KNIGHT CANCER Institute

OHSU

# Radiation-assisted Amplification Sequencing (RAMP-Seq)

# Radiation-induced enrichment of circulating tumor DNA for early-stage disease detection and monitoring

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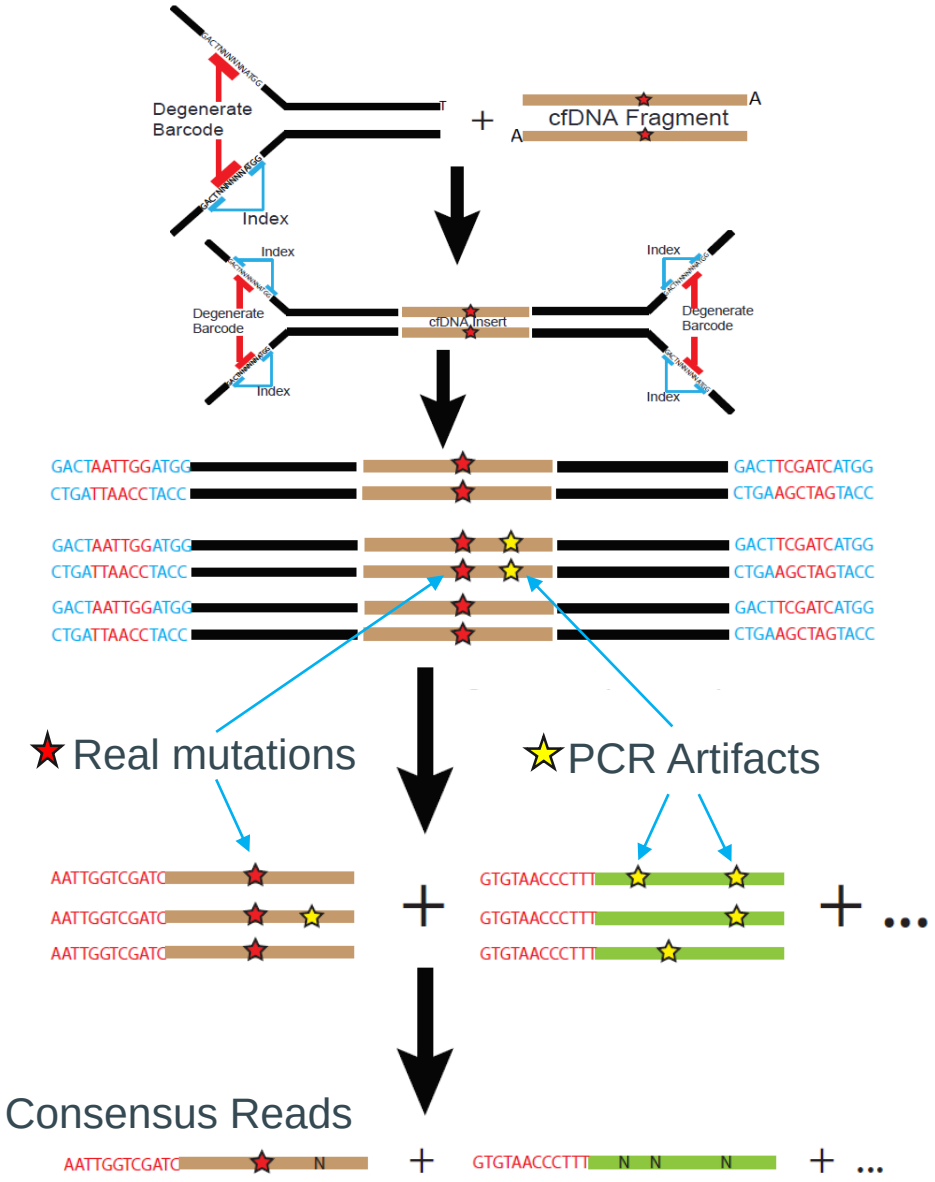
# Fundamental challenge of detecting circulating tumor DNA (ctDNA) in early-stage cancer:

