Liquid Biopsy for Diagnosis and Treatment Monitoring in Esophageal Adenocarcinoma

Tony E. Godfrey, PhD
Associate Chair-Research
Department of Surgery
Boston University School of Medicine

UPMC
University of Pittsburgh Medical Center

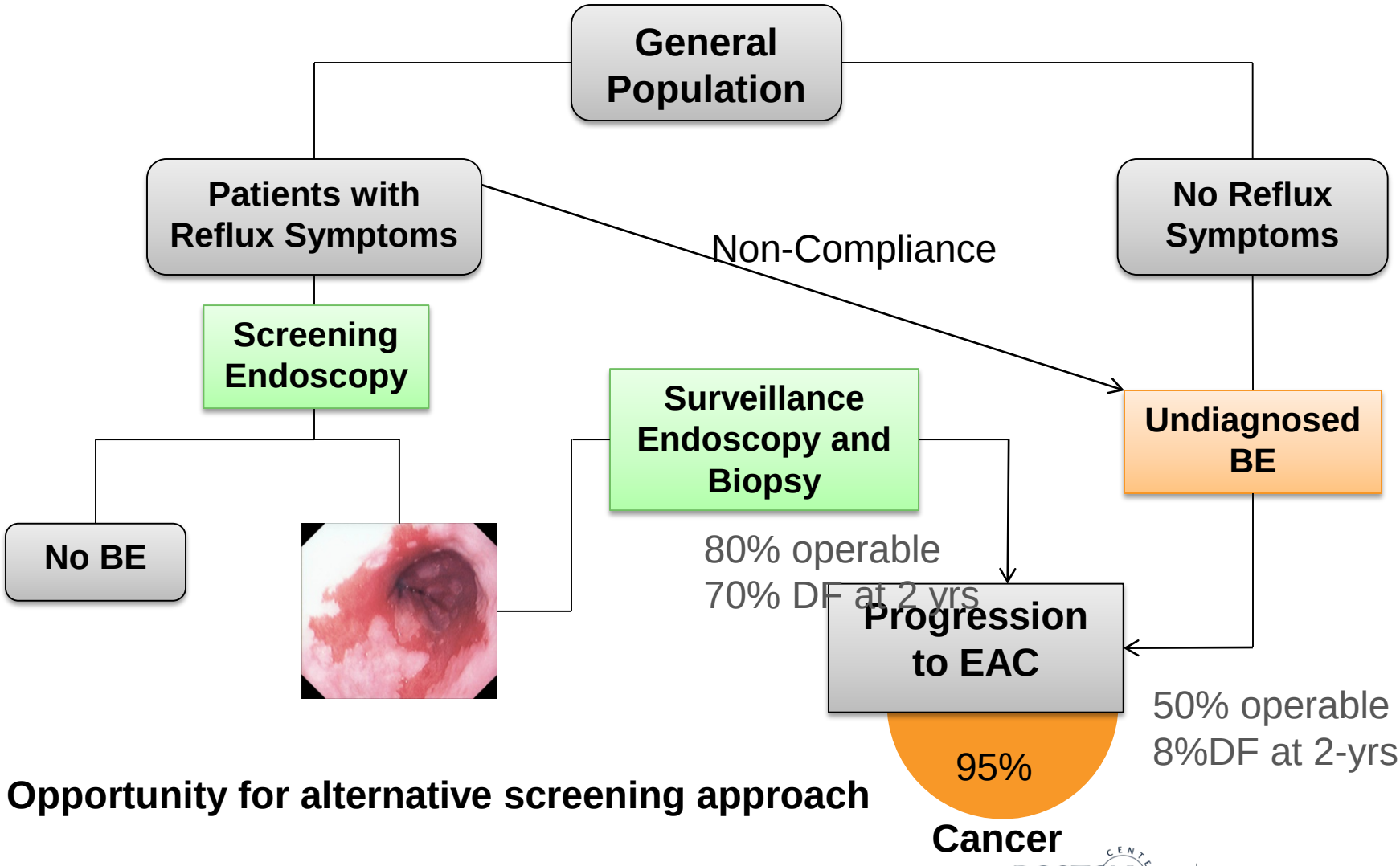
OICR
Ontario Institute for Cancer Research

UNIVERSITY of ROCHESTER

BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

Why ctDNA in Esophageal Adenocarcinoma?

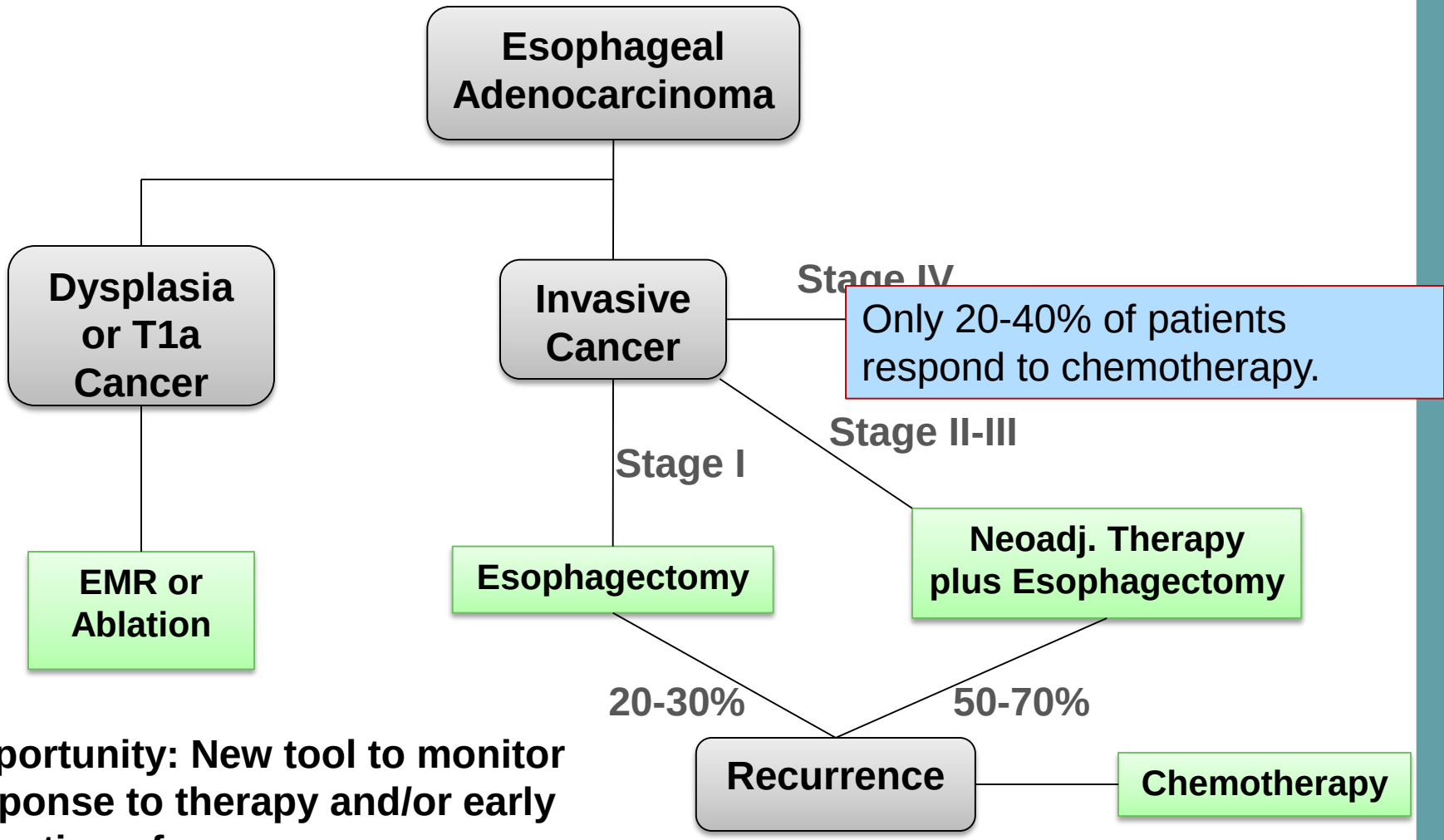


Opportunity for alternative screening approach

BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

Why ctDNA in Esophageal Adenocarcinoma?



