CERTIS™ ONCOLOGY INTELLIGENCE

Monitoring Drug Response With Circulating Tumor DNA in a Non-Small Cell Lung Cancer PDX

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Circulating tumor DNA (ctDNA) has been shown as a clinically relevant biomarker for non-invasive monitoring of therapy response, disease burden and disease progression in cancer patients. Patient-derived xenograft (PDX) mice are essential preclinical models to evaluate therapeutic response. Patients with a KRAS G12 mutation in Non-Small Cell Lung Cancer (NSCLC) have been shown to be resistant to EGFR inhibitors. Here, Certis reports Whole Genome Sequencing (WGS) of ctDNA from PDX for the monitoring of therapeutic response and demonstrate measurable ctDNA changes concordant with therapeutic response. Altogether, the isolation of ctDNA from PDX models is a robust methodology for interrogating therapeutic efficacy, response and resistance in a preclinical setting that can be translated as a viable biomarker for non-invasive monitoring in patients.

METHODS

PDX Pharmacology: Patient biopsies were surgically implanted into subcutaneous and orthotopic sites of female NOG mice. Animals were imaged with the M3^{TM} compact MRI from Aspect Imaging to monitor tumor growth. For this study, an NSCLC subcutaneous PDX was dosed with EGFR inhibitors formulated and administered per the manufacturer's instructions or past publications. Following the end of study, whole blood and tumor tissue was collected for cell-free DNA (cfDNA) and RNA extraction, respectively.

cfDNA Sequencing: PDX whole blood was collected into PaxGene cfDNA tubes. Plasma was separated by double centrifugation, collected and stored at –80C. cfDNA were extracted using magnetic bead-based technology (MagMAX cfDNA), following manufacturer's instructions. Low-pass WGS was performed by Azenta Life Sciences.[2] Watchmaker DNA Library Prep Kit was used following the manufacturer's recommendations for cfDNA library preparation. Briefly, the unfragmented DNA was end repaired. Adapters were ligated after adenylation of the 3' ends followed by enrichment by limited cycle PCR. Cleanups were then performed to remove gDNA fragments > 400bp. Sequencing was performed on an Illumina® HiSeq 3000/4000 using a 2x150bp paired end configuration at 12G reads per sample (3-4x coverage).

Analysis: Paired-end reads from cfDNA WGS and bulk RNA-seq were pre-processed to remove mouse contamination (Xenome) and poor-quality reads (fastp). BWA (cfDNA WGS) and STAR (RNA-seq) was used to map reads to the Human GRCh38 (hg38) genome. For cfDNA analysis, we used NucTools for the analysis of chromatin feature occupancy. A random forest classifier was trained against 150bp sliding window of feature occupancy to classify human from cancer cfDNA, followed by Boruta to select significant occupancy regions. For RNA-seq, RSEM was used to quantify transcripts. R was used to perform Locally-Weighted Scatterplot Smoothing (LOWESS) and calculate correlation coefficients.