- Tumor-derived cell-free DNA molecules are very limited

# Potential Solution:

- Radiation treatment may increase their abundance

# Dual-Index Degenerate Adapter (DIDA)-Seq error correction



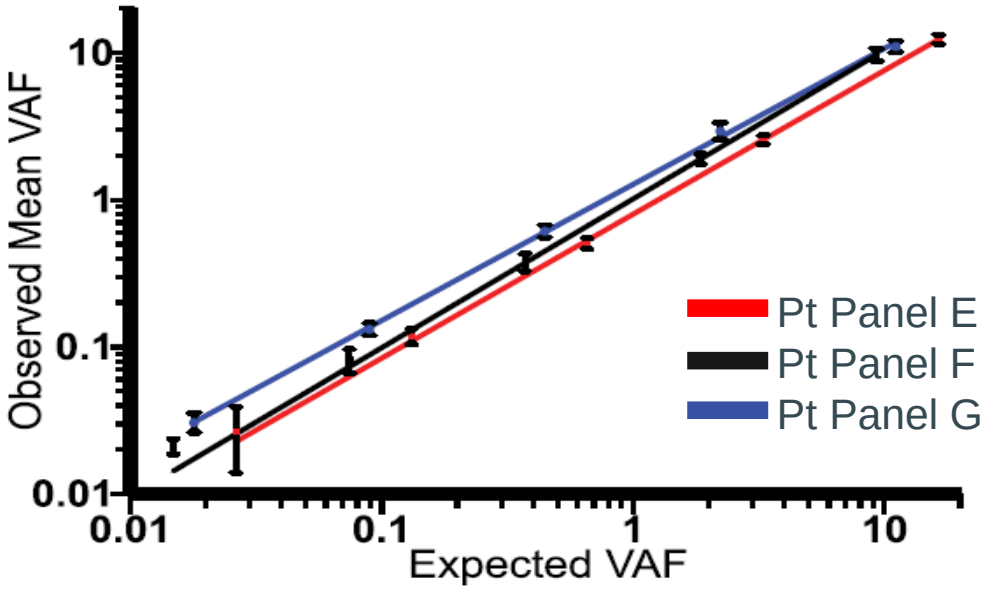

- Ligation of input cell-free DNA fragments to dual, degenerate UMI adaptors
- PCR amplification and custom-capture
- Next generation sequencing
- Consensus-based error correction