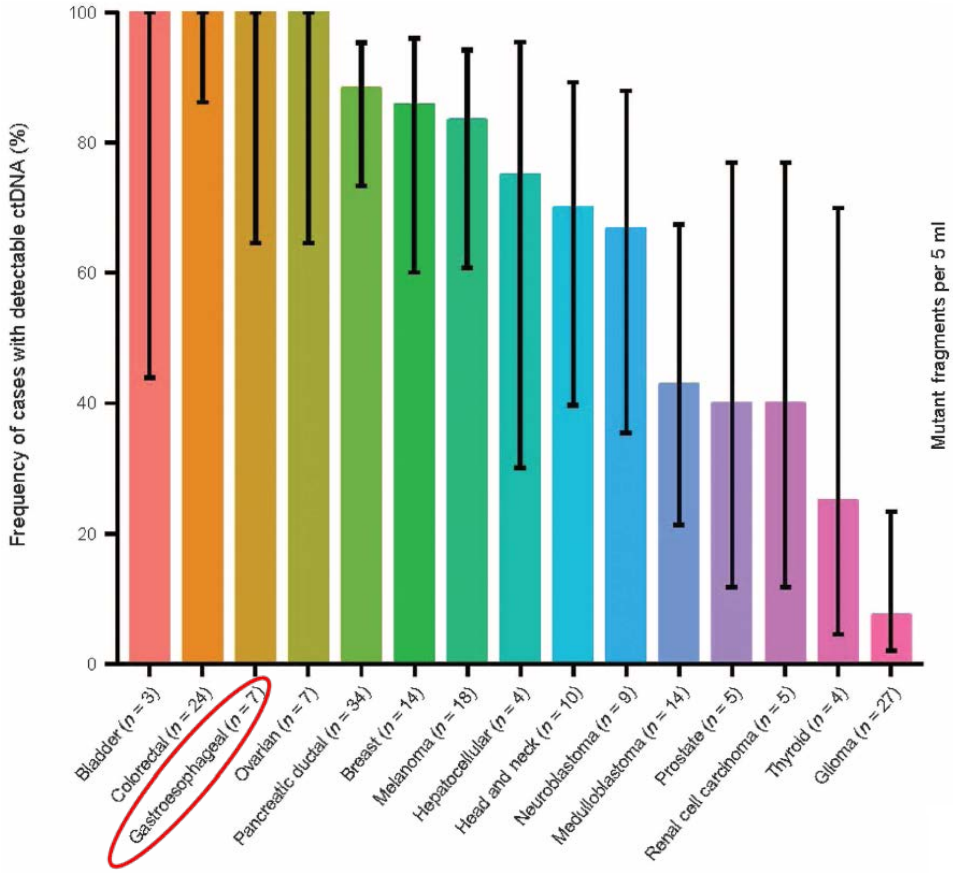
Opportunity: New tool to monitor response to therapy and/or early detection of recurrence

BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

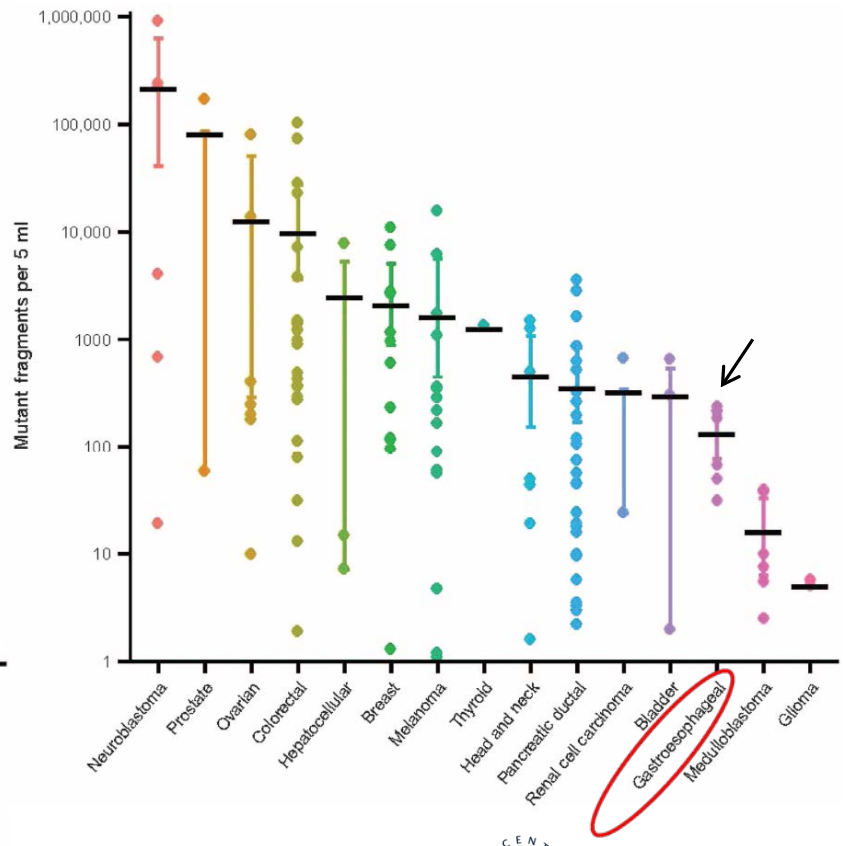
BU School of Medicine

Limited but Promising data on ctDNA in Gastroesophageal Cancer

ctDNA detected in 100% of metastatic cases (n=7)



ctDNA detected at ~10-100 copies/ml of plasma



Bettegowda *et al. Sci Transl Med* (2014)

BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

Circulating Tumor DNA in EAC

- **Open Questions:**
  - How does detection rate and quantity change with tumor stage?
  - Does ctDNA quantity change with response to therapy or disease progression/recurrence?

- **Challenges:**

- Need to reliably detect mutations at ~0.05% level.

- Very little DNA in plasma/serum (10-50ng/mL = 1-10 copies of tumor DNA) and it is very fragmented.

- **Developed modified version of SafeSeq-S using introduction of molecular barcodes (N_{10-14}) into NGS libraries using PCR.**

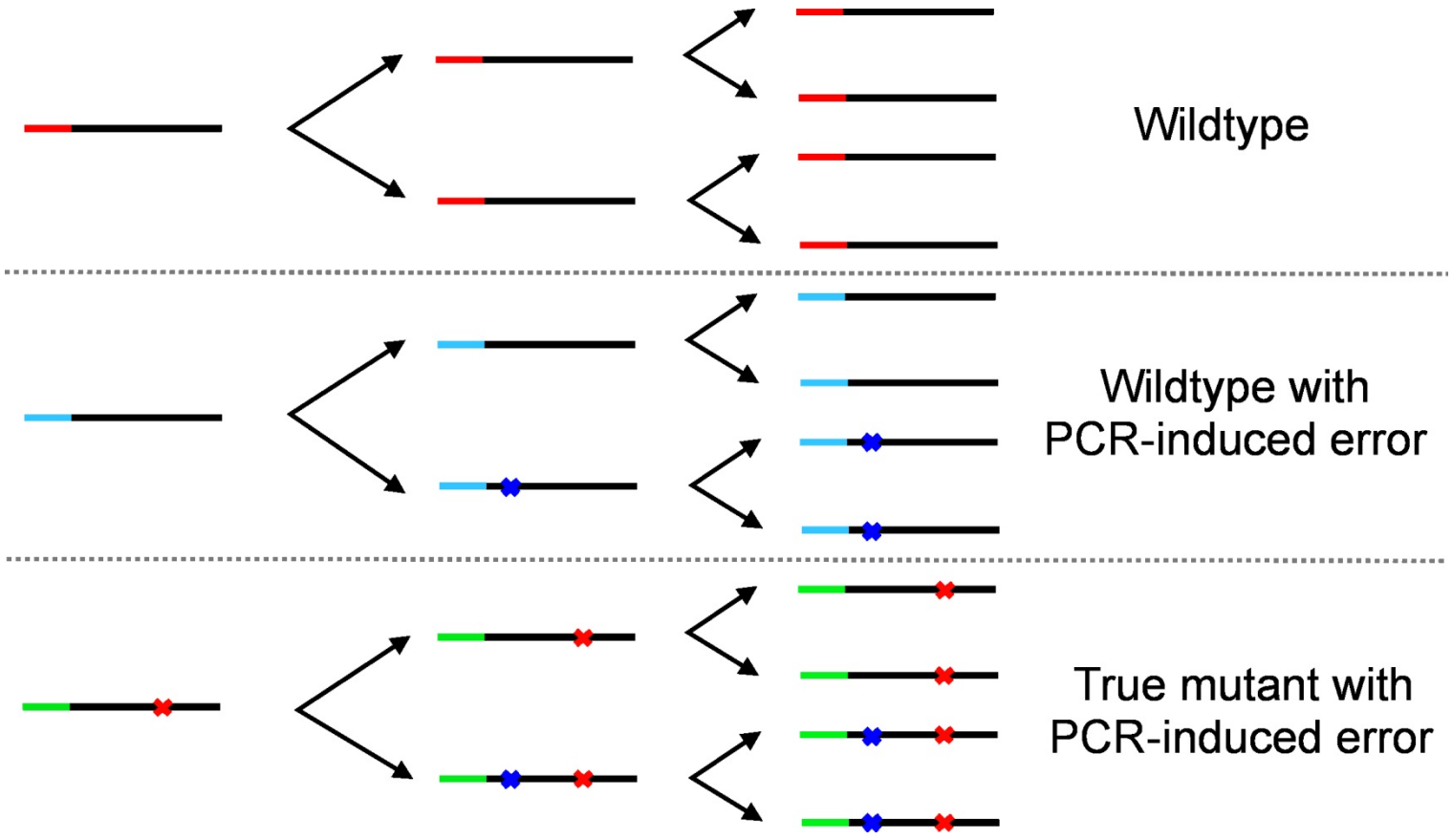
Kinde et al., Proc Natl Acad Sci U S A. 2011 Jun 7;108(23)