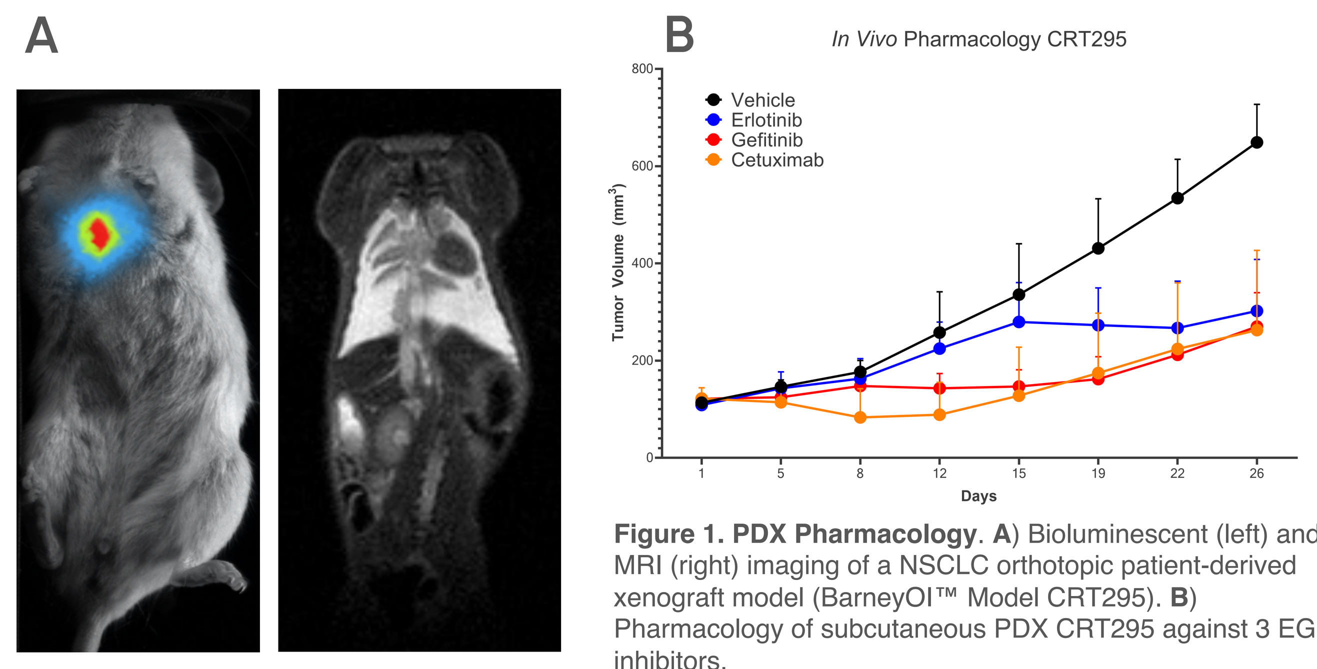


Figure 1. PDX Pharmacology. A) Bioluminescent (left) and MRI (right) imaging of a NSCLC orthotopic patient-derived xenograft model (BarneyOI™ Model CRT295). **B)** Pharmacology of subcutaneous PDX CRT295 against 3 EGFR inhibitors.

RESULTS

Figure 2. PDX ctDNA is concordant with human cfDNA. A) WGS ctDNA extracted from treated and untreated NSLC PDX models compared against human cfDNA from cancer and lung cancer patients as well as healthy individuals. LOWESS chromatin occupancy coverage against "Housekeeping" regions show high concordance of ctDNA samples from PDX against human cfDNA samples (Spearman $\rho > 0.92$ for all pairwise comparisons), but with varying levels of occupancy between samples. Treated PDX samples show lower occupancy ratios along the chromatin compared to untreated samples. **B)** Human cfDNA were sourced from published datasets[3,4,5,6]. **C)** Selected chromatin occupancy regions from a machine learning (ML) random forest classifier were used to distinguish human healthy and cancer cfDNA. Using these selected regions as prognostic biomarkers, the ML model classified both vehicle and treated PDX ctDNA as "Cancer" with probability 0.64 and 0.95, respectively.