T. Butler, C. Boniface, *et al*, Mol. Case Studies, 2019

# DIDA-Seq + custom hybrid capture =

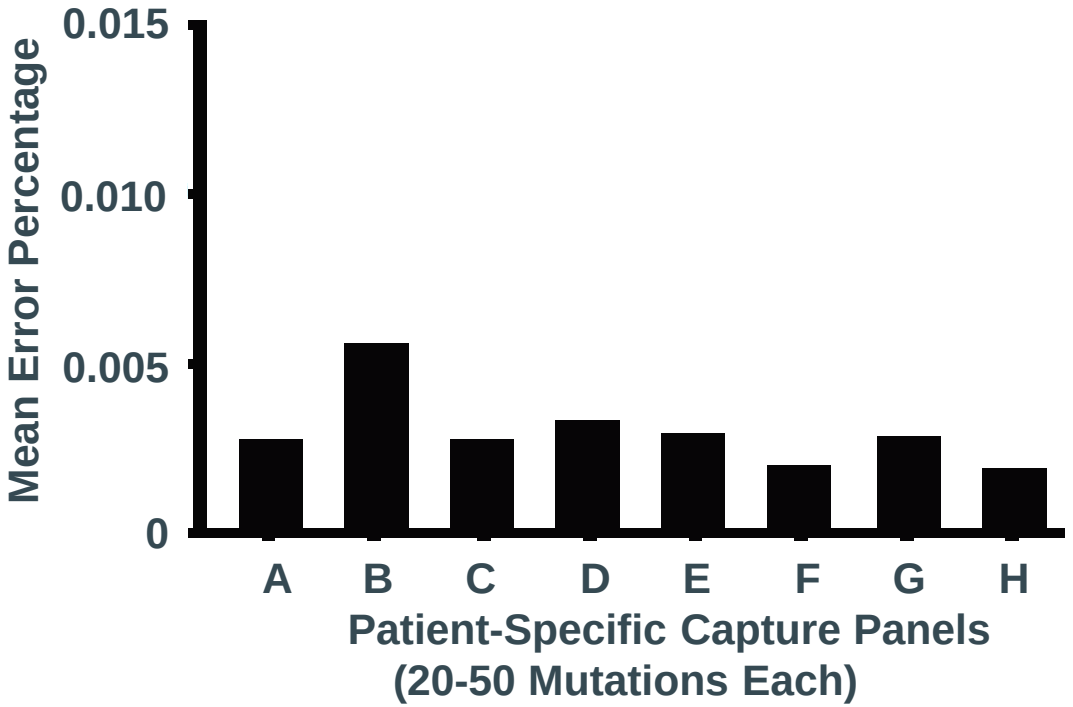
## Sensitive

0.01-0.1% VAF
Recovery



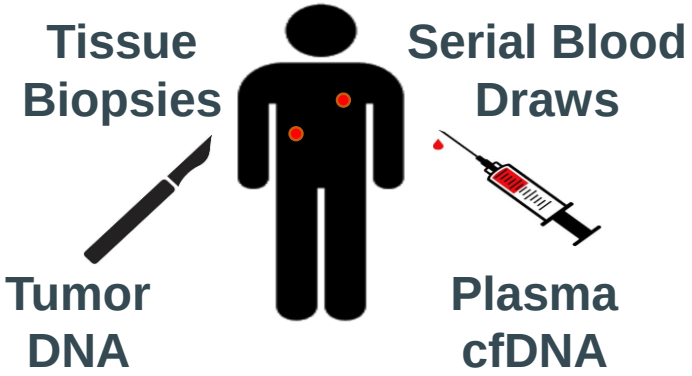

## Accurate

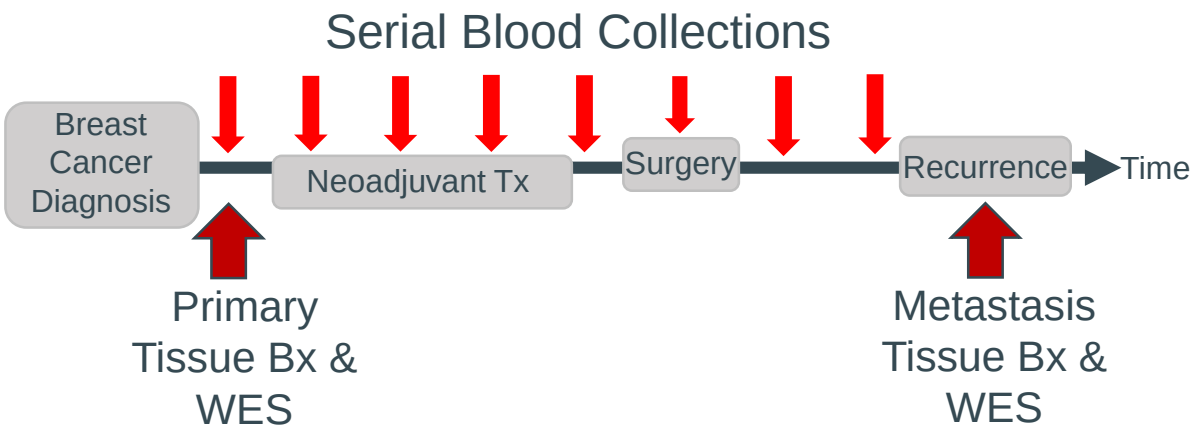
Error Rate = 1:10k-1:50k
Observations




T. Butler, C. Boniface, *et al*, Mol. Case Studies, 2019

# Solid tissue WES and custom capture DIDA-Seq of serial blood draws is used to monitor neoadjuvantly-treated breast cancer patients

