

Transcriptome-based Biomarker for guidance of Immune Checkpoint Inhibitor Therapy in Melanoma

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Introduction

Melanoma is the most aggressive form of skin cancer and shows an increasing incidence during the latest decades¹. The course as well as prognosis and treatment depends highly on the melanoma stages classified by the criteria of the American Joint Committee on Cancer (AJCC)². Immune checkpoint inhibitors (ICI) like Ipilimumab, a monoclonal antibody to cytotoxic T-lymphocyte antigen 4 (CTLA-4) or Nivolumab and Pembrolizumab, monoclonal antibodies directed against programmed cell death receptor 1 (PD-1), have revolutionized cancer treatment. ICIs now represent first-line therapies for various solid and liquid tumors, including melanoma. However, the substantial proportion of patients fail to respond to and benefit from ICI therapy. Further, immune-related adverse events complicate treatment.

Aim

Identification of biomarker patterns for ICI response using transcriptomic data from FFPE melanoma samples.

Material & Methods

We performed transcriptome-profiling, based on Next Generation Sequencing, flanked with bioinformatics/statistical approaches for molecular feature determination of 135 FFPE melanoma samples of overall 57 patients., from which 2 patients were excluded due to lack of follow up data. Partially, multiple samples were gathered out of those patients. In total, there were 21 Responder- and 34 Non-Responder patients stratified according to the RECIST 1.1 criteria.

FFPE Melanoma samples were selected at the Dermatology of Helios Klinikum Krefeld. Samples were inspected and graded by an experienced Dermatologist (CA). According to the newest and eighth AJCC (American Joint Committee on Cancer) classification from 2018, patients’ characteristics were determined as shown below (Tab. 1).

RNA Extraction, Quality control and Quantification

RNA was extracted from 10 µm sections using the Quick-DNA/RNA FFPE Mini Prep Kit from Zymo Research. 4 µm section/FFPE-block were used for HE-staining, according to standard procedure. RNA Quality control was performed by Electropherogram analysis (Fragment Analyzer, Agilent Technologies). Quantification was performed by Qubit Fluorometer; BR Kit. Real time PCR was performed with target-specific assays and Step One Plus real time PCR Cycler (Thermo Fisher Scientific), according to standard procedure.

MACE-Seq

Rapid MACE-seq³ was used to prepare 3’ RNA sequencing libraries (Fig.1). Samples of 100 ng of DNA-depleted RNA were used for library preparation, using the Rapid MACE-Seq kit (GenXPro GmbH, Germany). Fragmented RNA underwent reverse transcription using barcoded oligo(dT) primers containing TrueQuant unique molecular identifiers, followed by template switching. PCR amplified libraries were purified by solid phase reversible immobilization beads and subsequent sequencing was performed using the Illumina platform NextSeq 500 (Fig. 1).

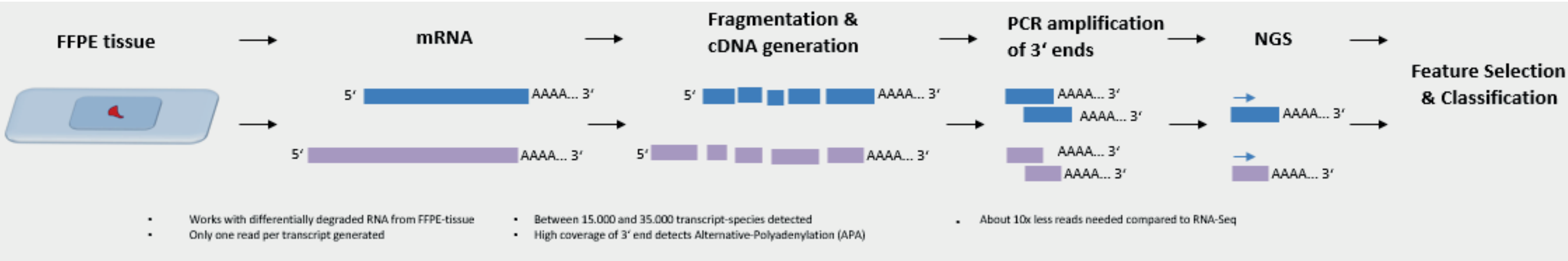


Figure 1: MACE sequence scheme

Bioinformatics

Raw Sequencing reads have been adapter-trimmed using Cutadapt⁴ and UMI-Deduplications has been performed using in-house tools. Processed reads were mapped to GRCh38 using Bowtie⁵. Mapped reads were quantified using HSeq⁶ and normalized by tags-per-million. We developed feature extraction and selection methods to identify a predictive set of binary features as a score from the normalized gene expression counts. The values of the feature set were summarized to a single score value for each sample.

