

ANTI-PD1 TREATMENT RESPONSE PREDICTION AND MONITORING IN NON-SMALL CELL LUNG CANCER PATIENTS USING PLASMA CELL-FREE DNA HYDROXYMETHYLATION

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Background Treatment with immune checkpoint inhibitors (ICIs) targeting programmed death-1 (PD-1) can yield remarkable anti-tumor responses in lung cancer patients, however, not all patients respond to ICIs. Biomarkers currently used in clinical practice are insufficient in determining patient populations who may benefit from anti-PD-1 treatment. Non-invasive liquid biopsy approaches for therapy response prediction present key advantages over methods that rely on tumor biopsy, especially in cancer types such as lung cancer, where the location of the tumor may impact both patient compliance and the health risks involved with the procedure. Liquid biopsies offer a simple and convenient alternative and can also help overcome challenges hampering tumor biopsy dependent biomarker discovery and development such as tumor heterogeneity and difficulty in longitudinal sampling over the course of treatment.

Methods 151 whole blood samples were collected from 31 non-small cell lung cancer (NSCLC) patients, prior to therapy start and while on anti-PD1 therapy, namely pembrolizumab or nivolumab (up to 5 timepoints). cfDNA was extracted from plasma, followed by fragment enrichment using 5-hydroxymethyl-cytosine (5hmC)-based epigenomic platform technology. As part of this analysis, both 5hmC and low pass whole genome libraries were generated and sequenced.

Results Here, we investigated the utility of 5-hydroxymethyl-cytosine (5hmC) signatures obtained from plasma-derived cell free DNA (cfDNA) for non-invasive prediction and monitoring of anti-PD-1 treatment response. Comparison of pre-treatment 5hmC profiles from responders to non-responders revealed differential hydroxymethylation, many of which localized to genes in pathways important for immune response. While anti-PD1 treatment induced changes in hydroxymethylomes of both responding and non-responding patients, these changes were distinct. Upon anti-PD1 treatment, 5hmC further accumulated over genes involved in immune activation such as IFN γ and IFN α response, inflammatory response and TNF α signaling, in responding patients but strikingly not in non-responders. On the other hand, non-responder plasma cfDNA had 5hmC increase over genes involved in epithelial to mesenchymal transition, which is associated with metastasis and drug resistance. These pathway-based changes in responders and non-responders are consistent with previous data from tumor tissue analysis. Furthermore, anti-PD1 treatment induced differential 5hmC changes in responders relative to non-responders starting from the first cycle of treatment, suggesting that 5hmC can help monitor treatment response.

Conclusions Our results demonstrate that 5hmC profiling can capture tumor-associated immune response signals in plasma without the need for tissue biopsy. Altogether, these findings show that 5hmC profiling of plasma-derived cfDNA enables non-invasive monitoring of immunotherapy response and can provide putative patient selection biomarkers from blood.

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