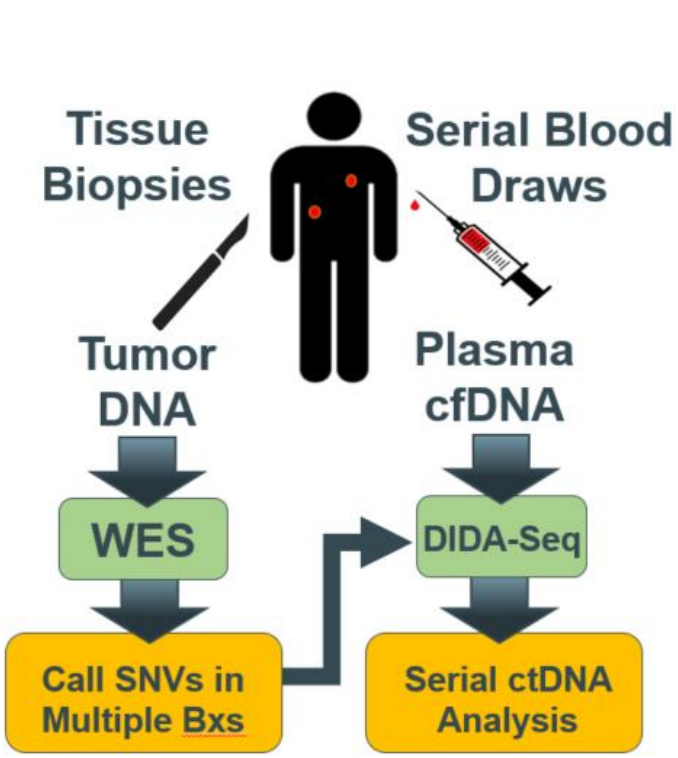


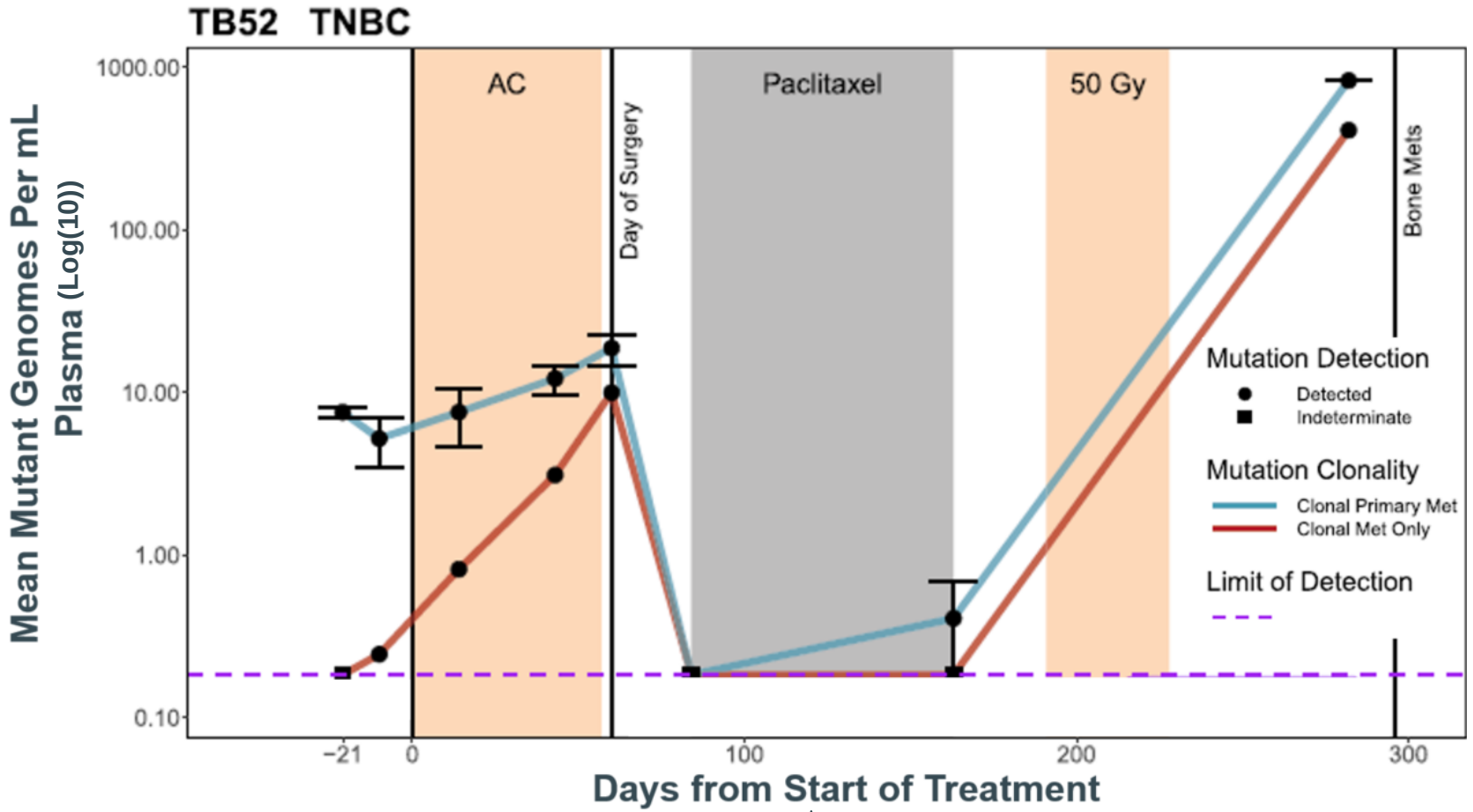




- Call mutations from solid tissue WES
- Design custom-capture panel based on 20-50 of those mutations and carryout DIDA-Seq on cfDNA samples
- Evaluate association between ctDNA levels, treatment response and overall patient outcome

T. Butler, C. Boniface, *et al*, Mol. Case Studies, 2019

# Patient-specific monitoring suggests that ctDNA levels are associated with treatment response and the post-surgery presence of ctDNA foreshadows recurrence

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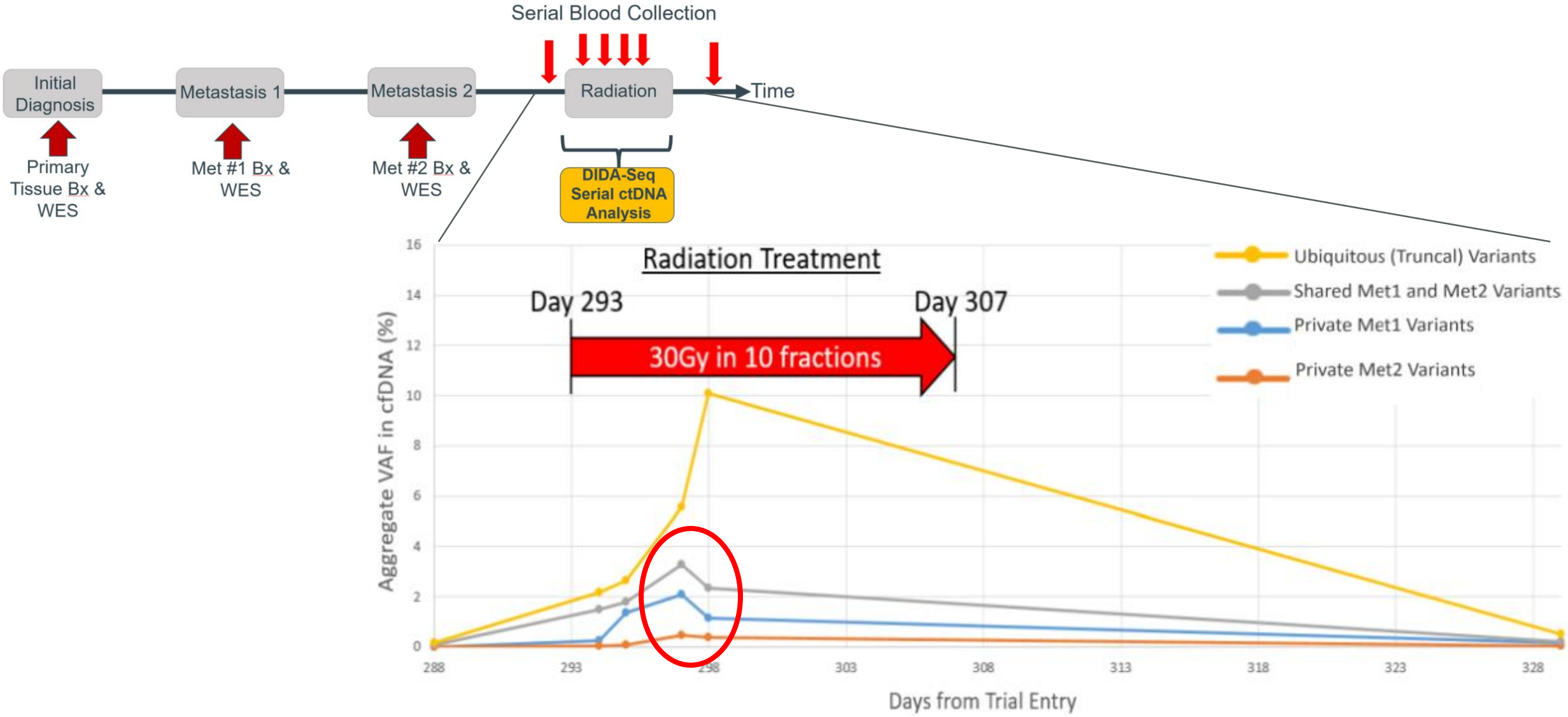
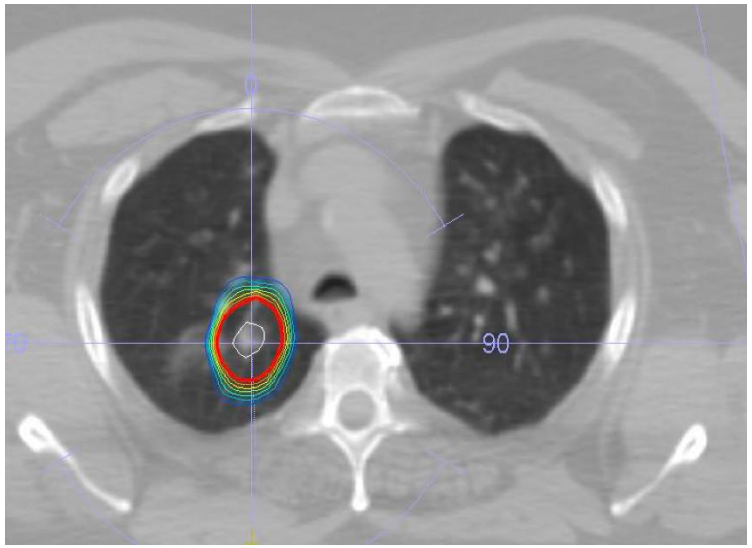
# CtDNA enrichment following XRT in advanced metastatic disease suggests that ctDNA abundance could be potentiated by radiation exposure



