I. Introduction

Circulating tumor DNA (ctDNA) has been detected in the blood of patients with a variety of cancer types and is thought to reflect the combined genetic profile of the primary tumor and metastases. ctDNA analysis is used clinically to monitor response to treatment in cancer patients and putative CNA events were identified. ctDNA concentrations were determined for all patients in this study, and CNA events were detected in ctDNA from 43% of patients (43/102), multiple collections were available (range: 2-17) to enable longitudinal monitoring of patients (43/102), multiple collections were available (range: 2-17) to enable longitudinal monitoring of patients (43/102). These data suggest that GIN values derived from low-coverage sequencing may be useful alone or as a complement to standard variant detection strategies in the development of liquid biopsy technologies.

II. Methods

Whole blood (10 ml) was collected from 102 patients diagnosed with a variety of solid tumors, prior to the initiation of therapy, and processed to isolate plasma and white blood cells. For almost half of the patients (43/102), multiple collections were available (range: 2-17) to enable longitudinal monitoring of treatment response. In total, 287 aliquots of blood from multiple collaborative studies were used as part of this study.

ctDNA from the plasma of each sample was extracted using a bead-based method and genomic DNA was extracted from white blood cells using a standard silica membrane-based protocol (Qiagen) for use as a germline control. Total ctDNA load was quantified using a single-locus droplet digital PCR assay for each plasma sample. All DNA samples were subjected to optimized library preparation and targeted next-generation sequencing (Illumina, San Diego, CA). Genome-wide CNA profiles were generated for each sample and CNA events were detected using an algorithm originally developed for non-invasive prenatal testing. Two variations of ctDNA that are present in paired ctDNA and white blood cell samples were considered to be derived from the germline and thus removed from further analysis. A genomic instability number (GIN) representing the cumulative difference between total ctDNA load and the frequency and magnitude of CNAs across all patients was defined. This metric, termed the genomic instability number (GIN), is strongly correlated with the number of genome-wide CNAs detected in a patient with colorectal cancer and liver metastases and may be a useful biomarker for clinical response. In total, 35.9% (103/287) of blood samples showed evidence of one or more genome-wide CNAs in ctDNA and 47.1% (48/102) of patients in this study showed similar evidence in at least one sample. In patients with longitudinal monitoring, 41.8% (18/43) of individuals showed evidence of CNAs at the most recent sampling point. Collectively, multiple CNAs were detected on all autosomes and overlapped 94% of the addressable genome, suggesting that genome-wide profiling is necessary to fully assess ctDNA distributions. Figure 3 shows the distributions of CNAs detected across all autosomes. Measures of CNA frequency alone may be informative, but fail to adequately capture changes in magnitude of detected CNAs. We devised a metric that quantified the level of genomic instability as inferred through CNA frequency and magnitude. This metric, termed the genomic instability number (GIN), is associated with the number of CNAs detected in a patient. Figure 4 shows the relationship between total cfDNA load and the frequency and magnitude of CNAs across all subjects. In patients capable of longitudinal monitoring, GIN values were observed varied both between patients and across longitudinal samples from individual patients. These data suggest that GIN values derived from low-coverage sequencing track with clinical response in cases for which CNA events are detected. This study suggests that genome-wide CNA profiling may be useful alone or as a complement to standard variant detection strategies in the development of liquid biopsy technologies.

III. Results

Low coverage genome-wide sequencing (median 13.3x) was performed for all 287 samples from 102 cancer patients and putative CNA events were identified. ctDNA concentrations were determined for all samples and total ctDNA load was found to be significantly elevated in cancer patients relative to healthy controls. Although there was a poor correlation between total ctDNA load and the frequency and magnitude of CNAs across all patients, a strong correlation was observed within individual patients, suggesting that the CNAs detected in the ctDNA were derived from additional genetic material contributed by the tumor. In total, 35.9% (103/287) of blood samples showed evidence of one or more genome-wide CNAs in ctDNA and 47.1% (48/102) of patients in this study showed similar evidence in at least one sample. Among patients with longitudinal monitoring, 41.8% (18/43) of individuals showed evidence of CNAs at the most recent sampling point. Collectively, multiple CNAs were detected on all autosomes and overlapped 94% of the addressable genome, suggesting that genome-wide profiling is necessary to fully assess ctDNA distributions. Figure 3 shows the distributions of CNAs detected across all autosomes. Measures of CNA frequency alone may be informative, but fail to adequately capture changes in magnitude of detected CNAs. We devised a metric that quantified the level of genomic instability as inferred through CNA frequency and magnitude. This metric, termed the genomic instability number (GIN), is associated with the number of CNAs detected in a patient. Figure 4 shows the relationship between the count of CNA events and the GIN metric across all subjects. In patients capable of longitudinal monitoring, GIN values were observed varied both between patients and across longitudinal samples from individual patients. These data suggest that GIN values derived from low-coverage sequencing track with clinical response in cases for which CNA events are detected. This study suggests that genome-wide CNA profiling may be useful alone or as a complement to standard variant detection strategies in the development of liquid biopsy technologies.

IV. Conclusion

Low coverage, genome-wide sequencing of ctDNA from the plasma of cancer patients enables the detection of large CNAs. These data demonstrate the feasibility of detecting ctDNA in some cancer patients solely on the basis of CNA profiling and more specifically using a numeric quantity, the GIN. Further, the nature of CNA events observed varied both between patients and across longitudinal samples from individual patients. These data suggest that GIN values derived from low-coverage sequencing track with clinical response in cases for which CNA events are detected. This study suggests that genome-wide CNA profiling may be useful alone or as a complement to standard variant detection strategies in the development of liquid biopsy technologies.

V. References