OICR Ontario Institute for Cancer Research

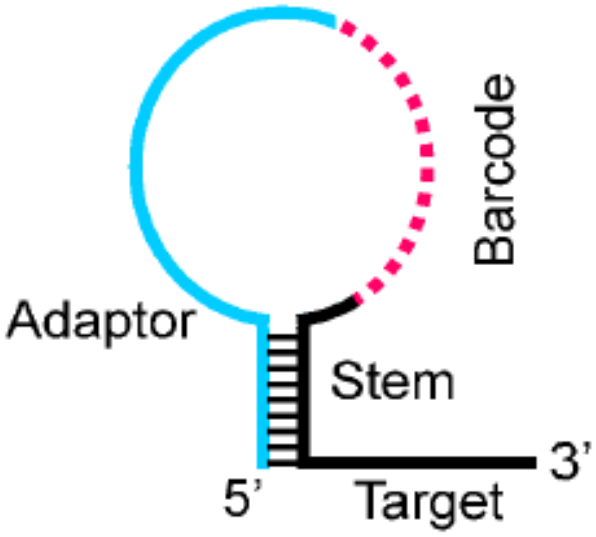
BOSTON MEDICAL CENTER EXCEPTIONAL CARE. WITHOUT EXCEPTION.

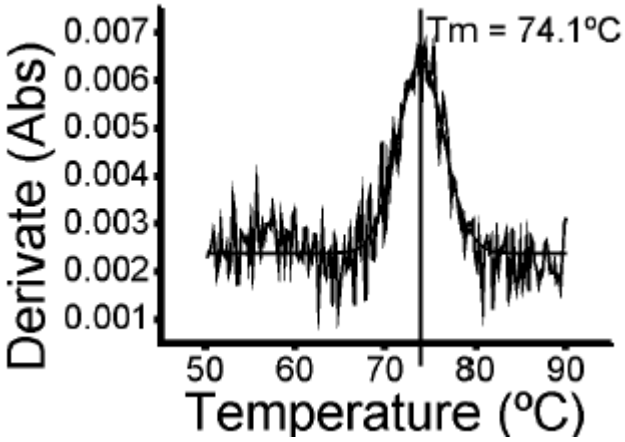
BU School of Medicine

Barcoding Enables Identification of True Mutations vs Polymerase errors



Simple,Multiplexed, PCR-based barcoding of DNA for Sensitive mutation detection using Sequencing (SiMSen-Seq)



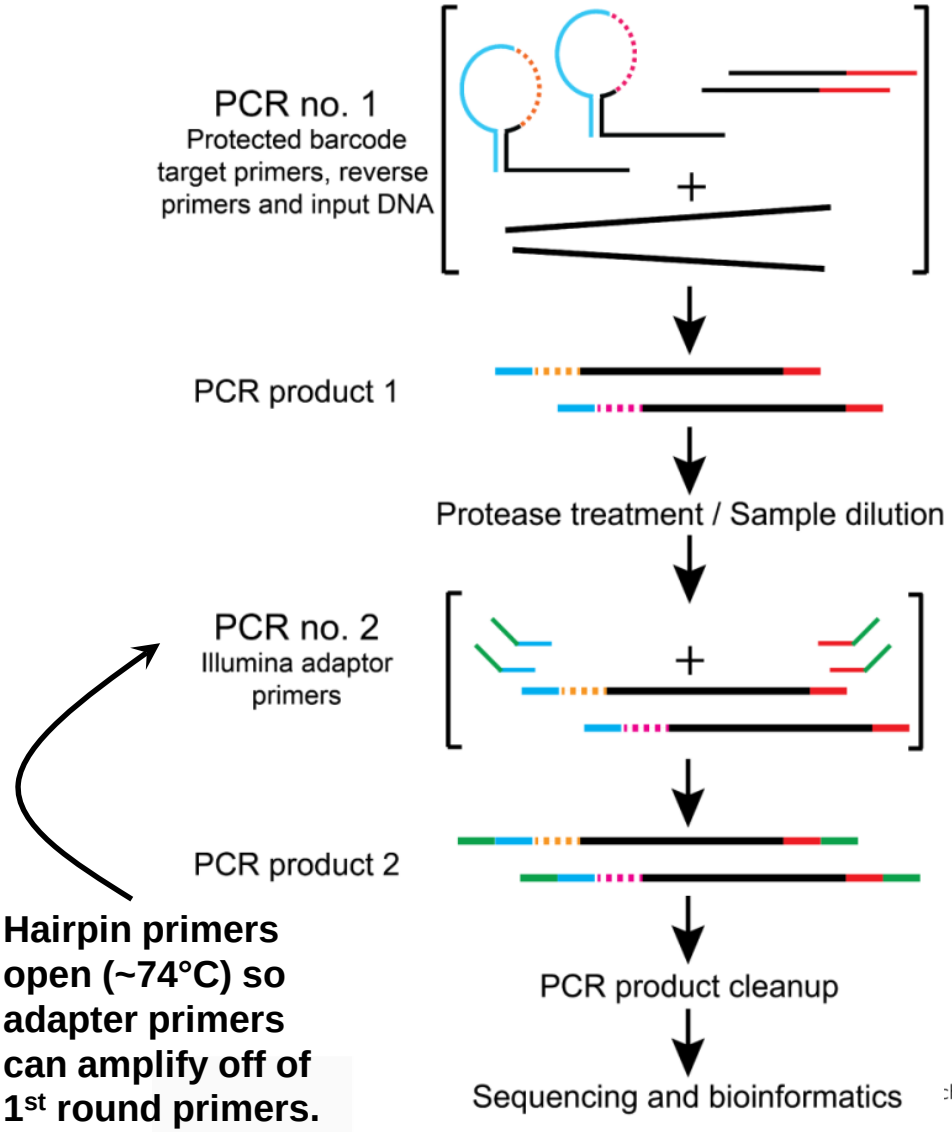


- Target primers designed with internal hairpin.
  - Protects random barcode sequence during PCR and increases specificity

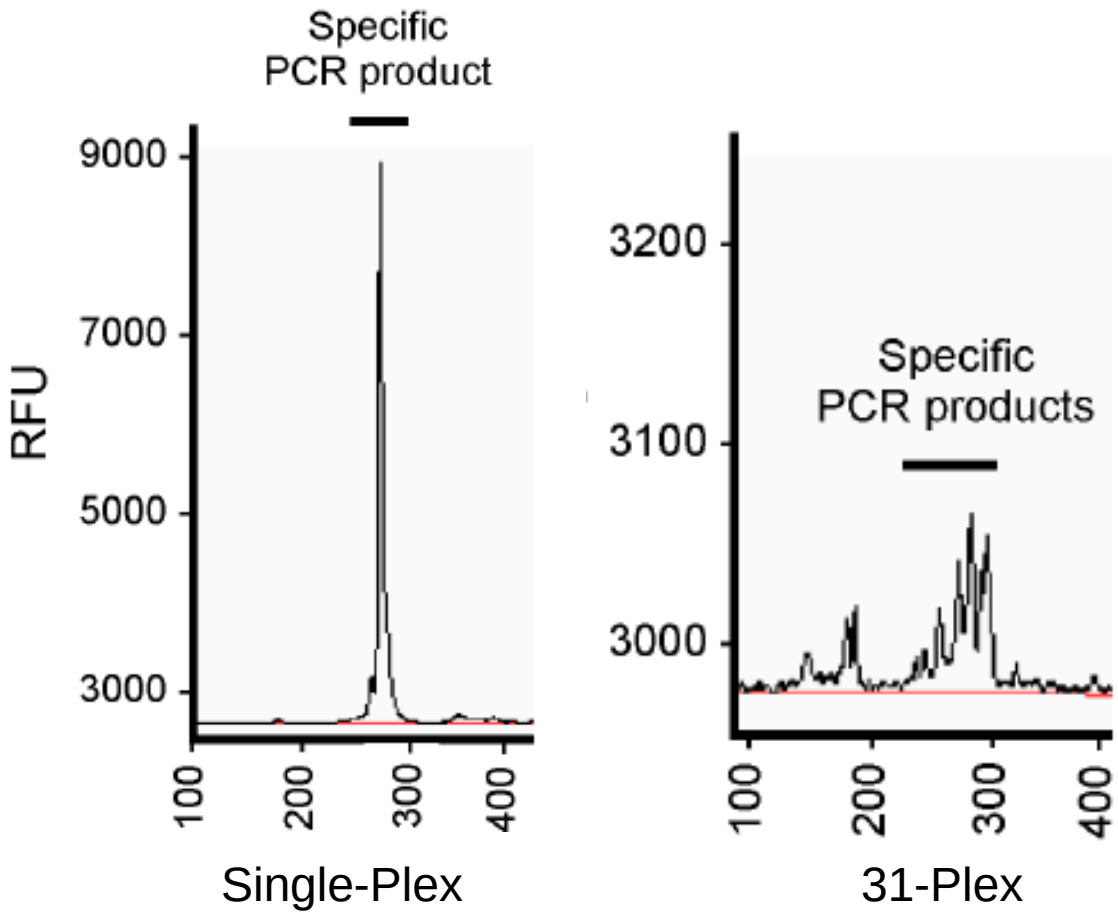
Stahlberg et al., Nucleic Acids Res. 2016 Jun 20;44(11)

Library Construction is Extremely Quick and Simple

- Two rounds of PCR
- Single purification step.
- Three hours from start to sequence-ready.