Results

We generated a predictive score as a highly discriminative classifier for responders and non-responders to Immune-Checkpoint-Inhibition therapies of melanomas. Real-Time PCR analysis was used to verify results exemplarily, which is still ongoing. The set of 25 features can retrospectively separate ICI-responders from non-responders of 100 samples, which were selected based on sequencing quality out of the 135 samples (Fig. 2, Fig.3).

characteristics	
number of patients	57
patients after exclusion of no follow-ups	55
age (years)	
average age (sd)	69.3 (14.4)
median (min-max)	71 (29-93)
gender, n (%)	
male	27 (49.1)
female	28 (50.9)
stage according to AJCC 2018 (n)	
IIIA	1
IIIB	3
IIIC	5
IIID	1
IV	45
Pembrolizumab mono	50
Nivolumab mono	11
Ipilimumab/Nivolumab	18
complete remission	9 (16.4)
stable disease	9 (16.4)
partial remission	3 (5.5)
progressive disease	34 (61.8)
progression-free survival (PFS)/ overall survival (OS), (months)	
average PFS	12.3
average OS since therapy	30
average OS since initial diagnosis	59.9
average LDH before therapy (U/l)	258.6
BRAF mutation, (n)	
positive	17
negative	36
unknown	2

Table 1: Baseline characteristics of patients (N = 57)