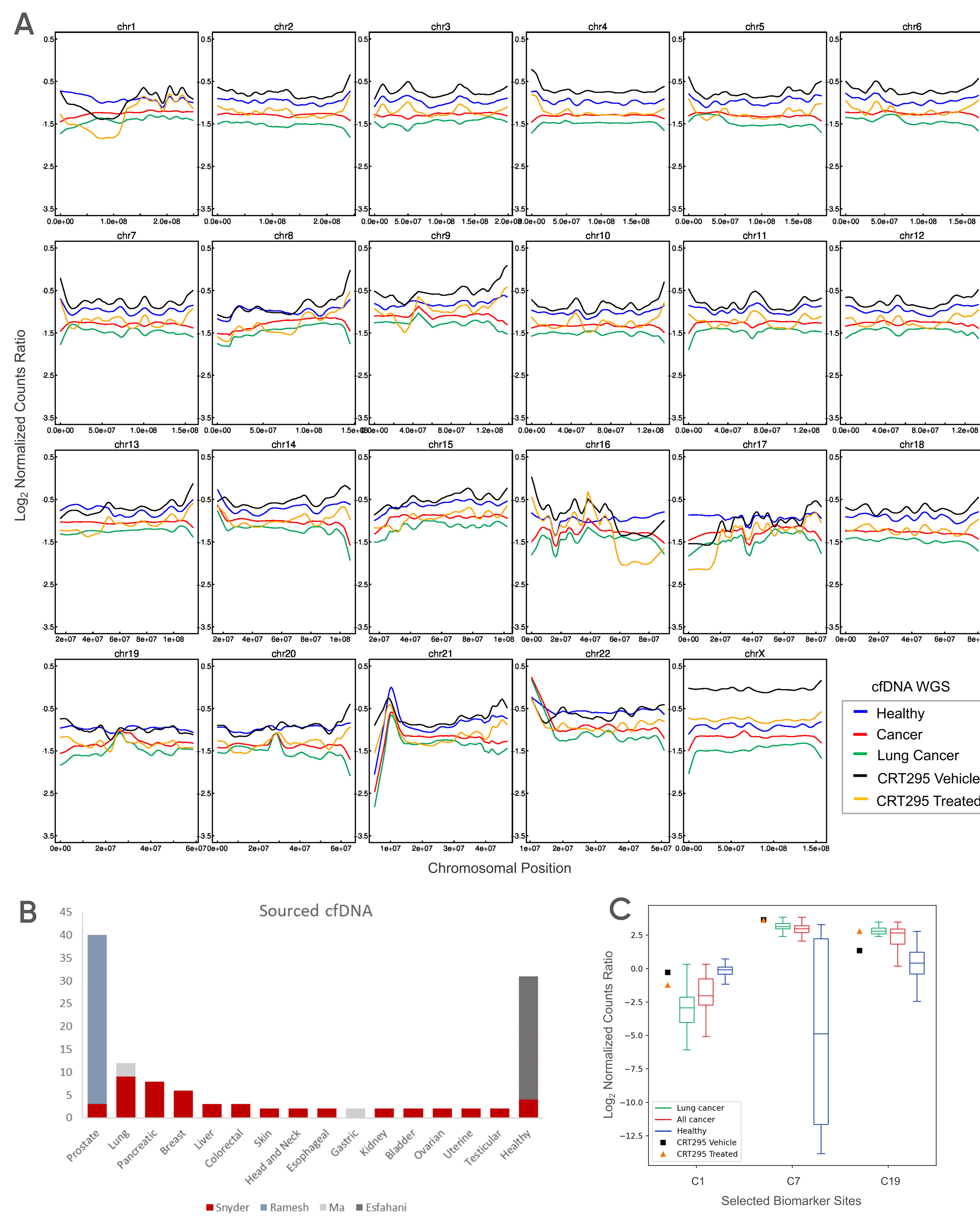


Figure 3. PDX ctDNA is concordant with gene expression. Tumor RNA-seq and ctDNA WGS reads spanning both WGS and RNA-seq are represented as the LOWESS line of normalized read counts. WGS of ctDNA from PDX models show high concordance with RNA-seq gene expression from PDX solid tumors in both vehicle and treated samples. Spearman correlation of LOWESS ctDNA vs. RNA-seq are $\rho = 0.85$ in vehicle samples and $\rho = 0.75$ in treated samples.

