

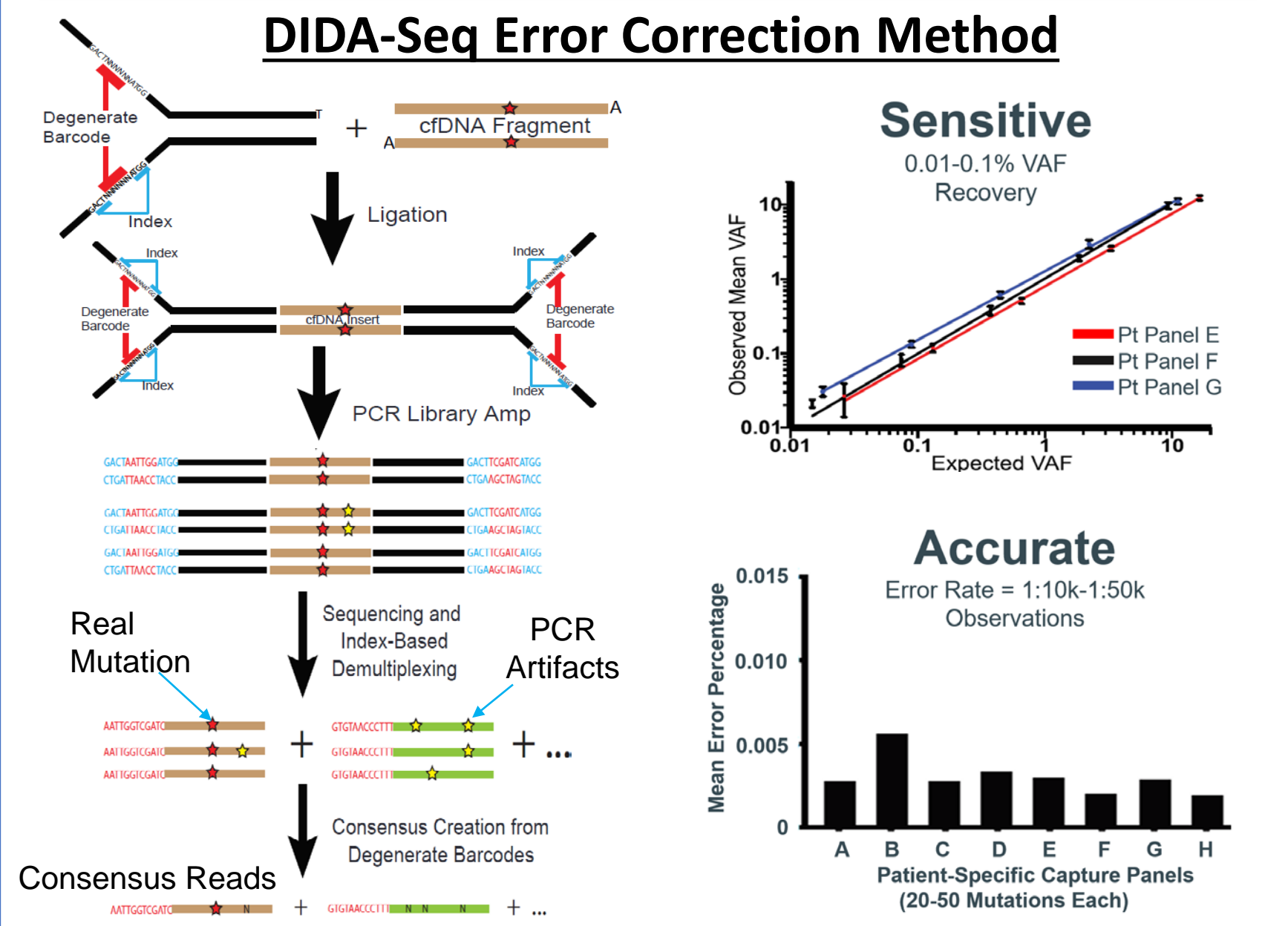
# Radiation-assisted Amplification Sequencing (RAMP-Seq): Evaluating the use of Stereotactic Body Radiation Therapy (SBRT) for Enriching Circulating Tumor DNA in Liquid Biopsies