SiMSen-Seq Enables Flexible Multiplexing



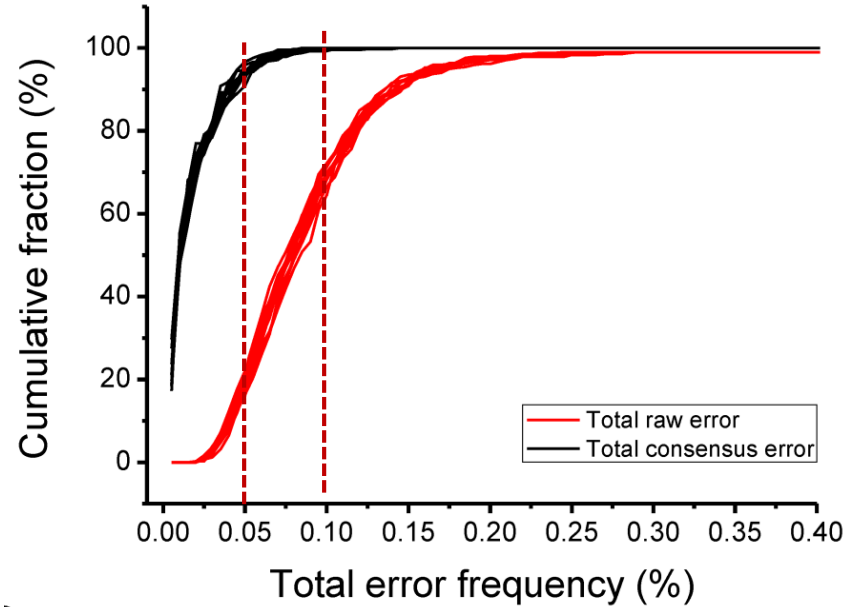
Single-Plex

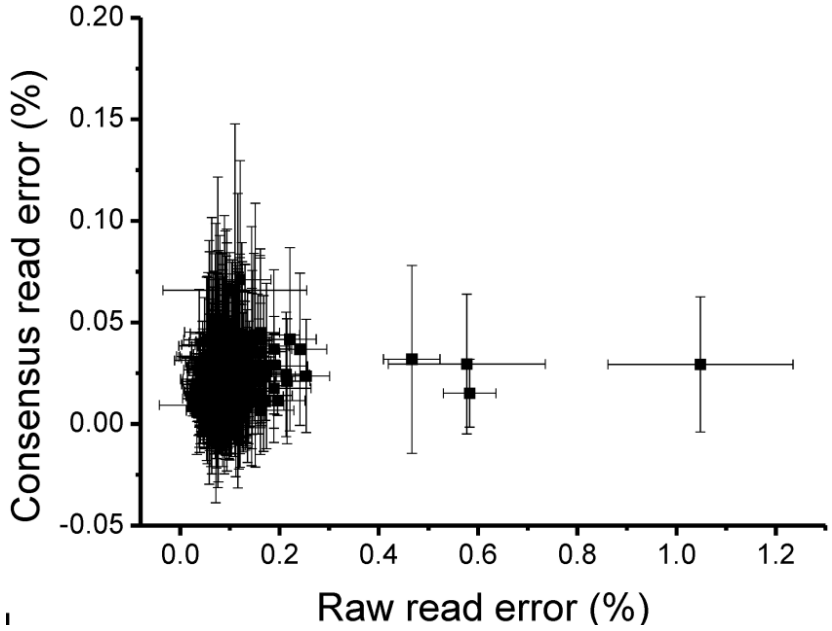
31-Plex

Background Consensus Error is Consistently Below 0.1%

12 replicates of 5-plex library covering 417 nucleotides

>90% of all nucleotides displayed consensus read error <0.05% and 99.3% of nucleotides showed consensus error <0.1% with 95% confidence.

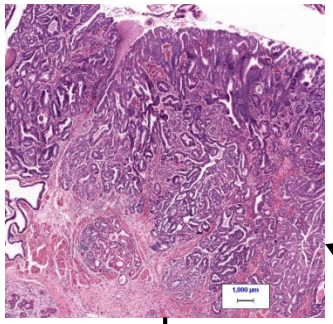




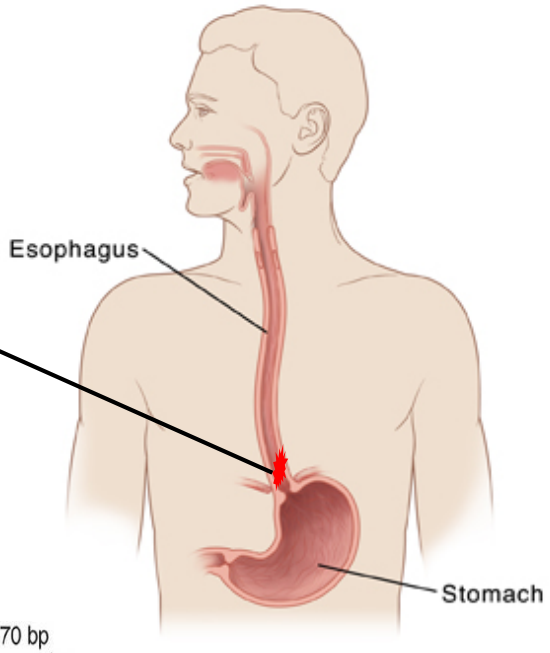
BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

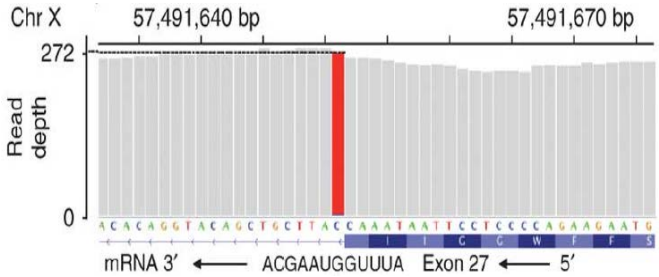
Approach



Sequence Tumor DNA



Isolate Plasma DNA



Identify Mutations

Barcoded Plasma DNA Sequencing

OICR Ontario Institute for Cancer Research

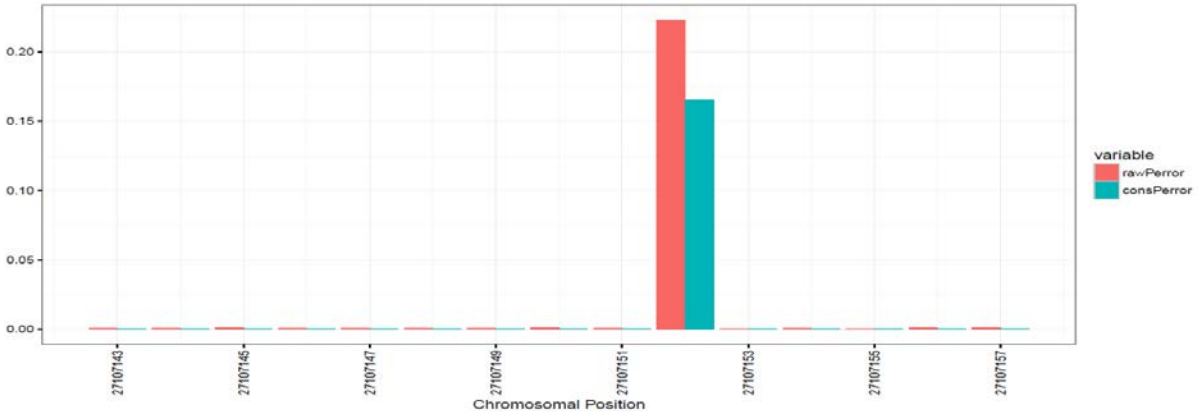
Boston Medical Center — Exceptional care. Without exception.