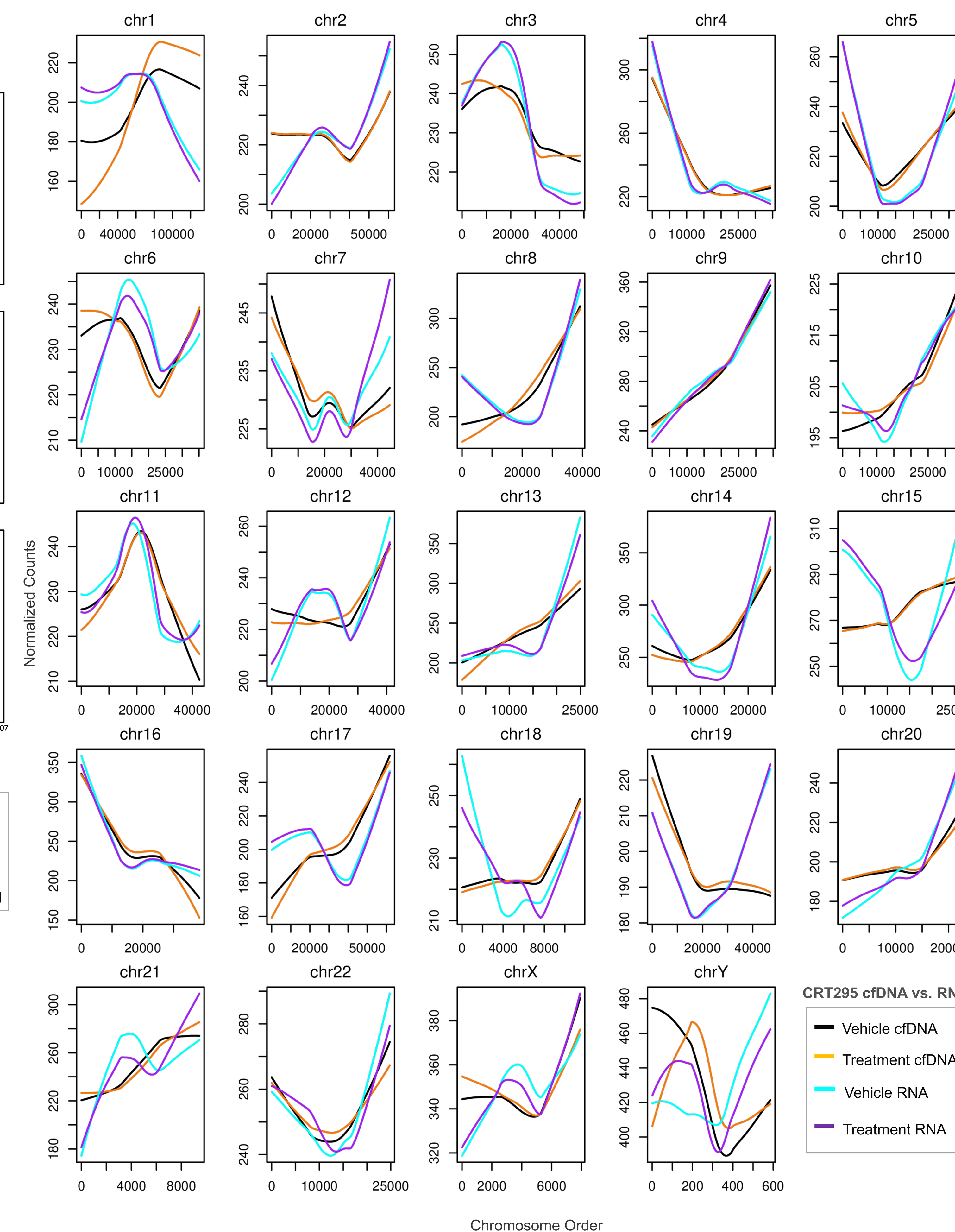
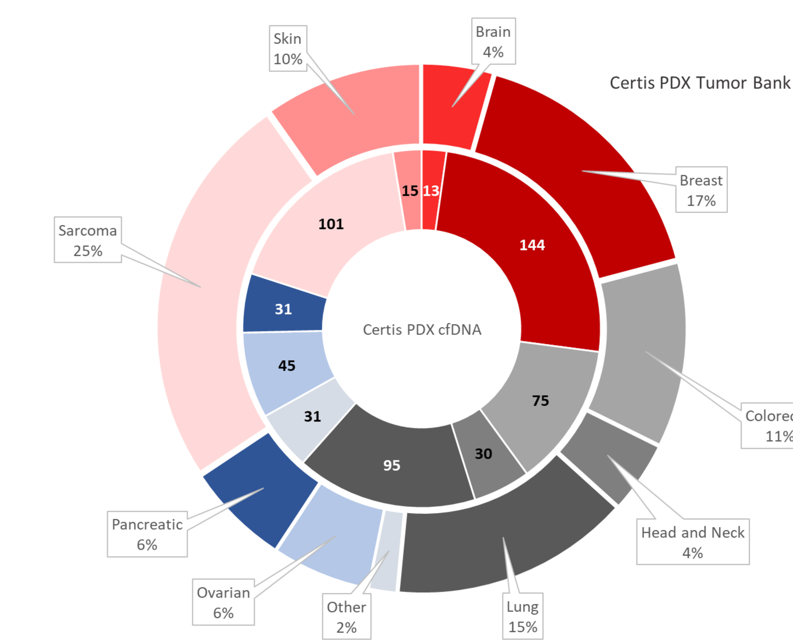


Figure 4. cfDNA sample collection from Certis PDX models in the BarneyOI Cancer Model Database™. Certis has more than 600 cfDNA samples from a collection of diverse solid tumor PDX models (295).



SUMMARY

Certis reports here the use of WGS to observe therapy response of ctDNA in PDX models. While WGS of PDX models have been previously reported[7,8], PDX ctDNA therapy response has not been extensively characterized. The analysis of NSCLC PDX showed reduced ctDNA levels following therapy, as seen in previous studies of patients[9]. Certis also developed an ML classifier to distinguish healthy from cancer cfDNA, and both untreated and treated PDX ctDNA were classified as "Cancer". Additionally, we found strong correlation between ctDNA chromatin occupancy and RNA expression ($\rho > 0.75$), suggesting cfDNA may be a suitable surrogate for tumor gene expression. These findings suggest that ctDNA isolation from PDX models is a promising method for studying therapeutic efficacy, response and resistance in a preclinical setting, and may be used as a viable biomarker for non-invasive monitoring in patients.

CITATIONS & ACKNOWLEDGEMENTS

1. Certis Oncology Solutions, San Diego, CA. 2. Azenta Life Sciences, Boston, MA. 3. Ma et al. *PLoS ONE* (2017). 4. Ramesh et al. *Genome Biol* (2020). 5. Snyder et al. *Cell* (2016). 6. Esfahani et al. *Nat Biotechnol* (2022). 7. Cristiano et al. *Nature* (2019). 8. Sauer et al. *EMBO Mol Med* (2022). 9. Chen et al. *Clin Cancer Res* (2019).

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