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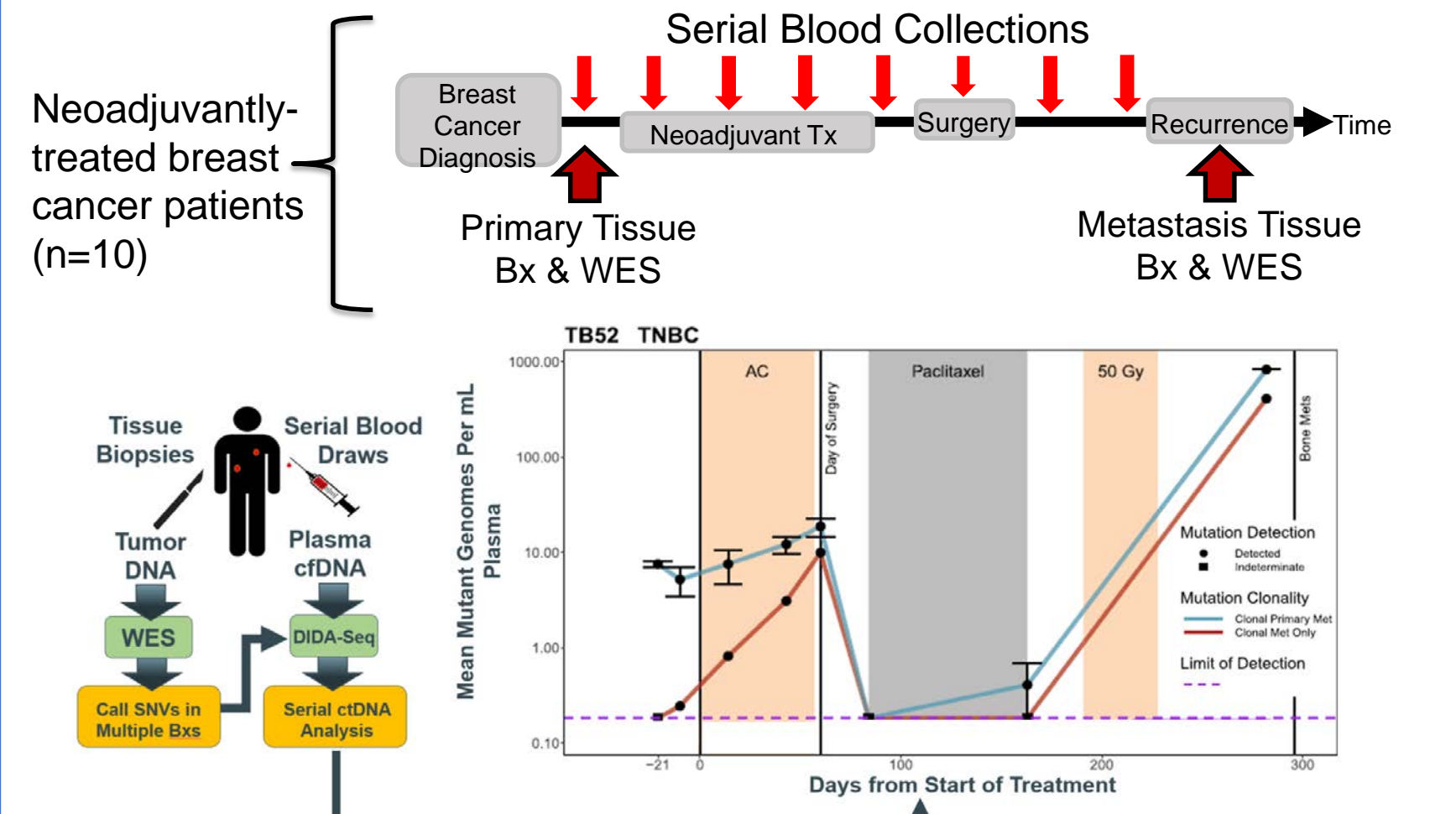