BU School of Medicine

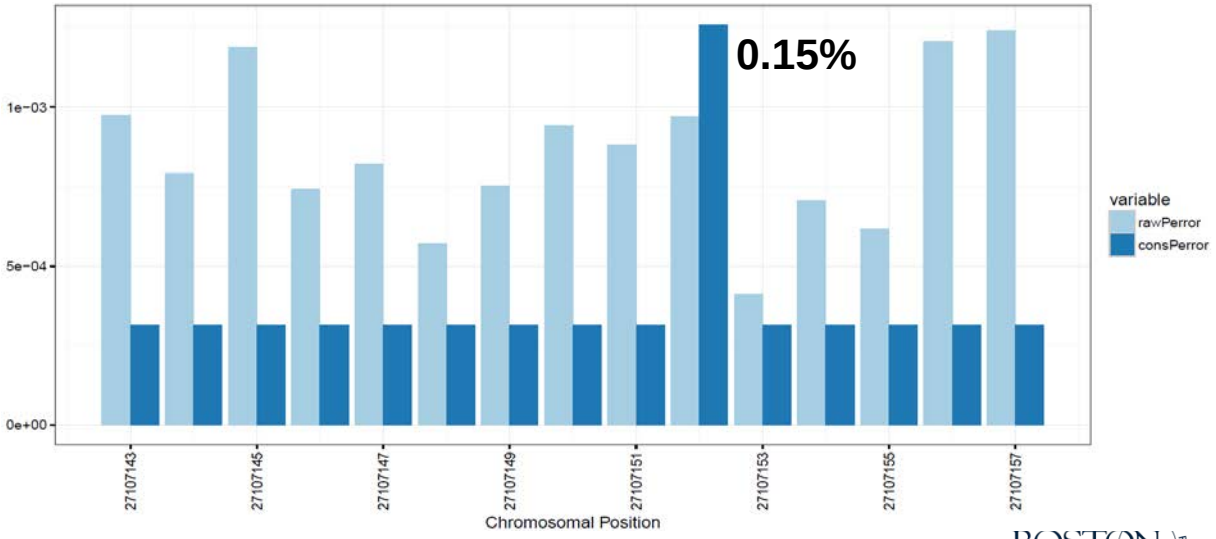
Pitt 07: T2N0M0, Stage IB

Gene	Chr	Position	Allele 1	Allele 2	Tumor allele freq	Mutation consequence
ARID1A	Chr1	27107152	G	T	0.2	Nonsense

Tumor



Plasma



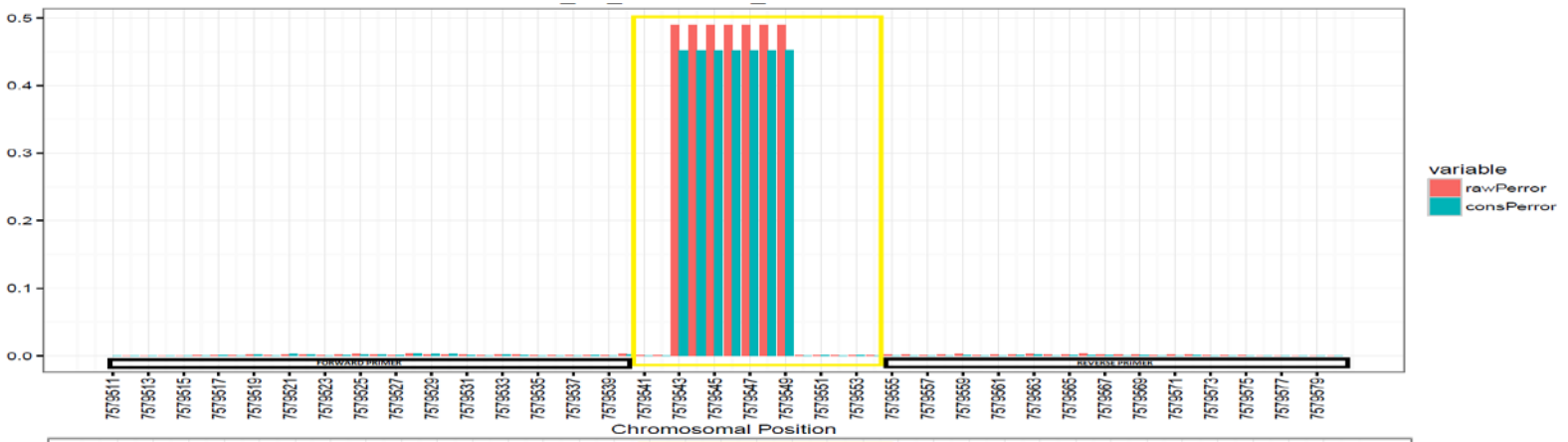
BOSTON MEDICAL
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

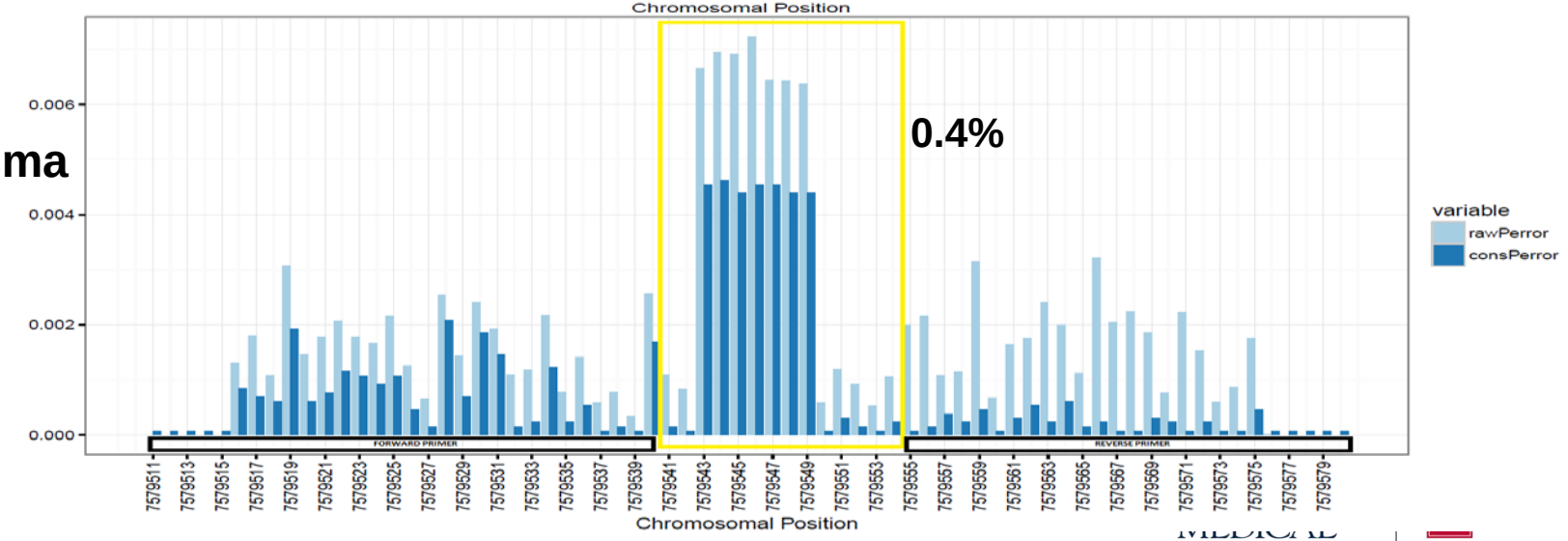
Pitt 29: T2N0M0, Stage IIB

Gene		Start Position	Allele 1	Allele 2	Tumor allele freq	Mutation consequence
TP53	Chr17	7579542	CGTCCGGG	C	0.45	Deletion