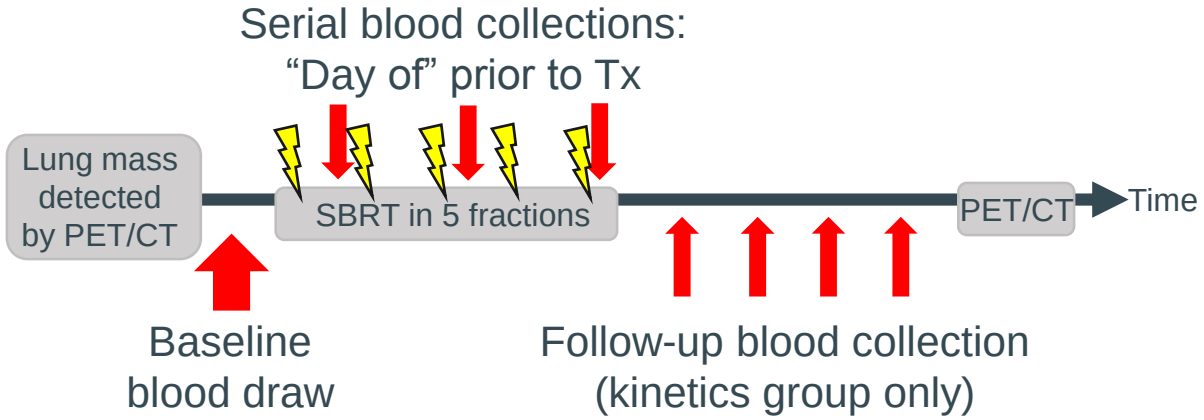
# Hypothesis:

- Radiation treatment of solid tumor tissue induces release of circulating tumor DNA
- Based on the very short half-life off cell free DNA and the biology of radiation-induced cell death, radiation-induced ctDNA enrichment is likely transient
- Peak enrichment of ctDNA after the first fraction of radiation treatment appears to occur after a minimum of 72 hours but may take up to two weeks

# Stereotactic body radiation therapy (SBRT) is highly conformational radiation used to target solid tissue tumors in NSCLC