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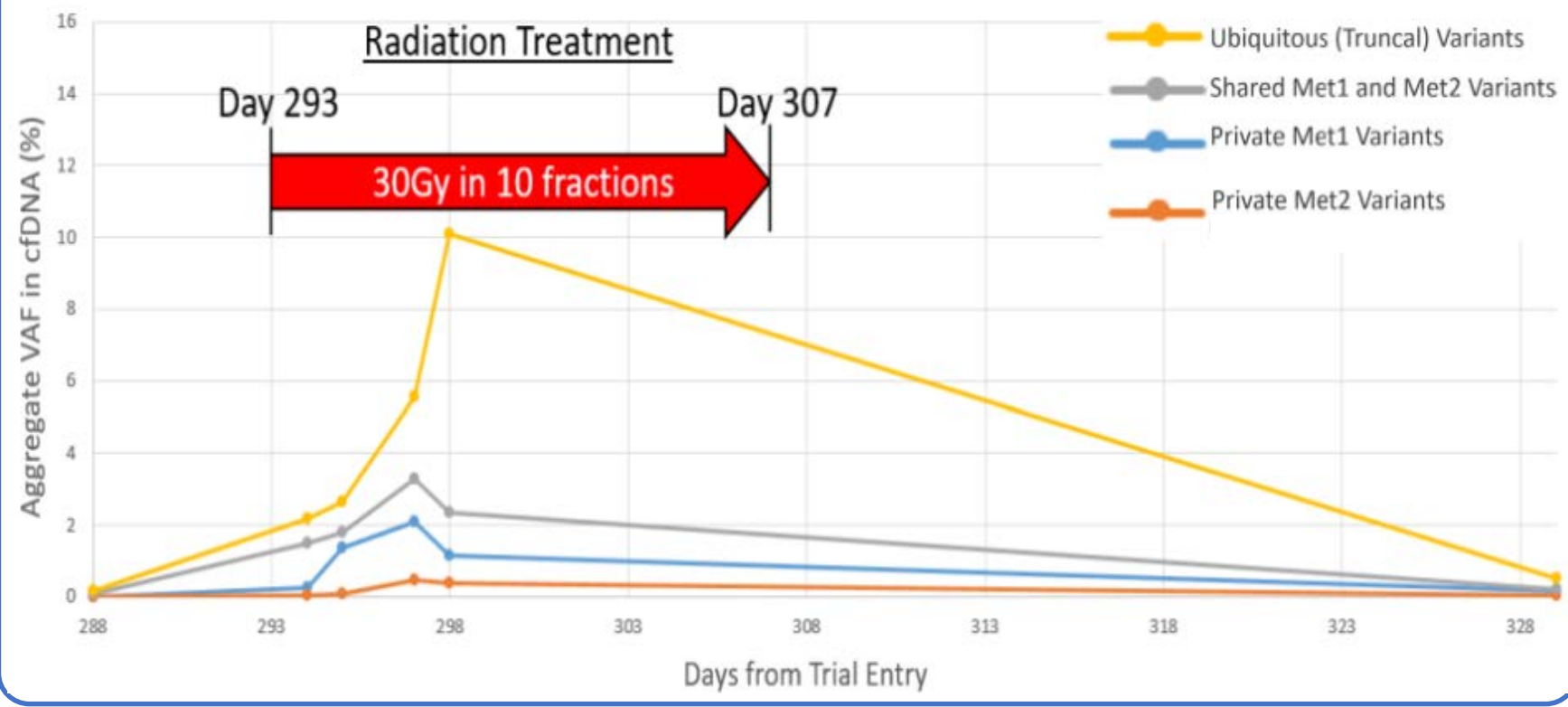
## Background