Tumor



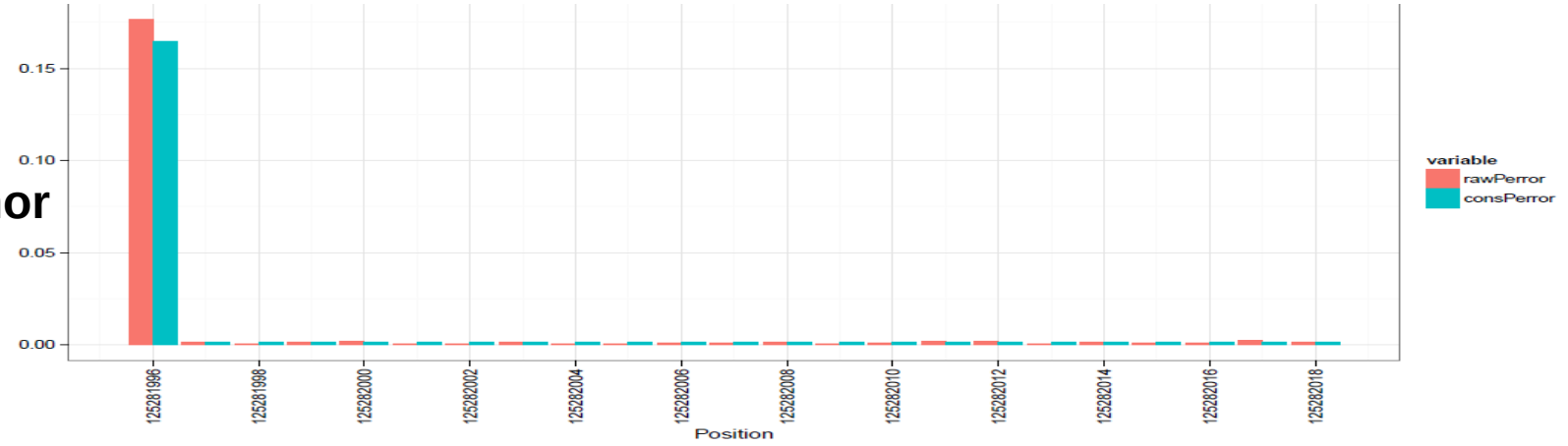
Plasma



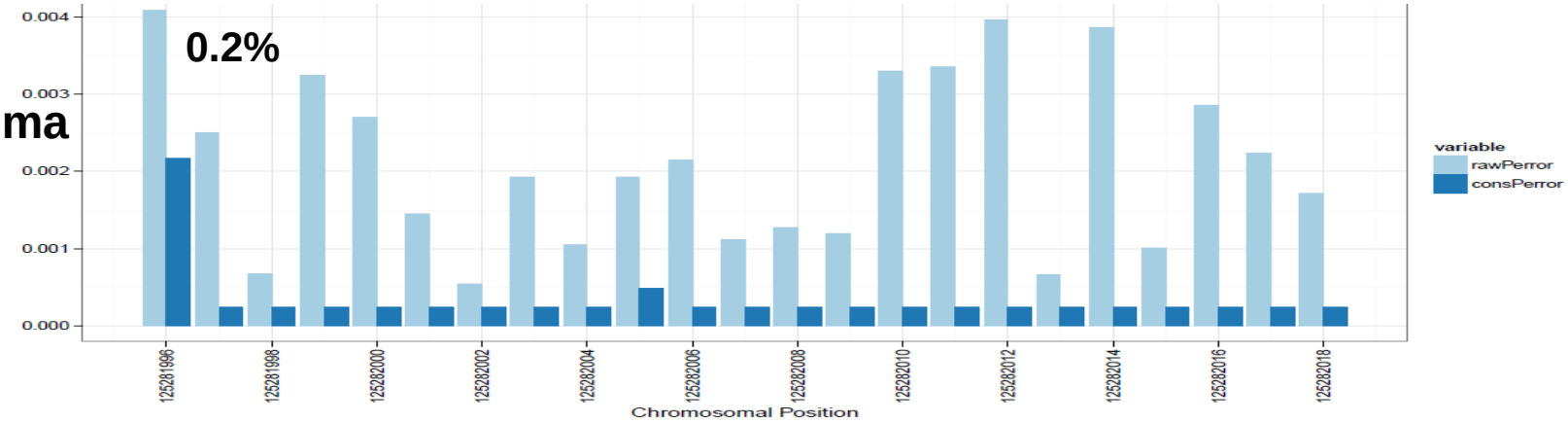
Pitt 27: T3N2MX, Stage IIIB

Gene	Chr	Position	Allele 1	Allele 2	Tumor allele freq	Mutation consequence
CNTNAP5	Chr2	125281996	C	A	0.16	Missense

Tumor



Plasma



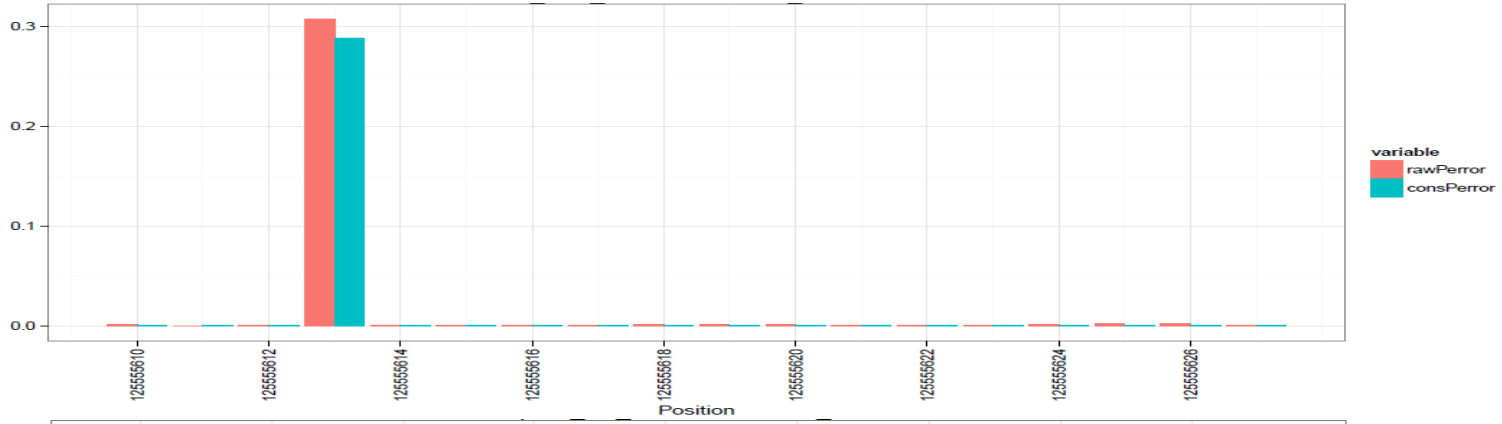
BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

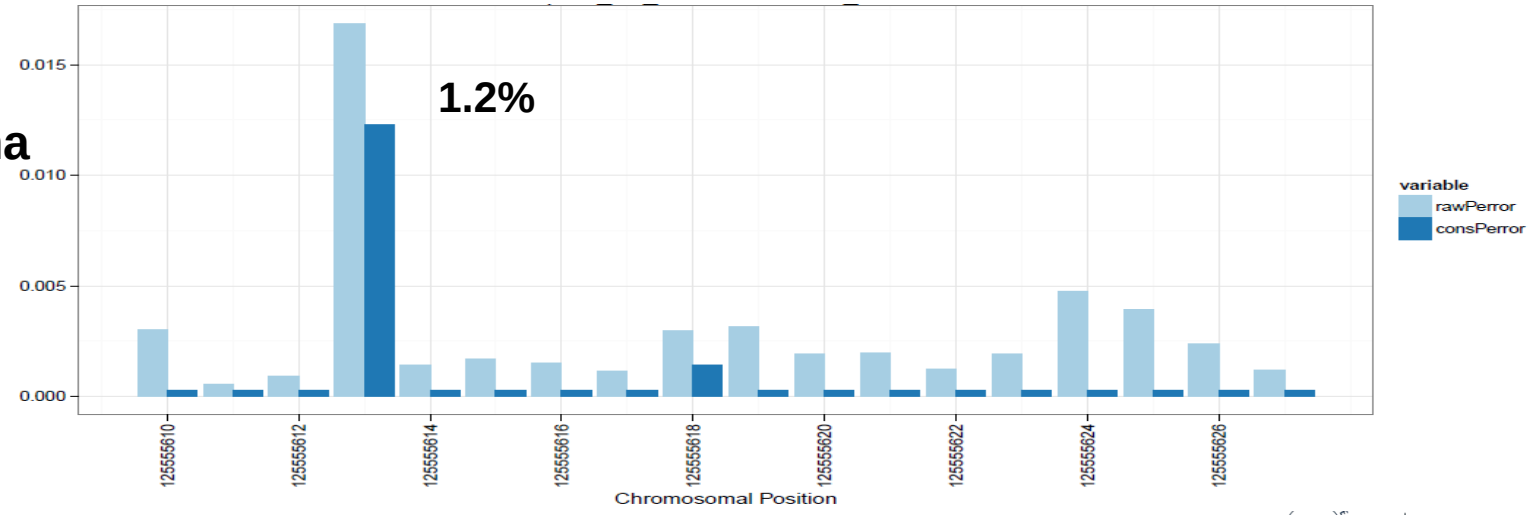
Pitt 25: TxN2M1, Stage IV

Gene	Chr	Position	Allele 1	Allele 2	Tumor allele freq	Mutation consequence
CNTNAP5	Chr2	125281996	T	G	0.31	Intron