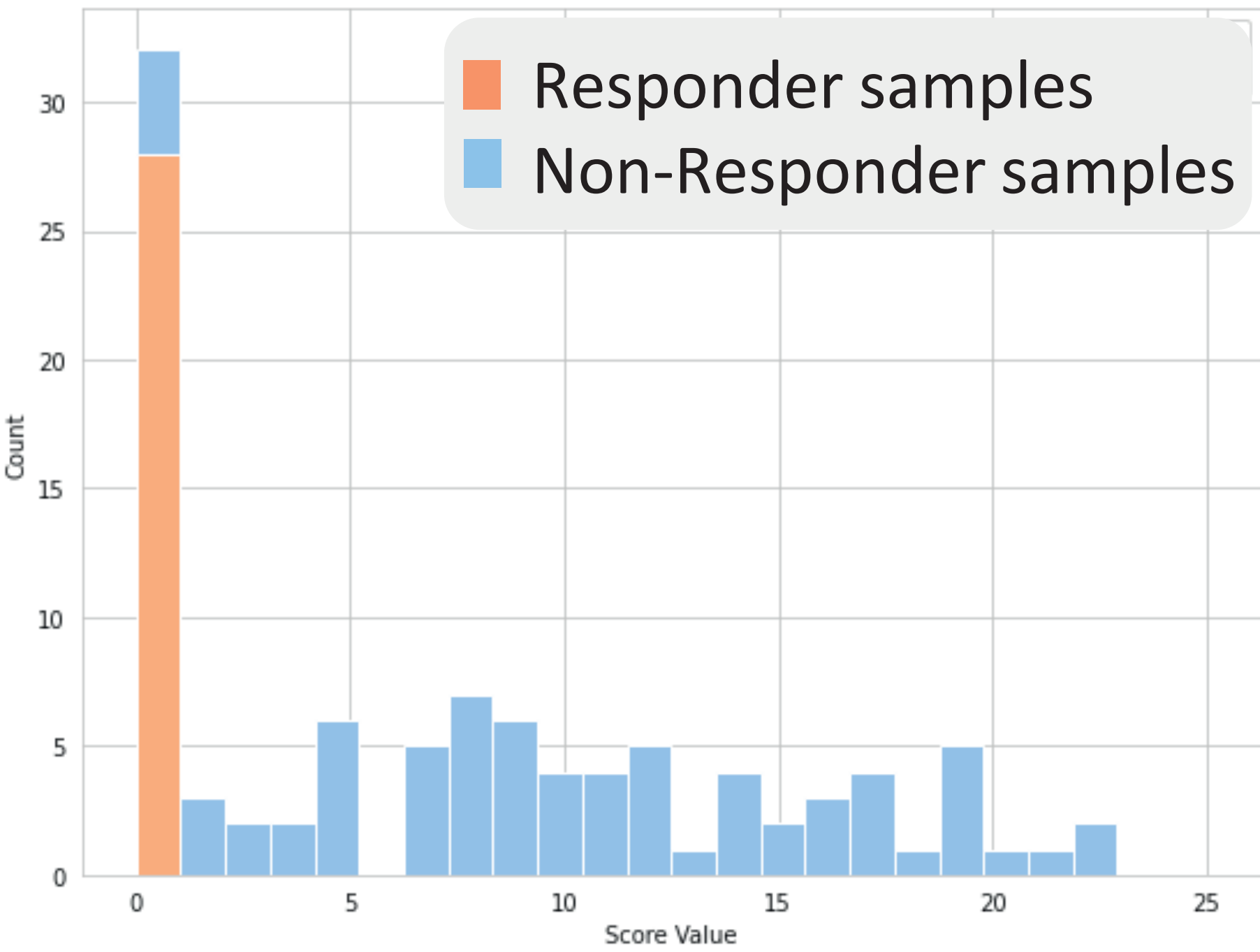


Figure 2: Histogram showing the score values between 0 and 25 for the dataset of 28 Responder and 72 Non-Responder samples. All responders reveal a score value of 0.

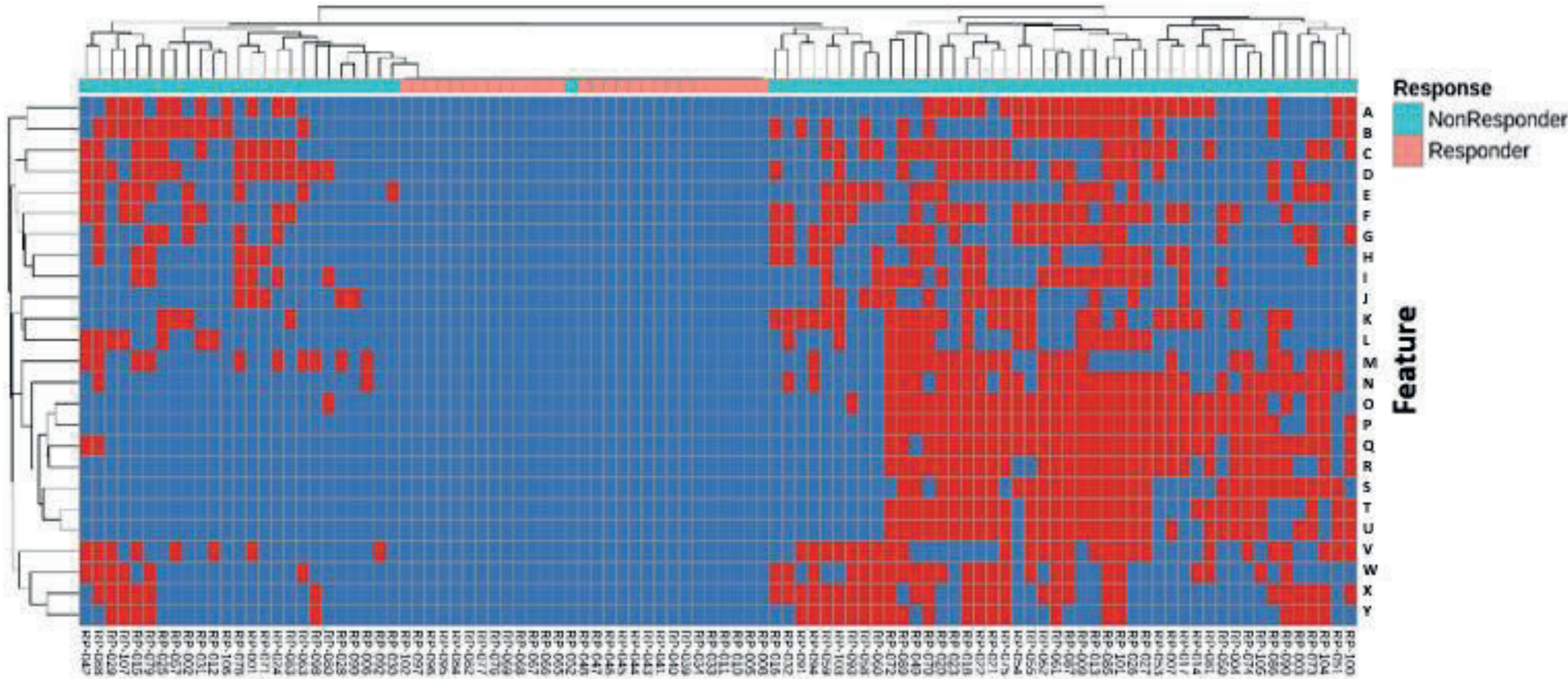


Figure 3: Heatmap showing the individual 25 features for the dataset of 28 Responder and 72 Non-Responder samples.

Conclusion

The *ICI Predict Score* identifies 100% of responders and 97,2% of non-responders to ICI-therapy. The biomarker panel enables guidance of ICI-therapy treatment decisions for melanoma patients. The analyses are going to be extended to an independent cohort of Melanoma samples to verify our results.

References

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