## CtDNA Levels in Early Breast Cancer were Associated with Treatment Response and Recurrence



## CtDNA Monitoring in Metastatic Disease Shows Enrichment with Radiation Treatment



## Hypotheses

- Administration of stereotactic body radiation therapy (SBRT) to known or suspected tumor masses will temporarily elevate levels of circulating tumor DNA (ctDNA)
- This will allow identification an optimal period of peak ctDNA enrichment for liquid biopsy (kinetics cohort).
- RAMP-Seq can help distinguish true NSCLC from non-cancerous masses and may be able to predict treatment response

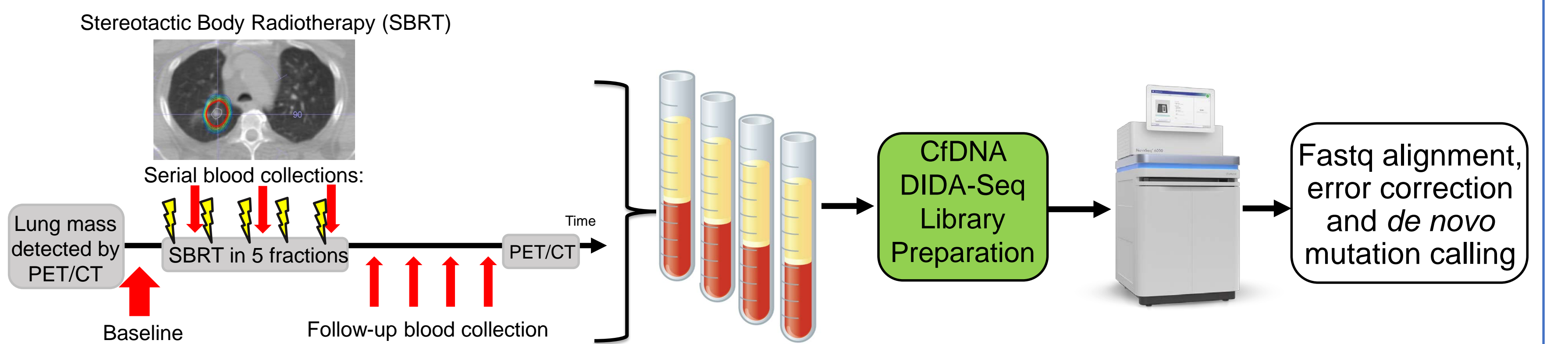
## Methods and Cohort

- DIDA-Seq custom-capture sequencing was carried out using a 150kb panel on cell free DNA (cfDNA) of individuals undergoing SBRT as standard-of-care to an average depth of 5k-20k X coverage.
- Blood draws were collected every 24-72 hours from each patient prior to and during SBRT treatment (n=8), as well as for two weeks following the final dose (n=3). CfDNA and genomic DNA were isolated from serial blood draws and subjected to custom hybridization capture and NGS.
- Sequencing data was aligned and error-corrected. Mutations were called *de novo* using GATK4/Mutect2 pipelines an hand curated in IGV.
- Genomic DNA extracted from buffy coat was prepared and used as a matched normal in mutation calling.
- Variant allele frequencies (VAF) of Mutect calls were compared between baseline and post-treatment samples to determine enrichment levels.
- Limit of detection and site-specific error rates were determined using unrelated patients as a negative control.