Tumor



Plasma



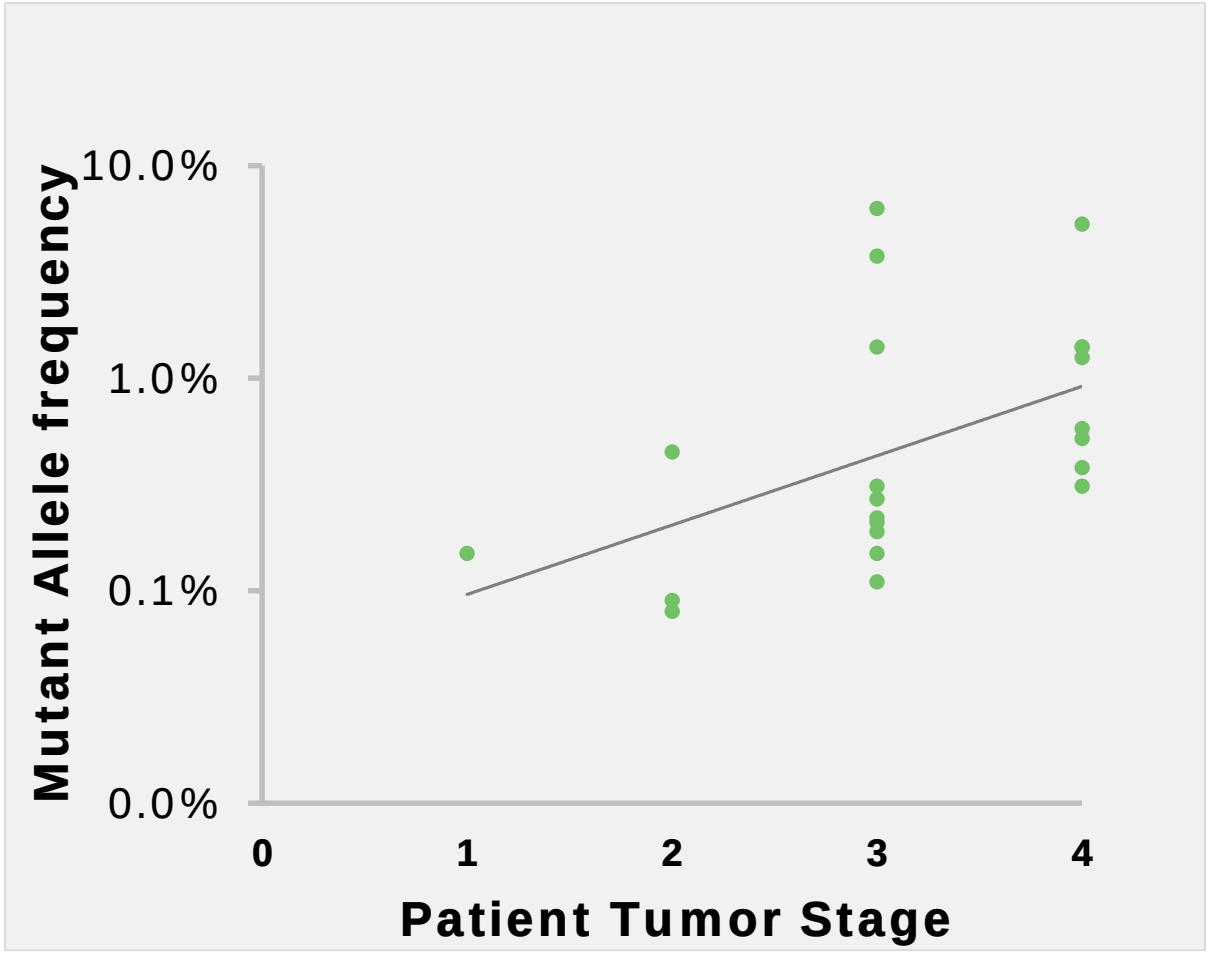
BOSTON MEDICAL
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

Circulating Tumor DNA Detection Frequency Increases with Stage

Stage	Total Pts	Pts w/ tumor mutations	Plasma Sequenced	Plasma mutations detected
I	9	5	4	1 (25%)
II	16	11	5	3 (60%)
III	18	16	12	6 (50%)
IV	7	5	5	4 (80%)
Total	**50**	**37**	**26**	**14 (54%)**

Mutant Allele Frequency in Plasma Increases with Tumor Stage



Improving Detection Sensitivity

- Evolution of SiMSen-Seq:
  - Shorter amplicon sizes

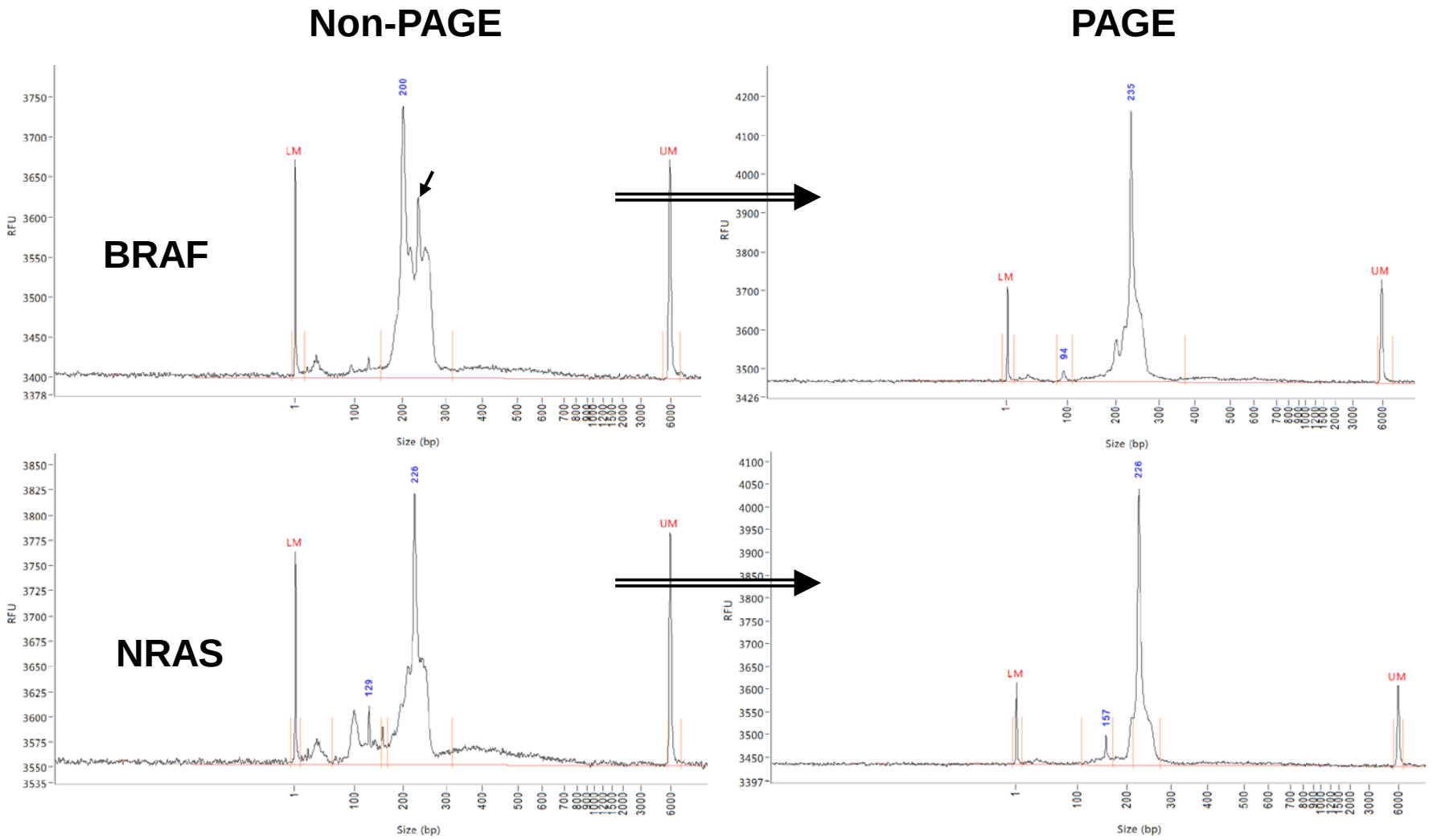
Amplicons <80bp Give Greater Sensitivity

Stage	Pts with tumor mutations	Plasma Sequenced	Plasma mutations detected	Long Amplicons Only	Short Amplicons Only
I	5	4	1 (25%)	0/1 (0%)	1/3 (33%)
II	11	5	3 (60%)	0/1 (0%)	3/4 (75%)
III	16	12	6 (50%)	3/7 (43%)	3/5 (60%)
IV	5	5	4 (80%)	0/1 (0%	4/4 (100%
Total	**37**	**26**	**14 (54%)**	**3/10 (30%)**	**11/16 (69%)**

Improving Detection Sensitivity

- Evolution of SiMSen-Seq:
  - Shorter amplicon sizes
  - PAGE purified hairpin/adapter primers in first round PCR