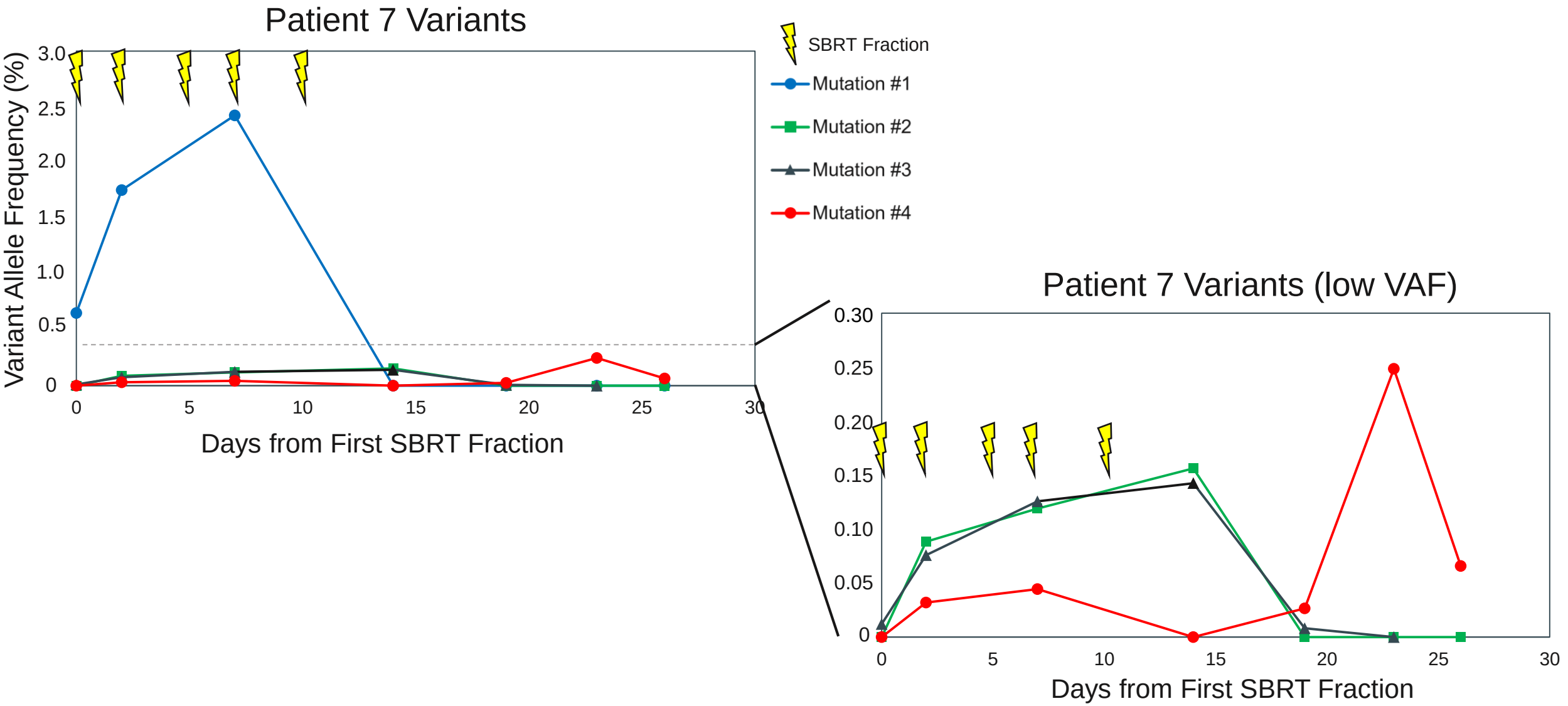



Stereotactic Body Radiation Therapy (SBRT)