## NSCLC Cohort Details and Sequencing Results:

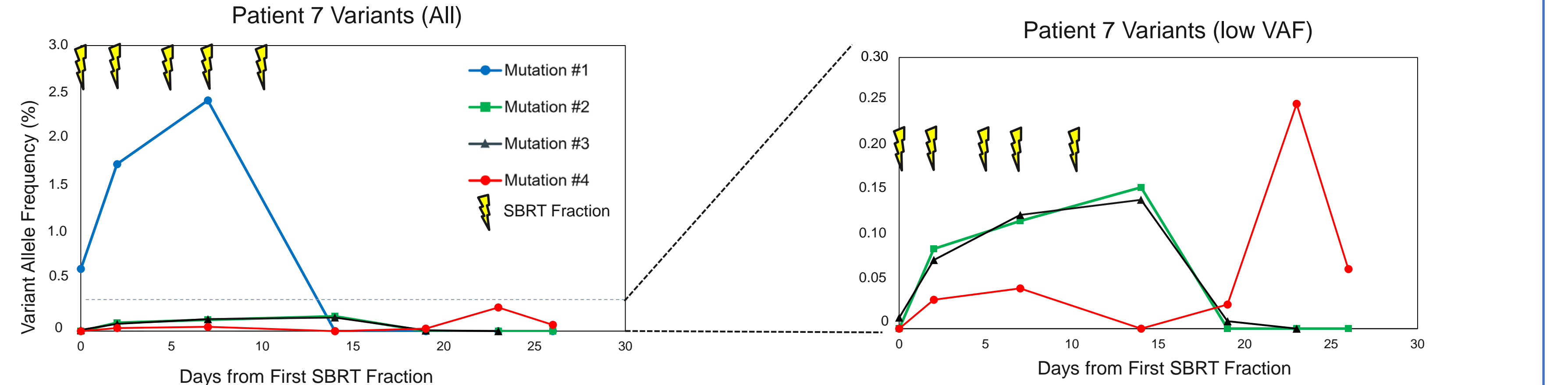
| Patient ID | Age | Gender | Diagnosis | Stage | Biopsy-Proven | Average Error-Corrected Coverage | Number of Overlapping COSMIC SNVs | Peak Post-SBRT VAF (%) |
|------------|-----|--------|-----------|-------|---------------|----------------------------------|-----------------------------------|------------------------|
| Pt1        | 80  | M      | NSCLC     | I     | No            | 14,518                           | 2                                 | 2.81                   |
| Pt2        | 68  | M      | NSCLC     | I     | No            | 7,970                            | 5                                 | 0.26                   |
| Pt3        | 70  | M      | NSCLC     | I     | No            | 9,014                            | 3                                 | 2.92                   |
| Pt4        | 68  | M      | NSCLC     | I     | No            | 12,476                           | 8                                 | 1.45                   |
| Pt5        | 80  | M      | NSCLC     | I     | No            | 7,769                            | 1                                 | 1.23                   |
| Pt6        | 82  | M      | NSCLC     | I     | Yes           | 15,094                           | 0                                 | 0.65                   |
| Pt7        | 80  | M      | NSCLC     | I     | No            | 11,785                           | 1                                 | 2.42                   |
| Pt8        | 68  | M      | NSCLC     | I     | No            | 9,563                            | 2                                 | 1.74                   |