PAGE Purified Primers give Cleaner Libraries



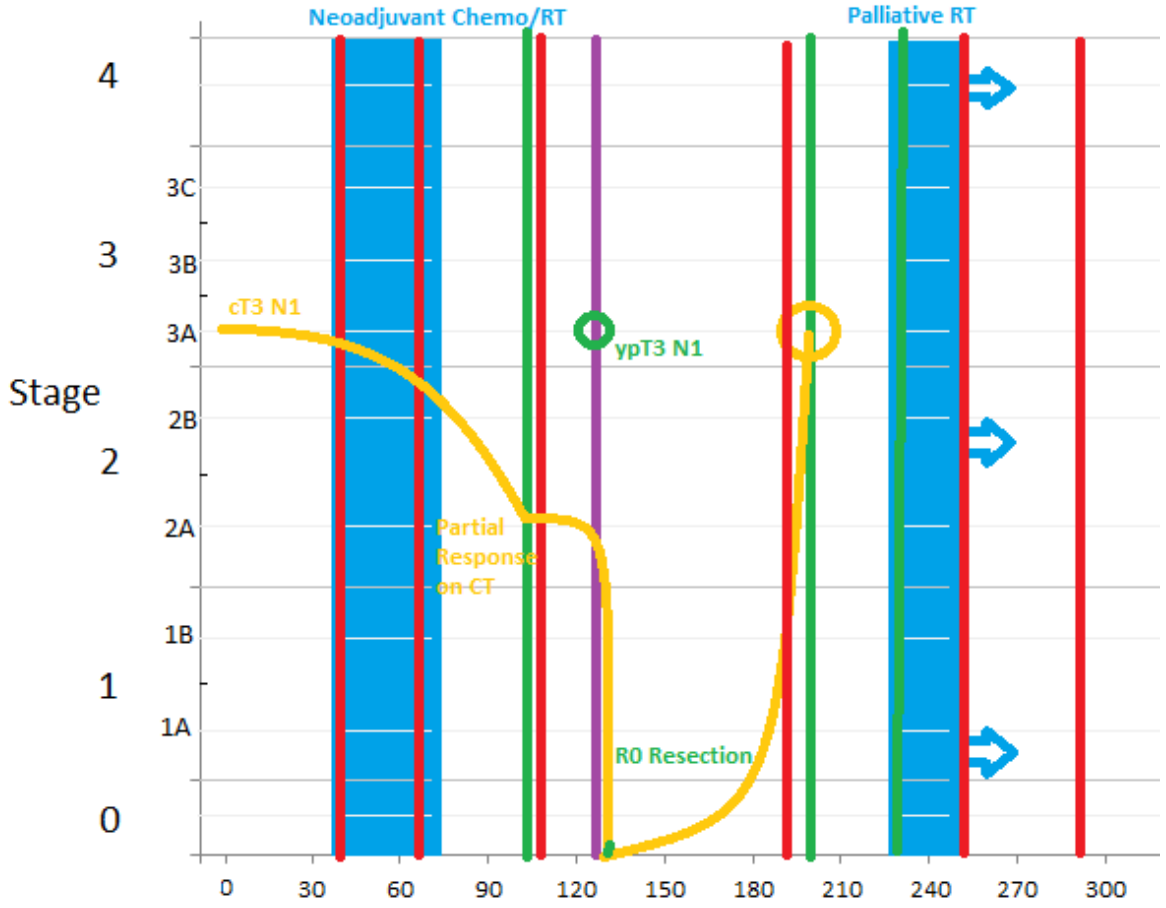
BOSTON MEDICAL
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

Improving Detection Sensitivity

- Evolution of SiMSen-Seq:
  - Shorter amplicon sizes
  - PAGE purified hairpin/adapter primers in first round PCR
    - Higher on-target reads and higher consensus depth
  - True Hi-fidelity polymerase in first round PCR
    - Phusion polymerase reduces background error to <0.02%

Does ctDNA quantity change with Disease Status?



Stage IIIA patient

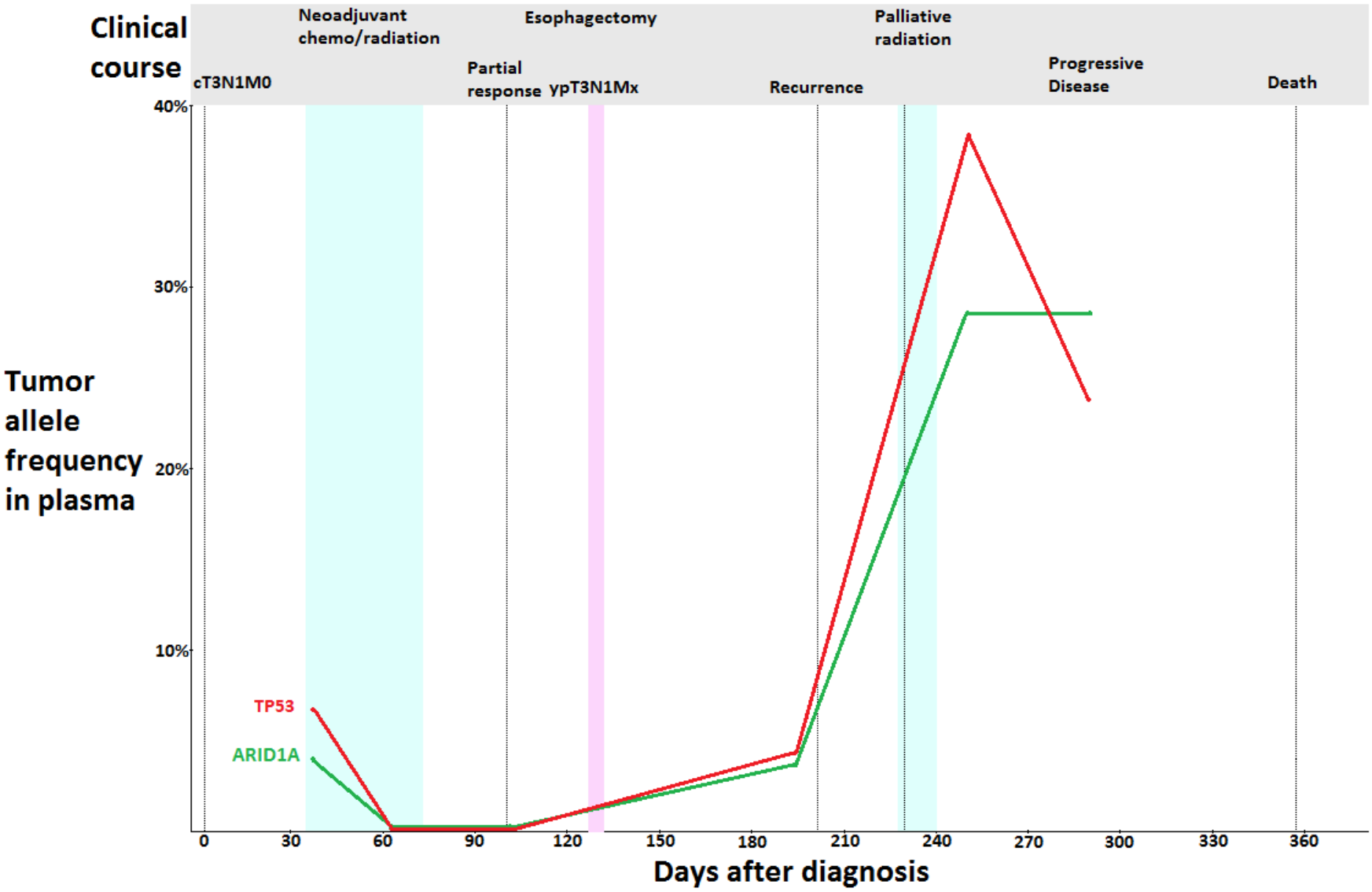
Point Mutations in:
ARID1A
TP53

Blood draws
Imaging Date
Surgery date
Chemo/radiation dates
Clinical Stage
Pathological Stage
Recurrence
Death

BOSTON MEDICAL CENTER
EXCEPTIONAL CARE. WITHOUT EXCEPTION.

BU School of Medicine

ctDNA Level Correlates with Clinical Disease Burden



SiMSen-Seq: Strengths and Weaknesses

- Strengths
  - Easy, fast library construction and relatively simple bioinformatics
    - Implement in individual research and clinical labs
  - Low DNA input requirement (<5ng)
  - Flexible library content
    - Most useful for 1-1000bp coverage
    - Content can be customized easily (individual patient panels, cancer-specific panels, therapeutic panels, companion diagnostics etc.)
  - Low cost for NGS approach (only sequencing regions of interest)
  - Fits perfectly between dPCR and large-scale NGS approaches.
- Weaknesses
  - Up-front assay development needed
  - Limited content relative to other NGS approaches
  - Sensitivity not quite as good as reported for some approaches

ctDNA as a Biomarker in Esophageal Adenocarcinoma

- Early Detection
  - Unclear if sensitivity will be high enough for stage I/II disease
    - But: High risk group known and current paradigm is failing
- Treatment response and recurrence monitoring
  - May have value for rapid identification of response to therapy
  - May identify pre-clinical recurrence
  - May identify residual disease following “curative” treatment
- Other
  - Prognostic biomarker in stage I/II disease

Acknowledgments

Jennifer Jackson
Virginia Litle
Anders Staahlberg
Emma Pinjic
Rhobi Marwa
Daniel Choi
Emiko Yamada
Juan Munoz

Lincoln Stein
Paul Krzyzanowski
Irina Kalatskaya

James Luketich
Arjun Pennathur
Cornelia Smith

David Zhou
Mohammed Tejani
Seher Gul