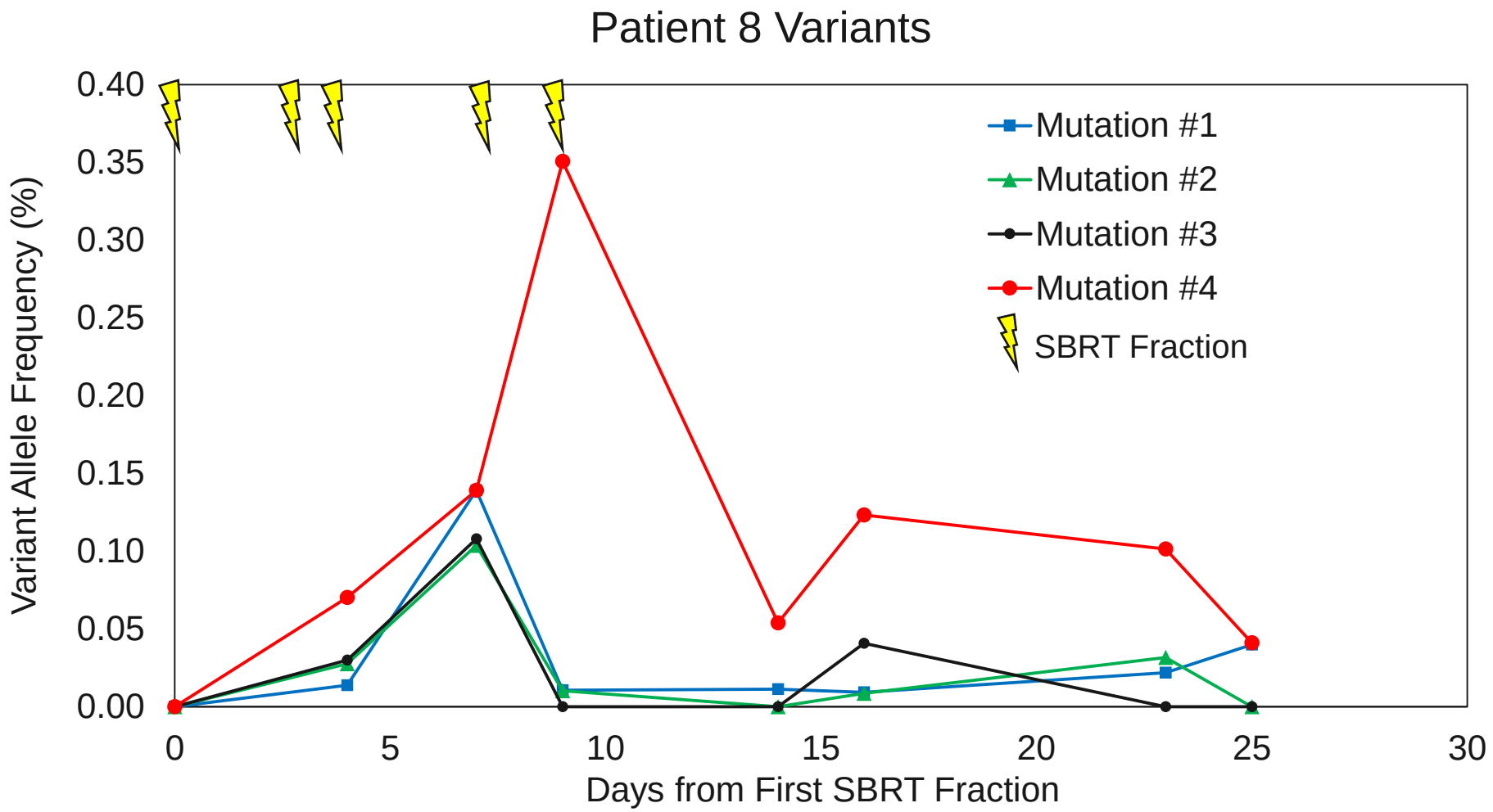



- Consent ~20 patients suspicious for NSCLC and receiving SBRT for serial blood collection every 24-48 hours
- Select 3-5 patients for follow-up blood draws to evaluate ctDNA kinetics
- Perform targeted DIDA-Seq to 5k-20k X coverage w/*de novo* mutation calling and infer optimal peak sampling period
- Select time points for remaining patients based on optimal collection period
- Targeted DIDA-Seq w/*de novo* mutation calling on optimal tps

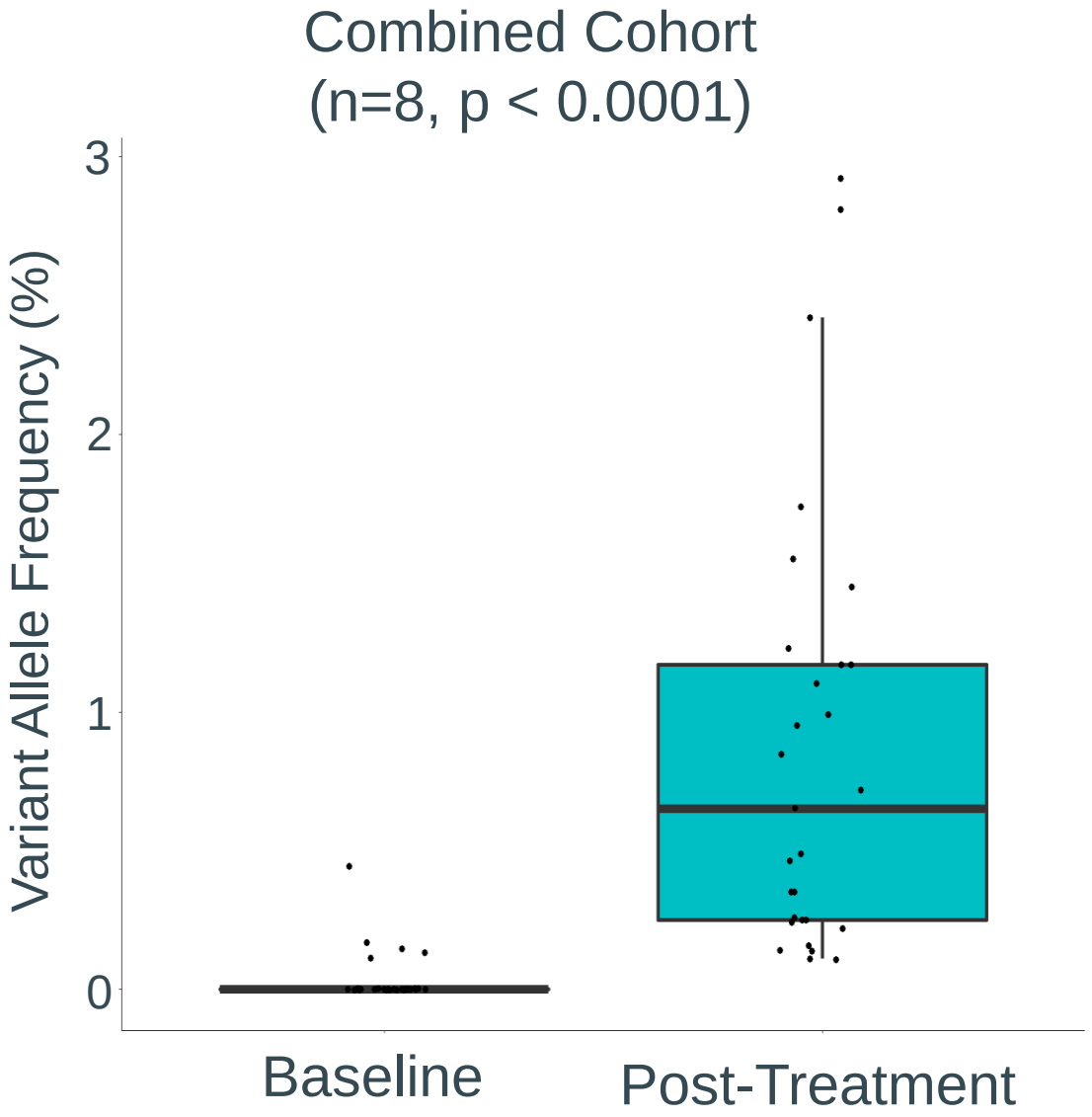
# Period of CtDNA enrichment, after the initial SBRT fraction, extends well beyond 72 hours in Patient 7



