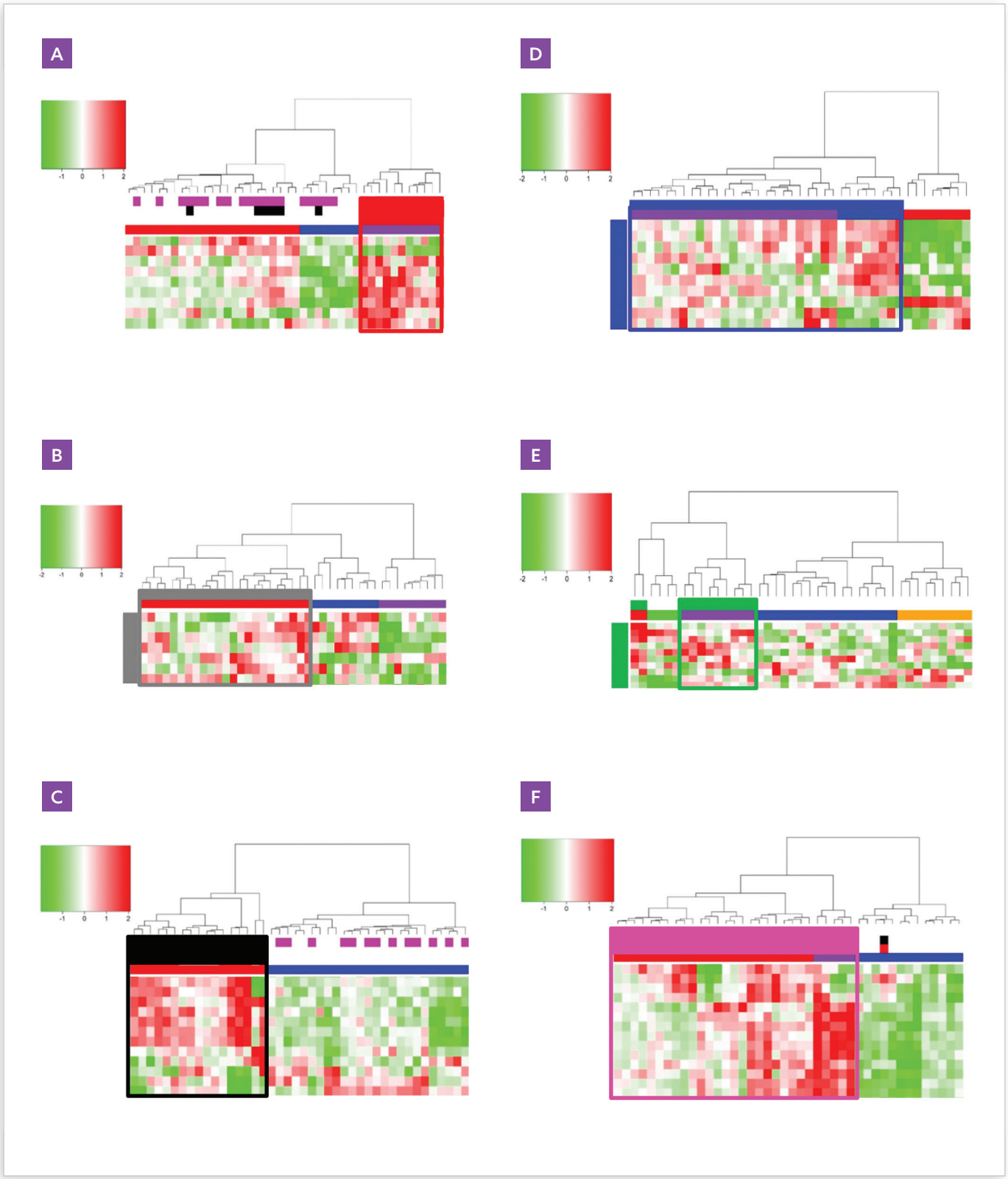

Figure 4: Single biology clustering with **Angiogenesis (A), Cell Death (B), EMT (C), Genome Instability (D), Proliferation (E) and Immuno-oncology (F),** gene expression signatures separately in an Ipilimumab treated dataset.

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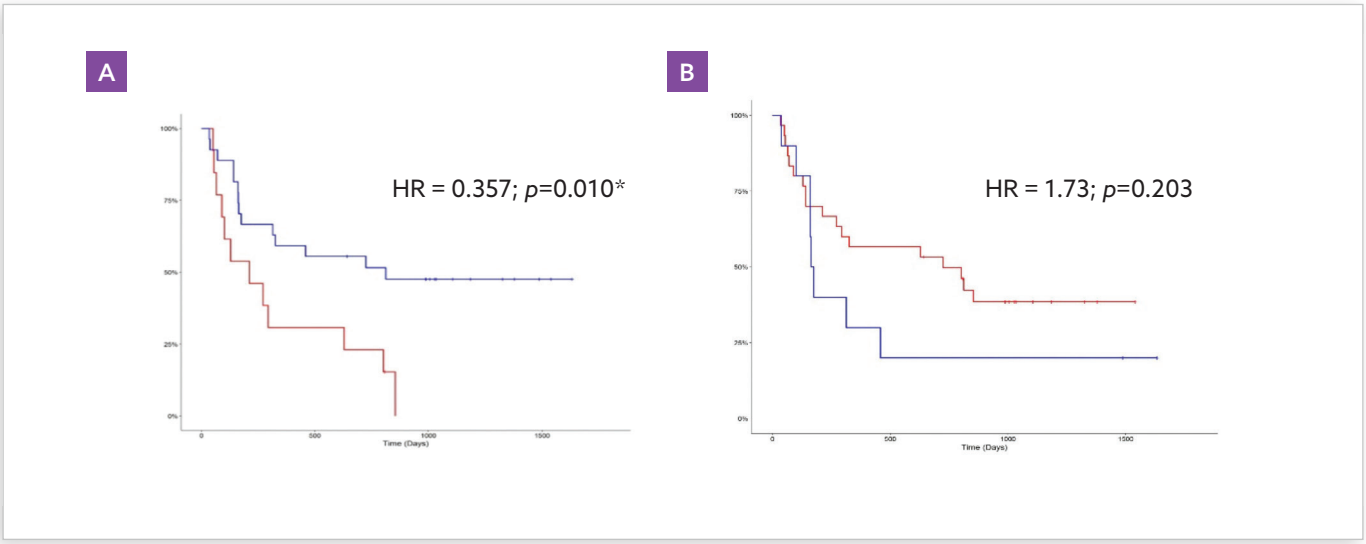

Figure 5: Single biology clustering with gene expression signatures shows immune-active tumours have improved OS **(A)**, angiogenesis-active tumours show a trend towards poorer OS **(B)** whilst EMT-active tumours show no survival benefit **(C)** in an Ipilimumab treated dataset.

Overall Conclusion

This white paper retrospective study demonstrates how simultaneous analysis of multiple gene expression signatures (GES) (*n*=62 in this study) can identify robust biologies through consensus readouts. Almac's clara^{T*} reporting solution may have value in the identification of reliable biomarkers for clinical trials and could inform combination strategies for targeting key biologies.

We believe that clara^{T}:

- Represents a significant step forward in the analysis of RNA expression data.
- Provides reproducible Hallmark classification in an automated fashion.
- Standardises and facilitates comparisons between different datasets.
- Removes variation that can occur due to the different GES discovery approaches.
- Helps to identify novel molecular subtypes with relevance to therapeutic outcomes.

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Want to know more about how clara^{T} can help your biomarker discovery and translational research?

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