INTRODUCTION
Tumor mutational analysis provides insight into patient drug response, prognosis, and tumor biology. A key limitation to this process is the availability of tumor tissue that adequately represents the current disease status. This study presents a Next Generation Sequencing (NGS) workflow utilizing enriched circulating tumor cells (CTCs) as an input. The CTC enrichment is performed using the IsoFlux System that accommodates multiple capture antibody cocktails and has been shown to be effective across multiple indications.

METHODS
Sample Collection
2 x 10cc EDTA blood tubes were collected and shipped overnight at room temperature.

Bead Coupling
RBCs removed with FICOLL EpCAM and EGFR magnetic beads added Sample loaded into microfluidic cartridge

CTC Enrichment
Cells pass through microfluidic isolation zone Target cells with beads captured on roof of channel Up to 4 samples/run, 12 samples/day, 4ºC controlled

NGS Analysis
CTCs transferred in low volume (5µL) Purity enhancement with IsoFlux NGS Kit (WGA optional) Ampliseq Cancer Hotspot v2 library prep (50 genes) Sequencing on Ion Torrent PGM (Thermo Fisher) Variant calling and filtering using Ion Reporter

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CONCLUSIONS
The IsoFlux System employs multiple capture antibodies to recover CTCs from multiple indications using a routine blood draw IsoFlux currently being used in numerous translational studies to monitor patients and profile tumor cells using NGS analysis

NGS workflow has been developed and validated to produce high-confidence somatic variants

ENRICHMENT OF CTCs FROM CLINICAL SAMPLES
The IsoFlux System has been used to enrich CTC samples from hundreds of cancer patients across multiple indications. With magnetic bead isolation, the IsoFlux System can utilize a variety of surface markers for CTC capture, including EpCAM, EGFR, Her2, and other disease/drug-specific targets. Users can define their own capture cocktails with straightforward protocols.

Kidney
Blood samples were collected from 8 kidney cancer patients at UT Southwestern pre-surgery (Time #1) and during one or more post-surgery follow-ups. Low CTC counts (<25) and significant drops in CTCs are highlighted in green. High CTC counts (>25) and significant increases in CTCs are highlighted in red. Disease Status: NED = no evidence of disease, AWD = alive with disease, DOD = dead of disease (all as of last clinical follow up - study still in progress)

NGS ANALYSIS
NGS analytical validation: A model tumor cell line (MDA-MB-231) was spiked into healthy donor blood, resulting in final CTC concentrations ranging from 0-12 CTC/mL blood. The cell line has 3 known somatic variants in the BRAF, KRAS, and TP53 genes. The NGS workflow was able to detect each of these variants in 17/18 attempts (Site 1) and 23/24 attempts (Site 2) with only 1 false positive call made across both test groups (N=18 samples).

High Purity Enhancement: Bladder cancer samples (N=15) were processed using the IsoFlux NGS Kit that contains a purity-enhancement column. The mean purity in CTC-positive (>10) samples went from 5% to 15%, a level that is compatible with NGS analysis.

NGS on clinical samples: Bladder cancer samples (N=4 neoadjuvant, N=4 metastatic) were enriched for CTCs, lysed, amplified, and sent through the NGS analysis workflow. Somatic variants were detected in 6/8 (75%) of samples.