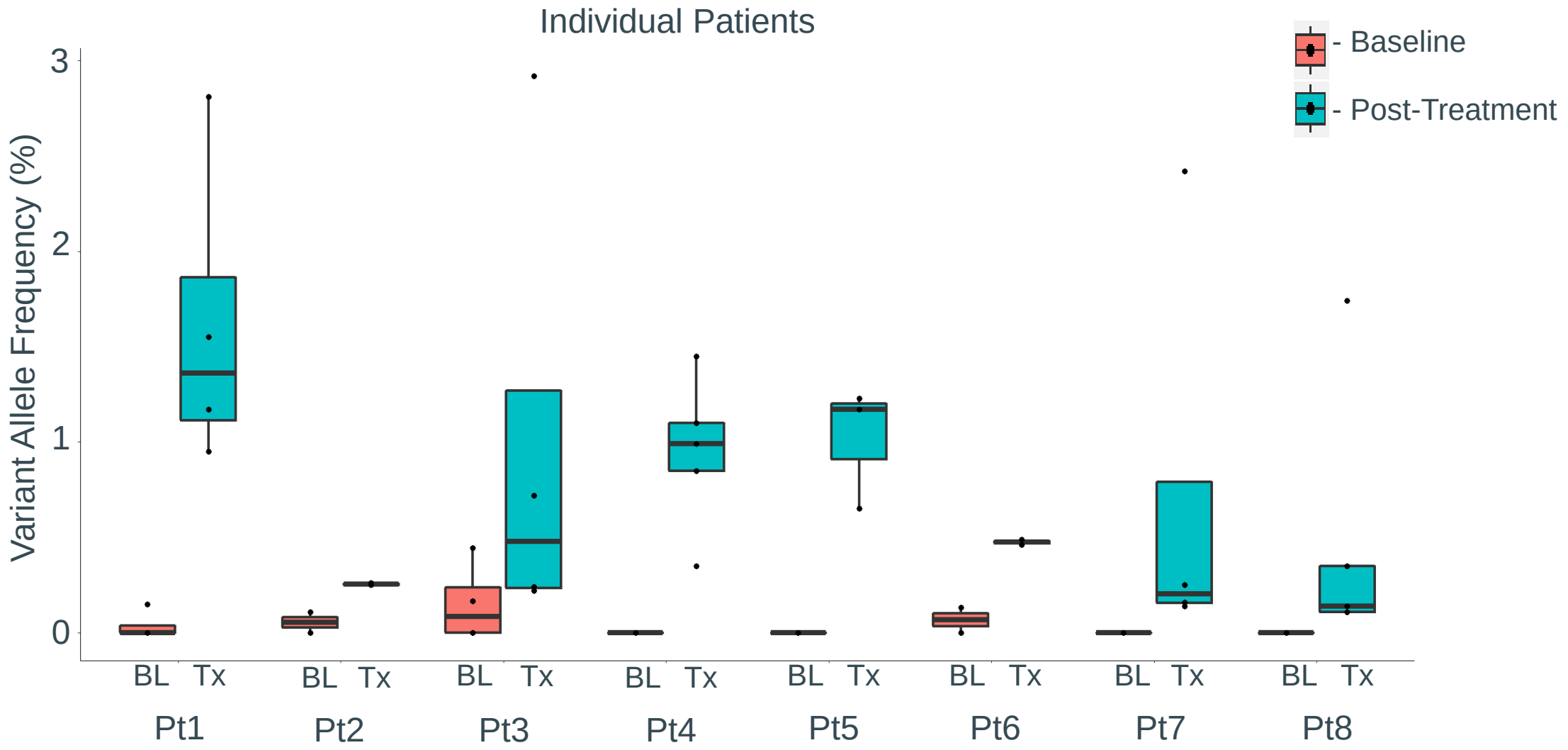
# Period of CtDNA enrichment peaks between 7 and 10 days after the initial SBRT fraction in Patient 8



# Radiation induces a 25-fold average ctDNA enrichment across entire Stage I NSCLC cohort



# CtDNA enrichment is not uniform across Stage I NSCLC cohort



# Recap and ongoing efforts

- 55-fold enrichment of ctDNA from SBRT within days of initial fraction from n=8 patients suspicious for stage I NSCLC
- Biopsy acquisition underway for tissue WES to validate *de novo* calls made with cfDNA DIDA-Seq
- Continue to sequence growing cohort samples
- Study continues to enroll patients and should exceed initial target of 20 participants

# Clinical and diagnostic utilization

- Ideal for early-detection and mutation profiling, particularly in cases of un-characterized masses and unbiopsiable tumors

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