## Experimental Workflow

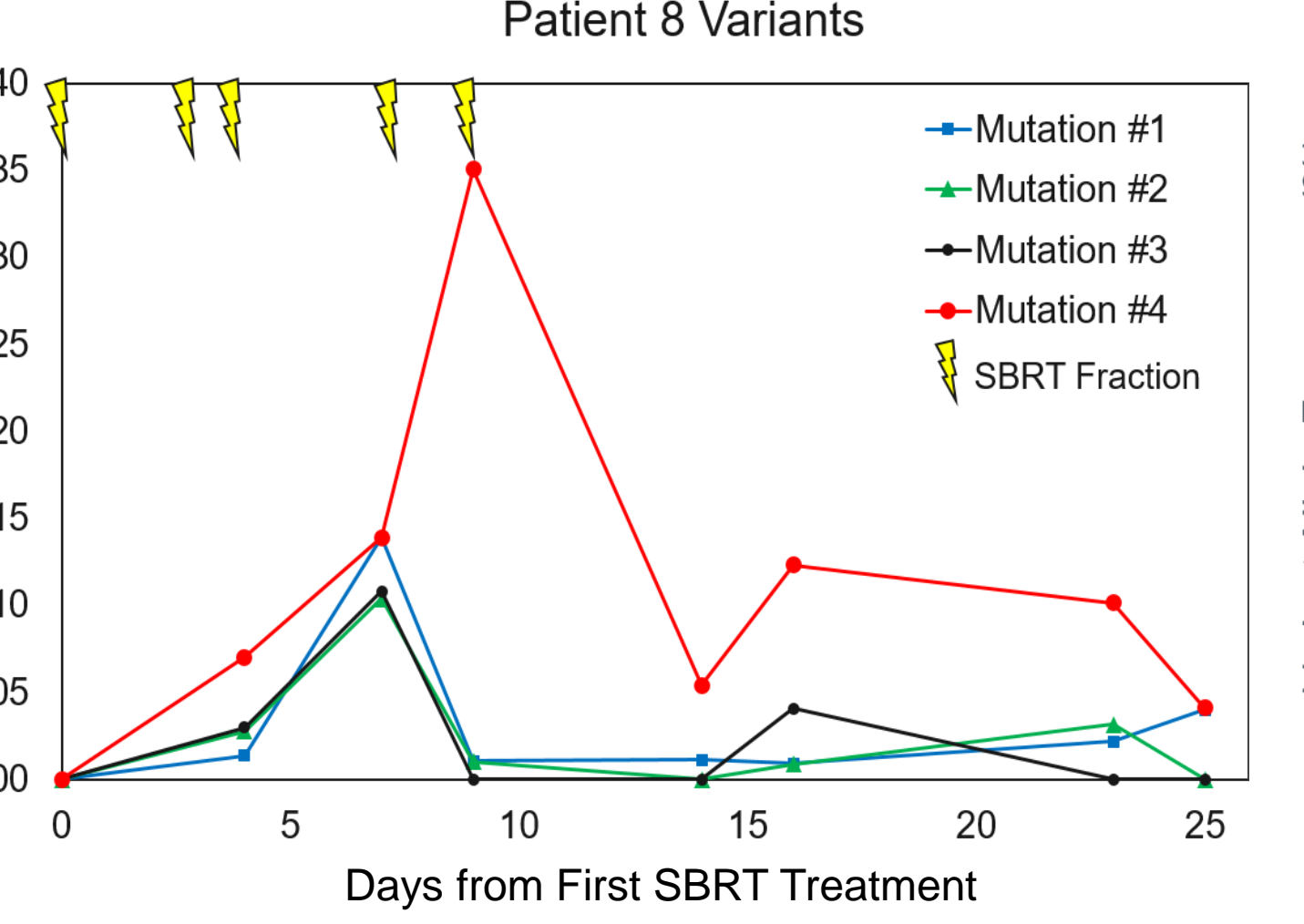


## Results

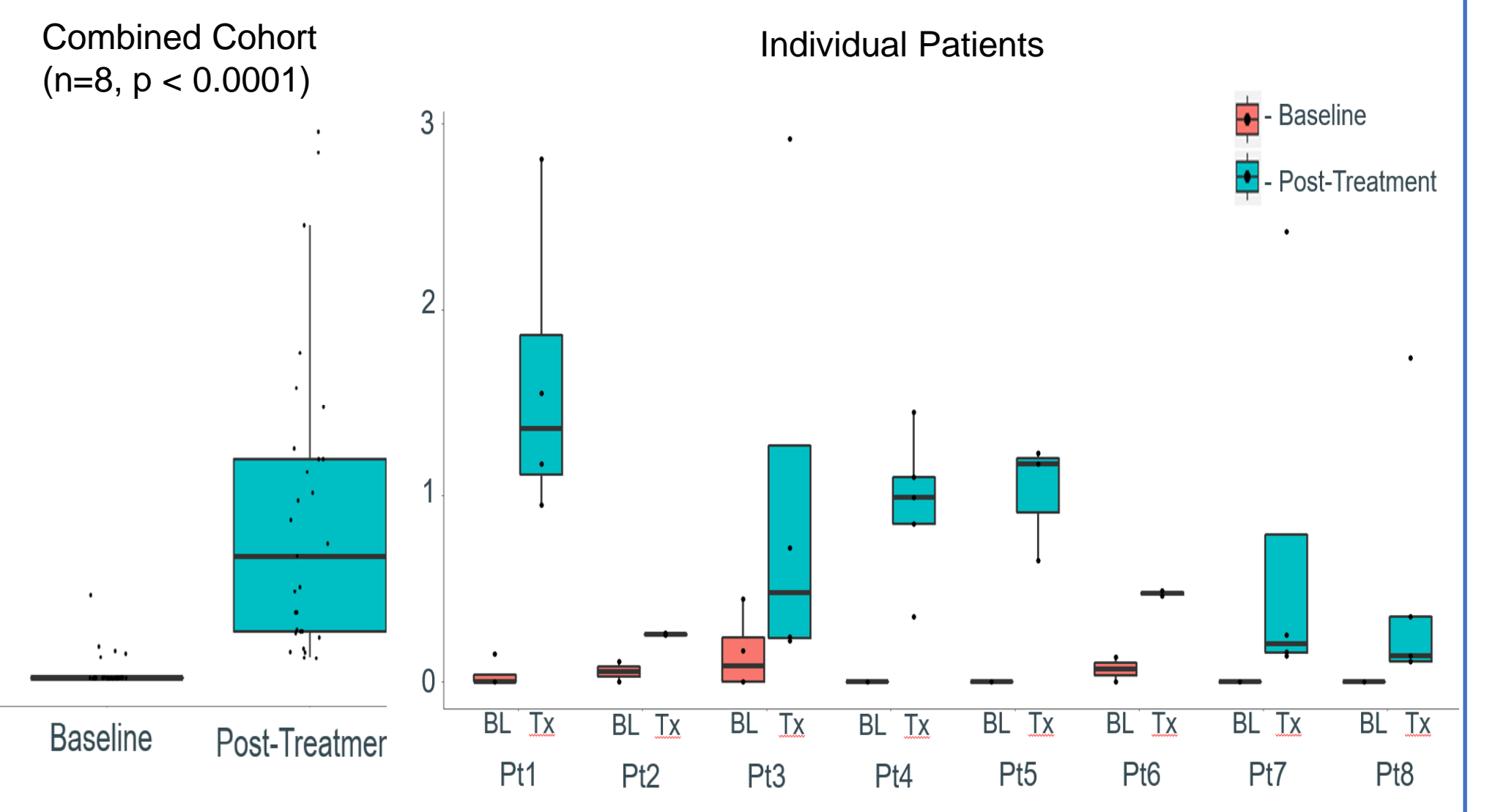
### Period of CtDNA enrichment extends beyond 72 hours after initial SBRT Fraction in Patient 7



### CtDNA enrichment peaks between 7 and 10 days after the initial SBRT fraction in Patient 8



### Radiation induces a 25-fold average ctDNA enrichment but varies between patients in Stage I NSCLC cohort



## Conclusions

- RAMP-Seq utilizes highly-conformational radiation to induce ctDNA enrichment
- On average, VAF increased 25-fold from baseline to treatment
- Kinetic curves of identified variants demonstrate that ctDNA abundance peaks after a minimum of 96 hours from initial treatments in our current cohort of 8 patients
- Biopsy acquisition underway for tissue WES to validate *de novo* calls made with cfDNA DIDA-Seq
- Study continues to enroll patients and should exceed initial target of 20 participants
- Our approach has possible applications such as diagnosis of early-stage cancer and genotyping lesions normally inaccessible by a traditional biopsy.

## References

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